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From contradiction to prediction: when is hybridization helpful or harmful to invaders?

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Abstract

It is important to understand how and why invasive species become established. Evidence suggests that hybridization plays an important role in the success of some invasive species. In this dissertation, I investigate in-depth the role of hybridization during invasion using two *Cakile* species, *C. edentula* (American searocket) and *C. maritima* (European searocket) (Brassicaceae) and their hybrids. These plant species offer a rare opportunity to investigate the role of hybridization, with naturally occurring, replicated hybrids found on two geographically isolated continents (western North America and Australia). In each invasive range the pattern of invasion, hybridization and replacement is similar, wherein *C. edentula* invaded the new habitat first, followed by an invasion of *C. maritima* and subsequent hybridization of the two parental species and a replacement of *C. edentula* by *C. maritima.* I used two different datasets. First, genome-wide markers were used to identify hybrids, their generation and geographic spread in each invaded range. I found that hybridization occurred in both invaded ranges, although the rate is higher in Australia. Parts of the *C. edentula* genome are retained in samples that are phenotypically *C. maritima*, even long after hybridization has taken place and morphological evidence has disappeared. I also identified evidence for multiple introductions of *C. maritima* in Australia, and multiple introductions of *C. edentula* in western North America. Secondly, a multifaceted approach involving a controlled greenhouse experiment and whole-genome sequencing of these samples was used to answer two main questions: (1) Is there evidence of convergent or divergent patterns of evolution during invasion, between the ranges and species? (2) Is there evidence that rapid adaptive evolution occurred through selection on novel variation generated by hybridization? I found that parallel latitudinal clines evolved in the invasive ranges mirroring those of the home ranges for phenology and size-related traits. Greenhouse data also indicate convergent patterns of enhanced herbivore damage in the invasive range of both species. The genomic results reveal stronger signals of parallel adaptation within and between species. I also found several candidates with signatures of parallel adaptive introgression in invasive *C. maritima*. It appears that these candidate regions are notably involved in defence, chilling response and circadian rhythm. These data suggest that adaptive introgression has contributed to the rapid adaptation of *C. maritima* during its recent range expansion, perhaps even contributing to the local extinction of the donor species, *C. edentula*.

Publications during enrolment

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Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes one original paper published in peer reviewed journals and zero submitted publications. The core theme of the thesis is From contradiction to prediction: when is hybridization helpful or harmful to invaders?. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the School of Biological Sciences, Monash University, under the supervision of Dr Kathryn Hodgins.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.
In the case of *Chapter 2, 3 and 4* my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status	Nature and % of student contribution	Co-author name(s) Nature and % of Co-author's contribution*	Co-author(s), Monash student
2	The tip of the iceberg: genome wide marker analysis reveals hidden hybridization during invasion.	Published	60%. Analysation of data, writing and editing of paper.	1. Geraldes, A., Analyzed data 5% 2. Nurkowski, K.A. Conducted molecular work 5% 3. Battlay, P., analyzed data 5% 4. Cousens, R. D., Conceived study, paper writing and editing, supervision 10% 5. Rieseberg, L. H Conceived study, paper writing and editing 10% 6. Hodgins, K. A. Conceived study, paper writing and editing, supervision 15%	No
3	Convergent and divergent trait evolution during the global range expansion of two co-occurring invaders	In preparation	60%. Sampling, greenhouse experiment, data analysis, writing and editing of paper.	1. Battlay, P. Data analysis, paper writing and editing 7.5% 2. Geraldes, A. Sampling 5% 3. Lee, C. Conducted molecular work 2.5% 4. Wilson, J. Conducted molecular work 2.5% 5. Monro, K. Statistical analysis 2.5% 6. Rieseberg, L. Conceived study, study design 5%	Yes

				7. Cousens, R. Conceived study, sampling, study design, paper writing and editing 5% 8. Hodgins, K. Conceived study, data analysis, paper writing and editing 10%	
4	Introgression contributes to parallel patterns of rapid adaptation in co-occurring global invaders	In preparation	60%. Sampling, greenhouse experiment, data analysis, writing and editing of paper.	1. Battlay, P. Data analysis, paper writing and editing 8% 2. Geraldes, A. Sampling 7% 3. Wilson, J. Conducted molecular work, genome annotation 3% 4. Lee, C. Conducted molecular work 2% 5. Rieseberg, L. Conceived study, study design 5% 6. Cousens, R. Conceived study, sampling, study design, paper writing and editing 5% 7. Hodgins, K. Conceived study, study design, data analysis, paper writing and editing 10%	Yes

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student name: Hanna Sarah Rosinger
Student signature: **Date:** **09/2/2022**

I hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor name: Kathryn Hodgins

Main Supervisor signature: **Date:** **09/2/2022**

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Chapter 1 - Introduction

It can be challenging to observe evolution in nature, but invasive species offer the rare chance to observe evolution on a contemporary timescale (Bock et al., 2015). Through these natural experiments, biologists can gain new insight into ecological and evolutionary processes as species experience novel biotic interactions and must contend with new environments, often distinct in many ways from their home ranges (Bock et al., 2015). Understanding invasions is not only of academic value, it is also important for the economy, the preservation of ecosystems, and human health. The cost of invasive weeds within Australian agricultural areas alone was estimated as $4 billion annually (Hoffmann & Broadhurst, 2016) and in the US, the loss in crop and forest production by alien species was estimated to be US$40 billion (Paini et al., 2016). Further, alien species can reduce biodiversity and threaten ecosystems (Sakai et al., 2001), while humans can also suffer directly from invasive species, such as through allergies and toxic reactions (Vitousek et al., 1996).

Range expansion creates opportunities for novel interactions between previously allopatric species. In some instances, these species are cross-compatible, leading to opportunities for hybridization. A famous example is the expansion of modern humans into Europe, where they came in contact with the Neanderthals, eventually replacing them (Papagianni & Morse, 2015). However, the traces of this interaction can still be seen in the genomes of Europeans, where 2-4% of an individual's genome can be traced to Neanderthal ancestry (Harris & Nielsen, 2016). Traits such as hair colour and immunity (Harris & Nielsen, 2016 and citations within), which may have an adaptive benefit, are associated with such regions of Neanderthal ancestry, but so are predispositions to certain diseases (Harris & Nielsen, 2016).

In this thesis, I used two invasive plant species, *Cakile edentul*a and *C. maritim*a, whose invasion history is similar to the modern human-Neanderthal hybridization. The two alien species hybridize and eventually the resident (alien) species (*C. edentul*a) is replaced by the newcomer (*C. maritima*) (Barbour & Rodman, 1970; Cousens et al., 2013; Rodman, 1974, 1986). By leveraging the invasion history of these species, replicated on two continents, I was able to examine the role of

hybridization during invasion with population genomic analyses, as well as an extensive phenotypic dataset to link phenotype to genotype.

1.1 Evolutionary changes during invasion

1.1.1 The impacts of range expansion on genetic variation

During invasion important evolutionary processes take place, which impact the success of the invasion. Founder effects, which are associated with initial colonization, can reduce genetic diversity significantly as only a fraction of genetic variants of the source populations are established in the new location (Barrett & Husband, 1990; Dlugosch & Parker, 2008; Nei et al., 1975). Colonization events may also involve population bottlenecks, as a result of a small number of initial colonists (Sakai et al., 2001). Therefore, the newly established population is unlikely to be as genetically diverse as the population from which it is derived (Barrett & Kohn, 1991; Dlugosch & Parker, 2008). Theory predicts that thereby their capacity to evolve and adapt to novel conditions is limited (Sakai et al., 2001). Additionally, it also can lead to inbreeding depression, limiting propagule production and population growth (Ellstrand & Elam, 1993; Sakai et al., 2001).

Range expansion following introduction can influence spatial patterns in allele frequencies and quantitative traits. Theoretical work has shown that extreme drift on the wave front of expanding populations can occur, because population density is low and growth rate is high (Edmonds et al., 2004). At the wave front new and standing mutations can increase (i.e., allele surfing) to high frequency, whether they are neutral, deleterious or beneficial (Klopfstein et al., 2006; Peischl et al., 2013). Therefore, range expansion can increase the frequency of deleterious alleles (i.e., expansion load), which can spread and be fixed locally even if they lead to reduced competitive ability and/ or reproduction rates (Peischl et al., 2013; Travis et al., 2007). Expansion load can result from new mutations, maladaptive alleles introduced via hybridization, and standing variation (Peischl & Excoffier, 2015). It can reduce fitness, slow the rate of spread, or even limit a species' range, and can persist for thousands of generations (Bock et al., 2015; Peischl et al., 2013).

Introduced species become abundant despite the negative consequences of the demographic bottlenecks commonly experienced during introduction. This phenomenon is known as the genetic

paradox of invasion (Estoup et al., 2016; Sax & Brown, 2000). However, genetic diversity is not always a requirement for successful plant invasion (Ward et al., 2008). Further, studies have shown that some invasive plants possess significant genetic diversity within the invaded range, whereas other successful invaders have little or no genetic diversity (Dlugosch & Parker, 2008; Ward et al., 2008). Most species lose a moderate amount of genetic diversity as they invade (10-20%) (Dlugosch & Parker, 2008), but extreme reductions are possible (e.g., Hollingsworth & Bailey, 2000). Losses of genetic diversity can be ameliorated by hybridization, and by multiple introductions and subsequent mixing (Barrett & Husband, 1990; Dlugosch & Parker, 2008; Ellstrand & Schierenbeck, 2000; Ward et al., 2008), as this can introduce a large amount of variation through novel genetic combinations. Indeed, despite a long history of research on genetic diversity in invaders, we still lack clear generalities that describe genetic changes over the course of invasion (Ward et al., 2008).

1.1.2 Adaptation during invasion

In the early stages of an invasion it is crucial for the species to adapt to the local environment, as biotic and/ or abiotic aspects might be novel (Atwater et al., 2018; Bock et al., 2015; Broennimann et al., 2007; Colautti & Lau, 2015). Shifts in the composition of enemies or competitors might occur (Colautti et al., 2004; Keane & Crawley, 2002) and one hypothesis posits that invasive species will evolve to divert resources away from defence, to growth or reproduction as a result. This hypothesis is known as the evolution of increased competitive ability (EICA) (Blossey & Notzold, 1995). Species invading a broad environmental gradient often adapt to local climatic conditions. Studies have shown that such adaptation can be rapid, with examples evolving in less than 50 years (Oduor et al., 2016; Whitney & Gabler, 2008). For example, in annual plants in temperate environments, a classic trade of between flowering time and plant size often leads to the evolution of clines in both traits in response to local growing season lengths (Colautti & Barrett, 2013; Griffith & Watson, 2006; Haggerty & Galloway, 2011; Hodgins & Rieseberg, 2011; Leiblein-Wild & Tackenberg, 2014; Santamaria et al., 2003; van Boheemen et al., 2019).

Adaptation strongly depends on genetic diversity on which selection can act (Bock et al., 2015), yet genetic bottlenecks, which are common during range expansion, decrease the standing variation and limit the species ability to adapt. Despite this decrease many invasive species are

successful (Allendorf & Lundquist, 2003) as multiple introductions and subsequent mixing or hybridization can ameliorate the loss of genetic diversity (Bossdorf et al., 2005; Dlugosch & Parker, 2008; Ellstrand & Schierenbeck, 2000). Nonetheless, it is unclear how often hybridization aids or obstructs adaptation during invasion (Bock et al., 2015). On top of its beneficial effects, hybridization can also lead to negative fitness consequences, and premating barriers (i.e., reinforcement) might evolve to oppose it. Such reinforcement can be caused by increased selfing or reductions in the overlap of flowering seasons between species (Comeault & Matute, 2016). Yet, assessing the evolution of such reinforcement has rarely been studied in the context of plant invasion, despite the novel species interactions that often occur (Alexander & Levine, 2019; Beans, 2014).

1.1.3 The role of hybridization during invasion

Hybridization is certainly not the sole evolutionary pathway to invasiveness, but it can catalyse the evolution of invasiveness (Ellstrand & Schierenbeck, 2000, Hovick & Whitney, 2014). Hybridization is the result of interspecific sexual reproduction, whereby the parental generations have been isolated (reproductively and/or geographically) and evolve to become genetically distinct (Rhymer & Simberloff, 1996; Ward et al., 2008). Anthropogenic activities can increase the likelihood of hybridization through long-distance dispersal that brings together previously isolated but closely-related taxa, disturbances that provide habitats suitable for hybrid progeny, or a combination of the two (Ellstrand & Schierenbeck, 2000).

There are several mechanisms by which hybridization can contribute to the evolution of invasiveness in hybrid-derived lineages. Mechanisms which can lead to a benefit though hybridization are:

(1) **Evolutionary novelty.** Novel and transgressive phenotypes are created through recombination, and some of these genotypes might be better adapted to a novel environment experienced during colonization (Stebbins, 1969). Evolutionary novelty may result from fixation of intermediate traits, from recombination of traits from both parents, or from traits that transgress the phenotype of both parents (Ellstrand & Schierenbeck, 2000). Non-additive trait expression is also possible, including extreme, novel or missing traits (Rieseberg & Ellstrand, 1993).

(2) **Increased genetic variation**. Recombination not only generates novel genotypes, but also genetic variation. On this variation selection can act, resulting in increased local adaptation and a potential rescue from maladaptation and/or genetically depauperate founding populations (i.e., evolutionary rescue) (Ellstrand & Schierenbeck, 2000; Hodgins et al., 2018).

(3) **Dumping genetic load** (i.e., genetic rescue). Genetic load and inbreeding depression can be caused by founding events and extreme drift (Peischl et al., 2013). Detrimental mutations can be fixed in small populations with a history of isolation, resulting in a gradual decrease of average fitness (Ellstrand & Schierenbeck, 2000). Hybridization between such populations can introduce superior alleles that can complement or replace deleterious variants (Conte et al., 2017; Ellstrand & Schierenbeck, 2000).

(4) **Demographic rescue.** Demographic factors, such as mate limitation, can lead to a reduced fitness in alien species. Populations which experience the Allee effect (i.e., reduced reproduction due to low density) can be rescued through hybridization without any other beneficial consequence of hybridization, and a theoretical study has shown that this might be the case for the plant species that are the topic of this thesis, *C. edentula* and *C. maritima* (Mesgaran et al., 2016).

(5) **Heterosis**. Hybrids often show an increase in vigor or heterosis, especially in early generations, which might be sufficient for a hybrid lineage to become invasive (Ellstrand & Schierenbeck, 2000). Heterosis is thought to be caused by the masking of recessive, deleterious alleles, causing hybrids to experience enhanced performance (Crow, 1948; Gowen, 1952; Shull, 1952).

However, not all the consequences of hybridization are beneficial and there can be significant costs associated with invasion such as:

(1) **Outbreeding depression**. Chromosomal rearrangements, genetic incompatibilities and/or disruption of adaptation to the local environment lead to outbreeding depression by hybridization (Baack et al., 2015). Strong outbreeding depression can, in extreme cases, lead to extinction, if population growth rates drop below the replacement rates (Hodgins et al., 2018).

(2) **Genetic swamping** (or genetic assimilation or genetic pollution). If the invading species has a relatively small founding population relative to the sympatric or parapatric congener, and the mating barriers are weak between the two species, the invader may experience genetic swamping

(i.e., genetic pollution) and lose their genetic or phenotypic identity (Hodgins et al., 2018; Todesco et al., 2016). The reverse is also possible, where material from the invader is transferred into the native species, resulting in genetic swamping of the native (Ward et al., 2008). Even with no introgression occurring between native and introduced species, native plants may still be swamped with pollen of the invader (Ward et al., 2008). Further, competition between co-occurring congeners has the possibility to contribute to negative competitive interactions, which potentially even impact evolutionary trajectories that may be observed through character displacement (Beans, 2014; Kooyers et al., 2017; Stuart et al., 2014).

It is still unknown why hybridization increases the colonization success of some species but not of others (Bock et al., 2015). One outstanding question is how frequently hybridization actually leads to a positive outcome, as hybridization can often have deleterious consequences (Pfennig et al., 2016). Hybrids can be less fit than their parental species (Barton & Hewitt, 1989; Coyne & Orr, 2004; Darwin, 1859 (2009); Pfennig et al., 2016) and deleterious hybridization has the potential to limit the geographical range of a species, by decreasing the fitness of vulnerable peripheral populations (Bridle & Vines, 2007; Holt & Gomulkiewicz, 1997; Pfennig et al., 2016; Sexton et al., 2009). Additionally, the study of hybridization is hamstrung by difficulties identifying hybrids due to morphologically cryptic evidence (especially in later generations of backcrosses, or when the amount of introgression is low; Pfennig et al., 2016). Whole-genome data are invaluable in studying hybridization, as they provide powerful evidence of introgression events, as well as the functional genetic changes involved in environmental tolerance and invasiveness (Chown et al., 2015).

1.2 The study species

Cakile edentula and *C. maritima*, the focal species of this thesis, exhibit a number of features that make them ideal for examining adaptation and hybridization during invasion. Below I discuss several of these features and provide background information relevant to the system.

The ecosystem in which both species occur is the top of the strandline, on lower foredunes of sandy beaches, and sometimes on edges of saline coastal lakes (Cousens et al., 2013; Rodman, 1974). Both species are salt tolerant, and their floating propagules survive weeks of immersion in sea water (Rodman, 1974). *Cakile* is thought to arrive in Australia and western North America in the

ballast of ships and is therefore an excellent example of an anthropogenic introduction (Barbour & Rodman, 1970; Ridley, 1930; Rodman, 1974). Both species are succulent herbaceous annual (facultative annuals) strand plants and have a chromosome number of 2n=18 (Rodman, 1974). The fact that both species are found in similar habitats, and have invaded the same regions of the globe, make them excellent study species to examine convergent evolution across replicate invasions. Further, genomic analyses are simplified by the fact they are diploid, have relatively small genomes, and are in the same family as the model plant *Arabidopsis thaliana*. Finally, although some reproductive barriers are evident (Li et al., 2019; Mesgaran et al., 2016), the species are cross-compatible and there is both theoretical (demographic rescue) (Mesgaran et al., 2016) and empirical evidence to support the occurrence of hybridization between these species during invasion (Ohadi et al., 2016).

1.2.1 Native range distributions

Cakile edentula is native to the Atlantic coast of North America (from Labrador to North Florida and the Great Lakes of America) and has two subspecies; subsp. *edentula* and subsp. *harperi* (additionally two variants are recognised in the subsp. *edentula*: var. *edentula* and var. *lacustris*; (Cousens et al., 2013; Rodman, 1974). The plant is an annual self-compatible species and is thought to be largely self-fertilizing (Rodman, 1974). *Cakile maritima* is native to Europe and Northern Africa (Rodman, 1974) and several subspecies exist. (1) subsp. *maritima* from the Mediterranean, (2) subsp. *baltica* form the Baltic, (3) subsp. *integrifolia* from the Atlantic Europe, (4) subsp. *euxina* from the Black sea and subsp. *islandica* from the sub-arctic (Marhold, 2011; Rodman, 1974). *Cakile maritima* is an outcrosser with a sporophytic self-incompatible system, but the level of self-incompatibility varies among plants (Rodman, 1974; Thrall et al., 2000). Although closely related and cross compatible, these species are allopatric, separated by the Atlantic Ocean and furthermore, they differ in their mating system.

1.2.2 Invasion history

Both species occur in a wide range of countries around the world. *Cakile edentula* not only occurs in its native range but is also invasive in Australia, New Zealand, western North America (Pacific coast) as well as Japan and Azores (Cousens et al., 2013). Australia, New Zealand, western North America were also colonized by *C. maritima*, as well as New Caledonia, eastern South America

and Iran (Caspian coast) (Cousens et al., 2013). The species' invasion and replacement history is reviewed in detail elsewhere (e.g., Barbour & Rodman, 1970; Cousens et al., 2013; Ohadi et al., 2016; Rodman, 1986), but I will outline briefly what is known based on historic records below.

1.2.2.1 Australia

The documented invasion of Australia is characterized by the rapid spread along the coastline of *C. edentula,* followed by the even faster spread and dominance of *C. maritima*. *Cakile edentula* (subsp. *edentula* var. *edentula*) was first recorded in Victoria in 1860 and since its introduction spread to New South Wales (1870), Queensland (1922), South Australia (1881), Tasmania (1893) and Western Australia (1862) (Rodman, 1974, 1986). Rodman (1986) calculated the migration rate of *C. edentula* at 48 km per year. The introduction of *C. maritima* (subsp. *maritima and* subsp. *baltica/integrifolia*) occurred first in Western Australia (1897), and Rodman (1986) assumed *C. maritima* spread from there through the rest of Australia. However, molecular studies (Cousens et al., 2013; Ohadi et al., 2016; Shaw et al., 2021), have shown that a second introduction of *C. maritima* occurred in South Australia (1918), which subsequently spread to Victoria (1922), New South Wales (1969), Tasmania (1979) and Queensland (2002). A survey in 2012 showed the most southerly *C. edentula* occurred in Hat Head (New South Wales) and the most northern *C. maritima* occurred at Moreton Bay (Queensland). The same survey showed that in Tasmania, *C. edentula* only remained in the south east-corner (Freycinet Peninsula southward to Bruny Island and sole *Cakile* species in D'Entrecasteaux Channel and the Derwent River area), and that *C. maritima* has invaded all of the island, with potential hybrids at the Seven Mile Beach (Cousens et al., 2013). The migration rate of *C. maritima* was calculated as 95 km per year according to Rodman (1986), but this was assuming only a single introduction of *C. maritima* in Western Australia and did not take into account a second introduction in South Australia. Potential hybrids between the two species were first recorded in 1979 in South Australia (Cousens et al., 2013). By 2012 *C. maritima* had replaced *C. edentula* in South Australia, Victoria and parts of New South Wales. The current hybrid zone is defined as New South Wales and Queensland, as well as the south east corner of Tasmania, where beaches are occupied by either species, or the species are found in sympatry (Cousens et al., 2013). In contrast to *C. edentula*, *C. maritima* is still spreading in Australia, including into areas which were previously occupied by *C. edentula*, replacing the latter on its way (Cousens et al., 2013).

1.2.2.2 Western North America

Similar to Australia, *C. edentula* quickly expanded its range up and down the coastline of western North America but was soon replaced by the rapid encroachment of *C. maritima. Cakile edentula* (subsp. *edentula* var. *edentula*) was introduced to western North America at San Francisco Bay in 1880/1882, and only 50 years later it had spread northward to Alaska (Kodiak Island 1931) and southward to the US/Mexico border. *Cakile maritima* reached western North America in 1935 at Stinson Bay (close to San Francisco), and was first observed sympatric with *C. edentula* (Barbour & Rodman, 1970). Within the first 35 years of its arrival, *C. maritima* had spread to British Columbia (1951) in the north and to Santa Barbara to the south (1952), increasing its range and abundance while *C. edentula*'s was reduced to near- extinction (Barbour & Rodman, 1970). In 1970 both species could be found in northern California, Oregon and Washington (Barbour & Rodman, 1970), and a field study in 1993 showed that *C. maritima* replaced *C. edentula* throughout most of coastal California except Oregon and Washington (Boyd & Barbour, 1993). The current hybrid zone is Oregon, Washington and British Columbia (based on field observations 2018, results).

1.2.3 Theories of replacement

The cause of the rapid invasion and replacement of *C. edentula* by *C. maritima* has been a mystery for decades. The fact that both species occupy the same habitat creates opportunities for species interactions, both positive (e.g., reduced Allee effects, adaptive introgression and heterosis) and negative (e.g., competition for resources, pollinators, outbreeding depression) for one or both species. There have been several, non-mutually exclusive hypotheses proposed for the replacement of *C. edentula* by *C. maritima*:

(1) **Direct competition** (Boyd & Barbour, 1993; Cody & Cody, 2004). Barbour and Rodman (1970) excluded the hypothesis of direct competition, as in mixed planting *C. edentula* outcompeted *C. maritima*. In contrast, a glasshouse experiment by Boyd and Barbour (1993) showed that *C. maritima* outcompeted *C. edentula* through an increased height, resulting in it

overshadowing *C. edentula*. However, no difference between the two species was detectable in natural habitats (Boyd & Barbour, 1993).

(2) **Lottery competition.** From a demographic perspective, the two species may undergo lottery competition for limited "safe sites" (Rodman, 1986). *Cakile maritima* may have a competitive advantage through greater longevity (sometimes living two years) and higher reproductive output, increasing the chance for *C. maritima* seeds to establish at those sites (Rodman, 1986). Indeed, Boyd and Barbour (1993) showed that in California, *C. maritima* had a reproductive advantage of 8.8-fold over *C. edentula*, and if *C. maritima* survived two seasons, this number increased to 18-fold (Boyd & Barbour, 1993).

(3) **Disease.** Both species are hosts of the fungal pathogen *Alternaria brassicicola* and studies have shown that there is no difference between species' susceptibility (Bock, 2008; Thrall et al., 2000). However, in theory the species' susceptibility might differ, as an inbreeder is potentially less able to compete in an evolutionary arms race with the disease, relative to the outcrosser (Antonovics et al., 2011). Another possibility is that *A. brassicicola* was introduced by *C. maritima*, which following coevolution with *C. maritima* made it especially damaging to *C. edentula*, and that the combination of increased competition and increased pathogen load on *C. edentula* lead to a net reproductive rate of less than one (Cousens et al., 2013; Linde et al., 2010).

(4) **Climate.** Rodman (1986) suggested that *C. maritima* might be more successful than *C. edentula* in certain regions of the invasive ranges because of climate matching. The Mediterranean-type climate of *C. maritima*'s origin may have aided its invasion of Australia and the southern regions of western North America. By contrast, *C. edentula* originates from more temperate environments in eastern North America and is still at higher densities in more temperate regions of the introduced ranges.

(5) **Coincidence of timing.** We cannot rule out the possibility that the demise of *C. edentula* and spread of *C. maritima* was just a coincidence. Indeed, Heyliger (2007) pointed out that *C. edentula* was already declining in South Australia and western Victoria before *C. maritima* arrived, leading Cousens et al., (2013) to conclude that the replacement might well be coincidence. However, Rodman 1986 also explored this hypothesis and deemed it less plausible than the others, as under this scenario the successful invasion of Australia by *C. edentula* would depend on continual

297 recruitment from native populations for several decades, which would then have had to cease for
298 no known reason. Coincidentally, at the same time *C. maritima* would need to be introduced and
299 its invasion bolstered by continual recruitment. Furthermore, in light of the replicated pattern of
300 replacement in North America, this scenario seems even less likely.

301 (6) **Hybridization.** Even though hybrids have been observed in Australia and western North
302 America, Rodman (1986) suggested this did not contribute to *C. edentula*'s replacement, based on
303 the rare identification of hybrids morphologically. Cody and Cody (2004) were the first authors to
304 conclude that in Australia hybridization may have played a role in the replacement of *C. edentula*.
305 Further, Mesgaran et al., (2016) showed with a model of species interactions that transient
306 hybridization could help *C. maritima* establish by overcoming the Allee effect. Invasive plant
307 populations might experience Allee effects by either low pollinator visitation or a low number of
308 compatible mates (Elam et al., 2007; Elliott & Irwin, 2009), and hybridization with a closely
309 related species can provide a higher number of suitable mates (Mesgaran et al., 2016). If such
310 transient hybridization was a key factor during establishment, I expect to observe signals of
311 introgression in most contemporary introduced *C. maritima* populations. There is also a possibility
312 of extinction by hybridization (Todesco et al., 2016), whereby the rare species (*C. edentula*) is
313 either demographically or genetically swamped by the other species (*C. maritima*) or their hybrids,
314 contributing to its extinction (Todesco et al., 2016). However, the extent of hybridization between
315 these species in the introduced ranges has not been investigated despite its hypothesised role in the
316 colonization and establishment of *C. maritima*.

317 Most of these hypotheses for the replacement of *C. edentula* propose significant ecological
318 interactions between the two species. In that case, I might expect to observe evolutionary responses
319 to these interactions. Few studies have examined the importance of novel species interactions
320 between co-invading species on evolutionary trajectories (but see Matsukura et al., 2016). These
321 replicate invasions leading to sympatry provide an important opportunity to examine such eco-
322 evolutionary processes.

323

324 **1.2.4 Genetic analysis of *C. maritima* and *C. edentula***

325 *Native range population structure*

Studies of genetic variation in *C. edentula* in its home range show three greater groups, mostly in line with the subspecies and variation distribution. One Great Lakes group (subsp. *edentula* var. *lacustris*, Lake Erie, Lake Ontario and northern New England), one North Atlantic coast group (North Atlantic coast, Lake Michigan, subsp. *edentula* var. *edentula*) and a third southern group (North Carolina to Georgia/Florida, subsp. *harperi*) (Gormally et al., 2011; Rodman, 1974, 1976).

In Europe (the native range of *C. maritima*), multiple genetic analyses have shown geographic structuring with rough groups in the Baltics (subsp. *baltica*), in Iceland and northern Norway (subsp. *islandica*), along the Atlantic coast (subsp. *integrifolia*), in the Mediterranean (subsp. *maritima*) and around the Black sea (subsp. *euxina*) (Clausing et al., 2000; Kadereit et al., 2005; Rodman, 1976; Shaw et al., 2021; Westberg & Kadereit, 2009). However, the results of those studies vary slightly in their clustering of populations and the degree of local population structure.

Invasive range population structure

Genetic population structure analysis can be indicative of the invasion history and has shown that *C. edentula* in Australia originated from the subspecies *C. edentula* subsp. *edentula* (Ohadi et al., 2016; Rodman, 1986). In contrast, multiple invasions of *C. maritima* occurred in Australia: one invasion by subsp. *baltica* or *integrifolia* in Western Australia, and a second in south-east Australia from the Mediterranean (Ohadi et al., 2016; Rodman, 1986). Ohadi et al., (2016) also produced the first genetic evidence of hybrids between the two species (using Microsatellites and CAPs makers), which was subsequently also demonstrated by Shaw et al., (2021).

Genetic investigation of the invasion history and hybridization rates in western North America is sparse. Barbour and Rodman (1970) documented the invasion and replacement history of the two species based on morphology, but only one study (Gormally et al., 2011) has used genetic markers (allozymes). This study investigated only *C. edentula* populations and found that *C. edentula* in Oregon (only one population investigated) originated from North Atlantic populations and contained evidence of introgression from *C. maritima*.

1.2 Knowledge gap

A major open question is why hybridization sometimes aids invasion, and why sometimes it does not (Bock et al., 2015). With *Cakile* I have the unique opportunity to investigate the costs and benefits of hybridization during invasion on two isolated continents (Australia and western North America). This study can add to the understanding of not only what evolutionary processes contribute to invasion, but also the outcome of hybridization. Therefore, it will contribute to the field of evolution as well as invasion biology, and furthermore inform management of invasive species by enhancing our understanding of how and why invaders evolve.

1.3 Thesis overview

My main aims were to investigate the extent of hybridization between *C. edentula* and *C. maritim*a during invasion, assess convergence and divergence in patterns of evolution during invasion between the species, and assess the role, if any, hybridization may have played during the successful range expansion of both species, and its potential contribution to the replacement of *C. edentula* by *C. maritima*. My central question was to assess if adaptive evolution occurs rapidly through selection on genetic variation generated by hybridization. To this end, I used genetic and phenotypic data of current populations. I first utilized a genotype-by-sequencing (GBS) dataset from leaf material sampled in the two native ranges and two invasive ranges from both species, to assess the invasion history and quantify the extent of hybridization in the sympatric introduced ranges (western North America and Australia) (Chapter 2). Secondly, I conducted extensive sampling of those four ranges and collected seeds and leaf material. Seeds from selected populations were raised in a common garden experiment. Phenotypes were recorded during the course of the experiment and the genomes of many of the common garden individuals were later re-sequenced (this time with whole-genome sequencing). This approach enabled me to connect phenotypic to genotypic data and formed the bases of Chapters 3 and 4. In Chapter 3, I examined the evidence for convergent or divergent patterns of evolution during invasion among ranges and species. Further, I tested for evidence that rapid, adaptive evolution occurred through selection on novel variation generated by hybridization. Specifically, signals of selection during range expansion and hybridization were investigated in the fourth chapter and the species ancestry of candidate loci was examined to assess evidence for adaptive introgression. The final chapter

385 summarises and discusses my findings in the context of invasion and hybrid biology and suggests
386 possible future research directions.

Chapter 2 - The tip of the iceberg: Genome wide marker analysis reveals hidden hybridization during invasion

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2.1 Abstract

Biological invasions are accelerating, and invasive species can have large economic impacts as well as severe consequences for biodiversity. During invasions, species can interact, potentially resulting in hybridization. Here, we examined two *Cakile* species, *C. edentula* and *C. maritima* (Brassicaceae), that co-occur and may hybridize during range expansion in separate regions of the globe. *Cakile edentula* invaded each location first, while *C. maritima* established later, apparently replacing the former. We assessed the evidence for hybridization in western North America and Australia, where both species have been introduced, and identified source populations with 4561 SNPs using Genotype-by-Sequencing. Our results indicate that *C. edentula* in Australia originated from one region of eastern North America while in western North America it is probably from multiple sources. *Cakile maritima* in Australia is derived from at least two different parts of Europe while the introduction in western North America is from one. Although morphological evidence of hybridization is generally limited to mixed species populations in Australia and virtually absent elsewhere, our genetic analysis revealed relatively high levels of hybridization in Australia (58% hybrids using Admixture) and supported the presence of hybrids in western North America (16% hybrids using Admixture) and New Zealand. Hybrids might be commonly overlooked in invaders, as identification based solely on morphological traits may represent only the tip of the iceberg. Our study reveals a repeated pattern of invasion, hybridization and apparent replacement of one species by another, which offers an opportunity to investigate the role of hybridization and introgression during invasion.

2.2 Introduction

Biogeographic barriers on a global, regional and local scale are often overcome by human activities, leading to biological invasions (Sax & Gaines, 2003; Simberloff, 2013; Vilatersana et al., 2016). Biological invasions can have a large economic impact (Hoffmann & Broadhurst, 2016; Pimentel et al., 2005), as well as severe negative consequences for biodiversity and ecosystems (Sakai et al., 2001). Most long-distance introductions of invasive species in historic times are directly (e.g., ornamentals) or indirectly the result of anthropogenic activities (e.g., via ballast on ships) (Baker, 1974; Ruiz et al., 2000; Sakai et al., 2001). Invasions can also lead to novel interactions between species that previously had not co-occurred and, where there are no strong

reproductive barriers, this may lead to instances of hybridization (Abbott, 1992; Ellstrand & Schierenbeck, 2000; Vallejo-Marín & Hiscock, 2016).

Rather than hybridization just being an incidental event, it could actually facilitate the success of invasive plant species, as invasive hybrid lineages can have increased fecundity and size (Hovick & Whitney, 2014). Various hypotheses have been proposed by which hybridization facilitates rapid range expansion (Bock et al., 2015; Ellstrand & Schierenbeck, 2000), including evolutionary novelty, increased genetic variation, heterosis, dumping genetic load (i.e., genetic rescue) (Ellstrand & Schierenbeck, 2000) and demographic rescue. However, convincing empirical data are limited. Hybridization is certainly not the sole evolutionary pathway to invasiveness, but can catalyze its evolution (Ellstrand & Schierenbeck, 2000). Not all of the potential consequences of hybridization are beneficial, however, and there can be significant costs associated with the phenomenon, such as outbreeding depression (Baack et al., 2015) and genetic swamping (Todesco et al., 2016). Our capacity to assess the role of hybridization during any particular invasion is hampered by the fact that it can be difficult to identify, especially when repeated backcrossing with one parental species has occurred rendering morphological identification difficult (Ward et al., 2008). However, genome-wide molecular markers can provide estimates of the extent of past hybridization and introgression across the genome (Payseur & Rieseberg, 2016).

On the beaches of Australia, the North Island of New Zealand and western North America a repeated pattern of invasion by two species of sea-rocket with contrasting mating systems (Barbour & Rodman, 1970; Cousens et al., 2013; Cousens & Cousens, 2011; Rodman, 1974, 1986) offers a rare opportunity to investigate the role of hybridization during invasion in distinct, geographically isolated regions. *Cakile edentula* (American sea-rocket), native to eastern North America, invaded each location first, while *Cakile maritima* (European sea-rocket) (Brassicaceae), native to Europe and northern Africa, arrived later. The invasion and replacement history in western North America and Australia are reviewed elsewhere (Barbour & Rodman, 1970; Cousens et al., 2013; Rodman, 1986), but is briefly outlined below.

In Australia, *C. edentula* was first recorded in Victoria in 1863 and subsequently spread along the coastline of Australia (Rodman, 1986). In 1897, *C. maritima* was recorded for the first time in

Western Australia, and a second introduction into South Australia (1918: see Cousens et al., 2013; Ohadi et al., 2016) spread from there to the east (Heyligers, 1984; Rodman, 1986). In contrast to *C. edentula*, *C. maritima* seems still to be actively spreading in Australia and appears to have replaced *C. edentula* throughout much of its initial introduced range (Cousens et al., 2013; Rodman, 1986). In western North America, a similar pattern of replacement occurred. *Cakile edentula* was found near San Francisco around 1880 (Barbour & Rodman, 1970), while *C. maritima* reached western North America by 1935 where it was found sympatric with *C. edentula* near San Francisco. The most recent published field study showed that *C. maritima* had replaced *C. edentula* throughout most of coastal California but not Oregon or Washington (Boyd & Barbour, 1993). In each case, there has been complete replacement of *C. edentula* by *C. maritima* over wide geographic areas (Barbour & Rodman, 1970; Cousens et al., 2013; Rodman, 1986), which was originally assumed to involve either direct or indirect competition (Rodman, 1986), although several additional mechanisms have been proposed such as disease (Bock, 2008; Cousens et al., 2013; Thrall et al., 2000), coincidence (Cousens et al., 2013; Rodman, 1986) or greater lifetime fecundity of *C. maritima* (Boyd & Barbour, 1993). However, the mechanism of the replacement remains unclear.

Cakile edentula and *C. maritima* are closely related and cross-compatible (Li et al., 2019; Mesgaran et al., 2016; Rodman, 1974). Both species are found in coastal strandline habitat, providing opportunities for hybridization in regions where they co-occur, but the species exhibit contrasting mating systems (Rodman, 1974). *Cakile edentula* (self-compatible) benefits from high levels of reproductive assurance as it is able to set seeds autonomously at high rates (Li et al., 2020); one of Baker's (1965) ideal weed traits. In contrast, the establishment of *C. maritima* (self-incompatible) may be initially hindered (during both initial establishment as well as subsequent range expansion) by a lack of compatible mates limiting sexual reproduction and resulting in strong Allee effects. The apparent presence of hybrids, based on an intermediate leaf and fruit shape of both parental species, in some sites in Australia led Mesgaran et al. (2016) to develop a model for the interacting species, with the novel outcome that transient hybridization could overcome Allee effects in *C. maritima*. As a consequence, we hypothesized that past hybridization with *C. edentula* could be a common feature of *C. maritima's* establishment and range expansion in western North America, Australia and New Zealand.

We used genome-wide markers derived from genotype-by-sequencing (GBS) to examine the invasion history of these two species in Australia and western North America and quantify the extent and distribution of hybridization. There have been several previous studies examining the population genetic structure of *C. edentula* and *C. maritima* in their native ranges in Europe (Clausing et al., 2000; Kadereit et al., 2005; Westberg, 2005), Africa (Gandour et al., 2008), eastern and western North America (Gormally et al., 2011) as well as in the introduced range of Australia (Ohadi et al., 2016). However, no study of the invasion history on two continents has been attempted nor has the extent of hybridization across multiple introductions been quantified. Specifically, we aimed to: (i) Identify probable source regions (from Europe and eastern North America); (ii) determine whether both recent and advanced generation hybrids occur in the introduced ranges and the extent of their geographic distribution; and (iii) determine if the change in levels of species ancestry post-invasion reflects a chronosequence along the direction of invasion of *C. maritima*. We predicted that early generation hybrids should be present at the leading edge of *C. maritima's* invasion into *C. edentula*-occupied areas, but later generation backcrosses with *C. maritima* should be more common in areas closer to where *C. maritima* first established. This should contribute to a gradient in species ancestry whereby *C. maritima* ancestry will be dominant in hybrids near the invasion source, while *C. edentula* ancestry will be more prevalent in hybrids identified in areas recently invaded by *C. maritima*. We predicted high levels of *C. maritima* ancestry in hybrids near the invasion source because *C. maritima* phenotypes are now exclusively present in the regions surrounding the invasion source, and studies of pollinators suggest preferential visitation of both hybrids and *C. maritima* over *C. edentula* which should facilitate backcrossing to *C. maritima* (Mesgaran et al., 2016).

2.3 Methods

2.3.1 Study species

Cakile maritima's native range extends over a wide climatic range from northern Norway to northern Africa. Current taxonomy recognizes subsp. *maritima* (Mediterranean), subsp. *baltica* (Baltic), subsp. *integrifolia* (Atlantic coast), subsp. *islandica* (Northern Europe and Northwestern Russia) and subsp. *euxina* (Black Sea) (Marhold, 2011). This is paralleled in the western Atlantic

by *C. edentula*, for which two subspecies are recognized in its native range (Rodman, 1974) subsp. *edentula* (Labrador to North Carolina) and subsp. *harperi* (North Carolina to Florida). Although *C. maritima* has a sporophytic self-incompatibility system, the level of self-incompatibility varies among plants (Thrall et al., 2000). *Cakile edentula* is self-compatible and can set seed autonomously at a high rate (Barbour, 1970; Rodman, 1974), although field estimates are suggestive of intermediate levels of autonomous selfing (Li et al., 2020). Both species are diploid (2n = 18) (Rodman, 1974). Hybrids are readily produced through artificial pollination (Rodman, 1974) with either parent as the pollen donor when emasculated (Li et al., 2019; Mesgaran et al., 2016), although crosses are more successful when *C. edentula* acts as the pollen recipient, consistent with the SI × SC rule (Harrison & Darby, 1955).

2.3.2 Samples

Samples of *Cakile spp.* were obtained from the native ranges (Europe and northern Africa, eastern North America) and the two introduced ranges (Australasia, western North America). We collected four of the five subspecies (subsp. *baltica*, subsp. *maritima*, subsp. *integrifolia* and subsp. *islandica*) of *C. maritima*. In the native range of *C. edentula* we sampled only *C. edentula* subsp. *edentula* as this subspecies is most likely the source of invasions in Australia and western North America (Cousens et al., 2013; Rodman, 1974). We obtained 214 samples of *C. maritima*, 137 samples of *C. edentula*, 17 putative hybrids (identified by morphology in the field) and two *C. lanceolata* samples from 92 locations in total (Figure 2-S1; Table 2-1 and Table 2-S1). Most samples were our own field collections of silica dried leaf tissue although a few samples were purified DNA from colleagues. We collected our samples along a transect through a population, ensuring that individuals were at least 2 m apart to avoid sampling close relatives or the same individual and collected individuals randomly with respect to their putative species based on morphology.

549 **Table 2- 1. Number of individuals and sampling locations as well as range is presented.**

550

Range	Phenotype	Number of individuals	Number of sampling locations	Mean number of individuals sampled per sampling location
Eastern North America	*C. edentula*	55	26	2.03
	C. lanceolata	2	2	1
Europe and northern Africa	*C. maritima* subsp. *integrifolia and baltica*	12	12	1
	C. maritima subsp. *maritima*	12	12	1
	C. maritima subsp. *islandica*	1	1	1
Western North America	*C. edentula*	39	4	4
	C. maritima	79	10	5.9
	Hybrids	2	1 (in mixed)	/
	Unknown	1	0 (in *C. edentula*)	/
	Mixed populations		3	15.6
	Total	120	17	7.05
New Zealand	Unknown	1	1	1
Australia	*C. edentula*	43	3	7.33
	C. maritima	110	11	8
	Hybrids	14	5 (in mixed)	/
	Mixed population		7	8.4
	Total	167	21	7.95

551

2.3.3 DNA extraction and genotype-by-sequencing

We performed DNA extractions from dried leaf material using a modified CCDB DNA Extraction Protocol following Whitlock et al. (2008). DNA quantity was assessed using a QuBit broadsensitivity DNA quantification system (Invitrogen, Carlsbad, CA, USA) and a double-digest GBS library preparation was carried out (using PstI-HF (NEB) and MspI (NEB) enzymes, see Appendix I for details). Sequencing (125 bp PE) was conducted on an Illumina HiSeq2500 (McGill University and Genome Quebec Innovation Centre) on two lanes.

2.3.4 SNP calling

Quality statistics of raw reads were assessed though FastQC (http:// hannonlab.cshl.edu/fastx_toolkit) and the reads were demultiplexed using STACKS process_radtags (Catchen et al., 2011). We removed adapter sequences and trimmed the reads using Sickle (N. A. Joshi & Fass, 2011) with a *Q*-score of ≥20 and read length of ≥20 base pair. FASTQ quality filter (http://hannonlab.cshl.edu/fastx_toolkit) was then used to filter for reads with a Q- score of 20 or greater for ≥90% of the read length. The filtered reads were aligned using the Burrows- Wheeler Aligner (BWA) (H. Li & Durbin, 2009) to a *C. maritima* draft genome. Early access to the draft genome was provided by S.I. Wright, University of Toronto (https://genome.jgi.doe.gov/portal/Cakma rStandDraft/CakmarStandDraft.info.html, GenBank: MK637688.1). The reference genome is found in 26,153 scaffolds with a scaffold N50 of 85,425. We assessed if there was a bias when mapping the reads of *C. edentula* to the reference genome of *C. maritima* but found limited evidence for such a bias (see Figures 2-S2 and 2-S3).

We called variants with GATK HaplotypeCaller (Poplin et al., 2017). We refer to this as the *unfiltered data set*. Using VCFtools (Danecek et al., 2011) we removed individuals with fewer than 25,000 reads, removed indels and restricted individual genotypes to have a depth between 5–100,000. Furthermore, we filtered for a minimum quality score of 20, a genotype quality of 20, and a minor allele frequency of 0.05. Subsequently, we kept only biallelic variants that were successfully genotyped in more than 50% of individuals and removed individuals that had more than 50% missing data. The above filtering steps resulted in 18,573 SNPs from 258 individuals. Additionally, we removed 121 SNPs which showed >80% observed heterozygosity, because such

high observed heterozygosity could be caused by paralogues. We refer to this as the *filtered data set*, which had a mean coverage of 39.21 (minimum coverage 9.18, maximum coverage 504.73).

2.3.5 Genetic clustering

Population genetic structure was inferred using Admixture (Alexander et al., 2009). For Admixture and most of our analysis we thinned our *filtered data set* for linkage using a single SNP per 1 kb window, resulting in a reduction to 4561 SNPs from 257 individuals (excluding the outgroup *C. lanceolata*). We refer to this as the *global thinned data set*. We ran Admixture using the *global thinned data set* with a major termination criterion of 1×10^{-9}, 1,000 bootstraps and 10-fold cross-validation for $K = 1–10$, where K equals the number of genetic groups. The K that produced the lowest cross-validation error was selected as the best K value. We refer to this as the *unsupervised run*. All following analyses were conducted in R v.3.5.2 (R Core Team, 2018) except where otherwise stated. The output of Admixture visualized with pophelper v.2.3.0 (Francis, 2017) and pie charts.

To complement the population clustering analysis and to provide further insight in the population differentiation, we conducted a principal component analysis (PCA) and an unrooted phylogenetic network analysis. Genetic differentiation between native and introduced populations was summarized in a PCA with an 95% confidence ellipse using the R package SNPRelate (Zheng et al., 2012), tidyverse (Hadley Wickham et al., 2019) and car (Fox & Weisberg, 2019) on the *global thinned data set*. We used SPLITSTREE5 (Huson & Bryant, 2006) to visualize the overall sample relatedness with an unrooted phylogenetic network. To do this, we created two data sets from our unfiltered data set (see details in Appendix I); (i) a global data set containing all samples (*global Splitstree data set*); and (ii) a native range data set containing samples from Europe and eastern North America (*native range Splitstree data set*).

2.3.6 Hybrid identification

We used three different approaches to identify hybrids using genetic data:

1. A *supervised run* of Admixture for $K = 2$ using the *global thinned data set*, by setting the samples from the two native ranges as reference individuals. Providing known ancestries allows the program to set some rows in the matrix Q to known constants and provides a

more accurate estimation of the ancestries of the remaining individuals, and of the ancestral allele frequencies (Alexander et al., 2009). The other settings were retained from the unsupervised run. We refer to this as the *supervised run* and used this run to classify individuals by their *Q*-scores as hybrid, or pure species. We used the highest standard error from the Q scores, resulting in individuals classified as hybrids if $0.025 < Q > 0.975$ of their genome was assigned to the *C. edentula* cluster.

2. We used the program NewHybrids (Anderson & Thompson, 2002) to identify early generation hybrids. It classifies their generation using a Bayesian model-based clustering framework to compute, by Markov chain Monte Carlo, the posterior probability that each individual belongs to each of the distinct first two generation hybrid classes (parental species, F1, F2, BC to species 1, BC to species 2). As the program is unable to deal with a large data set, we restricted our data to 63 SNPs that showed fixed differences between the two species obtained from individuals classified as parental species using the *supervised run* of Admixture. Details of the settings used are provided in the Appendix I.
3. We used the R package HIest (Fitzpatrick, 2012), which uses maximum likelihood to estimate ancestry and heterozygosity. For this package, we used the 471 loci that showed fixed differences between the individuals of the native ranges. Because it is possible that there is a low level of segregating variation within each species for these loci due to sampling error, particularly for SI *C. maritima* where the sample size is lower, we set the allele frequencies as 0.99 for *C. edentula* and 0.03 for *C maritima*. We also tested other SNP sets and allele frequencies. The details of the settings used and the hybrid assignments along with the results are provided in Appendix I.

We tested for a chronosequence by assessing if there was a correlation between the distance of each population from the first entry point of *C. maritima* (Adelaide in Australia, San Francisco in western North America) and the level of *C. maritima* and *C. edentula* ancestry using a Spearman's rank correlation test in R using the ggpubr package (Kassambara, 2020). We used the ranked order of populations from this origin point along the coastline for each range. In Australia, we only used the south-east mainland individuals (see Appendix I for details). We tested the correlation between the Q value of the *C. edentula* cluster of the *supervised run* for each population and the rank order of the sampling locations along the coastline to the first entry point of *C. maritima*. We used

individuals that were classified as hybrids by Admixture or all samples (including the parental species). We repeated this analysis using the S value from HIest and the hybrid classifications of this program.

Additionally, we used the program TreeMix (Pickrell & Pritchard, 2012) to identify evidence for hybridization in the introduced ranges using the *global thinned data set* for which we constructed maximum likelihood trees and calculated the f_3 statistic (for details see Appendix I).

2.3.7 Genetic diversity and differentiation

Genetic diversity and differentiation within the two native ranges and two introduced ranges were assessed for the 256 individuals (the New Zealand and *C. lanceolata* samples were excluded) using the *global thinned data set*. We calculated observed heterozygosity (H_O) and allelic richness (A_R) with the diveRsity package (Keenan et al., 2013). The 95% confidence intervals of A_R were calculated with 1000 bootstraps. We estimated differences in genetic diversity between the species and ranges because we expected self-fertilization in *C. edentula* and bottlenecks potentially experienced during introduction would reduce diversity. Because sampling at individual locations was limited in the native ranges, we grouped individuals based on their range, and their hybrid ancestry (pure parental or hybrid) using the supervised run *Q*-value assignments of the *global thinned data set* into eight groups. We used the *Q* value assignment of the *C. edentula* cluster and the highest standard error (0.024) of the *supervised run* to classify individuals. To determine regional differentiation, we calculated Weir and Cockerham's (1984) pairwise F_{ST} between the above eight groups using the *global thinned data set* with VCFtools (Danecek et al., 2011). Additionally, we calculated the F_{ST} for pure parental individuals, grouping individuals according to their Admixture cluster from the *unsupervised run* and range (see Appendix I).

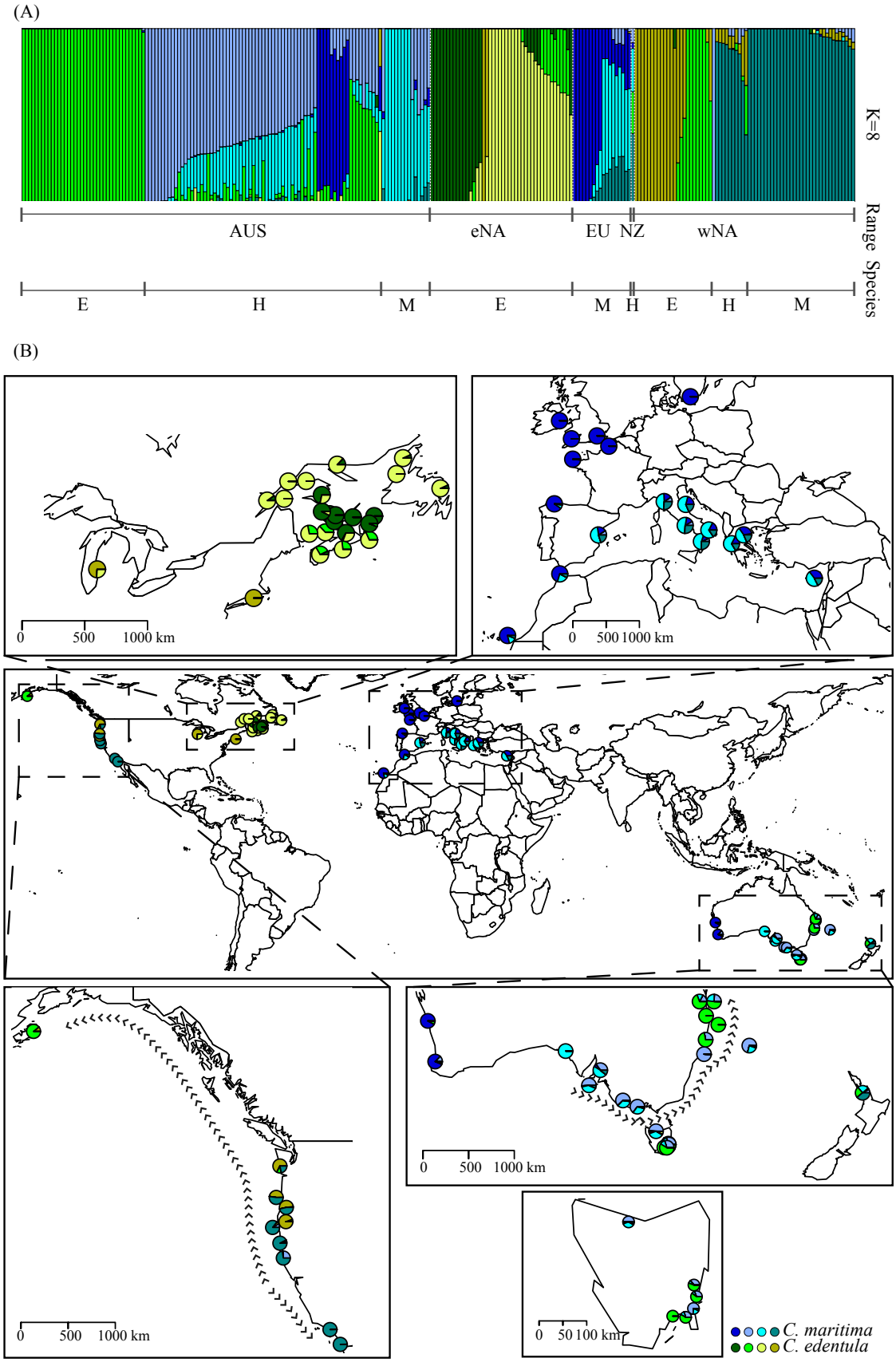
2.4 Results

2.4.1 Genetic structuring and differentiation

The Admixture analysis of the *unsupervised run* showed genetic structuring of *C. maritima*, *C. edentula* and hybrids with an optimal K value of 8 (Figure 2-1a,b and Table 2-S4). Genetic structure was present in the native range of *C. edentula*, where single samples from Lake Michigan

674 and Rhode Island constituted one group, samples from New Brunswick within the Gulf of St.
675 Lawrence a second group, samples from Newfoundland and Quebec (along the St Lawrence River)
676 a third group and samples from Nova Scotia a final group. As expected, for *C. maritima*, there
677 were two main groups: one group was largely from the Baltic and Atlantic coasts, which we termed
678 the "Atlantic" group (comprising mainly the dark blue cluster, Figure 2-1a,b) and a second
679 admixed group was associated with the Mediterranean, that we termed the "Mediterranean" group
680 (comprising mainly the light and medium blue clusters, Figure 2-1a,b). In Australia, several
681 genetic clusters were identified. First, in Queensland, New South Wales and Tasmania we
682 identified pure *C. edentula* individuals. Second, for populations along the west coast of Australia,
683 we identified a *C. maritima* cluster associated with the Atlantic coast in the native range. Third, in
684 South Australia, genetic clusters associated with the Mediterranean were found. In the south-east
685 of Australia there was evidence of hybrids between *C. maritima* and *C. edentula* (see below). In
686 the introduced range of western North America, we identified pure *C. edentula* along with pure *C.*
687 *maritima* (Figure 2-1a,b). A small number of samples from Washington, Oregon and California
688 showed evidence of hybridization (see below).
689
690 The PCA and SPLITSTREE5 analyses confirmed the findings of Admixture. There was clear
691 differentiation of *C. maritima* and *C. edentula* in the *global thinned data set*. The first eigenvector
692 (EV) (Figure 2-2a and Figure 2-S5A) explained 33.17% of the variation and clearly delineated the
693 species. The *C. edentula* group showed less variation than the *C. maritima* group along the first
694 two EVs. Two *C. maritima* groupings were also evident with one representing *C. maritima* from
695 Europe and Australia (EV1 < 0, EV2 < 0) and the other representing exclusively *C. maritima* from
696 western North America (EV1 < 0, EV2 > 0). In the SPLITSTREE5 network, using the *global*
697 *Splitstree data set*, *C. edentula* (as identified by the *supervised run*) formed a monophyletic group
698 without admixture. *Cakile maritima* samples were split into three groups (Figure 2-2b,c): *C.*
699 *maritima* (Mediterranean group), *C. maritima* (Atlantic group) and *C. maritima* in western North
700 America. Hybrids of the two species were scattered in between the *C. maritima* groups or between
701 the two-parental species along the network. The additional native range SPLITSTREE5 analysis
702 (Figure 2-S6) mirrored this pattern but provides clearer *C. edentula* grouping in the native range.
703 Pairwise F_{ST} (Table 2-S2) using the *global thinned data set* revealed clear genetic differentiation
704 between the two-parental species originating from the native range ($F_{ST} > 0.527$). Within the

introduced ranges the pairwise F_{ST} between the two species was similar to the comparison of the native ranges. Hybrids identified using Admixture in the introduced ranges showed higher genetic differentiation from *C. edentula* than from *C. maritima* (Table 2-S2).


(A)
K=8
AUS
eNA
EU
NZ
wNA
Range
E
H
M
E
M
H
E
H
M
Species
(B)
0 500 1000 km
0 500 1000 km
0 500 1000 km
0 500 1000 km
0 50 100 km
C. maritima
C. edentula

708

Figure 2- 1. Admixture results of the *unsupervised run* of the *global thinned data set.*
(a) A distruct plot for $K = 8$. Individuals are ordered according to their cluster association of the *supervised run*. AUS= Australia; eNA= eastern North America; EU= Europe and northern Africa; NZ= New Zealand; wNA= western North America; E=*C. edentula*; M=*C. maritime*; H= Hybrids. (b) Population pie charts for $K = 8$, Admixture proportions for each population are displayed. A global map is displayed as well as close ups of western North America, Europe, the Australian mainland and Tasmania. Colours correspond to the clusters in the distruct plot. Arrows indicates direction of invasion and direction of Spearman's rank correlation test.

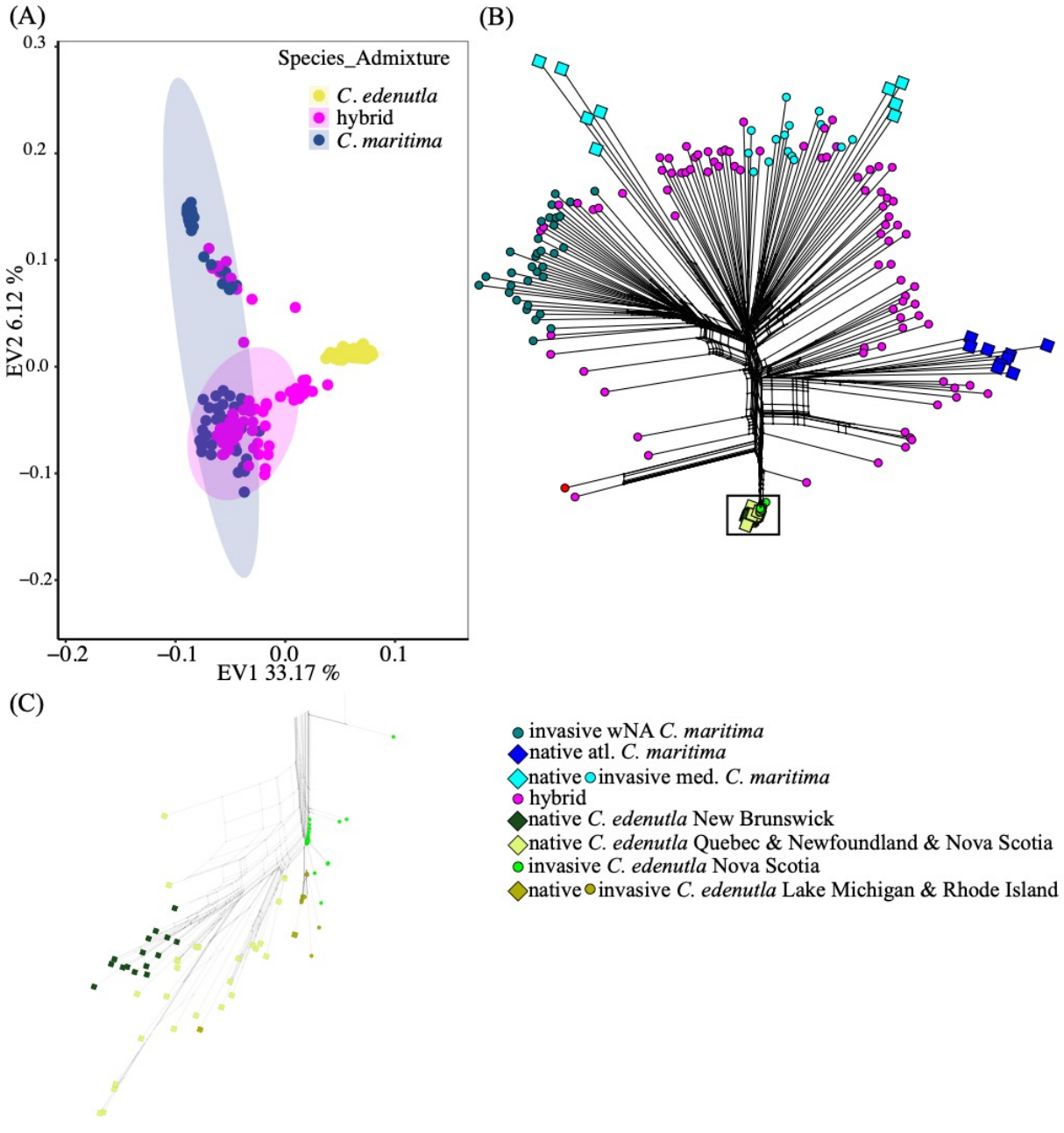


Figure 2- 2. Principal component analysis of the global thinned data set.
First two eigenvectors are presented. Individuals are coloured according to their species and hybrid status based on the supervised run of Admixture. Ellipses indicate the 95% confidence range of the cluster. (b) Splitstree network of the global 0.1 Splitstree data set. Individuals are coloured according to their predominant cluster of the *unsupervised run* of Admixture cluster 0.0 (K = 8 of the *global thinned data set*), with hybrids identified using the *supervised run*. The shapes indicate native vs. invasive −0.1 range.

2.4.2 Genetic diversity

Population statistics revealed that in their native ranges, *C. edentula*, the self-compatible species, has considerably less H_O than *C. maritima* and the hybrids of the two species (Table 2-S3). A_R was significantly reduced in *C. edentula* in comparison to *C. maritima*, the largely self-incompatible species. In the introduced ranges, no clear reduction of H_O or A_R was observed in either of the species. Hybrids of the two-species had higher H_O and A_R compared to both parental species.

2.4.3 Hybrid classification

The three approaches classified different proportions of individuals as hybrids, as expected due to their ability to detect recent hybrids (NewHybrid, HIest), vs. hybrid ancestry (Admixture, HIest). All hybrids identified by NewHybrids were also identified as hybrids with HIest and Admixture (Tables 2-S4 and 2-S5). The fourteen putative hybrids included in the samples as a result of morphological identification were assigned by all analyses as hybrids, providing evidence of the accuracy of the assignments. Furthermore, the NewHybrid analysis confirmed that these hybrids were probably the product of the first two generations of interbreeding. NewHybrids analysis revealed 19 hybrids (Figure 2-3; Table 2-S4) with 17 hybrids in Australia (13.49%), one in western North America (1.47%) and one in New Zealand. In Australia, F1 and F2 hybrids were detected in the current sympatric zones where individuals with both species' phenotypic traits were clearly identifiable in the populations. Hybrids (Figure 2-S5B) grouped in the PCA according to their generation, with F1 and F2 hybrids grouped between the parental species, and backcrosses grouped closer to species they backcrossed to. In this same PCA the advanced generation hybrids identified with the *supervised run* of Admixture as well as HIest frequently grouped with *C. maritima*, suggestive of further backcrossing to that species.

Classification of hybrids using the *supervised run* of Admixture revealed 73 hybrids in Australia (57.94%) from 15 locations, 11 hybrids in western North America (16.18%) from five locations and one hybrid from New Zealand (Figure 2-1; Table 2-S4). In western North America hybrids were found in each of two locations in California and Oregon and in one location in Washington.

All Admixture hybrids were also identified as hybrids in HIest and the ancestry assignments were highly correlated between the programs (Figure 2-S7). When the 471 loci that are fixed between

native range samples were used, and we allowed for a low level of polymorphism within each species (0.99 *C. edentula*, 0.03 *C. maritima*), a larger number of hybrids were identified using HIest than Admixture (138 vs. 85, Table 2-S6). Changing the allele frequencies and SNP set impacted the number of hybrids identified (see Appendix I), but this only influenced the classification of individuals with an apparent low level of ancestry from the alternate species. In all the runs, advanced generation hybrids were identified in this analysis with many in regions where *C. maritima* has not been recorded for many decades, but also in the current sympatric zone (New South Wales, Queensland and Tasmania).

We then examined if patterns of ancestry in Australia and western North America reflected the likely invasion route of *C. maritima*. Specifically, we tested if low levels of *C. edentula* ancestry were found in areas where *C. maritima* first arrived, and if high levels of *C. edentula* ancestry were found in regions *C. maritima* has more recently invaded and where *C. edentula* is still present. Using the supervised Admixture analysis, the mean *C. edentula* ancestry of hybrids at each location was correlated with the ranked distance from where *C. maritima* first arrived in south-eastern mainland Australia ($\rho = 0.82$, $p < .01$) (Table 2-2). This pattern was also significant when testing across all samples, including individuals identified as parental species ($\rho = 0.89$, $p < .05$). However, in western North America, although the direction of the correlation was as predicted, a geographic pattern in ancestry was only significant when using locations north of San Francisco as well as parental and hybrid individuals ($\rho = 0.72$, $p < .05$). The same pattern of significance was found when using the results of the HIest (Figures 2-4 and 2-5, Table 2-S8; Table 2-2).

Table 2- 2. Results of the Spearman's rank correlation test in the introduced ranges examining the association between species ancestry for *C. edentula*, *C. maritima* and hybrids or hybrids and the rank order of sampling locations based on the distance along the coastline from the first recorded case of *C. maritima* in western North America (San Francisco) or south-east mainland of Australia (Adelaide).

			Q			S	
Range	Species	# populations (# individuals)	ρ	p	# populations (# individuals)	ρ	p
south-east Australia	*C. edentula*, *C. maritima*, hybrids	10 (65)	0.815	**0.004**	10 (65)	0.815	**0.004**
	Hybrids	7 (30)	0.893	**0.012**	8 (38)	0.905	**0.005**
western North America	*C. edentula*, *C. maritima*, hybrids	10 (68)	0.511	0.132	10 (68)	0.576	0.088
all sampling locations	Hybrids	5 (11)	0.300	0.683	10 (50)	0.467	0.213
western North America	*C. edentula*, *C. maritima*, hybrids	8 (47)	0.719	**0.045**	8 (47)	0.810	**0.022**
north of San Francisco	Hybrids	5 (11)	0.300	0.683	7 (30)	0.679	0.110

Note: Spearman's Rank Correlation Coefficient ρ and p-values are presented for correlation between Q-value of the *supervised run* of the *C. edentula* cluster for each population in western North America and Australia and correlation between ancestry index (S) (Figure 2-4) and rank order of sampling locations.

Table 2- 3. Results of the f_3 statistic using TreeMix.

Range	Target	Source 1	Source 2	f_3	Standard error of f_3	Z-score
Australia	Australian hybrids	Australian *C. edentula*	Australian *C. maritima*	**-0.0058**	0.0002	**-31.9723**
w. North America	w. North American hybrids	w. North America *C. edentula*	w. North American *C. maritima*	**-0.0049**	0.0002	**-23.2228**

Note: Tests of admixture in the invasive range of Australia and western North America were done separately and both were based on three groups (hybrids, *C. edentula*, *C. maritima*). Hybrid classification was done according to the *supervised run* of Admixture. The f_3 statistic, the standard error of f_3 and the Z-score are reported.

We used TreeMix to assess geneflow between *C. edentula* and *C. maritima* within each introduced range. The maximum likelihood tree in both invasive ranges showed bidirectional gene flow (Figure 2-6). In Australia geneflow occurred from the *C. edentula* branch into Australian *C. maritima* (Mediterranean); a migration event also occurred from this group into the *C. edentula* branch (Figure 2-6b). In western North America the same pattern occurs. There is evidence of a migration event from the *C. edentula* branch into western North American *C. maritima* as well as a migration event from the western North American *C. maritima* branch into the western North American *C. edentula* (Figure 2-6a). The f_3 statistic of TreeMix (Table 2-3) confirmed that the hybrids (identified by the *supervised* Admixture *run*) in the introduced range are admixed from the *C. edentula* and *C. maritima* parental individuals within both introduced ranges (Australia f_3 = –0.006, Z = –31.97; western North America f_3 = –0.005, Z = –23.22).

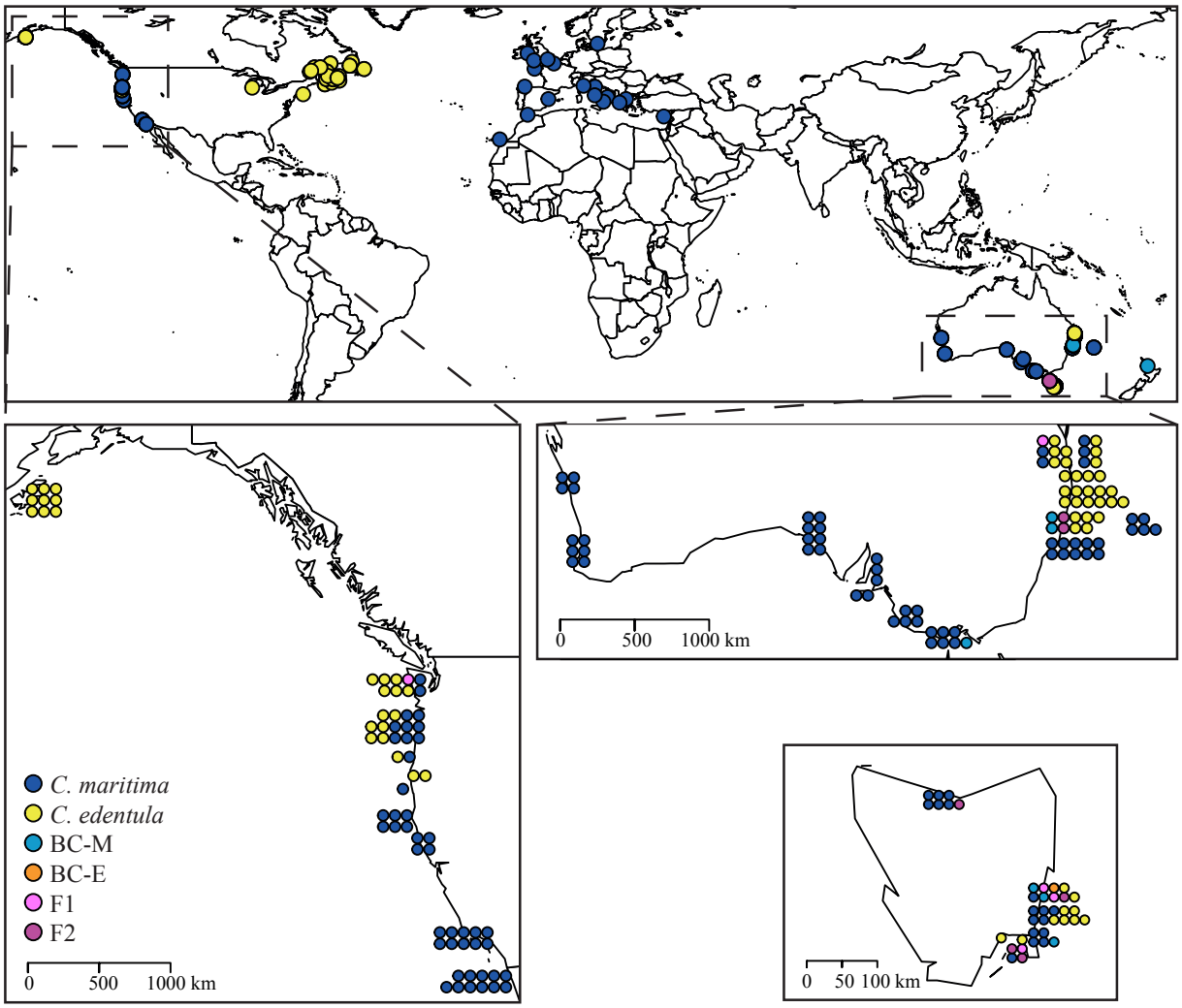
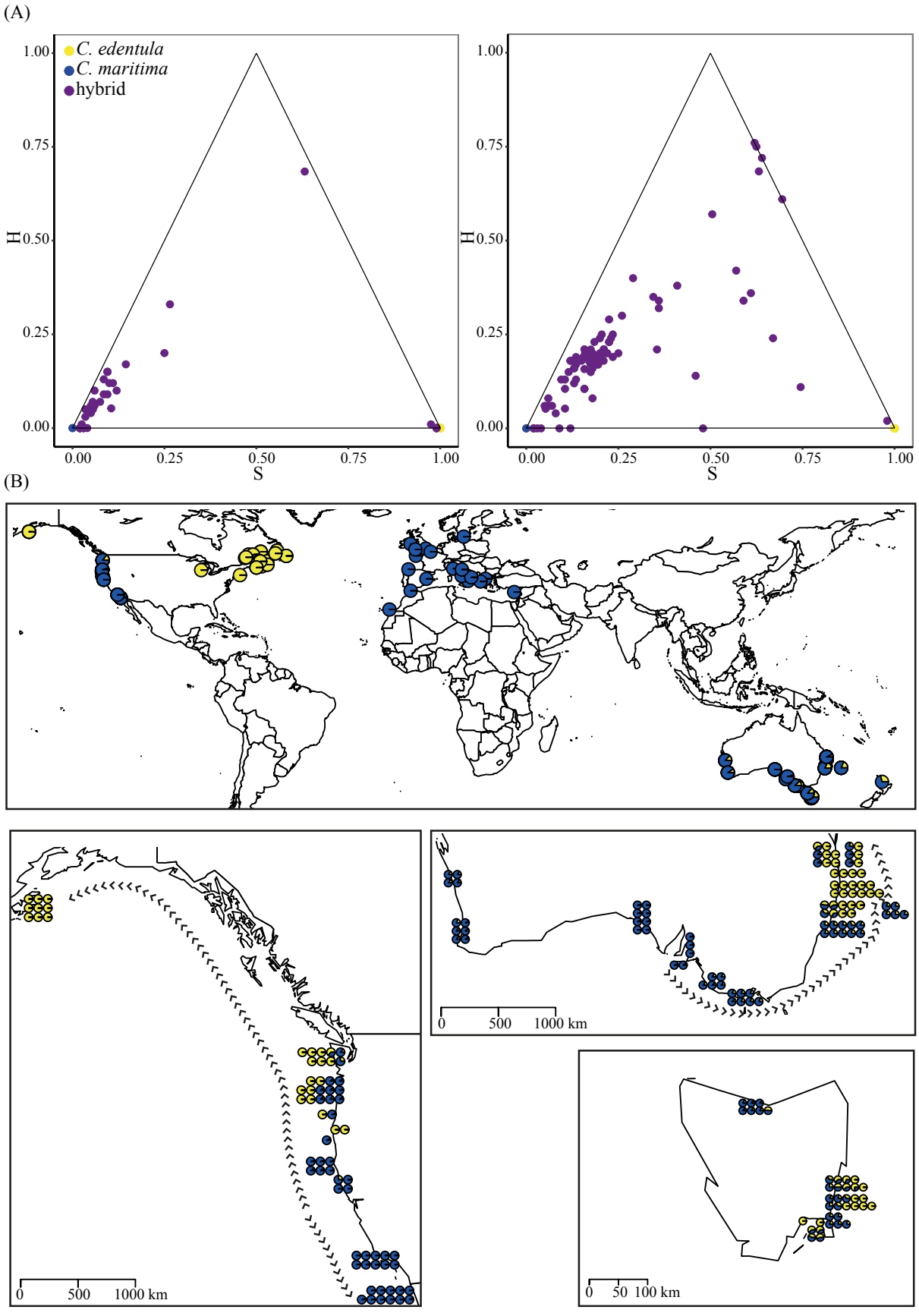


Figure 2- 3. Geographic distribution of the hybrid assignment test by NewHybrid.
Individuals are coloured according to their NewHybrid classification. A global map and close-ups of western North America, the Australian mainland and Tasmania are presented. BC-E= backcross to *C. edentula*, BC-M= backcross to *C. maritima*.


(A)
C. edentula
C. maritima
hybrid
H
S
0.00
0.25
0.50
0.75
1.00
(B)
0
500
1000 km
0
50
100 km

807

Figure 2- 4. Results of a hybridization assignment test implemented by HIest using 471 SNPs (0.99 and 0.03 *C. edentula* and *C. maritima* respectively).
(a) Association of ancestry index (S) and interclass heterozygosity (H) are given for western North America (left) and Australia (right). Individuals are coloured according to their HIest classification. For hybrids the continuous model was a better fit than the hybrid classes. (b) The geographic distribution of individuals and their S index; yellow= *C. edentula* proportion; blue= *C. maritima* proportion. A global map and close-ups of western North America, the Australian mainland and Tasmania are presented. Arrows indicates direction of invasion and direction of Spearman's rank correlation test.

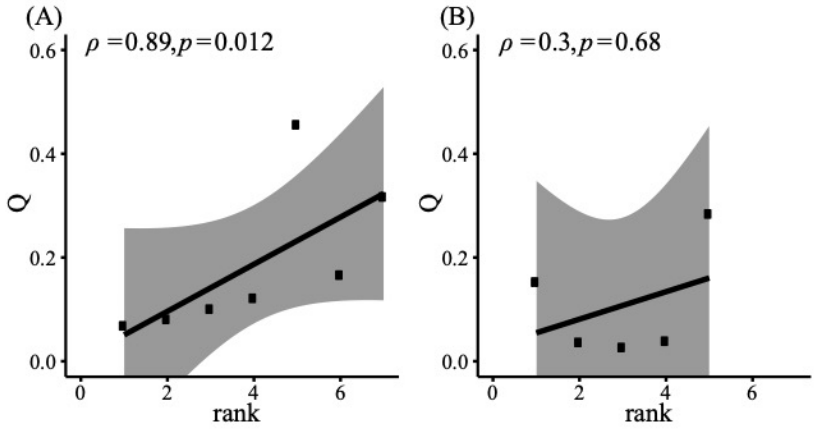


Figure 2- 5. Results of the Spearman's correlation test displayed (Table 2-2).
The associations between population mean *Q* values of hybrids identified using the *supervised* Admixture *run* and the ranked order of populations from the first entry point of *C. maritima* (a) in south-eastern Australia and (b) western North America

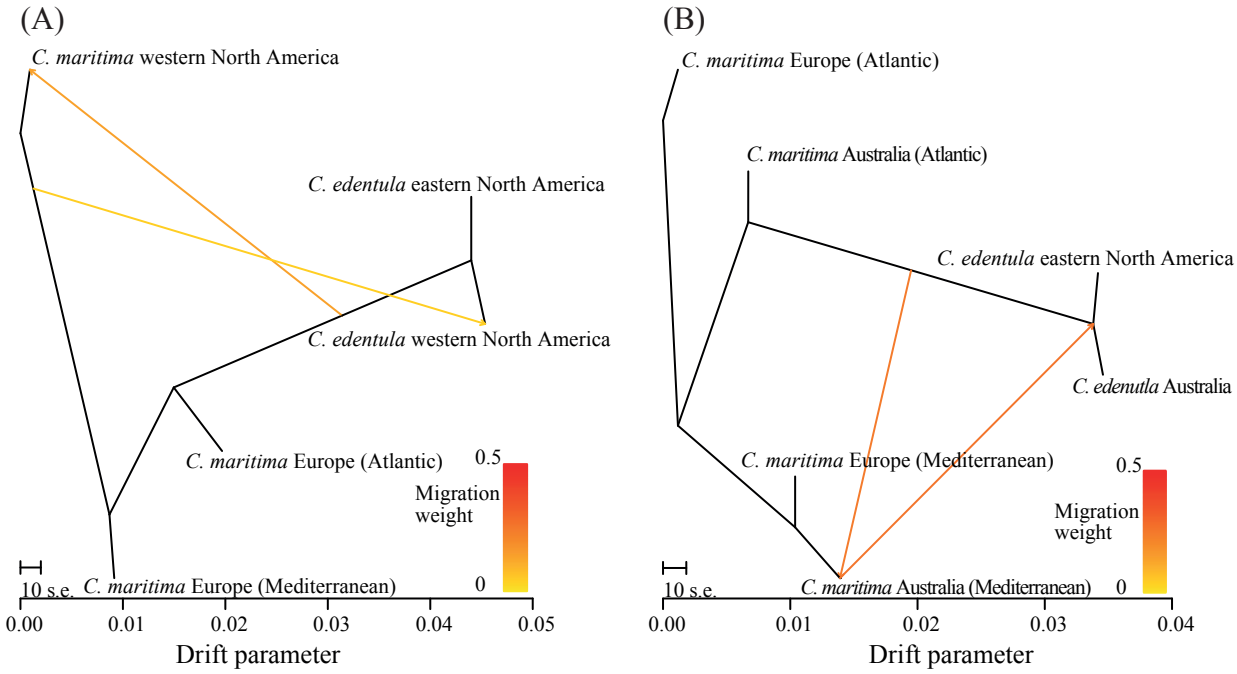


Figure 2- 6. Maximum likelihood trees with two migration events generated by TreeMix.
Native ranges and (a) western North America, (b) Australia. Individuals are grouped by species (identified morphologically), probably subspecies and geographic origin.

2.5 Discussion

Our analysis sheds light on the origin and extent of hybridization of two introduced species in two separate invasions, which experienced a parallel pattern of invasion and apparent replacement of one species by another. Except at places where the two species are currently sympatric and new hybrids are still being formed, it would be difficult to determine morphologically that hybridization has ever taken place, since backcrossing soon hides its phenotypic evidence. *Cakile maritima* is highly variable within and between populations in its native range and hybrids in the introduced range could easily be overlooked (e.g., Cousens et al., 2013) without the use of molecular methods. However, our analysis identified extensive hybrid ancestry in the introductions, particularly in Australia. It is therefore an intriguing possibility that hybridization may be commonly overlooked in a much wider range of invasive taxa, especially where morphological trait indicators of hybridization are more cryptic. Alien floras commonly include many congeneric species whose capacity for interbreeding is yet to be established. While previous authors (Ellstrand & Schierenbeck, 2000) have raised our attention to obvious hybrid species and allopolyploids, perhaps the impacts of hybridization are often more insidious. It is thus important – though not an easy task – to determine in future the extent to which such non-apparent introgression has been beneficial during invasion.

2.5.1 Native range patterns

One of our primary goals was to identify the source regions for the invasions for each species and our analysis provided evidence of geographic structuring in the *C. edentula* native range, at a much finer grain than currently recognized taxonomically (Figure 2-1). Samples from Quebec, Newfoundland, Nova Scotia and New Brunswick contain separate Admixture clusters, probably within *C. edentula* subsp. *edentula* var. *edentula* as this subspecies is the only one described in this region of the North American Atlantic coast (Rodman, 1974). Two single samples from Lake Michigan and Rhode Island grouped together in one cluster of the Admixture analysis; those samples might belong to the Atlantic coast variety of *C. edentula* subsp. *edentula* var. *edentula* as it is known to have invaded Lake Michigan in historical times (Huebner, 2009; Rodman, 1974), where it now coexists with the Great Lakes endemic var. *lacustris*. A second possibility, suggested by Gormally et al. (2011), but without morphological evidence, is that var. *lacustris* has dispersed to the Atlantic. Genetically distinct regional variation is not surprising, as the directions of currents

and the influences of geological features on seed dispersal can be highly predictable (Lapointe, 2000). Similar conclusions have been reached in the Mediterranean by Westberg (2005) and Gandour et al. (2008). *Cakile edentula* subsp. *harperi* occurs in areas south of the populations sampled in our study (Rodman, 1974), but comprehensive studies of herbarium samples by Rodman (1974) and Cousens et al. (2013) have found no morphological evidence that subsp. *harperi* has been introduced anywhere outside its native range.

Our analyses revealed clustering of *C. maritima* in its native Europe largely consistent with the accepted taxonomic distributions (Ball, 1964; Marhold, 2011; Rodman, 1974) as well as one previous population genetic analysis (Clausing et al., 2000). Other genetic studies with greater sampling intensity, however, showed more differentiation on a local level (Kadereit et al., 2005; Westberg, 2005). The absence of fine-grain local differentiation in our study might be driven by the limited number of native range samples for this species and restricted sampling of the Baltic area.

Cakile edentula showed lower genetic diversity than *C. maritima* in their native ranges as measured by A_R and H_O (Table 2-S3) and showed less variation along the EVs and in the SPLITSTREE network analysis (Figure 2-2). Higher selfing rates in *C. edentula* would be expected to reduce the effective population size compared to the largely self-in- compatible *C. maritima* (Pollak, 1987).

2.5.2 Introduced range patterns

2.5.2.1 Australia and New Zealand

Although *C. edentula* has now disappeared from much of its original introduced range in Australia, some pure *C. edentula* populations still remain. Our analyses show that they probably originate from populations located in Nova Scotia as they contained an Admixture cluster found exclusively in this region of the native range and showed the lowest genetic differentiation from this region (Figure 2-1; Table 2-S7). *Cakile edentula's* A_R and H_O did not change considerably in Australia compared to the native range (Table 2-S3), which is inconsistent with a strong invasion bottleneck. The genetic structure of the Australian *C. maritima* samples is consistent with a history of multiple introductions. This is in accordance with previous morphological and genetic studies of invasion

history in Australia (Cousens et al., 2013; Ohadi et al., 2016; Rodman, 1976, 1986). In particular, the cluster associated with the Atlantic European group is found in western Australia, while a Mediterranean cluster predominates in southern and eastern Australia (Figure 2-1; Table 2-S8). Similarly, analysis of microsatellite markers indicated that that western and south-eastern populations of *C. maritima* in Australia were genetically distinct and most likely resulted from independent introductions with severely limited gene flow from west to east (Ohadi et al., 2016). Finally, Australian *C. maritima* showed higher A_R and H_O values than its native range, consistent with admixture of multiple source populations and/or hybridization with *C. edentula*. Many successful invasions are sourced from multiple introductions (e.g., Vallejo-Marín et al., 2020; van Boheemen et al., 2017) and both hybridization and multiple introductions and admixture may spur successful invasions (Dlugosch & Parker, 2008; Ellstrand & Schierenbeck, 2000; Hodgins et al., 2018).

Our data provides substantial evidence for extensive hybridization in Australia between the two species. TreeMix supported bidirectional gene flow between the parental species (identified morphologically) (Figure 2-6). This was confirmed by the Admixture global analysis (Figure 2-1), the PCA and Splitstree analysis, as many Australian samples fell in-between the native range samples of both species (Figure 2-2), and the f_3 test (Table 2-3). Further support is provided by three separate analyses which specifically detect hybrid individuals (Figures 2-1, 2-3, 2-4, 2-5; Tables 2-S4 and 2-S8). As expected, Australian hybrids (*supervised* Admixture *run*) had higher genetic diversity than both parental species (Table 2-S3). Furthermore, the pattern of hybrid ancestry was geographically structured and reflected the historical invasion route of *C. maritima* in south-eastern Australia. This pattern was consistent across two separate approaches (*supervised* Admixture *run*, HIest) to identify hybrid ancestry (Figures 2-1 and 2-4; Table 2-2). NewHybrids confirmed the presence of a small number of early generation hybrids (within two generations) where both species still co-occur and some mixed populations show pure genotypes of both parental species and early generation hybrids, demonstrating on-going hybridization of the two taxa (Figure 2-3). In areas where *C. edentula* still persists, backcrossing to *C. edentula* has also occurred, but is rare, and recent backcrosses to *C. maritima* appear to be more common. In those parts of Australia where *C. maritima* has already appeared to have replaced *C. edentula* (i.e., where no *C. edentula* phenotypes remain; (Cousens et al., 2013; Rodman, 1986), evidence is consistent

with past hybridization between the species and repeated backcrossing to *C. maritima* (Figures 2-1, 4 and 6). In areas of Western Australia, where *C. edentula* has never been identified, evidence of hybridization with *C. edentula* was also found, confirming a previous observation by Ohadi et al. (2016). The sample from New Zealand was identified as a hybrid where the same replacement of *C. edentula* by *C. maritima* has also taken place (Cousens & Cousens, 2011).

2.5.2.2 Western North America

Our results revealed that *C. edentula* in western North America most likely originated from two sources in eastern North America. We also found that western North American *C. maritima* potentially originated from the Mediterranean region, as *C. maritima* in western North America contained the same Admixture clusters as the Mediterranean and showed the lowest differentiation from this region (Figure 2-1; Tables 2-S7 and 2-S8). However, these populations were genetically distinct (Figure 2-2 and Figure 2-S5) suggesting the possibility of an unknown source for this invasion, or the impact of an invasion bottleneck. *Cakile edentula* and *C. maritima* in western North America showed, as in Australia, no reduction of H_O and A_R, which may reflect the impacts of undetected hybridization, large founding populations, or multiple introductions.

Like Australia, hybridization was identified between the two species in western North America, although the proportion of hybrids was less (e.g., 58% vs. 16% using the *supervised* Admixture *run*). TreeMix identified bidirectional gene flow between the species in western North America (Figure 2-6; Table 2-3), and evidence consistent with hybridization was apparent in the global Admixture analysis (Figure 2-1), the PCA and Splitstree analysis (Figure 2-2). Furthermore, we employed three independent methods to specifically identify hybrid individuals and their likely generation. From this we identified 11 hybrid samples (all 11 were identified by both HIest and Admixture and one as an F2 by NewHybrids) from five locations in western North America. Specimens of hybrids based on morphological identification are largely unknown for this region, either in herbaria or in the field (Rodman, 1974). But more recently, Cody and Cody (2004) reported a small percentage of hybrids in a population from British Columbia. Although the fitness and demographic consequences of hybridization during introduction require further investigation, the lower incidence of hybrids in western North America compared to Australia suggests that hybridization could have facilitated the establishment and rapid spread of *C. maritima* to a greater

degree in Australia. In support of this hypothesis, the complete replacement of *C. edentula* by *C. maritima* phenotypes has not progressed as far north in western North America compared to Australia, where few northern populations of *C. edentula* remain. Indeed, although the introduction of *C. maritima* in western North America is more recent than Australia, migration rates for this species based on herbarium records are much lower in western North America (Barbour & Rodman, 1970; Rodman, 1986). However, the mechanism driving differences in hybridization rates in western North America compared to Australia is unclear and requires further investigation.

2.5.3 Hybrid identification and significance

The pattern of invasion first by *C. edentula*, then by *C. maritima*, has been repeated in three regions. Prior to this study, hybrids were known only from Australia. However, we also identified clear evidence of hybridization in western North America and in New Zealand. Hybrids between the two species can be produced readily by handcrossing (e.g., Li et al., 2019; Mesgaran et al., 2016; Rodman, 1974) and our data demonstrate that recent and advanced generation hybrids are at least partially fertile in natural populations. Our results show backcrossing to both parental species, although backcrossing to *C. maritima* was much more frequent. This pattern of biased backcrossing towards *C. maritima* was predicted based on field observations of pollinator visitations (Mesgaran et al., 2016), the morphological replacement of *C. edentula* by *C. maritima*, and previous genetic studies (Mesgaran et al., 2016; Ohadi et al., 2016). It is also consistent with expected mating asymmetries between these species and their hybrids caused by the inheritance of the self- incompatibility system and traits associated with pollinator attraction in hybrids (C. Li et al., 2019). In artificial crosses, early generation hybrids inherited mostly (but not exclusively) self-incompatibility, as well as larger floral displays, similar to *C. maritima* (Li et al., 2019). This suggests that F1 hybrids will often need to rely on outcrossing, and that larger floral displays should facilitate this. Consequently, these traits in the hybrids should further contribute to backcrossing to the self-incompatible parent (*C. maritima*). A similar asymmetric pattern of species ancestry has been identified in hybrids of other species with such differences in mating system (Brandvain et al., 2014; Pickup et al., 2019; Ruhsam et al., 2011).

Our identification of advanced generation backcrosses to *C. maritima* means that portions of the *C. edentula* genome have been retained in a largely *C. maritima* background (i.e., introgression),

long after morphological evidence of hybridization has gone from a population. The role of selection and neutral evolutionary processes in governing patterns of introgression across the genome, however, remains to be investigated in this system. Theory suggests that regions of the genome that are not introgressed will harbour incompatibilities or a high number of additive deleterious alleles in the introgressing species (Harris & Nielsen, 2016; Juric et al., 2016). A greater fixation rate of weakly deleterious alleles is predicted in the *C. edentula* due to its higher level of inbreeding, and indeed, the low levels of genetic variability in this species relative to *C. maritima* support a lower effective population size in this species. Selection against a higher genetic load originating from *C. edentula* in hybrids should more rapidly lead to the reconstitution of a *C. maritima* genome following transient hybridization during range expansion. In line with the expectation of selection against selfing ancestry in outcrossers, in *Mimulus gutattus* (outcrossing) genomic regions with high recombination rates have reduced levels of ancestry from the selfing species *Mimulus nasutus* (Brandvain et al., 2014). However, several remarkable examples in plants have demonstrated the infusion of favorable alleles via hybridization (adaptive introgression), including the transfer of herbivore resistance in *Helianthus* (Whitney et al., 2006). Indeed, Cody and Cody (2004) proposed the intriguing possibility of adaptive introgression in the *Cakile* system but this remains to be investigated. Our identification of replicated patterns of hybridization, replacement and invasion in *Cakile* provide an exciting opportunity for further investigation of the beneficial and detrimental consequences of hybridization during range expansion.

2.6 Conclusion

Here we confirm that, particularly in Australia, the apparent replacement of *C. edentula* by *C. maritima* is not complete and remnants of the *C. edentula* genome are evident in contemporary *C. maritima* populations. Furthermore, it appears that both early and later generation hybrids are at least partially fertile in natural populations and that there is a higher frequency of back- crossing to *C. maritima*. The patterns of hybridization we identified is consistent with the hypothesis that mating among these cross-compatible invaders has facilitated the establishment of the self-incompatible *C. maritima* whose range expansion may otherwise be limited due to Allee effects, as has been observed in other potential self-incompatible invaders (Uesugi et al., 2020). The

evolutionary consequence of hybridization for both species remains unclear, as is its role, if any, in the rapid expansion of one invader at the expense of another.

Acknowledgements

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Author contributions

K.H., R.C., and L.R. conceived and designed the study. K.H., K.N., and R.C. carried out sampling. K.N. conducted the molecular laboratory work. H.R. carried out the bioinformatics analyses with significant input from A.G., P.B., and K.H. A.G., K.H., L.R., P.B., R.C., and H.R. contributed to the writing and approved the final manuscript.

Data availability statement

Sequence data are available at the National Center for Biotechnology Information Sequence Read Archive under Bioproject PRJNA637114. Unfiltered data set available on https://bridges.monash.edu/articles/ dataset/GBSCAK_vcf_gz/12526220/1; filtered data set on https:// bridges.monash.edu/articles/dataset/filtered_dataset_GBS_Cakil e/12996854. Scripts are available on https://github.com/HannaRos/ Cakile-GBS-scripts.

2.7 Appendix I - Supplemental Information for: The tip of the iceberg: genome wide marker analysis reveals hidden hybridization during invasion

DNA extraction and GBS protocol

We performed DNA extraction and GBS library preparation following (Whitlock et al., 2008). DNA quantity was assessed (> 8.5 ng/µl) using a QuBit broad-sensitivity DNA quantification system (Invitrogen, Carlsbad, CA, USA). We performed double-digest genotype-by-sequencing library preparation by adding 200 ng of high-quality DNA in 7.2 µL water to 2.0 µL CutSmart Buffer 10x, 0.4 µL PstI-HF (NEB), 0.4 µL MspI (NEB). Samples were digested for 8h at 37°C, 20 minutes at 65°C with 2.0 µL 10x CutSmart Buffer, 4.0 µL 10mM ATP, 0.5µL T4 DNA Ligase, 8 µL H2O, 1µL 10mM common adaptor and 5µL 0.6ng/µL barcoded adaptor. Samples were ligated for 3h at 22°C and 20 minutes at 65°C, and all samples were mixed with 6144 µL Sera-Mag beads (Thermo Fisher). After 15-minute incubation at room temperature, we allotted samples to seven 1.5mL tubes and placed these in Dyna-Mag 2 (Thermo Fisher) magnet for 4 minutes. Clear liquid was then removed and washed three times using 80% EtOH and once with 100% EtOH and eluted in 150 µL 10mM Tris pH 8.0. We amplified eight reactions each with 3µL of elution and 7.5uL H2O, 12.5 µL KAPA 2x MasterMix, 1µL of 12.5mM each PCR primers f & r. Reaction cycle was 98°C for 1 minute, followed by 20s at 62°C and 30s at 72°C. Following 16 cycles, we additionally kept samples at 72°C for 5 minutes. After amplification, we cleaned up 30 µL from each well using the Bioline PCR and Gel kit (Bioline) and eluted the purified product in 30µL buffer. Size selection was performed by running the cleaned PCR product on a 2% agarose gel and removing the 400-600bp fragment. This gel fragment was cleaned up using the Bioline PCR and Gel Kit (Biolin1) and eluted in 20 µL H2O.

Methods to detect a reference bias when mapping reads

We assessed if there was a bias when mapping the reads of *C. edentula* to the reference genome of *C. maritima* but found limited evidence for such bias. In addition to using Burrows-Wheeler Aligner (H. Li & Durbin, 2009), we also aligned the filtered reads with NextGenMap (Sedlazeck et al., 2013), which has been shown to be superior at aligning reads to a more distantly related reference compared to the Burrows-Wheeler Aligner. To assess if there was a large bias when mapping the reads of *C. edentula* to the reference genome of *C. maritima*, we examined the proportion of missing data for each individual per species of both aligners and could not find any

evidence for higher levels of missing data in *C. edentula* versus *C. maritima* (Figure 2-S2). Additionally, we plotted the percentage of aligned high quality reads (mapQ > 20) per species (Figure 2-S3) and again found limited evidence for a reference bias. In fact, the percent of missing data in the filtered file using the NextGenMap aligner was lower for *C. edentula* (12.17%) than *C. maritima* (18.65%), and hybrids (17.96%). The same pattern was found to the BWA-aligner (*C. edentula* 11.62%, *C. maritima* 20.32% and hybrids 19.03%; Figure 2-S2, Figure 2-S3)

Splitstree analysis

The global Splitstree data set and the native range Splitstree data set were created by filtering the unfiltered data set for a minor allele count of 2, a minimum genotype quality of 20 and a maximum missing value of 1. This approach kept variants specific to the *C. lanceolata* lineage, which would have been removed by the previous filtering steps. VCFtools (Danecek et al., 2011) and Mesquite (Maddison & Maddison, 2019) were used for filtering and data conversion.

New Hybrids analysis

This program is designed to identify hybrids from the first two generations of interbreeding based on classification into six genotype classes and does not require the loci to be fixed between the species, although a large number of highly differentiated loci aids hybrid identification (Anderson & Thompson, 2002). As the program is unable to deal with a large data set, we restricted our data to 63 SNPs that showed fixed differences between the two pure species based on the supervised Admixture run. To obtain this data set, we calculated the F_{ST} between the pure species (using the global thinned data set) with VCFtools (Danecek et al., 2011) and selected SNPs which showed a F_{ST} value of one and considered those as fixed differences between the species. We ran NewHybrids (Anderson & Thompson, 2002) using the native range individuals as parental species and let NewHybrids (Anderson & Thompson, 2002) assign all individuals of the introduced range to the six classes (pure *C. edentula*, pure *C. maritima*, F1, F2, backcross to *C. edentula* (BC-E), backcross to C. maritima (BC-M) according to their posterior probability (> 50% class assignment). The settings for the three independent runs of NewHybrids (Anderson & Thompson, 2002) were as follows: Jeffries prior, 10,000 burn-in, and 50,000 number of sweeps.

HIest analysis

First, we used the function HIest of the HIest package (Fitzpatrick, 2012) to calculate the ancestry coefficient S and the interclass heterozygosity H with a startgrid of 20, 99 iterations and the native range individuals as parental populations. This method jointly considers ancestry together with interclass heterozygosity and without the assumption that only two generations of admixture have transpired. It specifically tests the assumption that discrete classification (i.e., pure species or early generation hybrids) rather than continuous distribution of hybrid genotypes best describes each individual. The simple likelihood approach it employs is relatively robust to small errors in the assumed parental allele frequencies, especially if the errors are unbiased. For the data set containing 471 SNPs we set the allele count of *C. edentula* to 0.99 and of *C. maritima* to 0.03 or 0.06; for the data set containing 63 SNPs we set the allele frequency of *C. edentula* to 1 and of *C. maritima* to 0 (Table 2-S5, Table 2-S6). We did this because our limited sampling of the native range, particularly for *C. maritima*, may mean that some SNPs with apparent fixed differences in our sample were actually SNPs that were segregating at low frequencies. We then used the function HIclass to assign each individual to the same six categories as in (3) above. We first tested if the continuous model was a better fit than a discrete model of hybrid classes produced in the first two generations using the function HItest. If the discrete classification AIC was lower than the AIC of the MLE for the continuous model (which was equivalent to a criterion of within 1.0 log-likelihood units of the MLE) we concluded the discrete genotypic clusters were a better fit. If this was the case, we then referred to the assigned hybrid class. We used the function HItest to determine whether the assigned hybrid class was over 2 units greater than the log-likelihood of the second best-fit class (Table 2-S5).

When the 471 loci that are fixed between native range samples were used, and we allowed for a low level of polymorphism within each species (0.99 *C. edentula*, 0.03 *C. maritima*), a larger number of hybrids were identified using HIest than Admixture (138 versus 85, Table 2-S6). When we increased the allele frequency of *C. maritima* (0.99 *C. edentula*, 0.06 *C. maritima*) we identified slightly fewer hybrids (132). In both cases the additional hybrids were exclusively found in the introduced ranges and were identified as advance generation hybrids with most showing a greater proportion of ancestry to *C. maritima* than *C. edentula* (Figure 2-4). When 63 SNPs that were fixed between all parental individuals based on the supervised Admixture analysis were used, the identification of parental and hybrids was identical between Admixture and HIest.

Chronosequence analysis

In Australia, we only used the south-east mainland individuals, as the introduction history and pattern of replacement based on herbarium records led us to predict a gradient in species ancestry in hybrids from high levels of *C. maritima* in South Australia to high levels of *C. edentula* further north in Queensland. In western North America we predicted this pattern to the north of San Francisco as *C. edentula* has only recently been replaced in parts of Oregon and Washington and *C. edentula* is common in British Columbia.

TreeMix analysis

First, we constructed maximum likelihood trees, allowing up to four migration events. We grouped our samples according to their species and origin. For *C. maritima*, we kept the Atlantic and Mediterranean *C. maritima* samples separate because they were likely different subspecies (Rodman, 1974, 1976, 1986) and these groups appeared well differentiated from one another (e.g., Figure 2-2 B). We excluded morphological hybrids to assess evidence for admixture between the species in the introduced ranges, which may not apparent phenotypically. Our groupings for the maximum likelihood trees were as followed.: 1) Australian *C. edentula*; 2) Australian *C. maritima* (Mediterranean); 3) Australian *C. maritima* (Atlantic); 4) western North American *C. edentula*, 5) western North American *C. maritima*; 6) eastern North American *C. edentula*; 7) European *C. maritima* (Mediterranean); and 8) European *C. maritima* (Atlantic). We tested for admixture in Australia separately from western North America but included native range samples in both analyses. We used the f_3 statistic (Pickrell & Pritchard, 2012; Reich et al., 2009), which is part of the TreeMix package, to test for evidence of admixture in the invasive ranges in putative hybrids. We grouped the samples according to their Admixture classification (supervised run). For south-east Australia we had three groups: 1) Australian *C. edentula*, 2) Australian *C. maritima*; and 3) Australian hybrids. For western North America we had three groups: 1) western North American *C. edentula*; 2) western North American *C. maritima*; and 3) western North American hybrids. No SNP blocking was used for TreeMix as the data set had been trimmed for linkage disequilibrium.

F_{ST} analysis of pure species individuals

We calculated the Weir and Cockerham's (1984) pairwise F_{ST} for pure *C. edentula* and pure *C.*

maritima individuals identified by the supervised Admixture run in the native and invasive ranges (Figure 2-1) using the global thinned data set with VCFtools (Danecek et al., 2011). For *C. edentula* we grouped the individuals according to their dominant cluster (Q value ≥ 50%) except nine samples from Nova Scotia, which showed 23-37% of their Q value of the light green cluster as this cluster was unique to this geographic region (Figure 2-1); 1) *C. edentula* with the dominant cluster present in Quebec and Newfoundland; 2) *C. edentula* with the dominant cluster present in New Brunswick; 3) *C. edentula* with the dominant cluster present in Lake Michigan/ Rhode Island; 4) nine *C. edentula* samples from Nova Scotia; 5) Australian *C. edentula*; 6) western North American *C. edentula* with the dominant cluster associated with Lake Michigan/Rhode Island; and 7) western North American *C. edentula* with the dominant cluster associated with the light green Nova Scotia cluster. *Cakile maritima* individuals were grouped according to their dominant cluster (Q value ≥ 46%). 1) *C. maritima* from Europe (Atlantic); 2) *C. maritima* from Europe (Mediterranean); 3) Australian *C. maritima*; and 4) western North American *C. maritima*. When comparing to the home range groups, the Australian *C. edentula*, is least differentiated from the nine samples from Nova Scotia and the same applies for the western North American samples from Kodiak Island (all except one) (Table 2-S7). Western North American *C. edentula* south of Kodiak Island shows the lowest genetic differentiation from Lake Michigan/ Rhode Island. *Cakile maritima* samples from Australia and western North America are more genetically similar to the European Mediterranean cluster than to the European Atlantic cluster (Figure 2-1; Table 2-S8).

1178 Table 2-S1 Sample ID, Original ID, Sample origin (Country, state, sampling location, range), collectors and classification of each individual (original morphological
1179 identification, Admixture, NewHybrids, HIest (for hybrids continuous classification was a better fit than hybrid classes), Population statistic pooling) are given.

1180

Sample_ID	Collector	Original ID	Country	State	Sampling location	Lat	Long	Range	Original Species	NewHybrids Species 63 SNPs	HIest Species 471 (0.99 E,0.03 M)	Admixture Species	Population statistik	Reason why not used in study
cak105	Sara Ohadi, Roger Cousens	Ape 2 (I)	Australia	QLD	Stradbroke Island, Amity	-27.4	153.5	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E	
cak114	Sara Ohadi	Slop E 21	Australia	TAS	Sloping Main	-43	147.7	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E	
cak116	Sara Ohadi	Ras E 5	Australia	TAS	Raspins Beach	-42.5	147.9	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E	
cak117	Sara Ohadi	FLbe 2 (I)	Australia	QLD	Flinders Beach, North Stradbroke Island	-27.4	153.5	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E	
cak123	Sara Ohadi	Ras E 11	Australia	TAS	Raspins Beach	-42.5	147.9	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E	
cak130	Sara Ohadi, Roger Cousens	POT 15	Australia	NSW	Fingal Head	-28.2	153.6	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E	
cak133	Sara Ohadi	RHE E 15	Australia	TAS	Rhebans Beach	-42.6	148	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E	

cak135	Sara Ohadi	FLbe B (I)	Australia	QLD	Flinders Beach, North Stradbroke Island	-27.4	153.5	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak149	Roger Cousens	SWR 15	Australia	NSW	South West Rocks	-30.9	153	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak175	Sara Ohadi, Roger Cousens	POT 9	Australia	NSW	Fingal Head	-28.2	153.6	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak18	Sara Ohadi, Roger Cousens	AH14	Australia	QLD	Stradbroke Island	-27.4	153.5	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak21	Sara Ohadi	RHE E 23	Australia	TAS	Rhebans Beach	-42.6	148	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak225	Sara Ohadi	CUR 18	Australia	QLD	Currumbin	-28.1	153.5	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak238	Sara Ohadi	CUR 19	Australia	QLD	Currumbin	-28.1	153.5	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak254	Sara Ohadi	CUR 7	Australia	QLD	Currumbin	-28.1	153.5	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak26	Sara Ohadi	RHE E 10	Australia	TAS	Rhebans Beach	-42.6	148	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak28	Sara Ohadi	RHE E 4	Australia	TAS	Rhebans Beach	-42.6	148	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak301	Roger Cousens	SWR9	Australia	NSW	South West Rocks	-30.9	153	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak302	Sara Ohadi,	POT6	Australia	NSW	Fingal Head	-28.2	153.6	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E

	Roger Cousens												
cak31	Sara Ohadi, Roger Cousens	AH15	Australia	QLD	Stradbroke Island	-27.4	153.5	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak336	Sara Ohadi, Roger Cousens	POT20	Australia	NSW	Fingal Head	-28.2	153.6	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak344	Roger Cousens	SWR23	Australia	NSW	South West Rocks	-30.9	153	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak348	Louise Emmerson	a20515	Australia	TAS	Tyndall Beach	-43	147.3	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak352	Sara Ohadi, Roger Cousens	POT21	Australia	NSW	Fingal Head	-28.2	153.6	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak364	Sara Ohadi	CUR 14	Australia	QLD	Currumbin	-28.1	153.5	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak370	Sara Ohadi, Roger Cousens	POT24	Australia	NSW	Fingal Head	-28.2	153.6	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak49	Sara Ohadi	RHE E 3	Australia	TAS	Rhebans Beach	-42.6	148	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak50	Roger Cousens	SWR 24	Australia	NSW	South West Rocks	-30.9	153	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak62	Sara Ohadi	RHE E 8	Australia	TAS	Rhebans Beach	-42.6	148	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak68	Roger Cousens	SWR 7	Australia	NSW	South West Rocks	-30.9	153	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E

cak69	Sara Ohadi, Roger Cousens	AH12	Australia	QLD	Stradbroke Island	-27.4	153.5	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak7	Sara Ohadi, Roger Cousens	POT 27	Australia	NSW	Fingal Head	-28.2	153.6	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak72	Sara Ohadi, Roger Cousens	POT 29	Australia	NSW	Fingal Head	-28.2	153.6	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak74	Sara Ohadi, Roger Cousens	POT 1	Australia	NSW	Fingal Head	-28.2	153.6	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak78	Sara Ohadi, Roger Cousens	POT 11	Australia	NSW	Fingal Head	-28.2	153.6	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak81	Sara Ohadi, Roger Cousens	POT 16	Australia	NSW	Fingal Head	-28.2	153.6	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak98	Sara Ohadi, Roger Cousens	Ape 3	Australia	QLD	Stradbroke Island, Amity	-27.4	153.5	AUS	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	AUS_E	AUS_E
cak112	Sara Ohadi	FLbe 3 (I)	Australia	QLD	Flinders Beach, North Stradbroke Island	-27.4	153.5	AUS	*Cakile edentula*	*Cakile edentula*	Hybrid	AUS_E	AUS_E
cak3	Sara Ohadi	Ras M 3 (I)	Australia	TAS	Raspins Beach	-42.5	147.9	AUS	*Cakile maritima*	BC_M	Hybrid	AUS_H	AUS_H
cak324	Sara Ohadi	PF27B	Australia	VIC	Port Fairy	-38.4	142.3	AUS	*Cakile maritima*	BC_M	Hybrid	AUS_H	AUS_H

cak37	Roger Cousens	MB 3	Australia	TAS	Marion Bay	-42.8	147.9	AUS	*Cakile maritima*	BC_M	Hybrid	AUS_H	AUS_H
cak83	Roger Cousens	SWR 18	Australia	NSW	South West Rocks	-30.9	153	AUS	*Cakile maritima*	BC_M	Hybrid	AUS_H	AUS_H
cak89	Roger Cousens	SWR 12	Australia	NSW	South West Rocks	-30.9	153	AUS	*Cakile maritima*	BC_M	Hybrid	AUS_H	AUS_H
cak35	Sara Ohadi	Ras H 20	Australia	TAS	Raspins Beach	-42.5	147.9	AUS	Hybrid	F1	Hybrid	AUS_H	AUS_H
cak80	Sara Ohadi, Roger Cousens	AH2	Australia	QLD	Stradbroke Island	-27.4	153.5	AUS	Hybrid	F1	Hybrid	AUS_H	AUS_H
cak4	Sara Ohadi	Slop H 13	Australia	TAS	Sloping Main	-43	147.7	AUS	Hybrid	F2	Hybrid	AUS_H	AUS_H
cak43	Roger Cousens	SWR 13	Australia	NSW	South West Rocks	-30.9	153	AUS	Hybrid	F2	Hybrid	AUS_H	AUS_H
cak82	Roger Cousens	SWR 22	Australia	NSW	South West Rocks	-30.9	153	AUS	Hybrid	F2	Hybrid	AUS_H	AUS_H
cak250	J. Chitty	B17	Australia	WA	Bunbury	-33.3	115.6	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak256	J. Chitty	B7	Australia	WA	Bunbury	-33.3	115.6	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak265	J. Chitty	B4	Australia	WA	Bunbury	-33.3	115.6	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak274	Sara Ohadi	P30	Australia	VIC	Port Fairy	-38.4	142.3	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak289	Sara Ohadi	EXPL SERR 2	Australia	WA	Geraldton	-28.8	114.6	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H

cak292	Sara Ohadi	ENTIRE6	Australia	WA	Geraldton	-28.8	114.6	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak295	Sara Ohadi	EXPL SERR 1	Australia	WA	Geraldton	-28.8	114.6	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak297	Sara Ohadi	SERR17	Australia	WA	Geralton	-28.8	114.6	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak30	Roger Cousens	MB 14	Australia	TAS	Marion Bay	-42.8	147.9	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak306	J. Chitty	B5	Australia	WA	Bunbury	-33.3	115.6	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak307	Sara Ohadi	P26	Australia	VIC	Port Fairy	-38.4	142.3	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak315	Roger Cousens	DUN24	Australia	NSW	Dunbogan Beach	-31.7	152.8	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak317	Sara Ohadi	P17	Australia	VIC	Port Fairy	-38.4	142.3	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak32	Roger Cousens	VIV 18	Australia	TAS	Ulverstone	-41.2	146.2	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak322	Sara Ohadi	PF29A	Australia	VIC	Port Fairy	-38.4	142.3	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak323	Sara Ohadi	D14	Australia	VIC	Discovery Bay	-38.2	141.3	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak329	Sara Ohadi	D5	Australia	VIC	Discovery Bay	-38.2	141.3	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak33	Roger Cousens	LH 12	Australia	NSW	Lord Howe Island	-31.5	159.1	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H

cak338	Sara Ohadi	P24	Australia	VIC	Port Fairy	-38.4	142.3	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak340	Roger Cousens	DUN23	Australia	NSW	Dunbogan Beach	-31.7	152.8	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak346	J. Chitty	B20	Australia	WA	Bunbury	-33.3	115.6	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak347	Sara Ohadi	PF16	Australia	VIC	Port Fairy	-38.4	142.3	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak353	Sara Ohadi	D9	Australia	VIC	Discovery Bay	-38.2	141.3	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak361	J. Chitty	B12	Australia	WA	Bunbury	-33.3	115.6	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak362	Sara Ohadi	D7	Australia	VIC	Discovery Bay	-38.2	141.3	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak379	Sara Ohadi	D6	Australia	VIC	Discovery Bay	-38.2	141.3	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak44	Sara Ohadi	RHE M 24	Australia	TAS	Rhebans Beach	-42.6	148	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak53	Sara Ohadi	RHE M 26	Australia	TAS	Rhebans Beach	-42.6	148	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak56	Roger Cousens	DUN 4	Australia	NSW	Dunbogan Beach	-31.7	152.8	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak58	Sara Ohadi	RHE M 29	Australia	TAS	Rhebans Beach	-42.6	148	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak59	Roger Cousens	DUN 14	Australia	NSW	Dunbogan Beach	-31.7	152.8	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H

cak6	Sara Ohadi	RHE M 30	Australia	TAS	Rhebans Beach	-42.6	148	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak63	Roger Cousens	DUN 18	Australia	NSW	Dunbogan Beach	-31.7	152.8	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak76	Roger Cousens	DUN 12	Australia	NSW	Dunbogan Beach	-31.7	152.8	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak79	Roger Cousens	DUN 10	Australia	NSW	Dunbogan Beach	-31.7	152.8	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak8	Sara Ohadi	Ras M 6	Australia	TAS	Raspins Beach	-42.5	147.9	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak87	Sara Ohadi, Roger Cousens	AH1	Australia	QLD	Stradbroke Island	-27.4	153.5	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak99	Roger Cousens	LH 3	Australia	NSW	Lord Howe Island	-31.5	159.1	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak111	Sara Ohadi	Ras H 18 (II)	Australia	TAS	Raspins Beach	-42.5	147.9	AUS	Hybrid	BC_E	Hybrid	AUS_H	AUS_H
cak119	Sara Ohadi	Ras H 19	Australia	TAS	Raspins Beach	-42.5	147.9	AUS	Hybrid	BC_M	Hybrid	AUS_H	AUS_H
cak113	Sara Ohadi	Ras H 16 (I)	Australia	TAS	Raspins Beach	-42.5	147.9	AUS	Hybrid	F1	Hybrid	AUS_H	AUS_H
cak120	Sara Ohadi	Slop H 12	Australia	TAS	Sloping Main	-43	147.7	AUS	Hybrid	F1	Hybrid	AUS_H	AUS_H
cak125	Sara Ohadi	Ras H 21	Australia	TAS	Raspins Beach	-42.5	147.9	AUS	Hybrid	F2	Hybrid	AUS_H	AUS_H
cak19	Sara Ohadi	Slop H 9	Australia	TAS	Sloping Main	-43	147.7	AUS	Hybrid	F2	Hybrid	AUS_H	AUS_H

cak221	Roger Cousens	UI7	Australia	TAS	Ulverstone	-41.2	146.2	AUS	Hybrid	F2	Hybrid	AUS_H	AUS_H
cak10	Sara Ohadi	RHE M 25	Australia	TAS	Rhebans Beach	-42.6	148	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak100	Roger Cousens	VIV 9	Australia	TAS	Ulverstone	-41.2	146.2	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak102	Roger Cousens	DUN 20	Australia	NSW	Dunbogan Beach	-31.7	152.8	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak103	Roger Cousens	MB 10	Australia	TAS	Marion Bay	-42.8	147.9	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak104	Roger Cousens	LH 17	Australia	NSW	Lord Howe Island	-31.5	159.1	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak107	Roger Cousens	LH 15	Australia	NSW	Lord Howe Island	-31.5	159.1	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak108	Roger Cousens	LH 14	Australia	NSW	Lord Howe Island	-31.5	159.1	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak109	Roger Cousens	MB 6	Australia	TAS	Marion Bay	-42.8	147.9	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak11	Sara Ohadi	FLbm M (I)	Australia	QLD	Flinders Beach, North Stradbroke Island	-27.4	153.5	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak110	Sara Ohadi	Slop H 11	Australia	TAS	Sloping Main	-43	147.7	AUS	*Hybrid*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak12	Roger Cousens	VIV 19	Australia	TAS	Ulverstone	-41.2	146.2	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak121	Roger Cousens	VIV 17	Australia	TAS	Ulverstone	-41.2	146.2	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H

cak122	Roger Cousens	MB 16	Australia	TAS	Marion Bay	-42.8	147.9	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak136	Roger Cousens	VIV 16	Australia	TAS	Ulverstone	-41.2	146.2	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak143	Roger Cousens	DUN 2	Australia	NSW	Dunbogan Beach	-31.7	152.8	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak16	Roger Cousens	VIV 13	Australia	TAS	Ulverstone	-41.2	146.2	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak176	Roger Cousens	DUN 9	Australia	NSW	Dunbogan Beach	-31.7	152.8	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak242	Sara Ohadi	KG 7	Australia	SA	Brown Beach, Kangaroo Island	-35.8	137.8	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_H	AUS_H
cak247	Sara Ohadi	KG 1	Australia	SA	Brown Beach, Kangaroo Island	-35.8	137.8	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_M	AUS_M
cak251	Roger Cousens	CJB 7	Australia	SA	West Lake Shore	-34.9	138.5	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_M	AUS_M
cak281	Roger Cousens	CJB 18	Australia	SA	West Lake Shore	-34.9	138.5	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_M	AUS_M
cak303	Roger Cousens	CJB 5	Australia	SA	West Lake Shore	-34.9	138.5	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_M	AUS_M
cak46	Sara Ohadi, Roger Cousens	Apm 1 (I)	Australia	QLD	Stradbroke Island	-27.4	153.5	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_M	AUS_M
cak153	Sara Ohadi	BOS 15	Australia	SA	Bosanquet Bay	-32.2	133.7	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_M	AUS_M

cak156	Sara Ohadi	BOS 6	Australia	SA	Bosanquet Bay	-32.2	133.7	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_M	AUS_M
cak158	Sara Ohadi	BOS 11	Australia	SA	Bosanquet Bay	-32.2	133.7	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_M	AUS_M
cak159	Sara Ohadi	BOS 4	Australia	SA	Bosanquet Bay	-32.2	133.7	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_M	AUS_M
cak165	Sara Ohadi	BOS 13	Australia	SA	Bosanquet Bay	-32.2	133.7	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_M	AUS_M
cak189	Sara Ohadi	BOS 5	Australia	SA	Bosanquet Bay	-32.2	133.7	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_M	AUS_M
cak2	Sara Ohadi	FLbm 2 (I)	Australia	QLD	Flinders Beach, North Stradbroke Island	-27.4	153.5	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_M	AUS_M
cak22	Sara Ohadi	FLbm 5 (I)	Australia	QLD	Flinders Beach, North Stradbroke Island	-27.4	153.5	AUS	*Cakile maritima*	*Cakile maritima*	Hybrid	AUS_M	AUS_M
cak155	Sara Ohadi	BOS 16	Australia	SA	Bosanquet Bay	-32.2	133.7	AUS	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	AUS_M	AUS_M
cak167	Sara Ohadi	BOS 2	Australia	SA	Bosanquet Bay	-32.2	133.7	AUS	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	AUS_M	AUS_M
cak193	Karen Samis	PS3	Canada	NL	Port Saunders	50.6	-57.29	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak194	Karen Samis	MS28	Canada	NB	Miscou, Marks Point South	47.9	-64.58	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak195	Karen Samis	SA1	Canada	NB	Kouch-Pointe Sapin	46.9	-64.87	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E

cak196	Karen Samis	GM16	Canada	QC	Mingan Archipelago	50.2	-63.55	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak197	Karen Samis	WM5	Canada	NS	West Mabou	46.1	-61.48	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak198	Karen Samis	MS4	Canada	NB	Miscou, Marks Point South	47.9	-64.58	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak199	Karen Samis	WM14	Canada	NS	West Mabou	46.1	-61.48	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak200	Karen Samis	PC7	Canada	NS	Pictou	45.7	-62.71	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak201	Karen Samis	MP10	Canada	NB	Mary`s Point	45.7	-64.75	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak205	Karen Samis	kj5	Canada	NS	Kejimkujik National Park	44.2	-65.17	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak207	Karen Samis	pc12	Canada	NS	Pictou	45.7	-62.71	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak208	Karen Samis	ps1	Canada	NL	Port Saunders	50.6	-57.29	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak209	Karen Samis	gm1	Canada	QC	Mingan Archipelago	50.2	-63.55	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak212	Karen Samis	MP1	Canada	NB	Mary`s Point	45.7	-64.75	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak214	Karen Samis	MC30	Canada	QC	Manicougan Peninsula	49.1	-68.2	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak215	Karen Samis	MC9	Canada	QC	Manicougan Peninsula	49.1	-68.2	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E

cak216	Karen Samis	GM27	Canada	QC	Mingan Archipelago	50.2	-63.55	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak217	Karen Samis	SAM14	Canada	QC	Sainte-Anne-des Monts	49.1	-66.5	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak218	Karen Samis	SA8	Canada	NB	Kouch-Pointe Sapin	46.9	-64.87	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak220	Karen Samis	MA7	Canada	NS	Martinique Beach	44.7	-63.14	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak223	Karen Samis	CJ5	Canada	NB	Cape Jourimaine	46.2	-63.83	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak224	Karen Samis	CA6	Canada	NS	Canso	45.3	-60.97	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak227	Helen Gilbert	e5	Canada	QC	Riveiere de Loup	47.8	-69.56	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak228	Feldstein	E3	Canada	NB	Prince Edward Island, Carls lane, South lake pei	46.4	-62.05	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak229	Karen Samis	CJ17	Canada	NB	Cape Jourimaine	46.2	-63.83	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak231	Karen Samis	cc6	Canada	NS	Point Cross	46.6	-61.04	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak232	Karen Samis	SAM29	Canada	QC	Sainte-Anne-des Monts	49.1	-66.5	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak234	Charlie Willis, K. Donohue	MB30	USA	MI	Muskegon	43.3	-86.36	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E

cak236	Karen Samis	SC9	Canada	NL	Pistoles, Sandy Cove	48.6	-53.74	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak237	Karen Samis	SA10	Canada	NB	Kouch-Pointe Sapin	46.9	-64.87	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak239	Charles Willis	PJ19	USA	RI	Fishermans memorial beach	41.4	-71.49	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak241	Karen Samis	HB3	Canada	NB	Hebron, Prince Edward Island	46.6	-64.26	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak244	Karen Samis	CJ25	Canada	NB	Cape Jourimaine	46.2	-63.83	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak246	Karen Samis	PC16	Canada	NS	Pictou	45.7	-62.71	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak257	Karen Samis	SC8	Canada	NL	Pistoles, Sandy Cove	48.6	-53.74	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak261	Karen Samis	DB7	Canada	NB	Darnley Basin, Prince Edward Island	46.6	-63.7	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak270	Karen Samis	RH7	Canada	NL	Rocky Harbour	49.6	-57.92	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak277	Karen Samis	RH3	Canada	NL	Rocky Harbour	49.6	-57.92	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak288	Karen Samis	MP12	Canada	NB	Harvey Bank	45.7	-64.67	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak311	Karen Samis	ca1	Canada	NS	Canso	45.3	-60.97	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E

cak337	Karen Samis	DB17	Canada	NB	Darnley Basin, Prince Edward Island	46.6	-63.7	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak363	Karen Samis	kj10	Canada	NS	Kejimkujik National Park	44.2	-65.17	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak366	Karen Samis	KJ8	Canada	NS	Kejimkujik National Park	44.2	-65.17	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak368	Karen Samis	TP4	Canada	QC	Trois-Pistoles	48.1	-69.18	eNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	eNA_E	eNA_E
cak203	Erik Westberg	5.236	Greece		Ni Poroi	40	22.65	EU	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	EU_M	EU_M
cak219	Erik Westberg	5.252	Sweden		Åhus	55.9	14.33	EU	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	EU_M	EU_M
cak226	Erik Westberg	5.275	Spain		Castellon de la Plana	40	0.01	EU	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	EU_M	EU_M
cak233	Erik Westberg	5.24	Greece		Preveza	39	20.76	EU	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	EU_M	EU_M
cak240	Erik Westberg	5.266	Italy		Longobardy Marina	39.2	16.06	EU	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	EU_M	EU_M
cak249	Erik Westberg	5.255	France		Ruguel	48.7	-4.013	EU	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	EU_M	EU_M
cak252	Erik Westberg	5.251	Fuerteventura		Plajandia	28.4	-14.17	EU	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	EU_M	EU_M
cak260	Erik Westberg	5.272	Spain		Pl. Porcia	43.6	-6.876	EU	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	EU_M	EU_M

cak268	Erik Westberg	5.29	Italy	Spiaggia della Lecciona	43.8	10.26	EU	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	EU_M	EU_M
cak272	Erik Westberg	5.243	England		51.4	-0.23	EU	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	EU_M	EU_M
cak278	Erik Westberg	5.254	France	Le Crotoy	50.2	1.62	EU	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	EU_M	EU_M
cak280	Erik Westberg	5.303	Ireland	Greystones	53.2	-6.07	EU	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	EU_M	EU_M
cak283	Erik Westberg	5.281	Italy	Mondragon	41.1	13.53	EU	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	EU_M	EU_M
cak308	Erik Westberg	5.245	England	Saunton Sands	51.1	-4.209	EU	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	EU_M	EU_M
cak332	Erik Westberg	5.295	Italy	Egnazia	40.5	17.22	EU	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	EU_M	EU_M
cak342	Erik Westberg	5.296	Italy	Marcelli	43.5	13.63	EU	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	EU_M	EU_M
cak343	Erik Westberg	5.512	Morocco	Asilah	35.5	-6.03	MA	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	EU_M	EU_M
cak350	Erik Westberg	5.307	Cyprus	Princess Beach	35	33.67	EU	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	EU_M	EU_M
cak380	Sara Ohadi	n1111	New Zealand	Auckland	-36.8	174.8	NZ	NA	BC_M	Hybrid	NZ_H	/
cak345	K. Hodgins, K. Nurkowski	35B	USA OR	Ophir Beach	42.6	-124.4	wNA	*Cakile edentula*	*Cakile edentula*	Hybrid	wNA_E	wNA_E

cak73	K. Hodgins, K. Nurkowski	OR1-9E	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile edentula*	*Cakile edentula*	Hybrid	wNA_E	wNA_E
cak9	K. Hodgins, K. Nurkowski	OR1-23E	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile edentula*	*Cakile edentula*	Hybrid	wNA_E	wNA_E
cak96	K. Hodgins, K. Nurkowski	OR1-22E	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile edentula*	*Cakile edentula*	Hybrid	wNA_E	wNA_E
cak97	Sally Aitken	KODIAK2	USA	AK	Kodiak	57.8	-152.4	wNA	*Cakile edentula*	*Cakile edentula*	Hybrid	wNA_E	wNA_E
cak129	Sally Aitken	KODIAK10	USA	AK	Kodiak	57.8	-152.4	wNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	wNA_E	wNA_E
cak13	Sally Aitken	KODIAK1	USA	AK	Kodiak	57.8	-152.4	wNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	wNA_E	wNA_E
cak181	K. Hodgins, K. Nurkowski	WA1-17E	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	wNA_E	wNA_E
cak183	K. Hodgins, K. Nurkowski	WA1-32E	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	wNA_E	wNA_E
cak184	K. Hodgins, K. Nurkowski	OR1-4E	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	wNA_E	wNA_E

cak24	Sally Aitken	KODIAK 3	USA	AK	Kodiak	57.8	-152.4	wNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	wNA_E	wNA_E
cak367	K. Hodgins, K. Nurkowski	33C	USA	OR	Ophir Beach	42.6	-124.4	wNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	wNA_E	wNA_E
cak384	K. Hodgins, K. Nurkowski	37A	USA	OR	Crooked Creek Beack	43.1	-124.4	wNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	wNA_E	wNA_E
cak47	Sally Aitken	KODIAK 8	USA	AK	Kodiak	57.8	-152.4	wNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	wNA_E	wNA_E
cak48	Sally Aitken	KODIAK 5	USA	AK	Kodiak	57.8	-152.4	wNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	wNA_E	wNA_E
cak5	K. Hodgins, K. Nurkowski	WA1-18E	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	wNA_E	wNA_E
cak52	Sally Aitken	KODIAK 9	USA	AK	Kodiak	57.8	-152.4	wNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	wNA_E	wNA_E
cak54	K. Hodgins, K. Nurkowski	OR1-31E	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	wNA_E	wNA_E
cak64	K. Hodgins, K. Nurkowski	WA1-31E	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	wNA_E	wNA_E
cak66	Sally Aitken	KODIAK 6	USA	AK	Kodiak	57.8	-152.4	wNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	wNA_E	wNA_E

cak71	Sally Aitken	KODIAK 4	USA	AK	Kodiak	57.8	-152.4	wNA	*Cakile edentula*	*Cakile edentula*	*Cakile edentula*	wNA_E	wNA_E
cak147	K. Hodgins, K. Nurkowski	WA1-3E	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile edentula*	*Cakile edentula*	Hybrid	wNA_E	wNA_E
cak170	K. Hodgins, K. Nurkowski	WA1-9E	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile edentula*	*Cakile edentula*	Hybrid	wNA_E	wNA_E
cak172	K. Hodgins, K. Nurkowski	OR1-37E	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile edentula*	*Cakile edentula*	Hybrid	wNA_E	wNA_E
cak375	K. Hodgins, K. Nurkowski	51a	USA	OR	Nesika Beack	42.5	-124.4	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_H	wNA_H
cak57	K. Hodgins, K. Nurkowski	WA1-26M	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_H	wNA_H
cak65	K. Hodgins, K. Nurkowski	CA1-11	USA	CA	Eureka	40.8	-124.2	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_H	wNA_H
cak75	K. Hodgins, K. Nurkowski	OR1-AM	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_H	wNA_H

cak85	K. Hodgins, K. Nurkowski	OR1-10M	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_H	wNA_H
cak171	K. Hodgins, K. Nurkowski	WA1-13M	USA	WA	Ocean shores	47	-124.2	wNA	Hybrid	F1	Hybrid	wNA_H	wNA_H
cak126	K. Hodgins, K. Nurkowski	WA1-23M	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_H	wNA_H
cak134	K. Hodgins, K. Nurkowski	CA2-25M	USA	CA	DeHaven	39.7	-123.8	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_H	wNA_H
cak137	K. Hodgins, K. Nurkowski	OR1-26M	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_H	wNA_H
cak15	K. Hodgins, K. Nurkowski	OR1-20M	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_H	wNA_H
cak178	K. Hodgins, K. Nurkowski	OR1-30M	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_H	wNA_H
cak354	K. Hodgins, K. Nurkowski	40B	USA	OR	Crooked Creek Beack	43.1	-124.4	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M

cak40	K. Hodgins, K. Nurkowski	OR1-21M	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak55	K. Hodgins, K. Nurkowski	CA2-12	USA	CA	DeHaven	39.7	-123.8	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak60	K. Hodgins, K. Nurkowski	CA1-30	USA	CA	Eureka	40.8	-124.2	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak67	K. Hodgins, K. Nurkowski	CA1-15	USA	CA	Eureka	40.8	-124.2	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak77	K. Hodgins, K. Nurkowski	CA1-27	USA	CA	Eureka	40.8	-124.2	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak88	K. Hodgins, K. Nurkowski	OR1-5M	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak91	K. Hodgins, K. Nurkowski	OR1-2M	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak118	K. Hodgins, K. Nurkowski	LAX 11	USA	CA	Los Angeles	33.9	-118.4	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M

cak128	K. Hodgins, K. Nurkowski	CA2-40	USA	CA	DeHaven	39.7	-123.8	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak139	K. Hodgins, K. Nurkowski	CA1-23M	USA	CA	Eureka	40.8	-124.2	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak142	K. Hodgins, K. Nurkowski	SAN 36	USA	CA	San Diego	32.7	-117.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak145	K. Hodgins, K. Nurkowski	SAN 38	USA	CA	San Diego	32.7	-117.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak148	K. Hodgins, K. Nurkowski	SAN 12	USA	CA	San Diego	32.7	-117.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak150	K. Hodgins, K. Nurkowski	LAX 41	USA	CA	Los Angeles	33.9	-118.4	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak151	K. Hodgins, K. Nurkowski	LAX 9	USA	CA	Los Angeles	33.9	-118.4	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak152	K. Hodgins, K. Nurkowski	SAN 39	USA	CA	San Diego	32.7	-117.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M

cak154	K. Hodgins, K. Nurkowski	LAX 10	USA	CA	Los Angeles	33.9	-118.4	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak160	K. Hodgins, K. Nurkowski	LAX 40	USA	CA	Los Angeles	33.9	-118.4	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak161	K. Hodgins, K. Nurkowski	SAN 24	USA	CA	San Diego	32.7	-117.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak164	K. Hodgins, K. Nurkowski	SAN 20	USA	CA	San Diego	32.7	-117.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak168	K. Hodgins, K. Nurkowski	SAN 18	USA	CA	San Diego	32.7	-117.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak169	K. Hodgins, K. Nurkowski	LAX 3	USA	CA	Los Angeles	33.9	-118.4	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak173	K. Hodgins, K. Nurkowski	SAN 21	USA	CA	San Diego	32.7	-117.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak174	K. Hodgins, K. Nurkowski	LAX 5	USA	CA	Los Angeles	33.9	-118.4	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M

cak177	K. Hodgins, K. Nurkowski	LAX 2	USA	CA	Los Angeles	33.9	-118.4	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak179	K. Hodgins, K. Nurkowski	SAN 3	USA	CA	San Diego	32.7	-117.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak182	K. Hodgins, K. Nurkowski	SAN 22	USA	CA	San Diego	32.7	-117.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak185	K. Hodgins, K. Nurkowski	LAX 1	USA	CA	Los Angeles	33.9	-118.4	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak187	K. Hodgins, K. Nurkowski	SAN 29	USA	CA	San Diego	32.7	-117.3	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak20	K. Hodgins, K. Nurkowski	CA1-24	USA	CA	Eureka	40.8	-124.2	wNA	*Cakile maritima*	*Cakile maritima*	Hybrid	wNA_M	wNA_M
cak157	K. Hodgins, K. Nurkowski	LAX 8	USA	CA	Los Angeles	33.9	-118.4	wNA	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	wNA_M	wNA_M
cak41	K. Hodgins, K. Nurkowski	CA2-2	USA	CA	DeHaven	39.7	-123.8	wNA	*Cakile maritima*	*Cakile maritima*	*Cakile maritima*	wNA_M	wNA_M

cak210	Charles Willis	C19	Mexico		Tulum	20.2	-87.44	eNA	*Cakile lanceolata*	/	/	/	/	
cak1	K. Hodgins, K. Nurkowski	WA1-35M	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile maritima*					Filter
cak101	K. Hodgins, K. Nurkowski	CA2-3	USA	CA	DeHaven	39.7	-123.8	wNA	*Cakile maritima*					Filter
cak106	K. Hodgins, K. Nurkowski	OR1-7E	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile edentula*					Filter
cak115	Sara Ohadi	Slop E 19	Australia	TAS	Sloping Main	-43	147.7	AUS	*Cakile edentula*					not enough reads
cak124	K. Hodgins, K. Nurkowski	WA1-22M	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile maritima*					Filter
cak127	K. Hodgins, K. Nurkowski	CA1-21M	USA	CA	Eureka	40.8	-124.2	wNA	*Cakile maritima*					Filter
cak131	K. Hodgins, K. Nurkowski	WA1-1E	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile edentula*					Filter
cak132	Sara Ohadi	Slop H 16	Australia	TAS	Sloping Main	-43	147.7	AUS	Hybrid					not enoug

										h reads
cak138	K. Hodgins, K. Nurkowski	CA1-20M	USA	CA	Eureka	40.8	-124.2	wNA	*Cakile maritima*	Filter
cak14	K. Hodgins, K. Nurkowski	WA1-20E	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile edentula*	Filter
cak140	K. Hodgins, K. Nurkowski	CA2-23M	USA	CA	DeHaven	39.7	-123.8	wNA	*Cakile maritima*	Filter
cak141	K. Hodgins, K. Nurkowski	WA1-BM	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile maritima*	Filter
cak144	K. Hodgins, K. Nurkowski	CA1-5M	USA	CA	Eureka	40.8	-124.2	wNA	*Cakile maritima*	Filter
cak146	K. Hodgins, K. Nurkowski	LAX 6	USA	CA	Los Angeles	33.9	-118.4	wNA	*Cakile maritima*	not enough reads
cak162	Sara Ohadi	BOS 12	Australia	SA	Bosanquet Bay	-32.2	133.7	AUS	*Cakile maritima*	not enough reads
cak163	K. Hodgins, K.	CA2-26M	USA	CA	DeHaven	39.7	-123.8	wNA	*Cakile maritima*	Filter

	Nurkowski									
cak166	K. Hodgins, K. Nurkowski	OR1-29E	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile edentula*	Filter
cak17	K. Hodgins, K. Nurkowski	CA2-10	USA	CA	DeHaven	39.7	-123.8	wNA	*Cakile maritima*	Filter
cak180	K. Hodgins, K. Nurkowski	OR1-8M	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile maritima*	not enough reads
cak186	K. Hodgins, K. Nurkowski	SAN 23	USA	CA	San Diego	32.7	-117.3	wNA	*Cakile maritima*	not enough reads
cak188	K. Hodgins, K. Nurkowski	OR1-29M	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile maritima*	not enough reads
cak190	K. Hodgins, K. Nurkowski	OR1-28M	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile maritima*	not enough reads
cak191	K. Hodgins, K. Nurkowski	LAX 4	USA	CA	Los Angeles	33.9	-118.4	wNA	*Cakile maritima*	not enough reads
cak192	K. Hodgins, K.	CA1-6M	USA	CA	Eureka	40.8	-124.2	wNA	*Cakile maritima*	Filter

	Nurkowski									
cak202	UBC Herbarium, DNA extraction: Allan Strand	23	USA	MD	Poplar Island	38.8	-76.38	eNA	*Cakile edentula*	Filter
cak204	Feldstein	e4	Canada	NB	Prince Eward Island Southlake	46.4	-62.05	eNA	*Cakile edentula*	not enough reads
cak206	Karen Samis	kb3	Canada	NB	Kouch-Pointe Sapin	46.8	-64.91	eNA	*Cakile edentula*	Filter
cak211	Karen Samis	mc17	Canada	QC	Manicougan Peninsula	49.1	-68.2	eNA	*Cakile edentula*	Filter
cak213	Sara Ohadi	KG 2	Australia	SA	Brown Beach, Kangaroo Island	-35.8	137.8	AUS	*Cakile maritima*	Filter
cak23	K. Hodgins, K. Nurkowski	CA1-17	USA	CA	Eureka	40.8	-124.2	wNA	*Cakile maritima*	not enough reads
cak230	Sara Ohadi	KG 19	Australia	SA	Brown Beach, Kangaroo Island	-35.8	137.8	AUS	*Cakile maritima*	Filter
cak235	Erik Westberg	TRUM5	Iceland		Kálfafell	64	-17.54	EU	*Cakile maritima*	Filter
cak245	Erik Westberg	5.263	Portugal		Ribamar	39.2	-9.346	EU	*Cakile maritima*	Filter
cak248	Sara Ohadi	KG 11	Australia	SA	Brown Beach, Kangaroo Island	-35.8	137.8	AUS	*Cakile maritima*	Filter

cak25	K. Hodgins, K. Nurkowski	CA2-6	USA	CA	DeHaven	39.7	-123.8	wNA	*Cakile maritima*	Filter
cak253	Sara Ohadi	KG 14	Australia	SA	Brown Beach, Kangaroo Island	-35.8	137.8	AUS	*Cakile maritima*	not enough reads
cak258	S.M. Wilson	1	USA	CA	Arcata	40.8	-124.1	wNA	*Cakile maritima*	not enough reads
cak259	Sara Ohadi	KG 5	Australia	SA	Brown Beach, Kangaroo Island	-35.8	137.8	AUS	*Cakile maritima*	Filter
cak262	Bryan Connoly	e1	USA	CT	Groton	41.1	-73.37	eNA	*Cakile edentula*	Filter
cak263	Karen Samis	PS7	Canada	NL	Port Saunders	50.6	-57.29	eNA	*Cakile edentula*	Filter
cak264	Sara Ohadi	KG 16	Australia	SA	Brown Beach, Kangaroo Island	-35.8	137.8	AUS	*Cakile maritima*	Filter
cak266	Karen Samis	TP5	Canada	QC	Trois-Pistoles	48.1	69.18	eNA	*Cakile edentula*	Filter
cak267	Sara Ohadi	KG 20	Australia	SA	Brown Beach, Kangaroo Island	-35.8	137.8	AUS	*Cakile maritima*	Filter
cak269	Erik Westberg	5.424	Denmark		Juelsmide	55.7	10.01	EU	*Cakile maritima*	Filter
cak27	K. Hodgins, K.	WA1-14M	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile maritima*	Filter

	Nurkowski									
cak271		13	Canada	BC	McNeilBay	48.4	-123.3	wNA	*Cakile maritima*	Filter
cak273	Sara Ohadi	KG 10	Australia	SA	Brown Beach, Kangaroo Island	-35.8	137.8	AUS	*Cakile maritima*	Filter
cak275	Erik Westberg	5.264	Italy		Spiaggia della Lecciona	43.8	10.26	EU	*Cakile maritima*	Filter
cak276	Erik Westberg	5.3	Greece		Olympiada	40.6	23.78	EU	*Cakile maritima*	not enough reads
cak279	Sara Ohadi	KG 9	Australia	SA	Brown Beach, Kangaroo Island	-35.8	137.8	AUS	*Cakile maritima*	Filter
cak282	J Rebman (22863; 2012) SD 222915, Sandiego Herbarium, DNA extraction: Allan Strand	9	USA	CA	Baja	29.9	-114.4	wNA	*Cakile maritima*	not enough reads
cak284	K. Hodgins, K. Nurkowski	35A	USA	OR	Ophir Beach	42.6	-124.4	wNA	NA	Filter

cak286	Erik Westberg	5.132	Turkey		Sile	41.2	29.6	EU	*Cakile maritima*	not enough reads
cak287	Erik Westberg	5.265	Portugal		Grande Porto Covo Beach	37.9	-8.794	EU	*Cakile maritima*	not enough reads
cak29	K. Hodgins, K. Nurkowski	CA1-33	USA	CA	Eureka	40.8	-124.2	wNA	*Cakile maritima*	Filter
cak290	UBC Herbarium, DNA extraction: Allan Strand	19	Canada	BC	Uclulet	48.9	-125.5	wNA	*Cakile edentula*	Filter
cak291	Sara Ohadi	SERR15	Australia	WA	Geralton	-28.8	114.6	AUS	*Cakile maritima*	Filter
cak293	Sara Ohadi	ENTIRE10	Australia	WA	Geralton	-28.8	114.6	AUS	*Cakile maritima*	Filter
cak294	Sara Ohadi	EXPL 3	Australia	WA	Geralton	-28.8	114.6	AUS	*Cakile maritima*	Filter
cak296	Sara Ohadi	ENTIRE9	Australia	WA	Geralton	-28.8	114.6	AUS	*Cakile maritima*	Filter
cak298	Sara Ohadi	SERR14	Australia	WA	Geralton	-28.8	114.6	AUS	*Cakile maritima*	Filter
cak299	Sara Ohadi, Roger Cousens	POT13	Australia	NSW	Fingal Head	-28.2	153.6	AUS	*Cakile edentula*	Filter

cak300	Charles Willis	C145	Puerto Rico		Puerto Nuevo Beach	18.5	-66.39	eNA	*Cakile lanceolata*	Filter
cak304	Karen Samis	HB2	Canada	NB	Hebron, Prince Edward Island	46.6	-64.26	eNA	*Cakile edentula*	Filter
cak312	K. Hodgins, K. Nurkowski	37B	USA	OR	Crooked Creek Beack	43.1	-124.4	wNA	*Cakile edentula*	Filter
cak318	J. Chitty	B3	Australia	WA	Bunbury	-33.3	115.6	AUS	*Cakile maritima*	Filter
cak319	Sara Ohadi	CUR 22	Australia	QLD	Currumbin	-28.1	153.5	AUS	*Cakile edentula*	Filter
cak320	Roger Cousens	DUN28	Australia	NSW	Dunbogan Beach	-31.7	152.8	AUS	*Cakile maritima*	Filter
cak321	K. Hodgins, K. Nurkowski	56/9s?	USA	OR	Oregon Dunes	43.7	-124.2	wNA	*Cakile maritima*	Filter
cak325	Roger Cousens	CJB 11	Australia	SA	West Lake Shore	-34.9	138.5	AUS	*Cakile maritima*	Filter
cak326	Karen Samis	KB2	Canada	NB	Kouch-Pointe Sapin	46.8	-64.91	eNA	*Cakile edentula*	not enough reads
cak327	Sara Ohadi	KG 4	Australia	SA	Brown Beach, Kangaroo Island	-35.8	137.8	AUS	*Cakile maritima*	not enough reads
cak328	Roger Cousens	CJB 10	Australia	SA	West Lake Shore	-34.9	138.5	AUS	*Cakile maritima*	Filter

cak330	Roger Cousens	SWR14	Australia	NSW	South West Rocks	-30.9	153	AUS	*Cakile maritima*	Filter
cak331	Sara Ohadi, Roger Cousens	WHA6	Australia	SA	Whyalla	-33	137.6	AUS	*Cakile maritima*	Filter
cak334		22	Canada	BC	Qualicum Beach	49.4	-124.4	wNA	*Cakile edentula*	Filter
cak335	J. Chitty	B10B	Australia	WA	Bunbury	-33.3	115.6	AUS	*Cakile maritima*	Filter
cak339	Sara Ohadi	P18	Australia	VIC	Port Fairy	-38.4	142.3	AUS	*Cakile maritima*	not enough reads
cak34	K. Hodgins, K. Nurkowski	CA1-10	USA	CA	Eureka	40.8	-124.2	wNA	*Cakile maritima*	Filter
cak341	Sara Ohadi	P25	Australia	VIC	Port Fairy	-38.4	142.3	AUS	*Cakile maritima*	Filter
cak349	Sara Ohadi, Roger Cousens	POT30	Australia	NSW	Fingal Head	-28.2	153.6	AUS	*Cakile edentula*	Filter
cak351	Sara Ohadi	D10	Australia	VIC	Discovery Bay	-38.2	141.3	AUS	*Cakile maritima*	Filter
cak355	Sara Ohadi	CUR 8	Australia	QLD	Currumbin	-28.1	153.5	AUS	*Cakile edentula*	Filter
cak356	Sara Ohadi	P27B	Australia	VIC	Port Fairy	-38.4	142.3	AUS	*Cakile maritima*	Filter

cak357	J. Chitty	B11	Australia	WA	Bunbury	-33.3	115.6	AUS	*Cakile maritima*	Filter
cak358	Sara Ohadi	P28	Australia	VIC	Port Fairy	-38.4	142.3	AUS	*Cakile maritima*	Filter
cak36	K. Hodgins, K. Nurkowski	WA1-2..E	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile edentula*	Filter
cak360	B. Munson (10; 2007) SD 193699, Sandeigo Herbarium, DNA extraction: Allan Strand	10	USA	CA	San Diego	32.7	-117.3	wNA	*Cakile maritima*	Filter
cak365	UBC Herbarium, DNA extraction: Allan Strand	20	Canada	BC	Uclulet	48.9	-125.5	wNA	*Cakile edentula*	Filter
cak369		8	USA	CA	San Diego	32.7	-117.3	wNA	*Cakile maritima*	Filter
cak371	Roger Cousens	CJB 13	Australia	SA	West Lake Shore	-34.9	138.5	AUS	*Cakile maritima*	not enough reads
cak372	Karen Samis	TP7	Canada	QC	Trois-Pistoles	48.1	69.18	eNA	*Cakile edentula*	not enoug

										h reads
cak373	Karen Samis	WM8	Canada	NS	West Mabou	46.1	-61.48	eNA	*Cakile edentula*	not enough reads
cak374	Sara Ohadi	11	Australia	WA	Geralton	-28.8	114.6	AUS	*Cakile maritima*	not enough reads
cak376	K. Hodgins, K. Nurkowski	32A-K	USA	OR	Ophir Beach	42.5	-124.4	wNA	*Cakile edentula*	Filter
cak377	Sara Ohadi	D13	Australia	VIC	Discovery Bay	-38.2	141.3	AUS	*Cakile maritima*	Filter
cak378	Roger Cousens	CJB 6	Australia	SA	West Lake Shore	-34.9	138.5	AUS	*Cakile maritima*	not enough reads
cak38	K. Hodgins, K. Nurkowski	CA1-28	USA	CA	Eureka	40.8	-124.2	wNA	*Cakile maritima*	Filter
cak381	K. Hodgins, K. Nurkowski	45a	USA	OR	Cressy Field State Park	42	-124.2	wNA	*Cakile maritima*	Filter
cak383	Sara Ohadi	D11	Australia	VIC	Discovery Bay	-38.2	141.3	AUS	*Cakile maritima*	not enough reads
cak39	K. Hodgins, K.	WA1-30M	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile maritima*	Filter

	Nurkowski									
cak42	K. Hodgins, K. Nurkowski	CA2-1	USA	CA	DeHaven	39.7	-123.8	wNA	*Cakile maritima*	Filter
cak45	K. Hodgins, K. Nurkowski	WA1-27E	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile edentula*	Filter
cak51	K. Hodgins, K. Nurkowski	CA2-11	USA	CA	DeHaven	39.7	-123.8	wNA	*Cakile maritima*	Filter
cak61	K. Hodgins, K. Nurkowski	OR1-27E	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile edentula*	Filter
cak70	K. Hodgins, K. Nurkowski	CA1-8	USA	CA	Eureka	40.8	-124.2	wNA	*Cakile maritima*	Filter
cak84	Sara Ohadi	RHE M 16	Australia	TAS	Rhebans Beach	-42.6	148	AUS	*Cakile maritima*	not enough reads
cak86	K. Hodgins, K. Nurkowski	CA2-9	USA	CA	DeHaven	39.7	-123.8	wNA	*Cakile maritima*	Filter
cak90	K. Hodgins, K.	OR1-35E	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile edentula*	not enough reads

	Nurkowski									
cak92	K. Hodgins, K. Nurkowski	WA1-28E	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile edentula*	Filter
cak93	K. Hodgins, K. Nurkowski	WA1-33M	USA	WA	Ocean shores	47	-124.2	wNA	*Cakile maritima*	Filter
cak94	K. Hodgins, K. Nurkowski	WA1-15H	USA	WA	Ocean shores	47	-124.2	wNA	Hybrid	not enough reads
cak95	K. Hodgins, K. Nurkowski	OR1-34E	USA	OR	Oregon,Empire	43.4	-124.3	wNA	*Cakile edentula*	not enough reads

Table 2-S2 Weir and Cockerham's (1984) pairwise F_{ST} between groups identified by the *supervised run* of Admixture using the *global thinned data set* in each region. Triangle below mean F_{ST}, triangle above weighted F_{ST}. ENA_E= eastern North American *C. edentula*, AUS_E= Australian *C. edentula*, wNA_E= western North American *C. edentula*, EU_M = European and northern African *C. maritima*, AUS_M= Australian *C. maritima*, wNA_M= western North American *C. maritima*, AUS_H= Australian hybrids, wNA_H= western North American hybrids

	AUS_E	**AUS_M**	**AUS_H**	**wNA_E**	**wNA_M**	**wNA_H**	**EU_M**	**eNA_E**
AUS_E	0	0.808	0.450	0.291	0.764	0.781	0.776	0.320
AUS_M	0.592	0	0.106	0.728	0.240	0.166	0.175	0.802
AUS_H	0.305	0.080	0	0.399	0.220	0.091	0.124	0.456
wNA_E	0.140	0.522	0.273	0	0.701	0.680	0.691	0.240
wNA_M	0.573	0.164	0.170	0.530	0	0.082	0.263	0.764
wNA_H	0.606	0.120	0.083	0.514	0.086	0	0.174	0.772
EU_M	0.525	0.119	0.097	0.457	0.184	0.128	0	0.771

eNA_E	0.131	0.590	0.311	0.135	0.576	0.610	0.527	0

Table 2-S3 Population diversity statistics of the *global thinned data set* (4561 SNPs, 256 individuals, exclusion of the New Zealand and *C. lanceolata* samples). The population groupings are based on the *supervised run* of Admixture. H_O = observed heterozygosity, A_R=allelic richness. Confidence intervals for A_R are presented: means with the same letters do not differ significantly.

Species	**Range**	**N**	**H_O**	**A_R**	**A_R_CI**
C. edentula	eastern North America	44	0.021	1.046	1.039-1.057a
	Australia	38	0.023	1.024	1.015-1.045a
	western North America	24	0.024	1.06	1.046-1.073a
C. maritima	Europe and northern Africa	18	0.157	1.643	1.546-1.701b
	Australia	15	0.203	1.665	1.572-1.725b
	Western North America	33	0.206	1.618	1.571-1.654b
Hybrid	Australia	73	0.246	1.854	1.816-1.881c
	Western North America	11	0.272	1.736	1.581-1.816b

Table 2-S4 Admixture, NewHybrids and HIest classification of hybrid ancestry for *Cakile* individuals sampled in Australia, western North America, New Zealand, eastern North America, Europe and northern Africa. The number classified as a pure species or to a hybrid generation (BC-E= backcross to *C. edentula*, BC-M= backcross to *C. maritima*) is shown and percentage per range is given. Note that Admixture does not identify the hybrid class.

Program	**Range**	***C. edentula***	***C. maritima***	**F1**	**F2**	**BC-E**	**BC-M**	**Advanced Generation Hybrids**	**Total Hybrids**
Admixture	Australia	38 (30.16%)	15 (11.09%)						73 (57.94%)
	western North America	24 (35.29%)	33 (48.53%)						11 (16.18%)
	New Zealand								1 (100%)

	eastern North America	44 (100%)							
	Europe and northern Africa		18 (100%)						
Total									85
NewHybrids	Australia	38 (30.16%)	71 (56.35%)	4(3.17%)	6(4.76%)	1(0.79%)	6(4.76%)		17(13.49%)
	western North America	24 (35.29%)	43(64.34%)	1(1.47%)					1(1.47%)
	New Zealand						1 (100%)		1 (100%)
	eastern North America	44 (100%)							
	Europe and northern Africa		18 (100%)						
Total				5	8	1	11		19
HIest	Australia	37 (29.37%)	2(1.59%)					87(69.05%)	87(69.05%)
	western North America	16 (23.53%)	2(2.94%)					50(73.53%)	50(73.53%)
	New Zealand							1 (100%)	1 (100%)
	eastern North America	44 (100%)							
	Europe and northern Africa		18 (100%)						
Total								138	138

Table 2-S5 Results of the HItest function of HIest using 471 SNPs (Allele frequency: 0.99 *C. edentula*, 0.03 *C. maritima*). Number of individuals are presented which best-fitted class was more than two log-likelihood units over the second best class (c1) and number of individuals was more than two log-likelihood units of the maximum likelihood estimate (c2) (column 1). The remainder are in column 2.

Data set		Number of individuals greater than threshold	Number of individuals within threshold
63 SNPs (0 *C. edentula*, 1 *C. maritima*)	c1	257	0
	c2	173	84
471 SNPS (0.99 *C. edentula*, 0.03 *C. maritima*)	c1	256	1
	c2	119	138
471 SNPS (0.99 C. *edentula*, 0.06 *C. maritima*)	c1	256	1
	c2	125	132

Table 2-S6 Summary of the results of the HIest function of HIest. E= *C. edentula*, M= *C. maritima*, BC-E= backcross to *C. edentula*, BC-M= backcross to *C. maritima.* W. North America= western North America, e. North America= eastern North America.

SNP set	Allele frequency	Range	E	M	F1	F2	BC-E	BC-M	continuous classification of hybrids	hybrid total
63 SNPs	1 E/0 M	Australia	38	15		1			72	73
		w. North America	24	33					11	11
		New Zealand							1	1
		e. North America	44							
		Europe		18						
		Total								85
471 SNPs	0.99 E/ 0.03 M	Australia	37	2					87	87
		w. North America	16	2					50	50
		New Zealand							1	1
		e. North America	44							
		Europe		18						
		Total								138
471 SNPs	0.99 E/ 0.06 M	Australia	37	4					85	85

	w. North America	15	7	46	46
	New Zealand			1	1
	e. North America	44			
	Europe		18		
	Total				132

Table 2-S7 Weir and Cockerham's (1984) pairwise F_{ST} between pure *C. edentula* groups, identified by the *unsupervised* Admixture *run* using the *global thinned data set* (Figure 2-1). Triangle below mean F_{ST}, triangle above weighted F_{ST}. W. North America = western North America, e. North America= eastern North America.

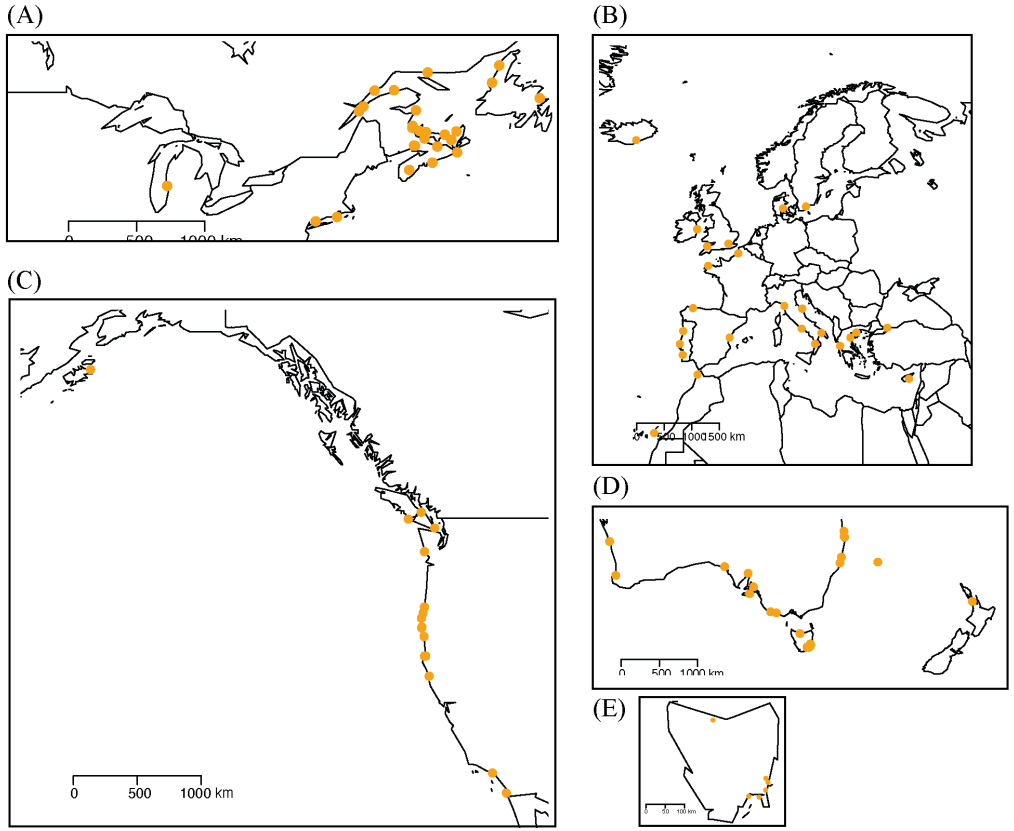
	Australia	**w. North America associated with Nova Scotia**	**w. North America associated with Lake Michigan/ Rhode Island**	**e. North America associated with Quebec and Newfoundland**	**e. North America associated with New Brunswick**	**e. North America associated with Nova Scotia**	**e. North America associated with Lake Michigan/ Rhode Island**
Australia	0	0.03	0.58	0.39	0.51	0.31	0.63
w. North America associated with Nova Scotia	0.03	0	0.46	0.31	0.46	0.24	0.53
w. North America Lake associated with Lake Michigan/ Rhode Island	0.24	0.21	0	0.45	0.52	0.42	0.13
e. North America associated with Quebec and Newfoundland	0.18	0.18	0.22	0	0.22	0.18	0.45
e. North America associated with New Brunswick	0.22	0.27	0.24	0.14	0	0.33	0.56
e. North America associated with Nova Scotia	0.14	0.13	0.18	0.12	0.21	0	0.44
e. North America associated with Lake Michigan/ Rhode Island	0.30	0.36	-0.08	0.23	0.35	0.23	0

Table 2-S8 Weir and Cockerham's (1984) pairwise F_{ST} between pure *C. maritima* groups, identified by the *unsupervised* Admixture *run* using the *global thinned data set* (Figure 2-1). Triangle below mean F_{ST}, triangle above weighted F_{ST}.

1214

	European Atlantic	European Mediterranean	Australian	western North American
European Atlantic	0	0.26	0.31	0.38
European Mediterranean	0.17	0	0.13	0.22
Australian	0.20	**0.08**	0	0.24
western North American	0.25	**0.16**	0.16	0

1215



1216

1217

Figure 2-S1 Sampling locations. (A) eastern North America, (B) Europe and northern Africa, (C) western North America, (D) Australia and New Zealand and (E) close up of Tasmania.

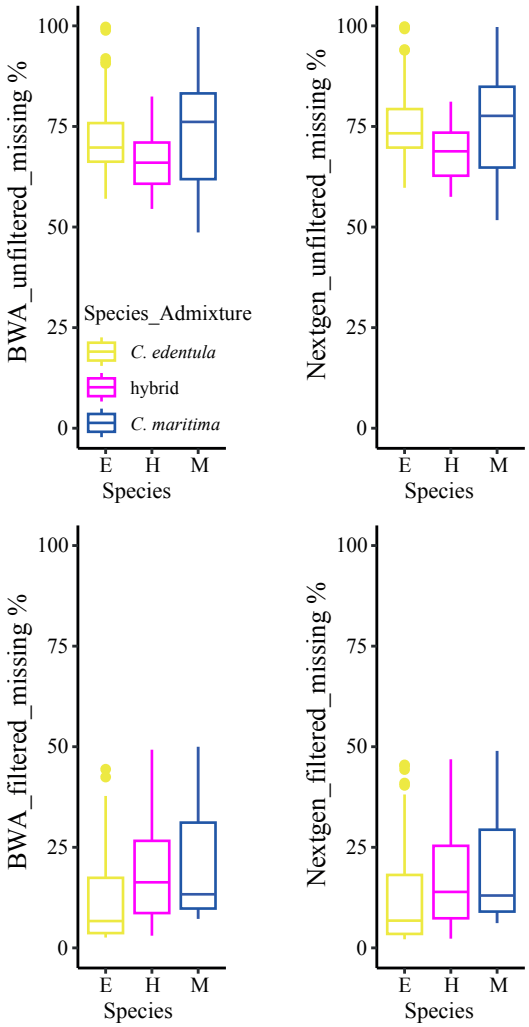


Figure 2-S2 Comparison of the Burrows-Wheeler aligner (BWA) and NextGenMap aligner (Nextgen). (A) The percentage of missing data per species using the Burrows-Wheeler aligner before filtering the data set. (B) The percentage of missing data per species using the NextGenMap aligner before filtering the data set. (C) Percentage of missing data per species using the Burrows-Wheeler aligner after filtering the data set (*filtered data set*). (D) Percentage of missing data per species using the NextGenMap aligner after filtering the data set. E= *C. edentula*, M= *C. maritima*, H= Hybrids.

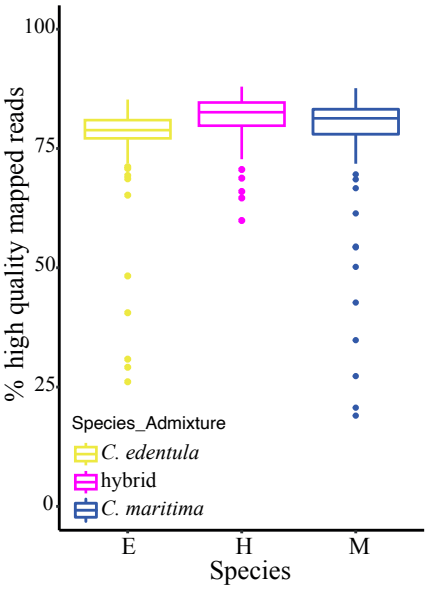


Figure 2-S3 Percentage of mapped high-quality reads (MQ=20, percentage of high quality reads mapped to the total reads mapped) per species, using the Burrows-Wheeler aligner. E= *C. edentula*, M= *C. maritima*, H= Hybrids.

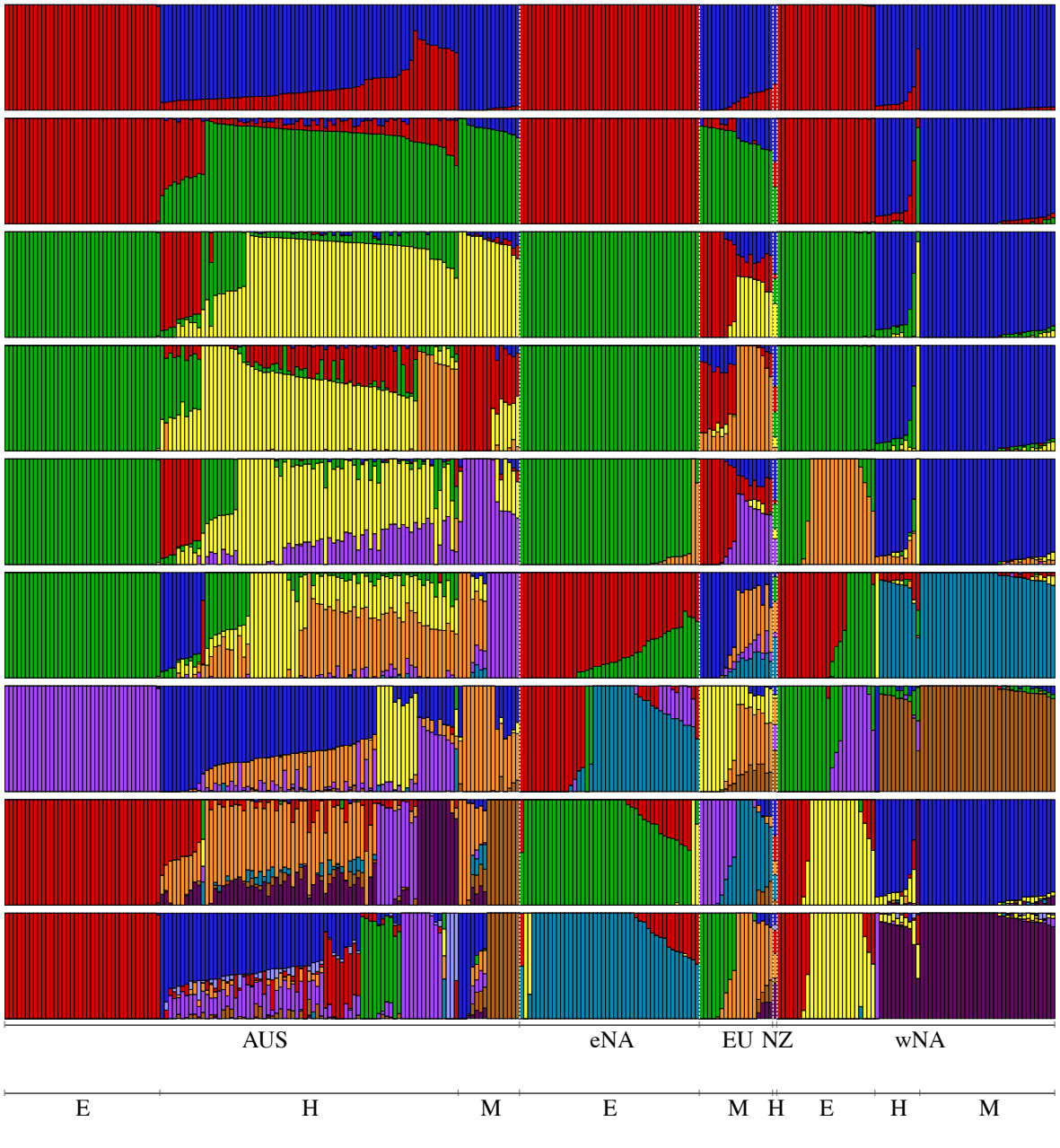


Figure 2-S4 Admixture results of the *global thinned data set*. Distruct plot for K=2-10. Individuals are ordered according to their cluster association of the *supervised run*. AUS=Australia, eNA= eastern North America, EU= Europe and northern Africa, NZ= New Zealand, wNA=western North America. E= *C. edentula*, M= *C. maritima*, H= Hybrid.

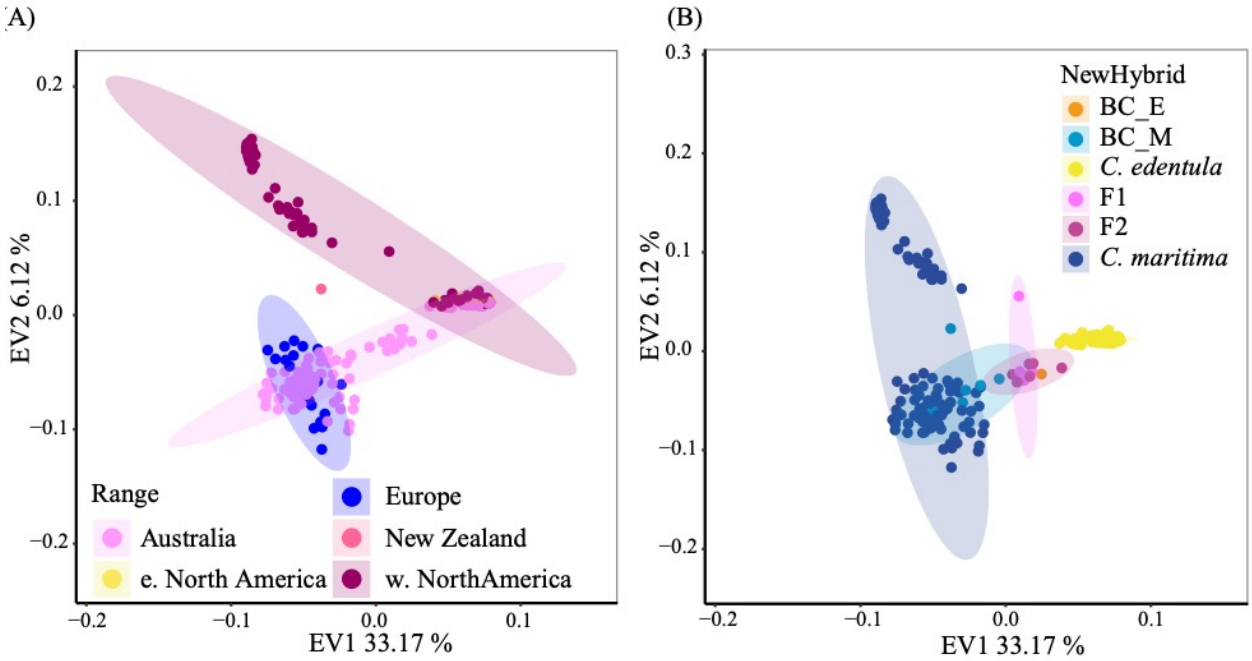


Figure 2-S5 Principal component analysis of the *global thinned data set*. The first two eigenvectors are presented. Ellipses indicate the 95 % confidence range of the cluster. (A) Individuals are coloured according to the origin of the samples (Australia, Europe and northern Africa, New Zealand, western North America (w. North America), eastern North America (e. North America). (B) Individuals are coloured according to NewHybrids classification of individuals. E= *C. edentula*, M= *C. maritima*, F1, F2, BC-E= backcross to *C. edentula*, BC-M= backcross to *C. maritima.*

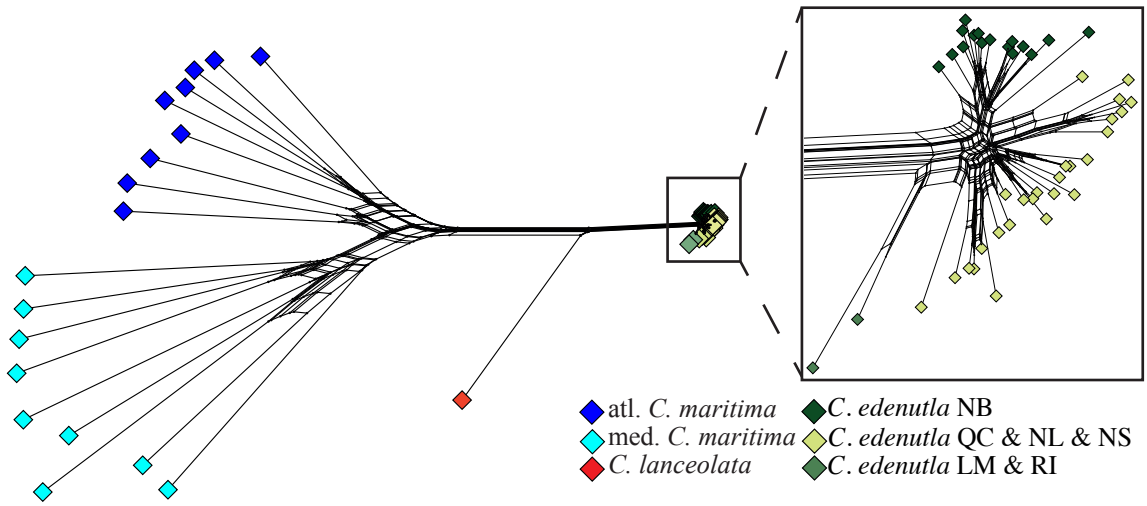


Figure 2-S6 Splitstree network of the *native range Splitstree data set*. Individuals are coloured after their cluster association from the *unsupervised run* of Admixture (using the *global thinned data set). C. maritima* from the Atlantic (atl. *C. maritima*), *C. maritima* from the Mediterranean (med. *C. maritima*), *C. edentula* from eastern North America (Figure 2-1) and outgroup *C. lanceolata* are presented. *C. edentula* coloured according to their predominant clusters; NB= New Brunswick, QC= Quebec, NL= Newfoundland, LM= Lake Michigan, RI= Rhode Island, NS= Nova Scotia.

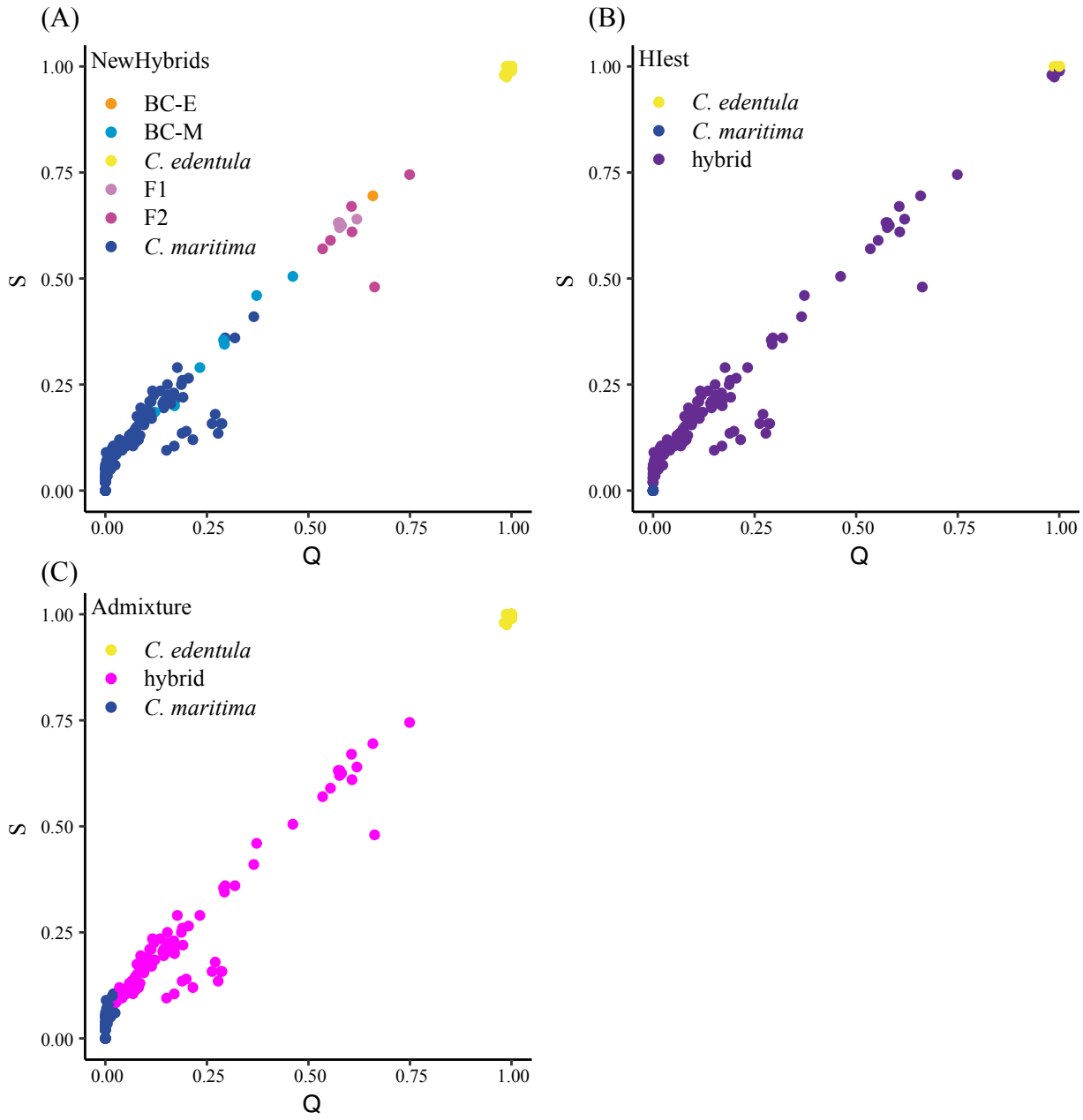


Figure 2-S7 Correlation of the Q-value of the *C. edentula* cluster of the *supervised run* of Admixture and the S-value of HIest (471 SNPs; Allele frequency: 0.99 C. edentula, 0.03 C. maritima). Classification of species and generation using (A) NewHybrid, (B) HIest (for hybrids continuous classification was a better fit than hybrid classes), and (C) Admixture. BC-E= Backcross to *C. edentula*, BC-M= Backcross to *C. maritima*.

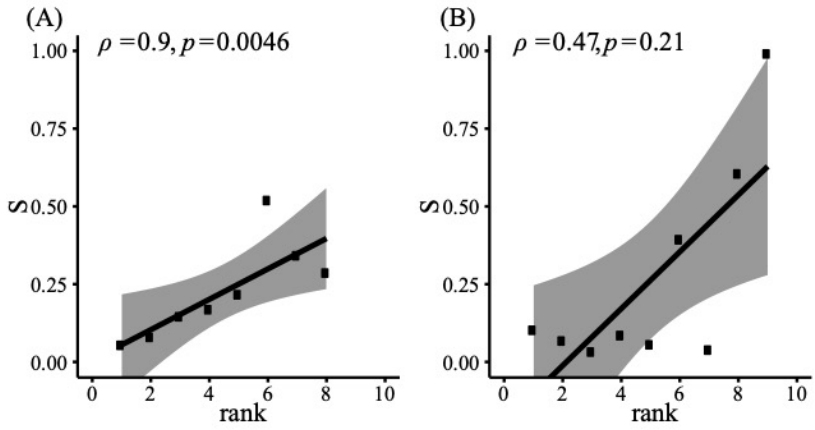


Figure 2-S8 Results of the Spearman correlation test displayed (Table 2-2). The associations between population mean S values of hybrids identified using HIest 471 SNPs (allele frequencies: 0.99 *C. edentula*, 0.03 *C. maritima*) and the ranked order of populations from the first entry point of *C. maritima* in (A) south-eastern Australia and (B) in western North America.

Chapter 3 - Convergent and divergent trait evolution during the global range expansion of two co-occurring invaders

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3.1 Abstract

During invasion it can be crucial for alien species to adapt to their new environment to establish and spread. Increasingly, invasions of the same geographic regions involve multiple closely related species, allowing previously allopatric species to interact. Such instances provide opportunities to examine patterns of trait divergence during invasion to assess the repeatability of evolution following range expansion to similar environments. To do this we used two *Cakile* species, *C. edentula* and *C. maritima*, which are native to opposite sides of the Atlantic, but co-occur and hybridize in their introduced ranges of western North America and Australia. To examine patterns of trait evolution between the species we grew 398 plants from 49 populations in a greenhouse common garden and measured traits related to phenology, defence, performance, physiology and morphology. We also conducted whole-genome resequencing of all of the samples to identify putative source populations, classify recent hybrids, and to assess population-level selfing rates. We identified convergent patterns of trait evolution for germination % and aphid damage for both species in both introduced ranges. Plants from the introduced range experienced greater damage from herbivores but enhanced germination %, consistent with theories predicting the evolution of reduced defence and enhanced performance during invasion. However, in the sympatric introduced ranges, *C. maritima* flowered much later (25 days later on average in invasive ranges) and at a larger size than *C. edentula,* while source populations of both species flowered at similar times and sizes. This evolution of divergent flowering times in sympatry is consistent with reproductive character displacement. Finally, we uncovered parallel latitudinal clines in flowering time and size that are apparent in both species, and rapidly evolved following range expansion along two broad climatic gradients in western North American and Australia. These parallel clines are likely caused by local adaptation to climate gradients and mirror native range patterns. Although non-adaptive explanations for the parallel trait changes cannot be completely ruled out, our data strongly support rapid adaptation (100-150 generations) in response to both biotic and abiotic environmental heterogeneity following invasion.

3.2 Introduction

Invasive species enable us to study ecological and evolutionary processes on contemporary time scales (Bock et al., 2015). Through them we can gain novel insights into evolutionary processes such as the rate of adaptation and the impact of demographic changes on adaptive

trajectories. Additionally, we can develop our understanding of the characteristics that define invasive populations, and what genetic changes underlie these characteristics (Bock et al., 2015; Lawson Handley et al., 2011; Lee, 2002). Species introduced to new ranges face many challenges in mounting a successful invasion. An invasive species' establishment is more likely if the environment is a close climatic match to the species' native range, but at least some aspects (biotic and/or abiotic) will be novel (Atwater et al., 2018; Bock et al., 2015; Broennimann et al., 2007; Colautti & Lau, 2015). Therefore, a crucial component of invasion can be adaptation to the local environment (Bock et al., 2015). As the rate of invasions increases globally (Ellstrand & Schierenbeck, 2000), so too do novel species interactions, and this can have evolutionary consequences. Increasingly, closely related species that were once allopatric in the native range are being found in sympatry, creating opportunities to study the impact of these novel species interactions on their evolutionary trajectories. Such instances can provide opportunities to examine if convergent or divergent evolutionary responses occur following introduction, shedding light on the repeatability of adaptation during range expansion.

During invasion, substantial changes in the biotic environment are expected, including shifts in the composition and abundance of enemies, competitors, and mutualists (Colautti et al., 2004; Keane & Crawley, 2002). One of the most influential hypotheses relating to trait evolution in invasive species is the evolution of increased competitive ability (EICA), which posits that an escape from natural enemies in the introduced range will shift allocation of resources away from defence, to growth or reproduction, facilitating the evolution of a more "invasive" phenotype (Blossey & Notzold, 1995). Studies have tested EICA using common garden comparisons of native and introduced populations (Blumenthal & Hufbauer, 2007; Bossdorf et al., 2005; Colautti et al., 2009; Felker-Quinn et al., 2013; Joshi et al., 2014; Orians & Ward, 2010). Overall evolutionary shifts in traits related to reproduction, growth, defence, and competitive ability in many invasive species are common, but not always in the direction predicted by EICA (Colautti & Lau, 2015; Felker-Quinn et al., 2013) and evolution of defence response may be specific to particular changes in the composition and abundance of the herbivore community and influenced by other aspects of the environment. Although the biotic environment is likely to shift during invasion and exert a selective pressure, convergent evolutionary changes, which can be a hallmark of selection, in defence-related traits during introduction have rarely been examined among multiple introduced ranges of the same species, or among species invading the same regions.

Many invasive species are found across broad environmental gradients. As a result, adaptation to local climate conditions is expected to evolve and even contribute to range expansion. Indeed, local adaptation can be rapid, with many examples occurring in under 50 years (Whitney & Gabler, 2008). In a meta-analysis, Oduor et al. (2016) showed that the signature of local adaptation in invasive species was at least as strong as in native species. Evolved clines in phenotypic traits have been identified in invasive ranges, consistent with rapid local adaptation to climate (reviewed in Colautti et al., 2009). In many annual plants, a trade-off between earlier flowering and plant size occurs: Earlier flowering at higher latitudes ensures reproduction in a shorter growing season at the cost of plant size (Colautti & Barrett, 2013; Griffith & Watson, 2006; Haggerty & Galloway, 2011; Hodgins & Rieseberg, 2011; Leiblein-Wild & Tackenberg, 2014; Santamaria et al., 2003; van Boheemen et al., 2019). For example, Colautti and Barrett (2013) convincingly demonstrated that invasive *Lythrum salicaria* populations have adapted to their local climate, where an earlier onset of flowering evolved at the cost of size in the north relative to the south of its introduced range. More recently parallel latitudinal clines in flowering time, size and many other traits have been identified across multiple introductions and the native range in *Ambrosia artemisiifolia*, alongside signatures of parallel adaptation to climate in genomic data (Hodgins & Rieseberg, 2011; van Boheemen et al., 2019). Together these data suggest that parallel patterns of climate adaptation are likely to evolve rapidly during invasion, especially for phenology and size, when annual plant species expand their range across similar climatically diverse regions.

The success of many invasive species despite the negative consequences of bottlenecks commonly experienced during introduction, is known as the genetic paradox of invasion (Allendorf & Lundquist, 2003). An introduced species' capacity to adapt to a novel environment relies on genetic variation, yet standing variation is expected to be frequently diminished upon introduction. New beneficial mutations are a potential alternative source of adaptive genetic variation (Bock et al., 2015; Hedrick, 2013), but the waiting time for new mutations could severely limit rapid adaptation via this mechanism. However, the loss of genetic diversity experienced during introduction can be ameliorated by hybridization, as well as by multiple introductions and subsequent mixing, as these processes can produce a large amount of variation and novel combinations of alleles (Bossdorf et al., 2005; Dlugosch & Parker, 2008; Ellstrand & Schierenbeck, 2000). As we begin to see the impacts of climate change on the planet, the role of hybridization during invasion is an important consideration. Evidence is accumulating that both invasion and climate change accelerate hybridization

(Chown et al., 2015; Garroway et al., 2010; Muhlfeld et al., 2014), and some studies reveal that genes of hybrid origin can be important for climate change adaptation (Becker et al., 2013; Chown et al., 2015; De La Torre et al., 2014). In some cases, hybridization can lead to adaptive introgression, which may facilitate climate adaptation during range expansion. For example, in Geraldes et al., (2014), introgression from *Populus balsamifera* into *Populus trichocarpa* influenced the geographic and climatic pattern of genetic variation: *P. balsamifera* genes associated with faster growth enabled admixed *P. trichocarpa* to colonize northern regions by compensating for short growing season at higher latitudes. Similarly, introgression of genes from *Rhododendron ponticum* into *Rhododendron. catawbiense* on the British Isles allowed the introduced species to better tolerate cold (Milne & Abbott, 2000). However, it is still an open question as to how frequently hybridization helps or hinders rapid adaptation to changing environments during invasion.

Multiple studies have revealed that invasive populations can evolve traits that may facilitate their invasiveness, including increased size, fecundity and colonization ability (Hovick & Whitney, 2014; Jelbert et al., 2015). Hybridization has been implicated as a driving factor behind invasions in some cases (Ellstrand & Schierenbeck, 2000). In support of this, a review by Hovick and Whitney (2014) has shown that hybridization is associated with the evolution of invasiveness, and hybrids tend to be larger and more fecund than their parental species. However, performance of hybrids depends on the hybrid class (F1, back crosses, etc). F1-generation hybrids can experience hybrid vigour caused by high heterozygosity, but subsequent generations can have decreasing fitness due to segregation (Arnold & Martin, 2010; Arnold & Hodges, 1995; Hooftman et al., 2007; Hovick & Whitney, 2014). The benefits of hybridization do not end with heterosis or adaptive introgression and include the dumping of genetic load Ellstrand & Schierenbeck, 2000) and even demographic rescue (Mesgaran et al., 2016). However, hybridization can impose its own costs, such as outbreeding depression, and lead to genetic or demographic swamping, which can result in population extinction of one or both species (Todesco et al., 2016). If reproductive barriers are incomplete, the evolution of premating barriers, which is known as reinforcement, might be expected. A common indicator of reinforcement is reproductive character displacement in sympatry, such as divergence in flowering time or increased rates of self-fertilization (Comeault & Matute, 2016). Divergence of traits in sympatric populations may evolve for other reasons as well, such as competition for pollinators, stochasticity, or divergent response to environmental change between the species.

Cakile edentula and *Cakile maritima* are two closely related, cross compatible species with contrasting mating systems, that have invaded multiple regions of the globe (Barbour & Rodman, 1970; Li et al., 2019; Rodman, 1974, 1986). Allopatric in their native ranges (eastern North America and Europe respectively), they are found in sympatry in portions of their introduced ranges, including in Australia and western North America. Both species are found in coastal strandline habitat, providing opportunities for hybridization in regions where they co-occur. Both early generation and advanced generation hybrids are present in the Australian and western North American invasions (Cousens et al., 2013; Ohadi et al., 2016; Rosinger et al., 2021; Chapter 2). The invasion history of these two species has followed a similar pattern in both of these invaded ranges. *Cakile edentula* arrived in each region first followed by subsequent colonization by *C. maritima,* hybridization between the two species, and apparent replacement of *C. edentula* by *C. maritima* (Barbour & Rodman, 1970; Cousens et al., 2013; Rodman, 1986; Rosinger et al., 2021; Chapter 2). *Cakile edentula* is self-compatible and is able to set seeds autonomously at high rates (Li et al., 2020; Rodman, 1974), and benefits from high levels of reproductive assurance. However, *C. maritima* is self-incompatible, and during colonization may be hindered (during both initial establishment as well as subsequent range expansion) by a lack of compatible mates, limiting sexual reproduction and possibly resulting in strong Allee effects. Mesgaran et al. (2016) developed a model for the interacting species, with the novel outcome that transient hybridization with *C. edentula* could overcome Allee effects in *C. maritima* (demographic rescue). Both *Cakile* species are distributed along broad climatic gradients within their native ranges and multiple introduced ranges. For *C. maritima*, common gardens carried out for populations across Great Britain and the Baltic showed significant geographic variation for the timing of flowering, as well as fruit and seed weight. Leaf morphology was also related to variation in precipitation and temperature, hinting at local adaptation (Petty, 2020). However, the extent of local adaptation in the native range or introduced range is not well understood.

Our aim was to examine patterns of trait evolution during invasion in *C. edentula*, *C. maritima* and their hybrids. To do this we conducted a common garden experiment using 69 populations from across the two native ranges (Europe and eastern North America), and the introduced ranges where both species co-occur (Australia and western North America). We measured traits related to invasiveness and climate adaptation including plant size measurements (above and below ground biomass, biovolume and growth rate), reproductive traits (flowering onset, fruit onset, fruit weight, reproductive production), physiology and morphology (SLA, leaf shape,

fruit shape), viability (germination %, pollen viability) and herbivory (aphid damage). We also conducted whole-genome resequencing of 398 samples to reexamine the population structure and likely origins of the introduced populations at an increased genomic resolution. It was important to identify the likely progenitors of the introductions in our trait analysis of evolutionary change as our interpretation of any genetic differentiation of traits observed in common gardens is based on the assumption that the initial invading populations were similar to the present day source populations (Shaw et al., 2021; Sotka et al., 2018). Additionally, knowing source areas provides us with information on their environment and together with the knowledge of the entry point of the alien species it may be possible to derive information on selective pressure experienced during invasion (Shaw et al., 2021).

We used these data to address the following specific questions:

1. Can we confirm our previous findings of the putative source populations for particular invasions and identify recent hybrid individuals with an increased genomic resolution?
2. Are there parallel or divergent patterns in trait differentiation between the species, and within species, between the source and introduced populations? We predicted convergent patterns in defence related and perhaps performance related traits should evolve if escape from enemies led to rapid evolutionary change in the introductions.
3. Is there evidence of parallel latitudinal patterns in traits, either between species or between ranges, that may be indicative of local adaptation to climate following range expansion? We predicted early flowering at a smaller size at higher latitudes should evolve in all ranges and species. We also examined the impact of species ancestry on trait divergence as geographic patterns in the prevalence of admixture were previously documented in the introduced ranges (Rosinger et al., 2021; Chapter 2).

3.3 Methods

3.3.1 Study species

C. maritima and *C. edentula* (Brassicaceae) are found in coastal strandline habitat, and at places where they co-occur, they can hybridize (Rodman, 1974). The two species can be distinguished by morphology, primarily based on fruit and leaf shape (Cousens et al., 2013). Both species are diploid (2n=18) (Rodman, 1974) and cross-compatible (Li et al., 2019; Mesgaran et al., 2016; Rodman, 1974). The two species exhibit contrasting mating systems. *Cakile edentula* is self-compatible and *C. maritima* is self-incompatible (Rodman, 1974). Although hybrids are easily produced through artificial pollination (Rodman, 1974) with either parent as the pollen donor

(Li et al., 2019; Mesgaran et al., 2016), post-mating reproductive barriers have also been identified (Li et al., 2019). Reduced pollen performance, fruit and seed set are evident in some early generation hybrid classes, with greater fitness reductions when self-compatible *C. edentula* is the pollen parent, consistent with the SI × SC rule (Harrison & Darby, 1955). Reduced pollen performance as well as fruit and seed set has also been identified for F1s and F2s. There is also evidence of asymmetrical premating barriers related to differences in their mating system. In artificial crosses, early generation hybrids inherited mostly (but not exclusively) self-incompatibility, as well as larger floral displays, similar to *C. maritima* (Li et al., 2019) and field observations support much higher pollinator visitation of *C. maritima* phenotypes (Mesgaran et al., 2016). This suggests that F1 hybrids will often need to rely on outcrossing, and that larger floral displays should facilitate this and favour asymmetrical backcrossing to *C. maritima*. In support of this nuclear asymmetry of hybrid ancestry towards *C. maritima* in the sympatric introduced ranges has been confirmed by previous genetic studies (Mesgaran et al., 2016; Ohadi et al., 2016; Rosinger et al., 2021; Chapter 2).

3.3.2 Field collection

The basis of this study is phenotypic data from a common garden experiment, and genotypic data from whole genome re-sequencing of individual samples collected from the two native ranges (eastern North America, Europe) and the two invasive ranges (western North America, Australia) in the years 2017 and 2018 (Appendix II Table 3-S1; Figure 3-S1). To explore possible fitness differences between species and hybrids, as well as trait differentiation within and among ranges indicative of climate adaptation or the evolution of invasiveness, we raised seedlings from 67 populations (13 eastern North America, 13 Europe, 20 western North America, 21 Australia) in a greenhouse common garden experiment in Australia and re-sequenced 398 individuals from 49 of those populations, along with two individuals from two populations of outgroup *C. geniculata*.

3.3.3 Experimental set-up

The greenhouse experiment was conducted on the Monash Clayton campus (Melbourne, Victoria, Australia; Figure 3-S2). Prior to germination the fruit coat of the seeds was removed using a razor blade. Germination of the seeds was conducted on four consecutive days. Seeds were washed in three steps: 1) 70% Ethanol; 2) 10% Bleach; and 3) ddH20 each for 30 sec. To break the dormancy a small part (flat side of the cotyledon) of the seed coat was scratched with tweezers. Seeds from each individual were placed on individual agar plates with PPM. The

agar plates were placed in clear plastic boxes. Boxes were wrapped in aluminium foil for a dark treatment to stimulate root growth and placed in a Phytotron (16h light/ 8h dark, 19°C). Aluminium foil was removed after one week to allow photosynthesis and agar plates were randomised every day until planting. In total, 4655 seeds of 846 mother plants were germinated (approximately 10 individuals per home range population, 15 individuals per invasive range population, 5 seeds per individual).

During the course of the experiment several traits were measured (Table 3-1). Two weeks after germination, germination proportion % was recorded (by count) and three seedlings (of the five germinated seeds) per maternal plant (if applicable) were chosen by random to be potted. Seedling size was measured in cm, and seedlings were potted in seedling trays (REKO 30-seedling tray with a soil mixture of 40L of fine washed sand, 10L pine bark, 50g dolomite, 75g wetting agent, 200g fertilizer). Following a further week, additional germination % was recorded, and replacement seedlings were planted (if the seedling number per mother fell below three). Germination % was calculated for each mother plant, for which we counted all germinated and ungerminated seeds after three weeks. We top-watered all plants and randomised the seedling trays every two weeks. Five weeks after germination (14/5/19-17/5/19) all individuals that survived were transplanted into larger pots (15 cm diameter). One of the surviving seedlings for each mother plant was chosen at random and haphazardly assigned to a tray (with 3 other pots, 4 pots per tray). The trays containing the pots were randomised every two weeks until 13 weeks after germination. At this point two pots of each tray were chosen at random and placed on a tray; the tray was then placed in a second greenhouse to provide more space for the growing plants. Greenhouse settings were adjusted during the experiment to encourage optimal growth and then flowering (1) 25-35°C, 12h dark/ 12h light, 22/4-6/5/2019 (2) 18-22°C, 12h dark/ 12h light, 6/5/2019-12/8/2019 (3) 18-22°C, 8h dark/ 16h light, 12/8/2019- 16/12/2019. We recorded the date of the onset of first branching, the onset of buds and the opening of first flowers (onset of open flowers), and the onset of fruits. At the onset of buds and opening of flowers, plant volume (using height and width measurements) was recorded as secondary biomass measurement. Biovolume was calculated using the volume formula of a cylinder:

$$V = \pi * \left(\frac{width}{2}\right)^2 * height$$

Equation 2- 1 Biovolume formula

Stem length was recorded once a week after repotting into big pots for the first 5 weeks and once at 7 weeks to measure growth rate. We used the package ggpubr (Kassambara, 2020) and ggplot2 (Wickham, 2016) in R to fit a linear regression with stem length as the response and time (in days) as the predictor. The slope of the linear regression was used for further analysis. Leaf harvest for DNA extractions and glucosinolate analysis took place 10 weeks after germination (18/6 – 21/6/2019), for which several leaves derived from the primary meristem were harvested and placed in an empty teabag and in silica gel for drying (for DNA extraction) and one additional leaf was placed in an Eppendorf tube and frozen at -70°C (for glucosinolate analysis). Twelve weeks (2/7- 5/7/2019) after germination we conducted a leaf harvest for specific leaf area (SLA) and leaf shape measurement. We harvested one young, fully expanded leaf per plant for SLA and additionally two or three more leaves for leaf shape analysis (if applicable) and placed them between a damp paper towel in a sealed bag. Plastic bags were stored in a fridge (4°C) until scans were conducted. For the scans, leaves were tapped dry onto a flat page and scanned at 300 dpi in colour three times. We used the scans to measure leaf area with Image J software (Schneider et al., 2012) and the dry weight of each leaf was then used to calculate the SLA by dividing the leaf area by the dry weight for the leaf with the highest area. After scanning, single leaves were placed in tea bags and all leaves of an individual were placed in a paper bag and dried in the oven (min. 70°C/ 72h). Dry weight of each leaf was measured to the closest milligram. During the duration of the greenhouse experiment aphids appeared (first appearance of single aphids 15/7/2019, insect bomb 19/7/2019, insecticide application 24/7/2019). The outbreak was rapidly controlled by applying an insecticide (Spectrum Systemic Insecticide 200SC (200g/L imidacloprid)). However, we also opportunistically measured the effect of aphids on the plants (classification: one-no/light effect, two-modest effect, three-severe effect and four-aphids likely caused death of the plant; see Table 3-S2 for further information on the aphid damage scoring method). We identified the aphids to be *Myzus persicae*, an insect known to occur worldwide and to feed on Brassicaceaes (https://entnemdept.ufl.edu/creatures/veg/aphid/green_peach_aphid.htm; https://cesaraustralia.com/pestnotes/aphids/green-peach-aphid/). Further, it has to be noted that the plants in both greenhouses seemed to be equally exposed. Pollen viability was conducted

by harvesting three stamens from the third open flower from the top (to standardize the age of the flowers) in the morning (7 am- 12 pm). Stamens were placed in a tube and pollen was counted on the same day as the harvest. Pollen was stained with Aniline blue (Lactophenol, 35 ul) and viewed using a compound microscope (21/8 - 4/9/2019, 19-21 weeks after germination). We deconstructed the plants for final biomass when the plant senesced or 14 weeks (98 days) after the onset of the first bud. Plants were divided into above and below ground biomass. Above ground biomass was placed in a paper bag after a count of flowers, buds, pedicels and fruits, and dried at 70°C for at least 72h before measuring the dry weight. Fruits were bagged separately and dried at 35°C for at least 72h before weighing. Three mature fruits of each plant (if applicable) were photographed for fruit shape analysis, and the dry weight of those 3 fruits was taken. Fruit weight was calculated as an average of 1 to 3 fruits per individual. Only fully developed fruits were weighed, for which both fruit parts were fully developed. Below ground biomass (roots) were washed, dried (70°C/ 72h) and dry weight was determined. We did not measure fitness in form of seed production as under the greenhouse conditions as we could not allow for natural cross-pollination. Self-compatible *C. edentula* would likely have an advantage under such conditions and this form of extreme pollinator limitation is not necessarily expected in the field. As such, we felt total female reproductive output in the form of the total number of flowers produced over the lifetime of the plant was a more relevant metric compared to seed set.

3.3.4 Reference genome sequencing and assembly

The *Cakile edentula* reference genome (*C. edentula* subsp. *edentula*) was assembled from a single individual taken from the native range. Field-collected seed from the NS1 population (Nova Scotia, GPS coordinates: 49.6915, -63.137444) was grown at the University of British Columbia greenhouse and selfed for two generations before a single individual from this line was selected for sequencing (ID: NS1-10A-2A). A fresh leaf was collected (August 2020) and flash frozen in liquid nitrogen and stored at -80℃. The sample was then shipped to Dovetail Genomics and the company completed the DNA extraction. High molecular weight DNA was obtained by grounding 1.8g leaf material with mortar and pestle to a fine powder to which 200ml of prewarmed CTAB and 100ul BME was added. The mixture was incubated at 68℃ for 15 minutes. This was followed by adding a mixture of 2x phenol chloroform, 1x isoamyl and 0.7x isopropanol and centrifugation step to form a pellet. The resulting pellet was combined with 9.5ml of G2, 200ul protease and 19ul RNase. An additional incubation step of 50℃ for

1h followed. The precipitated genomic DNA was used for the library constructions. DNA samples were quantified using Qubit 2.0.

The assembly was generated by using a combination of PacBio HiFi reads to generate the initial assembly and Dovetail Omni-C for scaffolding. Fluorometer (Life Technologies, Carlsbad, CA, USA). The PacBio SMRTbell library (~20kb) for PacBio Sequel was constructed using SMRTbell Express Template Prep Kit 2.0 (PacBio, Menlo Park, CA, USA) using the manufacturer recommended protocol. The library was bound to polymerase using the Sequel II Binding Kit 2.0 (PacBio) and loaded onto PacBio Sequel II. Sequencing was performed on PacBio Sequel II 8M SMRT cells generating 29Gb of data. These PacBio CCS reads were used as an input to Hifiasm (Cheng et al., 2021) with default parameters.

For each Dovetail Omni-C library, chromatin was fixed in place with formaldehyde in the nucleus and then extracted. Fixed chromatin was digested with DNAse I, chromatin ends were repaired and ligated to a biotinylated bridge adapter followed by proximity ligation of adapter containing ends. After proximity ligation, crosslinks were reversed, and the DNA purified. Purified DNA was treated to remove biotin that was not internal to ligated fragments. Sequencing libraries were generated using NEBNext Ultra enzymes and Illumina-compatible adapters. Biotin-containing fragments were isolated using streptavidin beads before PCR enrichment of each library. The library was sequenced on an Illumina HiSeqX platform to produce ~30x sequence coverage. Then HiRise used MQ>50 reads for scaffolding.

The *de novo* assembly and Dovetail OmniC library reads were used as input data for HiRise, a software pipeline designed specifically for using proximity ligation data to scaffold genome assemblies (Putnam et al., 2016). Dovetail OmniC library sequences were aligned to the draft input assembly using bwa (https://github.com/lh3/bwa). The separations of Dovetail OmniC read pairs mapped within draft scaffolds were analyzed by HiRise to produce a likelihood model for genomic distance between read pairs, and the model was used to identify and break putative misjoins, to score prospective joins, and make joins above a threshold.

3.3.5 WGS and SNP data preparation

We selected 400 individuals from 54 populations (16 Australia, 16 western North America, 10 eastern North America, 10 Europe, 2 outgroup populations) for whole genome sequencing

(phenotypically 214 *C. maritima* individuals, 159 *C. edentula* individuals and 2 *C. geniculata* individuals). The majority of the samples (375 samples) were chosen from the greenhouse experiment. If too few individuals survived from each population or if we were not able to obtain seeds from a geographically important location (e.g., certain native range populations to assess invasion source), we relied on field-collected leaf samples (25 individuals).

We performed a DNA extraction of dried leaf material (15-30 mg) using DNeasy Plant Mini Kit (QiaGen). DNA quantity was assessed using a QuBit broad-sensitivity DNA quantification system (Invitrogen, Carlsbad, CA, USA) and a WGS library preparation was carried out following the protocol of Carø et al., (2018). Sequencing was conducted by NovaSeq (Genewiz) on seven lanes (total 4796.21 Gb, 11 Gb +/- 6.16 on average).

We removed the adapters (AdapterRemoval v2; Schubert et al., 2016) and aligned the whole genome reads to the *C. edentula* reference genome using Burrows wheeler aligner (BWA-MEM) (Li & Durbin, 2009). Indels were realigned using GATK (IndelTargetCreator and IndelRealigner; Van der Auwera & O'Connor, 2020), and duplicate reads were marked with Picard (https://broadinstitute.github.io/picard/). SNPs were called with the GATK Unifiedgenotyper (DePristo et al., 2011) and we used hard filters following examination of the distributions of variant metrics and recommendations from GATK (McKenna et al., 2010). SNPs were filtered as follows: QD < 2.0, FS > 60.0, SOR > 3.0, MQ < 40.0, ReadPosRankSum < -8.0, MQRankSum < -12.5 and filtered for DP > 10644.21 (mean +1.5 sd) or DP < 235.70 (mean -1 sd), missing > 50%. This first filtering step produced a dataset of 20,386,265 SNPs. For imputation we combined the SNPs with the Indels, but removed indels which did not follow following criteria: QD < 2.0, FS > 200, SOR > 10, ReadPosRankSum < -20, InbreedingCoeff < -0.8, DP > 7152.98 (mean +1.5 sd) or DP < 80.41 (mean -1 sd), missing > 50%. Additionally, for SNPS we used vcftools (Danecek et al., 2011) to further filter the SNPs unless otherwise stated. We kept only SNPs which were biallelic, had a minimum genotype depth of 3 (bcftools; (Li et al., 2009) and filtered for a maximum missing rate of 30% and removed SNPs with heterozygosity higher than 0.8 and a minor allele count of 2 with vcftools (Danecek et al., 2011). We imputed missing genotypes with Beagle (Browning & Browning, 2007). We call this vcf file the *base file*. We also produced an unimputed filtered file which followed the exact filter criteria which we call *unimputed base file*.

For the data preparation for Admixture we filtered the *base file* for minor allele frequency (MAF) > 0.05, and LD pruned (window size 50, step size 5, r^2 0.5) it using PLINK 1.9 (Chang et al., 2015). We produced two files for Admixture, a data set with all SNPs - the *complete admixture file*- and downsampled file (10,000 SNPs) -*downsampled admixture file*- to speed up computational time. We also excluded the two outgroup individuals. For NewHybrid (Anderson & Thompson, 2002), we calculated the F_{ST} between the home ranges (using the *complete admixture file*) and considered SNPs with an F_{ST} of 1 as fixed differences. For HIest (Fitzpatrick, 2012) we used the fixed differences where the *C. edentula* samples were fixed for the reference allele (as the reference was *C. edentula*).

3.3.6 Genetic analysis

3.3.6.1 Population structuring and genetic diversity

We used the program Admixture (Alexander et al., 2009) to investigate population structuring, using the *downsampled admixture file.* Admixture was run for K=1-10 using a major termination criterion of $1x10^{-9}$, 1000 bootstraps and ten-fold cross-validation. The K value which produced the lowest cross-validation error was selected as the best K. We refer to this run as the *population admixture run*. The output of the Admixture run was visualized in R with pophelper v.2.3.0 (Francis, 2017) and pie charts. To summarize genetic differentiation between groups of the native and introduced range we use a principal component analysis (PCA) with an 95% confidence ellipse with the R packages SNPRelate (Zheng et al., 2012), tidyverse (Wickham et al., 2019) and car (Fox & Weisberg, 2019) on the *complete admixture file*. Genetic diversity and differentiation were assessed for 398 individuals (exclusion of outgroup) using the *downsampled admixture file*. We calculated expected heterozygosity (H_e), observed heterozygosity (H_O), inbreeding coefficient (F_{IS}) with the diveRsity package on species per population level (Keenan et al., 2013). Additionally, we calculated Tajima's D and nucleotide diversity $\boldsymbol{\pi}$ with ANGSD (Korneliussen et al., 2013; Korneliussen et al., 2014) using the bam files (doSaf1, GL 1, baq 2, minMapQ 30, minQ 20); the R package plotrix (Lemon, 2006) was used to calculate the standard error. Selfing rates were calculated on each population for each species and individuals were grouped into species according to their phenotypes (https://github.com/vmikk/Selfing-rate/blob/master/RMES_extract.R; David et al., 2007). We used the Kruskal-Wallis rank sum test to test for significant differences among groups (species and range) followed by pairwise Wilcoxon rank sum exact tests for F_{IS}, $\boldsymbol{\pi}$, Tajima's D and selfing rates. P-values were Bonferroni adjusted for comparison of interest (for $\boldsymbol{\pi}$, Tajima's D

and F_{IS}: eNA_E vs. AUS_E, eNA_E vs. wNA_E, eNA_E vs. EU_M, EU_M vs. AUS_M, EU_M vs. wNA_M, AUS_E vs. AUS_M, AUS_E vs. AUS_H, AUS_M vs. AUS_H, wNA_E vs. wNA_M, wNA_E vs. wNA_H, wNA_M vs. wNA_H; and for selfing rates: eNA_E vs. AUS_E, eNA_E vs. wNA_E, eNA_E vs. EU_M, EU_M vs. AUS_M, EU_M vs. wNA_M, AUS_E vs. AUS_M, wNA_E vs. wNA_M). Finally, we calculated the linkage disequilibrium (LD) decay with Plink. Here, the base file was filtered for MAF 0.05 per group and LD decay was calculated (ld-window-r2 0.2, ld-window 99999999, ld-window-kb 90000).

3.3.6.2 Hybrid identification

To identify hybrids, we used multiple approaches. First, we used a supervised run of Admixture (K=2) on the *complete admixture file* and set the samples from the native ranges as reference individuals and other settings were identical to the *population admixture run,* and we termed this run the *hybrid admixture run*. We then identified individuals with hybrid ancestry by the standard error of the Q scores, using the highest standard errors as a cut off (Q values 0.041-0.959). Secondly, we used the program NewHybrid (Anderson & Thompson, 2002) to identify early generation hybrids (F1, F2, backcross *C. edentula* (BC_E), backcross *C. maritima* (BC_M)). We ran NewHybrid eight times sampling without replacement 300 SNPs from all SNPs with fixed differences between the home ranges (2149 SNPs) and used the majority assignment to classify new generation hybrids. Finally, we used the R package HIest (Fitzpatrick, 2012) to visualize hybrid ancestry. Here we used the SNPs showing fixed differences between the home ranges, with an allele frequency of 0 for eastern North American individuals and 1 for European individuals. We then plotted the ancestry index (S) and the interclass heterozygosity (H) in triangle plots.

3.3.7 Statistical analysis of trait differentiation

All analyses were conducted in R v.3.5.2. (R core team, 2018) except otherwise stated. We only used phenotypic data from individuals which were also sequenced to ensure species identity.

3.3.7.1 Leaf and fruit shape

Leaf- and fruit shape analysis was conducted using the MOMOCS package (Bonhomme et al., 2014). Leaf scans and fruit photos were prepared for processing with Adobe Illustrator (Adobe Inc., 2019). We used the tracing tool of Adobe Illustrator to outline each leaf/fruit and coloured

each leaf/fruit black. Individual leaf or fruit outlines were exported as jpegs. The geometric information of each individual leaf or fruit was quantified with MOMOCs (Figure 3-S5, 3-S6). We used the elliptic Fourier series approach (efourier function). To select the appropriate number of harmonics for the Fourier analysis, we examined the power of the harmonics (harmonicpower_efourier function). Ten harmonics were sufficient to explain 99% of variation of fruit shape and 16 harmonics were sufficient to explain 99% of variation of the leaf shape. We conducted a principal component analysis using the efourier results using 10 (fruit) or 16 (leaf) harmonics and removed bilateral asymmetry. We retained the first four PCs for leaves and fruits. We then averaged each PC for each individual (fruit 1-3 fruit per individual, leaf 1-4 per individual, if applicable) and used those data for further analysis.

3.3.7.2 Multivariate Analysis of Trait Differences

We were interested in identifying if the species and hybrids, as well as ranges within species, differed in their multivariate trait distributions. To do this, we first used our hybrid Admixture run, NewHybrid and HIest to classify pure *C. edentula*, pure *C. maritima* and (early generation) hybrids. Because of the small number of early generation hybrids (five individuals, one F1, three F2, one E_BC_E), we excluded them from most downstream analyses. Phenotypic traits were summarized in a principal component analysis, for which we excluded highly correlated variables (Pearson's correlation coefficient > 0.70) by removing one of each pair of highly correlated traits (Appendix II Table 3-S3, 3-S4). Principal component analysis was conducted using the FactoMineR (Lê et al., 2008) and missMDA (Josse & Husson, 2016) package to account for missing data. Results were displayed with the FactoMinR (Lê et al., 2008) and ggplot2 (Wickham, 2016) package of R. Using population means we conducted a trait PCA and included all individuals except recent hybrids. Individuals were grouped by population and species using Admixture (*C. edentula*, *C. maritima*, hybrids), populations required to have a minimum number of two individuals. We used the Hotellingers T^2 (Curran & Hersh, 2021) test to investigate differences between groups within the PCA. We also conducted a second trait PCA using values of individuals (versus population means) from likely source populations and from the invasive range (exclusion of *C. edentula* subsp. *harperi*, *C. maritima* subsp. *islandica*, *C. maritima* subsp. *baltica*). For this analysis new hybrids were also included so we could explore patterns of trait variation in response to hybridization.

We explored patterns of absolute latitudinal trait divergence among ranges and species using population mean trait response to range (native, introduced wNA, introduced Australia), species (*C. edentula*, *C. maritima*), absolute latitude and their interaction in a multivariate model. From this point forward we will refer to absolute latitude as latitude for simplicity. Our species identification was defined by Admixture results but reflect the morphological identification of each species (native *C. edentula* subsp. *edentula*, native *C. maritima*, Australian *C. maritima*/backcrossed hybrids to *C. maritima*, Australian *C. edentula*, western North American *C. maritima*/hybrids, western North American *C. edentula*, Table 3-S5). A multivariate test was conducted using a Manova on population means of traits, with highly correlated traits removed as above (Table 3-S3), with which approximate F-statistics and Wilk`s λ were calculated. We excluded from this analysis *C. edentula* subsp. *harperi*, *C. maritima* subsp. *baltica* and *C. maritima* subsp. *islandica* as described above.

3.3.7.3 Univariate analysis of trait differentiation

We further explored trait differentiation among ranges and species using univariate tests by implementing linear mixed models (lme4) for continuous response variables. We excluded from this analysis *C. edentula* subsp. *harperi*, *C. maritima* subsp. *baltica* and *C. maritima* subsp. *islandica* as they did not contribute to the invasions. We also excluded early generation hybrids. Species (*C. edentula*, *C. maritima*-including those with some *C. edentula* ancestry), range (putative source regions, introduced Australia, introduced western North America) and their interaction were fixed effects in the model. We retained all main fixed effects in the model as well as the range:species interaction since these effects were integral to our experimental design. We included the population as a random effect. Data were log or square root-transformed to improve normality when needed. We tested each effect of our model using the Anova function in the car package with type III tests and F tests with Kenward-Roger degrees of freedom. We calculated least-square means and confidence intervals and conducted pairwise contrasts between levels for significant effects using the R packages emmeans. For categorical traits (germination %, viable pollen, aphid damage) a generalized linear mixed model (glmer function) was conducted using a binomial response. Type III tests using the car package were generated and emmeans was used to test for pairwise contrasts. Aphid damage was re-coded as a binary trait (0 representing or no/low damage, which was 1 on the ordinal scale in Figure 3-S7, and 1 representing greater damage, which was more than 1 on the ordinal scale). The results of an ordinal mixed model (not presented) provided identical patterns of significance.

We were interested in determining if parallel patterns of trait by environment variation had re-evolved in the introduced ranges, as this provides evidence for recent local adaptation to similar climate gradients following invasion. Divergence between source and introductions in traits observed in common gardens might also be caused by adaptation to the local climate (Colautti et al., 2010). Therefore, we repeated the above analyses but also included latitude as a covariate as well as all interactions among the main effects of range and species and latitude. Latitude is highly correlated with a range of environmental factors, particularly mean annual temperature, so we used this variable to capture this climatic variation. We reduced the higher order interactions with latitude using backward stepwise elimination, while retaining the three main effects and the interaction between range and species. If an interaction with latitude was significant, we used emtrends to identify significant slopes for each group, and ggemmeans to plot the predictions for the model as a function of latitude. In the case of an interaction with latitude the package Phia (De Rosario-Martinez et al., 2015) was also used to assess differences between the ranges and species and specific latitudes.

Since previous analysis (Rosinger et al., 2021; Chapter 2) has revealed a geographic pattern in species ancestry of *C. edentula* in phenotypic *C. maritima* in western North American and Australia, we wanted to determine if latitudinal patterns in traits of *C. maritima* could be explained by *C. edentula* ancestry in these ranges. To do this we conducted an analysis of trait variation for *C. maritima* in western North American and Australia and excluded all other species and ranges from this analysis. For these models we include range (western North American and Australia), latitude, the Q value from the supervised admixture run and all interactions among fixed effects. As above, we conducted a linear or generalized linear mixed model using lme4 and included population as a random effect. We reduced the higher order interactions using backward stepwise elimination, while retaining the three main effects. As non-linear relationships of traits with ancestry might be expected in some cases, we tested if second and third order polynomial models would better explain the data using the poly function and compared models with and without the higher order polynomial for either latitude or ancestry (Q) using likelihood ratio tests (using the anova function in R). Second or third order polynomials did not provide a better fit to the data, which is further supported by the scatter plots of the trait values versus Q values or latitude.

Table 3- 1. Phenotypic traits measured during greenhouse experiment.

Trait	Description
Germination %	Count of germinated/ not germinated seeds of the mother plant.
Seedling size individual	Total seedling length of each individual (cm)
Growth rate	Stem growth rate (cm per week)
SLA	Leaf area/leaf dry weight (1 leaf)
Leaf shape	Outline analysis of leaf (mean of 1-4 leaves), PC1-PC4
Fruit weight	Mature fruit average per individual (mean of 1-3 seeds) (g)
Fruit shape	Outline analysis of fruits (mean of 1-3 seeds), PC1-PC4
Onset branching	Date of first branching (days since germination)
Onset bud	Date of first bud development (days since germination)
Onset open flower	Date of first open flower (days since germination)
Onset seed	Date of first seed development (days since germination)
Biovolume bud	Biovolume at the onset of bud, measured as apex height and width (cm^3)
Biovolume open flower	Biovolume at the onset of open flower, measured as apex height and width (cm^3)
Above ground biomass	Oven dried above ground biomass at harvest date (g)
Below ground biomass	Oven dried below ground biomass at harvest date (g)
Total reproductive count	Total count of flowers, seeds, pedicels, buds at harvest date
Pollen viability	The proportion of viable pollen
Aphid damage	Classification of aphid damage (1 light, 2 modest, 3 severe, 4 death)

3.4 Results

3.4.1 Genome assembly

A draft genome of size 651.503 Mb was assembled *de novo* using 29 Gb of Hifi PacBio reads. This initial assembly consisted of 1314 scaffolds (Table 3-2). After scaffolding using the Omni-C data, the final assembly was 651.583 Mb in size and had a N50 length of 68,669,067 bp (Table 3-2). As the final assembly had a N90 represented in nine scaffolds (Table 3-2) and we assumed that these scaffolds represented the majority of the nine haploid chromosomes of the *Cakile edentula* (Bigelow) Hook. genome (Chinnappa & Chmielewski, 1987; Rice et al., 2014). The BUSCO (Benchmarking Universal Single-Copy Orthologs) analysis (Seppey et al., 2019; Simão et al., 2015) of the final assembly revealed that there are 155 single, 96 duplicated, 2 fragmented and 2 missing genes out of 255 total BUSCO genes (Table 3-2).

Table 3- 2. Statistics of the *Cakile edentula* genome assembly.

Assembly statistics	HiFi assembly	HiFi+Omni-C
N50/L50 (size(bp)/number)	1,387,598/ 115 scaffolds	68,669,067 / 5 scaffolds
N90/L90 (size(bp)/number)	309,490 / 501 scaffolds	54,597,944 / 9 scaffolds

Total genome size (Mb)	651.503	651.583
Largest scaffold	11,949,139	86,584,315
Number of scaffolds (all>1kbp)	1314	531
Number of gaps	0	794
Number of Ns per 100kbp	0	12.31
BUSCO* Single:Duplicated :Fragmented :Missing (Total =255)	144:108:11:2	155:96:2:2
BUSCO % Complete (Total =255)	98.82%	98.43%

*Number of BUSCO (Benchmarking Universal Single-Copy Ortholog) genes found in the assembly using the eukaryota odb10 dataset. Genes are split into four categories: complete and single-copy, complete and duplicated, fragmented, and missing

3.4.2 Population structure

3.4.2.1 Native ranges

We first explored population structuring with an unsupervised admixture run and the best K value present was K=8. In eastern North America three groups were present (Figure 3-1, Figure 3-2). Two of the three groups were identified phenotypically as *C. edentula* subsp. *edentula*. One group in the area spanning Maine, New Brunswick, Nova Scotia and Newfoundland we term the "Nova Scotia" cluster; a second group constituted samples from the Great lakes and Virginia/Maryland, named hereafter the "Great Lake" cluster. The third group was found in Georgia and Florida, representing the subspecies *C. edentula* subsp. *harperi.* The PCA results (Figure 3-S8) mirror the pattern of the Admixture analysis. For the PCA *Cakile edentula* samples clustered close together, but the *C. edentula* subsp. *harperi* was slightly differentiated from the *C. edentula* subsp. *edentula*. In Europe samples from Iceland (subsp. *islandica*) and the Baltic (subsp. *baltica*) grouped together (red cluster) in the Admixture analysis. Samples from the Mediterranean (subsp. *maritima*) (largely the blue cluster) grouped together with samples from the Black Sea (possible subsp. *euxenia,* based on the sampling location). Samples from the Atlantic coast (subsp. *integrifolia*) showed admixture between the blue (Mediterranean samples) and red (Baltic samples) clusters. The PCA found a similar structure (Figure 3-S8). Native *C. maritima* was differentiated by subspecies; *C. maritima* subsp. *islandica* and subsp. *baltica* grouped together, *C. maritima* subsp. *integrifolia* clustered in between the Baltic (subsp. *baltica*) and Mediterranean samples (subsp. *maritima*). Lastly, the Mediterranean and Black Sea (possibly subsp. *euxina*) samples clustered together but were still slightly differentiated. Similarly, the Splitstree (Figure 3-S9) analysis confirmed the Admixture and PCA analyses; the only exception was a more pronounced differentiation of the *C. maritima* subsp. *euxina* subspecies present in the tree.

3.4.2.2 Australian invasion

In Australia one Admixture cluster of *C. edentula* subsp. *edentula* ("Nova Scotia" cluster) was present in Tasmania and New South Wales and Queensland (Figure 3-1, 3-2), consistent with a single invasion source. Admixture identified multiple clusters in southeastern Australian *C. maritima*, which were predominantly associated with the Mediterranean samples (blue and purple clusters). However, in South Australia, the red cluster was also present, which was found exclusively in the Atlantic (subsp. *integrifolia*) or Baltic samples (subsp. *baltica*). This is consistent with a second source from this region in Europe. The PCA (Figure 3-S8) also placed these samples as intermediates between the Mediterranean (subsp. *maritima*) and Atlantic samples (subsp *integrifolia*), although a contribution from the Black Sea (subsp. *euxina*) also seems possible since the Black Sea and South Australian samples overlap on the PCA (Figure 3-S8). In the Splitree (Figure 3-S9), *C. maritima* from Australia grouped most closely to the Mediterranean samples and hybrids were dispersed from the Mediterranean *C. maritima* group to the *C. edentula* group. Overall, there is evidence for multiple introductions of *C. maritima* into southwest Australia, with contributions predominantly from the Mediterranean, but also from one more source (likely the Atlantic).

3.4.2.3 Western North American invasion

Admixture identified two different clusters of *C. edentula* in western North America (Figure 3-1, Figure 3-2). The first cluster is the Nova Scotia cluster which is prevalent in Alaska and northern British Columbia. The second cluster is the "Great Lake" cluster and is concentrated in the Pacific Northwest of British Columbia and the US. This is consistent with at least two separate introductions from eastern North America. The *C. maritima* present in western North America consists mainly of the light purple cluster, also found in the Mediterranean samples (subsp. *maritima*), and are clustered closest to the Mediterranean samples in the PCA.

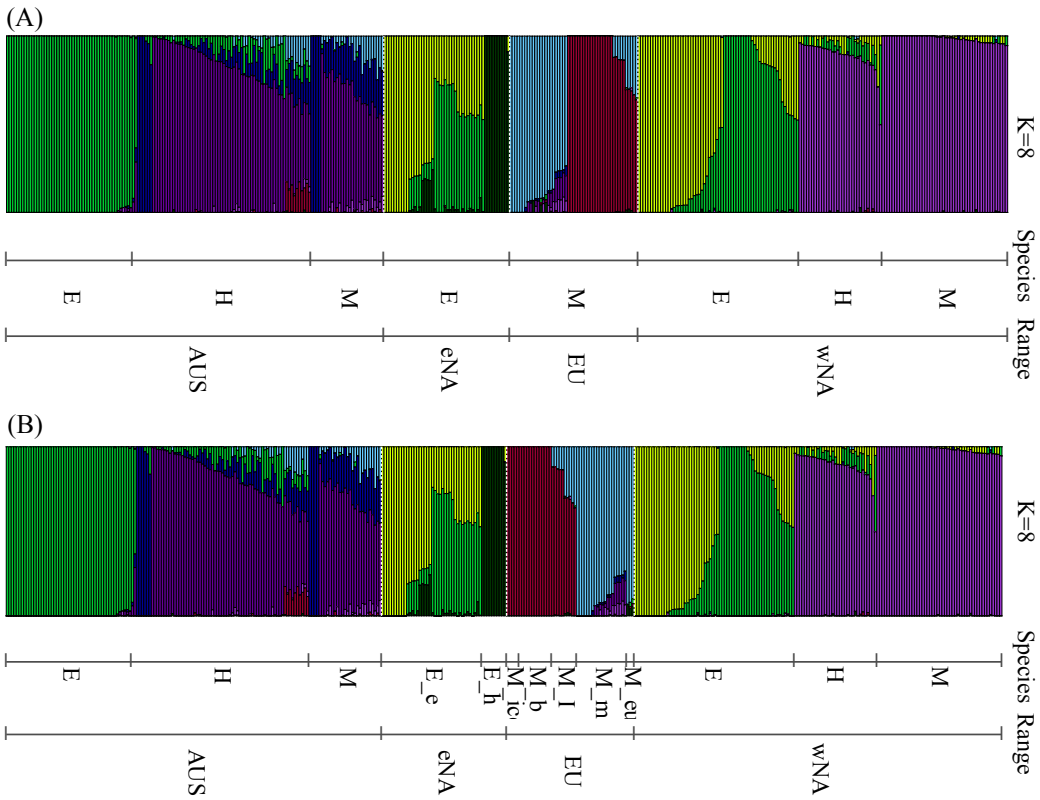


Figure 3- 1. Bar plots of the unsupervised Admixture run (K=8).
(A) Individuals ordered by species and range. (B) Individuals ordered according to the home range and sub-species. E= *C. edentula*, M= *C. maritima*, H= hybrid, E_e= *C. edentula* subsp. *edentula*, E_h= *C. edentula* subsp. *harperi,* M_ice= *C. maritima* subsp. *islandica*, M_b= *C. maritima* subsp. *baltica*, M_I= *C. maritima* subsp. *integrefolia*, M_m= *C. maritima* subsp. *maritima*, M_eu= *C. maritima* subsp. *euxina*.

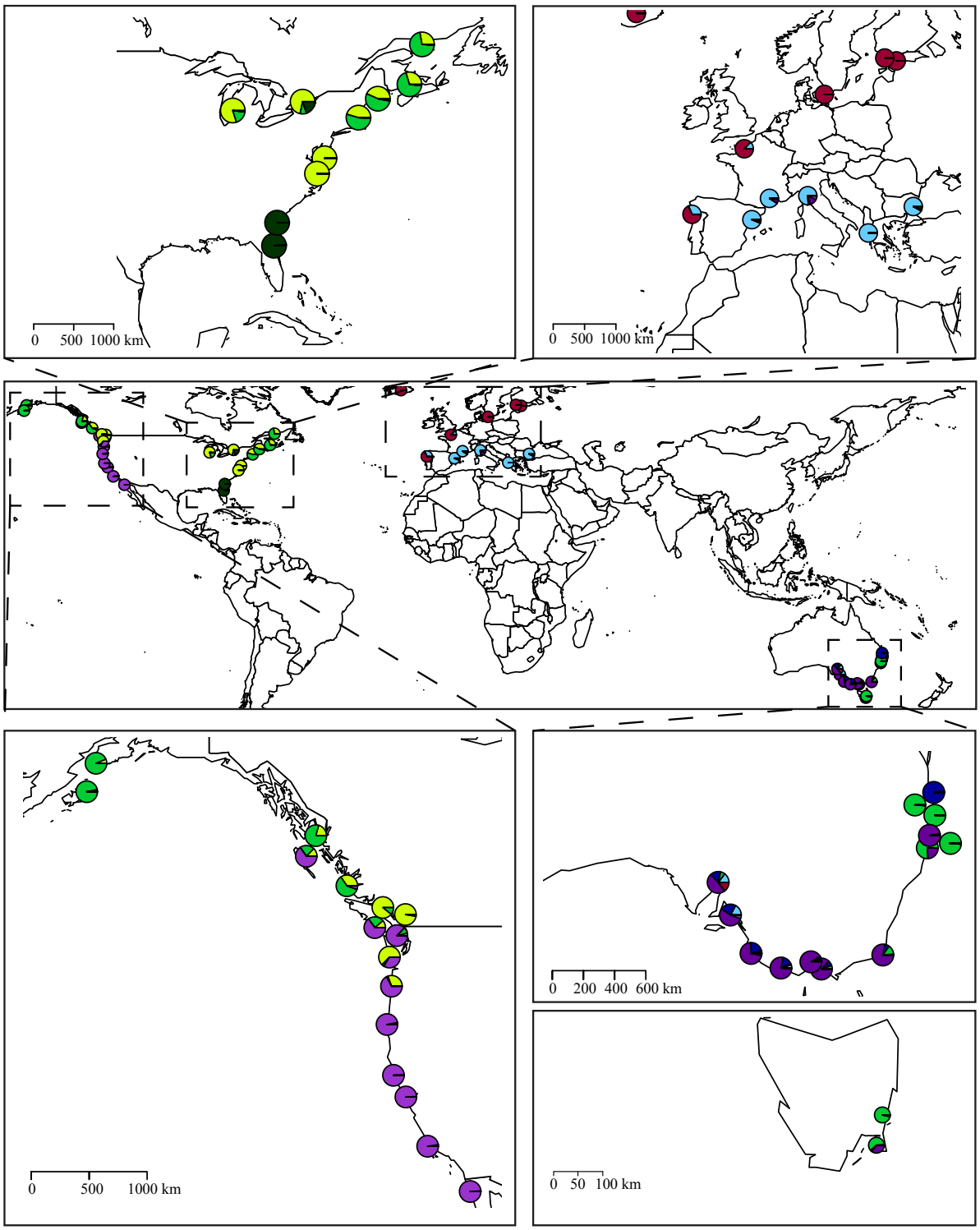


Figure 3- 2. Population pies of the unsupervised Admixture run (K=8).
Close ups of eastern North America (upper left), Europe (upper right), western North America (lower left) and mainland Australia (lower right) and Tasmania (lower right) are presented. Colours correspond to Figure 3-1.

3.4.2.4 Hybrid identification

To identify hybrids of *C. edentula* and *C. maritima* among our samples, we used the results of three separate analyses: Admixture, NewHybrid and HIest. The supervised Admixture run

identified 71 hybrids in Australia and 33 hybrids in western North America. This was complemented by the NewHybrid analysis which found 14 early generation hybrids in Australia (11 backcrossed to *C. maritima* BC_M, 3 F2s) and 7 early generation hybrids in western North America (6 BC_M, 1 F1). NewHybrid F2 and (BC_E) E individuals were found in New South Wales, Tasmania in Australia and in western North America the F1 individual was found in British Columbia, hence those early generation hybrids were found in the current sympatric regions. Further, in Australia back-crosses to *C. maritima* have been identified in New South Wales, Queensland and Victoria. In western North America, those back-crosses were identified in Washington and British Columbia. HIest results suggest that many introduced *C. maritima* that morphologically appear to be the parental species contain some *C. edentula* ancestry, indicative of advanced generation backcrosses. However, a single *C. edentula* individual had high levels of *C. maritima* ancestry (see Figure 3-3 arrow). In the Splistree analysis, *C. maritima* with some *C. edentula* ancestry grouped with the respective invasive *C. maritima* groups. Early-generation F1 and F2 hybrids were found inbetween the two species on the tree.

Table 3- 3. Hybrid identification per program. Admixture and NewHybrid results are presented per range, species and hybrid class. Note Admixture does not categories hybrid generation. BC_M= Back-cross to *C. maritima*.

				NewHybrid			Admixture
Program	**Range**	***C. edentula***	***C. maritima***	**F1**	**F2**	**BC_M**	**Hybrid ancestry total**
Admixture	Eastern North America	50					
	Europe		51				
	Australia	50	29		3	11	71 (47.34%)
	Western North America	64	50	1		6	33 (22.45%)

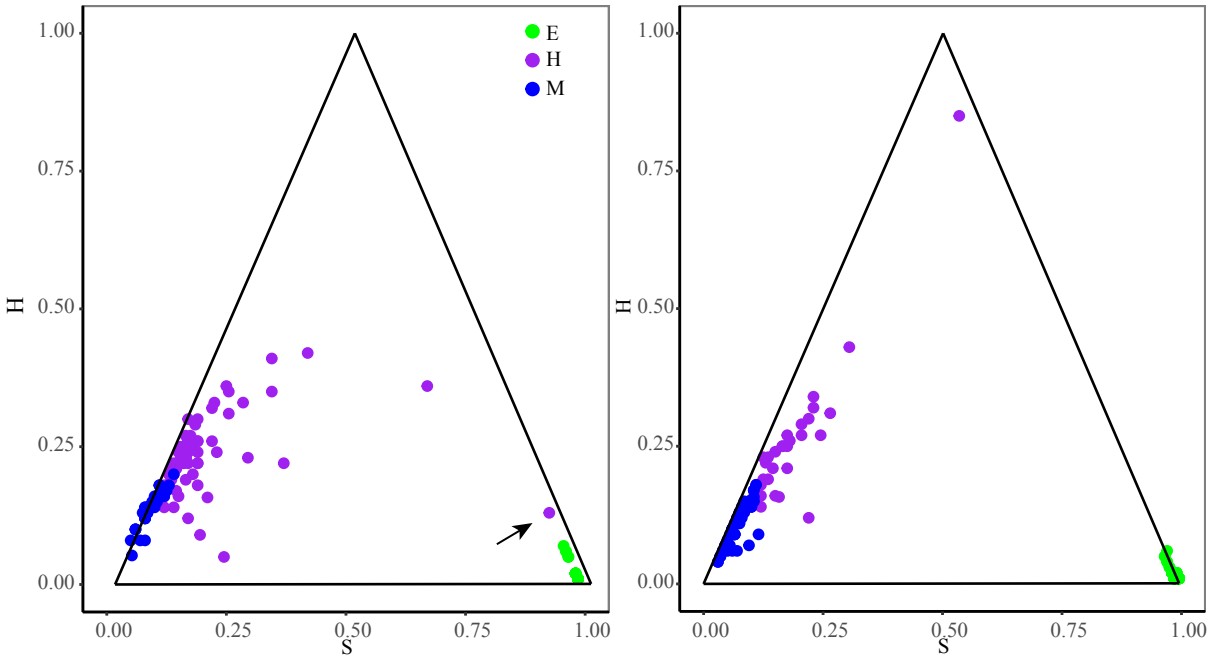


Figure 3- 3. HIest triangle of ancestry index (S, S=1 pure *C. edentula*, S=0 pure *C. maritima* ancestry) and interclass heterozygosity (H).
Individuals coloured according to their species status of the Admixture hybrid run (E= *C. edentula*, M= *C. maritima*, H= hybrid). (A) Australian individuals, (B) western North American individuals.

3.4.2.5 Population diversity and patterns of LD

In the home and invasive ranges, the self-compatible *C. edentula* showed lower genome-wide genetic diversity (nucleotide diversity) (Figure 3-4; Table 3-S6 to 3-S12) than the self-incompatible *C. maritima*. Introduced *C. maritima* with some hybrid ancestry had similar diversity to pure *C. maritima*. Comparing the introduced and native ranges, *C. edentula* had similar levels of nucleotide diversity, but lower Tajima's D in western North America and particularly in Australia, consistent with a population expansion following a bottleneck. By contrast, nucleotide diversity was similar for *C. maritima* when comparing native and introduced ranges. Tajima's D was not significantly different among the ranges for *C. maritima*, and was positive in western North America and Australian hybrids, perhaps reflecting admixed ancestry within and between species (Figure 3-4). Western North American *C. edentula* populations were substantially more inbred than all other groups, although there was substantial variability in F_{IS} within the *C. edentula* home range. *Cakile maritima* groups appear to have similar linkage disequilibrium (LD) decay in each range (groups: native (European) *C. maritima*, Australian *C. maritima*, western North American *C. maritima*). In contrast, *C. edentula* shows much lower LD decay in western North American, followed by native *C. edentula* and Australian *C. edentula* (Figure 3-S10), which is also consistent with greater inbreeding in western North America for this species. The selfing estimates were higher

for *C. edentula* than *C. maritima*, yet no significant difference between the native and invasive populations were detectable (Figure 3-4).

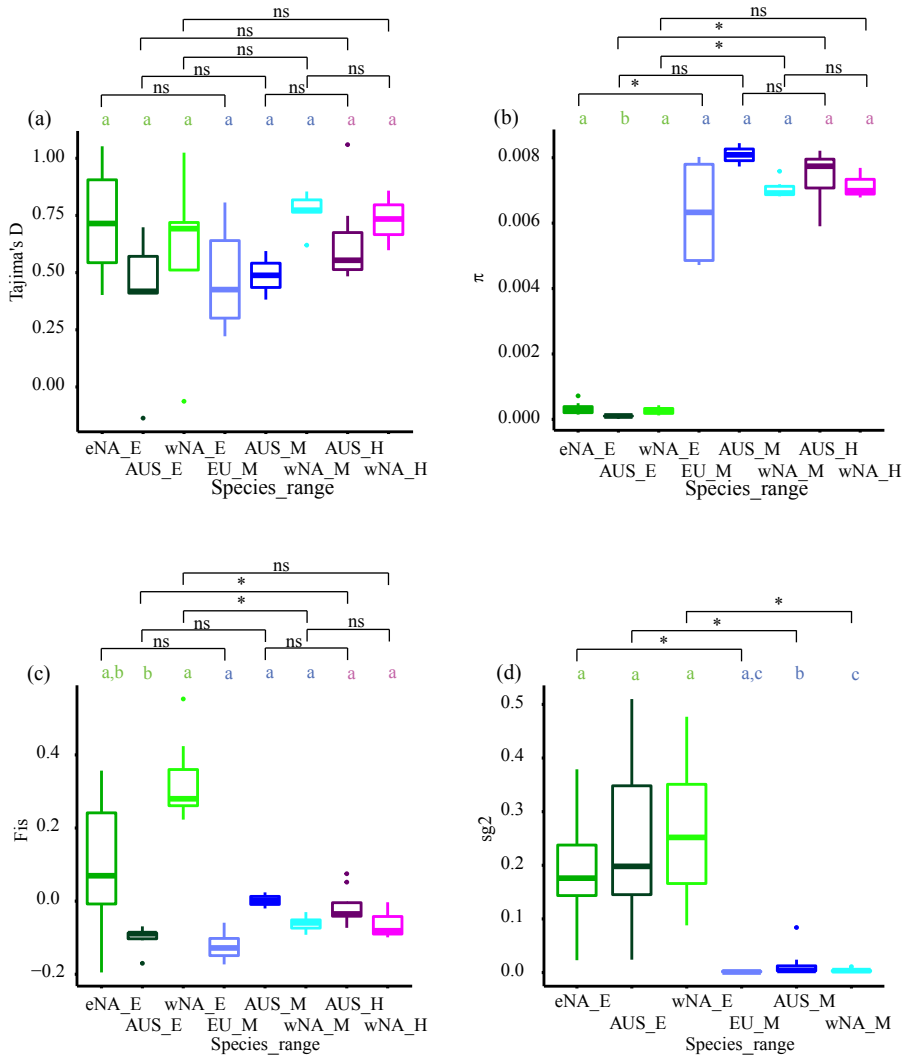


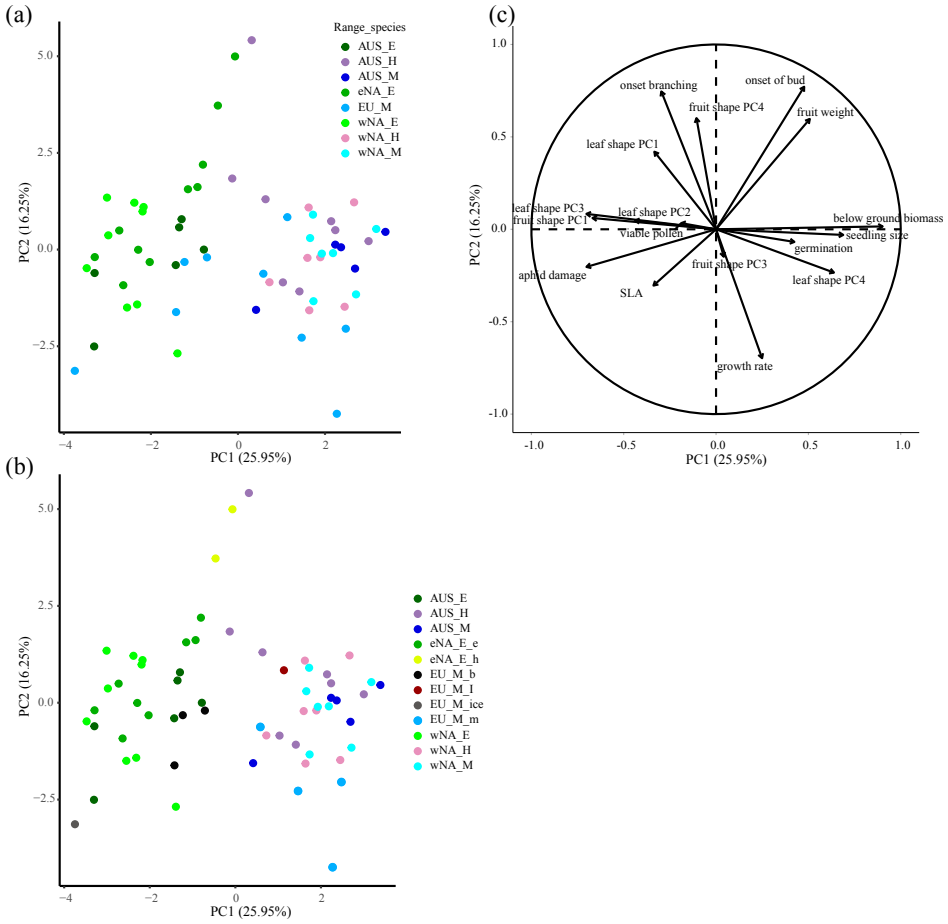
Figure 3- 4. Boxplots of median genome-wide (a) Tajima's D, (b) nucleotide diversity (π), (c) inbreeding coefficient (F_{IS}) per range and species and (d) selfing rate.
Box plots depict interquartile range, minimum and maximum values as well as outliers. P-values are Bonferroni adjusted for multiple testing of comparison of interest (Tajima's D, π and F_{IS} eleven comparisons; selfing rate seven comparisons). Groups that share the same letters are not significantly different between the ranges within each species. Pairwise comparisons with stars are significantly different between species within a range. ENA= eastern North America, EU= Europe, AUS= Australia, wNA= western North America, E= *C. edentula*, M= *C. maritima*, H= hybrids. Minimum of 5 individuals per population were used. Note that in (d) *C. maritima* and hybrids are grouped together and are labelled as M.

3.4.3 Trait differentiation among species and ranges

We first conducted a multivariate analysis of traits to examine differentiation between and within species. Our goal was to assess the evidence for convergent or divergent patterns of trait

evolution during invasion in *C. maritima* and *C. edentula*. Specifically, we were interested in if the putative source populations for each invasion, identified above, were phenotypically differentiated from the introduced populations. Using population means and a PCA of traits, we identified differentiation of *C. edentula* and *C. maritima* ($t^2_{2,43}$=104.61, $p < 0.001$; Figure 3-5; Table 3-S13). *Cakile maritima* was larger, flowered later, was damaged less by aphids, and had differences in seed and leaf shape. Interestingly, *C. maritima* subsp. *islandica* grouped closer to *C. edentula* than to any *C. maritima* group, although they were genetically clustered with *C. maritima* (Figure 3-5). Within the home ranges, the *C. edentula* subspecies tended to be differentiated from one another in their traits (*harperi* vs *edentula* $t^2_{2,7}$=24.485, $p < 0.01$) however the *C. maritima* subspecies were not (*baltica* vs *maritima* $t^2_{2,4}$=28.938, $p > 0.05$; *baltica* vs *integrifolia* $t^2_{2,1}$=41.406, $p > 0.05$; *maritima* vs *integrifolia* $t^2_{2,2}$=4.8181, $p > 0.05$; Figure 3-5; Table 3-S13). As *C. maritima* subsp. *islandica* and *baltica* and *C. edentula* subsp. *harperi* appeared not to have contributed to the invasions (above), we removed them from all further analyses of traits. Within *C. edentula* subsp. *edentula*, the different ranges largely clustered together in the PCA and were not significantly differentiated. Similarly, comparisons of the putative source populations of *C. maritima* (subsp. *maritima* and *integrifolia*) to the invasive range populations were not significant ($p > 0.05$ in all cases, Table 3-S13).

In our second PCA of traits we included recent hybrids and examined traits at an individual level (versus population means) and coloured the individuals according to their hybrid class from the Admixture supervised run and NewHybrid analysis (Figure 3-6). We only included putative source populations from each introduced range (subsp. *edentula*, *maritima* and *integrifolia*; no subsp. *euxina* phenotypes were available). We did this to determine the impacts of hybridization on the multivariate traits. Hybrids tended to group together between the two species, with traits shifted towards the parental species if there was higher ancestry of that species, which was *C. maritima* in most of the cases.

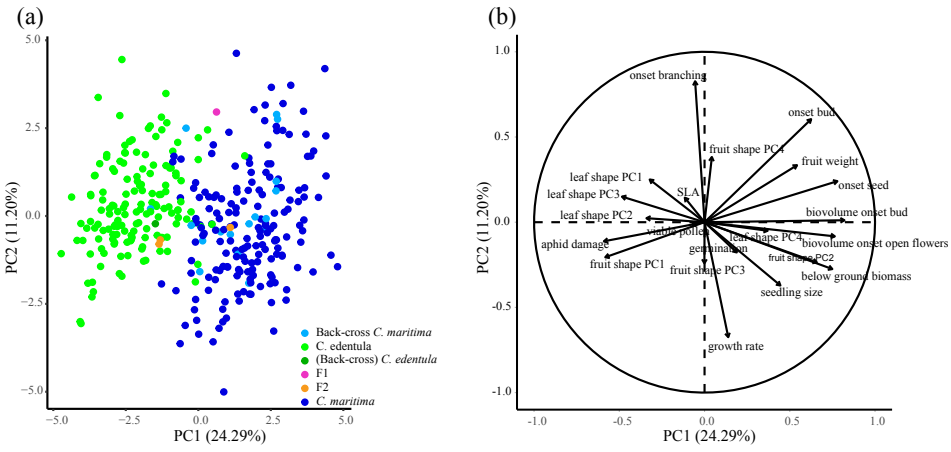


2015

2016 **Figure 3- 5. PCAs of traits using all sequenced populations. Traits and individuals included for details see**
2017 **Table 3-S3, Table 3-S4.**

2018

2019



2020

2021 **Figure 3- 6. PCA of traits at the individual level. Traits and individuals included for details see Table 3-S3.**

2022

We then conducted a univariate analysis of traits to examine evidence for parallel and divergent evolutionary responses of specific traits to invasion between the two species. Species and range (putative source populations, introduced western North America, introduced Australia) were the main effects and the interaction between these effects was also tested. We found a significant species:range interaction for several traits (days to bud, days to flower, biovolume at bud and flower, flower number, fruit weight and pollen viability) (Figure 3-7, Figure 3-S11; Table 3-4, Table 3-S14). Interactions revealed that differences between the ranges were dependent on the species and were possibly indicative of a contrasting evolutionary response to introduction between the species. Specifically, we found that *C. maritima* flowered later (days to bud and days to flower) and at a larger size (biovolume at bud and flowering) than *C. edentula* in the introduced ranges, but not when comparing the native source populations between the two species. The divergent patterns between the two species appeared to be driven by the evolution of a later flowering time at a larger size in introduced *C. maritima* relative to the native range. We discovered a reduction in flower number in the introduced ranges compared to the native range for *C. maritima,* potentially linked to its later flowering time in these regions. However, *C. maritima* substantially outperformed *C. edentula* for flower number in all ranges. Individual fruit weight was only significantly lower in *C. edentula* compared to *C. maritima* in Australia. Although pollen viability had a significant interaction between range and species, contrasts revealed only a marginally significant reduction in *C. maritima* versus *C. edentula* in Australia.

In the absence of a species:range interaction, a significant range effect is indicative of parallel evolutionary shifts in both species across the ranges (Figure 3-8, Figure 3-S12, Figure 3-S13; Table 3-4, Table 3-S14). SLA was significantly lower in Australia compared to western North America in both species, while seedling size was greater in Australia compared to western North America. Fruit shape also changed, with fruit PC2 decreasing in both introduced ranges, while fruit PC1 only increased in western North America. Germination % increased in both introduced ranges, while aphid damage increased. Many traits differed significantly between the species consistently across the ranges, as they exhibited a significant species effect, but no interaction with range. Specifically, *C. maritima* was significantly larger in size, as measured by above and below ground biomass and biovolume at flowering, and seedling length. As expected, most leaf and fruit shape PCs also differed between the species (leaf PC2-4 and fruit PC1-2). Germination % was also lower in *C. edentula* compared to *C. maritima*, while aphid

damage was greater. The number of days to seedset was greater in *C. maritima*, as was the growth rate. However, the number of days to branching was significantly greater in *C. edentula*.

Table 3- 4. The results of linear (or generalized linear) mixed models for traits of native source and introduced (western North America and Australia) populations of *Cakile maritima* and *Cakile edentula* measured in a common garden.

The native range for *C. maritima* is Europe and *C. edentula* is eastern North America (*C. maritima* subsp. *maritima* and subsp. *integrifolia, C. edentula* subsp. *edentula)*. Each trait (response) was modelled as a function of species, range, and their interaction. Population was included as a random effect in the model. Type III tests and Kenward-Rogers degrees of freedom were used for the linear models. F-values (linear mixed models) or chi-squared values (generalized linear mixed models) with degrees of freedom as subscript and symbols specifying significance of effect are reported for the continuous traits. Trait descriptions are given in Table 3-1. Significant pairwise contrasts are also reported (FDR corrected) (M= *Cakile maritima*, E= *Cakile edentula*).

Trait	Model R^2	Species	Range	Species:Range	Species contrasts	Range contrasts
Days to bud	0.28	$17.09_{1,45.42}$***	$4.43_{2,42.80}$*	$6.70_{2,42.80}$**	E < M (AU, wNA)	Native < (wNA, AU) (M)
Biovolume at bud (apex)	0.46	$70.32_{1,45.49}$***	$0.73_{2,42.49}$	$5.98_{1,42.49}$**	E < M (AU, wNA)	Native < AU (M)
Flower number	0.46	$158.59_{1,46.08}$***	$5.81_{2,43.48}$**	$11.24_{2,43.48}$***	E < M	Native > (wNA, AU) (M)
Fruit weight	0.28	$11.34_{1,43.56}$**	$0.55_{2,42.25}$	$3.61_{2,42.26}$*	E < M (AU)	-
SLA		$0.20_{1,46.98}$	$7.43_{2,43.73}$**	$0.56_{2,43.73}$	-	AU < wNA
Germination %		5.51_{1}*	11.86_{2}**	1.50_{2}	E < M	Native < (wNA, AU)
Aphid damage		11.28_{1}**	12.83_{2}**	2.61_{2}	E > M	Native < (wNA, AU)

ns p>0.1; # p<0.1; * p<0.05, ** p<0.01; *** p<0.

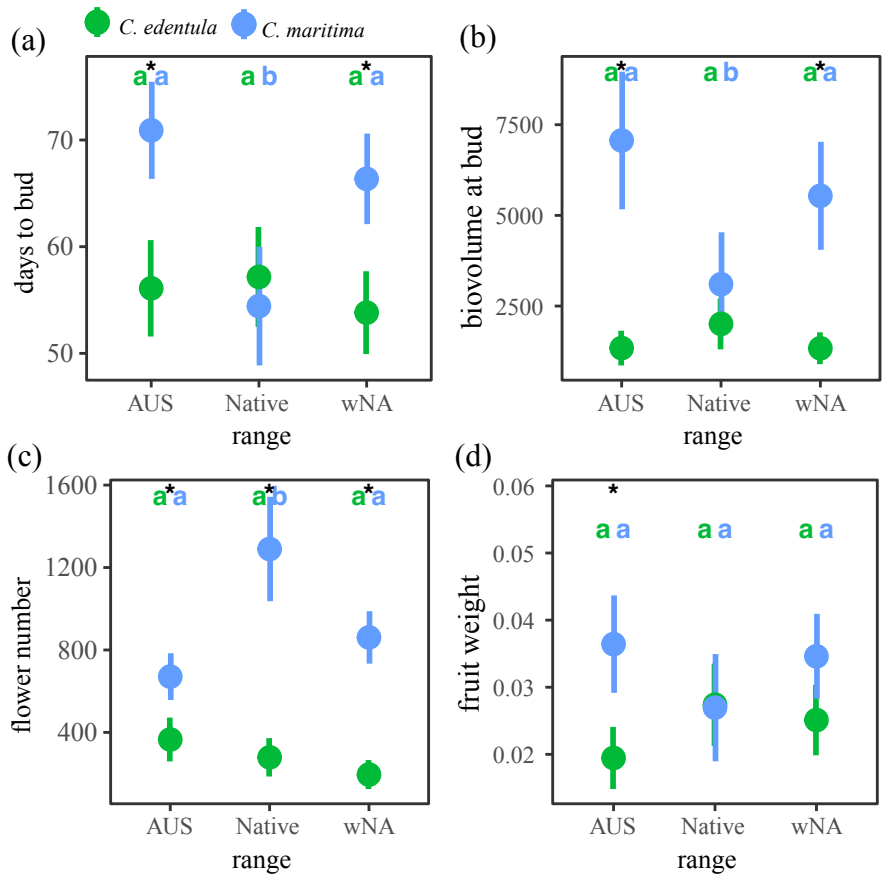


Figure 3- 7. The results of linear (or generalized linear) mixed models for traits of native source (*C. edentula* subsp. *edentula*, *C. maritima* subsp. *maritima* and subsp. *integrifolia)* and introduced (western North America and Australia) populations of *Cakile maritima* and *Cakile edentula* measured in a common garden. The native range for *C. maritima* is Europe and *C. edentula* is eastern North America. Each trait (response) was modelled as a function of species, range, and their interaction. Population was included as a random effect in the model. Trait descriptions are given in Table 3-1 and model results are in Table 3-4. Significant pairwise contrasts are reported ($p<0.05$, FDR adjusted), where different letters denote significant differences between the ranges within species and * denotes significant differences between the species within each range. Lsmeans and 95% confidence intervals are reported.

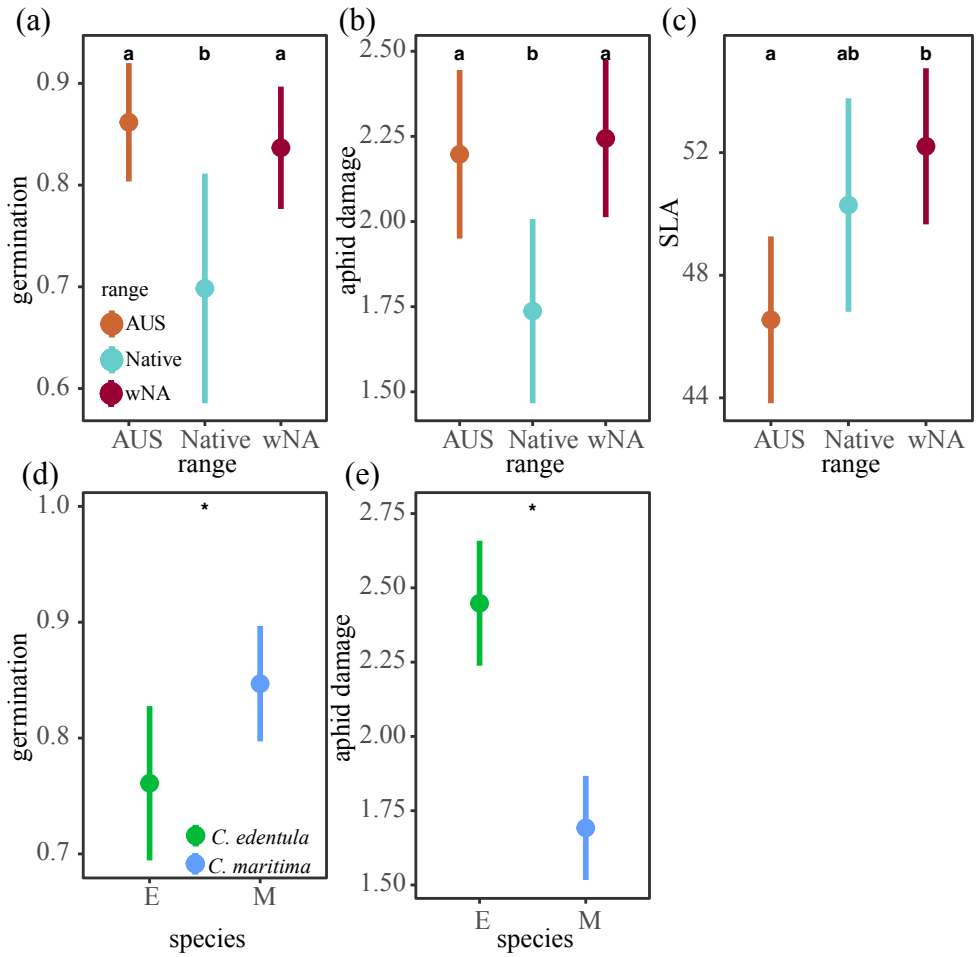


Figure 3- 8. The results of linear (or generalized linear) mixed models for traits of native source (*C. edentula* subsp. *edentula*, *C. maritima* subsp. *maritima* and subsp. *integrifolia)* and introduced (western North America and Australia) populations of *Cakile maritima* and *Cakile edentula* measured in a common garden. The native range for *C. maritima* is Europe and *C. edentula* is eastern North America. Each trait (response) was modelled as a function of species, range, and their interaction. Population was included as a random effect in the model. Trait descriptions are given in Table 3-1 and model results are in Table 3-4. Significant pairwise contrasts are reported ($p<0.05$, FDR adjusted), where different letters denote significant differences between the ranges (groups with shared letters are not significantly different) and * denotes significant differences between the species. Lsmeans and 95% confidence intervals are reported.

3.4.4 Clinal patterns

We were interested in determining if parallel patterns of trait by latitude associations had re-evolved in the introduced ranges, as this is strong evidence for recent local adaptation to climate following invasion. In some cases, divergence between ranges in traits observed in common gardens might also be caused by adaptation to the local climate and reflect differences in the range of climates samples in each range (Colautti & Barrett, 2013). To determine the importance of local adaptation to climate in governing divergence within and among ranges, we included latitude as a main effect in an analysis of the traits. Latitude is correlated with a range of environmental factors such as mean annual temperature and related to the length of

the growing season across large geographic regions, so we used this variable to capture climatic variation (Figure 3-S14; Table 3-S15). Using a Manova with group (species and range), latitude, and their interaction as main effects, we found that all interactions and main effects were significant, except the three-way interaction (Table 3-5). This suggests that traits were correlated with latitude and the relationship depended on the combination of species and range. Consequently, we tested each trait individually to examine their associations with latitude within each range and species.

Table 3- 5. Multivariate analysis of population trait means of *Cakile* in response to range, species, latitude and their interactions.

Approximate F-statistic with degrees of freedom as subscript and symbols depict significance effect, in addition to Wilk's (multivariate F-value). Exclusion of recent hybrids, *C. edentula* subsp. *harperi*, *C. maritima* subsp. *baltic* and *islandica*.

Effect	F	λ	p-value
Species	$53.43_{17,27}$	0.029	< 2.2e-16***
Range	$3.52_{34,54}$	0.097	1.85e-05 ***
Latitude	$7.61_{17.27}$	0.17	2.15e-06***
Species:Range	$2.78_{34,54}$	0.13	0.00038***
Species:Latitude	$2.78_{17,27}$	0.36	0.0086**
Range:Latitude	$1.95_{34,54}$	0.20	0.014*
Species:Range:Latitude	$1.09_{34,54}$	0.35	0.38

* p<0.05; ** p<0.01; *** p<0.0

Table 3- 6. The results of linear (or generalized linear) mixed models for traits of native source and introduced (western North America and Australia) populations of *Cakile maritima* and *Cakile edentula* measured in a common garden.

The native range for *C. maritima* is Europe and *C. edentula* is eastern North America (*C. maritima* subsp. *maritima* and subsp. *integrifolia, C. edentula* subsp. *edentula)*. Each trait (response) was modeled as a function of species, range, and their interaction as well as latitude and all two and three way interactions with latitude, species and range (non-significant interactions with latitude were removed in a stepwise manner). Population was included as a random effect in the model. Type III tests and Kenward-Rogers degrees of freedom were used for the linear mixed models. F-values (linear mixed models) or chi-squared values (generalized linear mixed models) with degrees of freedom as subscript and symbols specifying significance of effect are reported for the continuous traits. Trait descriptions are given in Table 3-1. Significant pairwise contrasts are also reported (FDR corrected) (M= *Cakile maritima*, E=*Cakile edentula*).

Trait	R2	Species	Range	Species: Range	Latitude	Specie: Latitude	Range: Latitude	Species:Range:Latitude	Species contrasts	Range contrasts
Days to bud	0.28	$12.52_{1,45.42}$***	$3.99_{2,45.42}$*	$8.51_{2,43.6}$***	$12.25_{1,36.85}$**	-	-	-	E<M (AU, wNA)	Native < (wNA, AU) (M)
Biovolume bud apex	0.40	$73.09_{1,44.98}$***	$2.07_{2,45.24}$	$9.17_{2,43.31}$***	$11.61_{1,36.64}$**	-	-	-	E<M (AU, wNA)	Native > AU (E)
Below-ground biomass	0.50	$125.55_{1,44.73}$***	$4.35_{2,45.89}$*	$0.65_{2,42.36}$	$34.47_{1,61.06}$***	-	$4.50_{1,46.55}$*	-	E<M	AU<Native (E)
Fruit weight	0.28	$12.87_{1,41.30}$***	$4.88_{2,42.83}$*	$6.27_{2,40.39}$***	$13.70_{1,50.63}$***	-	$5.71_{1,44.41}$**	-	E<M (AU)	AU < Native (E)

ns p>0.1; # p<0.1; * p<0.05, ** p<0.01; *** p<0.001

Table 3- 7. The results of linear (or generalized linear) mixed models for traits of introduced (western North America and Australia) populations of *Cakile maritima* measured in a common garden.

Each trait (response) was modeled as a function of range, latitude, Q value and all two and three way interactions (non-significant interactions with latitude were removed in a stepwise manner). Population was included as a random effect in the model. Type III tests and Kenward-Rogers degrees of freedom were used for the linear mixed models. F-values (linear mixed models) or chi-squared values (generalized linear mixed models) with degrees of freedom as subscript and symbols specifying significance of effect are reported for the continuous traits. Trait descriptions are given in Table 3-1. Slopes and standard errors for significant continuous predictors are shown below (significant slopes are bolded).

Trait	R2	Range	Latitude	Q	Range:Latitude	Range:Q	Latitude:Q	Range:Latitude:Q
Days to bud	0.10	$0.38_{1,15.68}$	$0.013_{1,27.10}$	$5.81_{1,88.59}$*	-	-	$4.92_{1,84.58}$*	-
Days to flower	0.11	$4.55_{1,15.04}$*	$0.38_{1,23.81}$	$5.39_{1,89.27}$*	$4.40_{1,14.29}$# (p=0.053)	-	$4.77_{1,83.05}$*	-

Above ground biomass	0.12	$0.70_{1,15.25}$	$0.74_{2,17.26}$	$9.54_{1,34.99}$** (**-3.72 +/-1.17**)	-	-	-	-
Below ground biomass	0.10	$1.08_{1,14.90}$	$6.22_{1,17.10}$* (**-0.0089 +/-0.004**)	$4.25_{1,31.85}$* (**-0.71 +/-0.33**)	-	-	-	-
Biovolume at flowering	0.04	$0.81_{1,14.65}$	$0.23_{1,16.88}$	$5.64_{1,30.81}$* (**-3.12 +/-1.26**)	-	-	-	-
Flower number	0.09	$0.27_{1,14.76}$	$0.18_{1,18.44}$	$1.85_{1,31.71}$	-	$5.21_{1,33.34}$* (AUS=**-44.8 +/-15.4** wNA=10.5 +/-19.4)	-	-
Pollen viability		2.45_{1}	6.03_{1}*	36.36_{1}***	-	-	36.36_{1}***	-
Aphid damage		0.02_{1}	2.59_{1}	3.97_{1}*	-	-	3.92_{1}*	-

2134 ns p>0.1; # p<0.1; * p<0.05, ** p<0.01; *** p<0.001

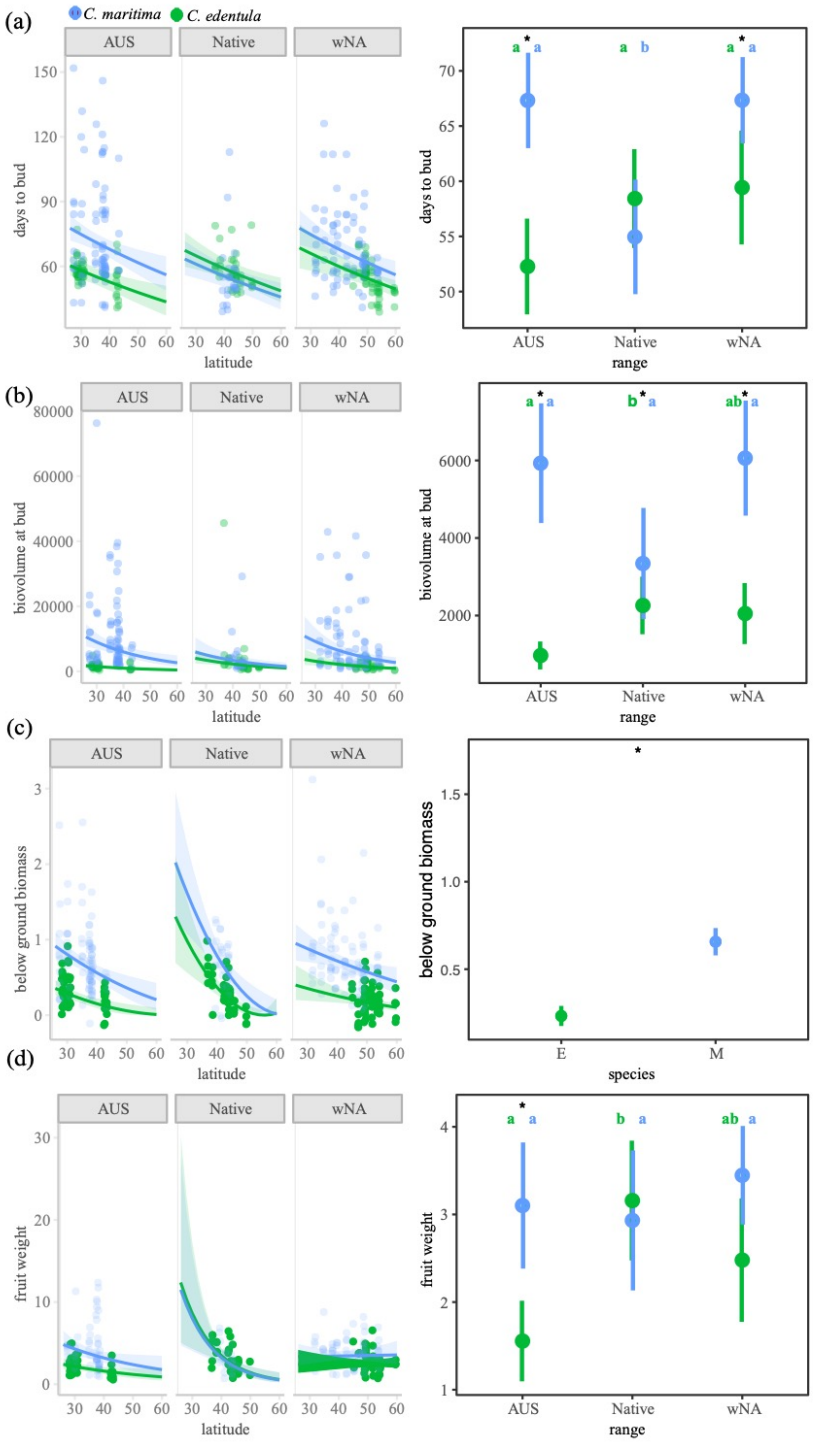


Figure 3- 9. The results of linear (or generalized linear) mixed models for traits of native source (*C. edentula* subsp. *edentula*, *C. maritima* subsp. *maritima* and subsp. *integrifolia)* and introduced (western North America and Australia) populations of *Cakile maritima* and *Cakile edentula* measured in a common garden. The native range for *C. maritima* is Europe and for *C. edentula* is eastern North America. Each trait (response) was modelled as a function of species, range, their interaction, as well as latitude and significant interactions with latitude. Population was included as a random effect in the model. Trait descriptions are given in Table 3-1 and model results are in Table 3-6, Table 3-S16. The raw data, predicted values and CI intervals are reported in the left panel for significant relationships with latitude. Lsmeans and 95% confidence intervals for are reported in the right for significant categorical predictor variables. Significant pairwise contrasts are reported, where different

letters denote significant differences between the ranges within species (groups with shared letters are not significantly different) and * denotes significant differences between the species within each range.

For the univariate analysis of individual traits, we found parallel associations of several flowering time and size-related traits within all species and ranges (days to bud, days to seed, biovolume at bud, below ground biomass, seedling length; Figure 3-9, Figure 3-S15; Table 3-6, Table 3-S16). As predicted, our data reveal that plants collected from higher latitudes tended to evolve an earlier onset of reproduction, but this came at a cost to size. Specifically, the main effect of latitude, but not the interaction between latitude and species or latitude and range was significant for days to buds, days to seed set, seedling length and biovolume at bud. For below ground biomass the interaction between latitude and range or species was significant, but the slopes for each group were all significantly different from zero and in the same direction (negative) indicating clines in the same direction (Table 3-S17). Above ground biomass was only significantly correlated with latitude in the native ranges, while growth rate and days to branching had complex, three way interactions and only had slopes significantly different from zero in one (days to branching; *C. edentula* wNA) or two (growth rate *C. maritima* AU and *C. edentula* native) groups. Fruit weight was negatively correlated with latitude in the native ranges and in Australia but showed no significant patterns in western North America. SLA, and most leaf and seed shape traits did not show significant relationships with latitude (results not presented).

When including latitude in the model, comparisons of the species and ranges remained largely unchanged. When controlling for latitude, *C. maritima* still set seed much later than *C. edentula* in all ranges. *Cakile maritima* flowered (days to bud and days to flowering) much later than *C. edentula* but only in the two introduced ranges (Figure 3-9, Figure 3-S15; Table 3-6, Table 3-S16). Although there was a significant species:latitude interaction for days to flowering, the CI intervals between the species in the introduced ranges did not overlap at latitudes both species were present at, but they did in the native range, consistent with the model without latitude. Similarly, for biovolume at bud, *C. maritima* was significantly larger than *C. edentula* but only in the introduced ranges. This was driven by a reduction in size of Australian *C. edentula* compared to the native range. Biovolume at flowering was greater in *C. maritima,* although a significant species:latitude interaction was apparent, but the CI intervals along the cline did not overlap for latitudes at which both species were present. For biomass measures, *C. edentula* was significantly smaller than *C. maritima*, even when taking latitude into account. Seedling

length was also lower in *C. edentula* in all ranges except wNA. Australian *C. edentula* populations tended to be smaller than the other two ranges and have lower fruit weights.

We conducted an analysis of *C. maritima* individuals in each introduced range to determine if the Admixture proportion (Q) was correlated with any traits, since species ancestry may explain any differences within and between the ranges. Since the relationship may be curved, we conducted a regression including higher-order polynomial terms. However, including second and third-order polynomials did not improve the model fit and were not included in the final models. For two flowering time-related traits (days to bud and days to flowering), the interaction between latitude and Q value was significant (Table 3-7). Interaction plots revealed that the slope of the relationship between the trait and latitude became more negative at higher Q values, consistent with admixture from *C. edentula* contributing to the evolution of later flowering times at higher latitudes (Figure 3-10). This interaction was also observed for pollen viability and aphid damage. Several size-related traits showed significant associations with Q value and all had negative slopes (above and below ground biomass, biovolume at flowering) revealing that increased *C. edentula* ancestry was associated with reduced size. Flower number also showed a significant negative association with ancestry, but this was only significant in Australia (Table 3-7). Latitudinal associations were still significant for below ground biomass, but not for above ground biomass or biovolume at flowering, suggesting that species ancestry was sufficient to explain these geographic patterns in size.

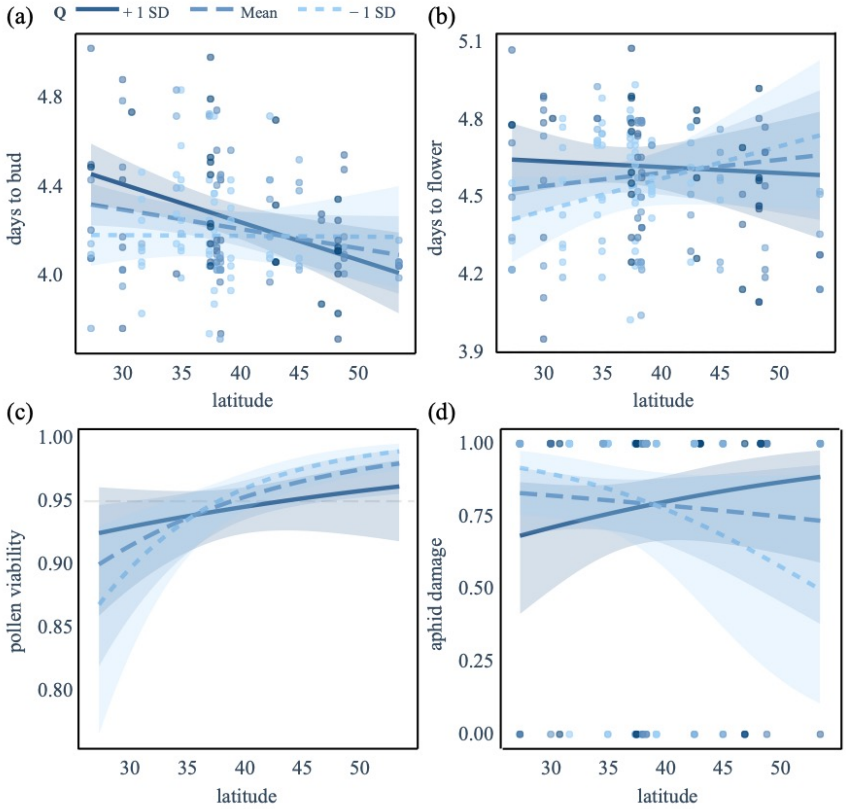


Figure 3- 10. Interaction plots for the results of linear (or generalized linear) mixed models for traits of introduced *Cakile maritima* (western North America and Australia) measured in a common garden. Each trait (response) was modelled as a function of latitude, range, Q value (a measure of species ancestry) and all significant interactions. Population was included as a random effect in the model. All traits depicted had a significant interaction between latitude and the Q value. Trait descriptions are given in Table 3-1 and model results are in Table 3-7. Predicted values and CI intervals are plotted for three Q values. Raw data and predicted relationships are colour coded by Q value (higher values are darker blue).

3.5 Discussion

We used a common garden experiment employing widespread accessions from four geographic regions coupled with genome sequencing to examine the evolution of two *Cakile* species during invasion. The use of multiple invasive ranges and species allowed us to examine the extent of convergent versus divergent trait evolution during invasion of similar climatic gradients and coastline habitats. Further, these species are allopatric in their native range but experience sympatry in the introduced range, leading to novel ecological interactions between the species as well as hybridization. We examined the population structure of these species to facilitate a comparison of the introductions to their putative source populations. To do this we assembled a reference-quality genome for *C. edentula* and called variants using 400 re-sequenced samples from home and invasive ranges. In both invasive ranges, populations of both species appear to have evolved greater susceptibility to herbivores than the source populations, consistent with hypotheses related to the evolution of defence during invasion. By contrast, while the allopatric

source populations experienced similar flowering times in the common garden, we discovered substantial divergence in flowering time between the species for the invasive populations with a current or recent history of sympatry for both ranges. The repeated and rapid evolution of divergent phenology in sympatry is consistent with reproductive character displacement and may be a reflection of incomplete reproductive barriers and known fitness reductions of hybrids, although this needs to be tested in the field. Finally, we identified parallel latitudinal clines for the timing of reproduction and size-related traits, likely reflecting repeated adaptation to local climates within each range. Many of these repeated patterns of trait evolution within and between species are consistent rapid adaptation of invasive populations in the past 140-160 years for *C. edentula* and 85-123 years for *C. maritima*.

3.5.1 Population structure and invasion history

Our findings of native-range population structure are largely consistent with our previous analysis based on a dataset that was more limited in its geographic sampling, total sample size and SNP number (Rosinger et al., 2021; Chapter 2). The native range of *C. edentula* is split into three clusters (Figure 3-1, Figure 3-2). The "Nova Scotia" cluster (middle green) and the "Great Lake" cluster (light green) both belong to *C. edentula* subsp. *edentula*. A third cluster was identified in Georgia and Florida belonging to *C. edentula* subsp. *harperi*. In Europe, native *C. maritima* populations were divided into three main groups, largely following the established taxonomy: 1) a Baltic (red) group representing the subsp. *baltica* and subsp. *islandica* (Finland, Estonia, Sweden and Iceland); 2) an Atlantic group representing subsp. *integrifolia* (France and Portugal) (admixed between the red and blue cluster); and 3) a Mediterranean group (blue cluster) representing subsp. *maritima* (Mediterranean and Black Sea) and possibly subsp. *euxina*. Neither the Black Sea subsp. *euxina* nor the Icelandic subsp. *islandica* clustered separately in the Admixture results, although they were somewhat differentiated genetically in other analyses (e.g., Figure 3-S8, 3-S9).

Consistent with our GBS-based analysis (Rosinger et al., 2021; Chapter 2), *C. edentula* in Australia likely originated from the "Nova Scotia" cluster. In western North America two sources are apparent: The "Nova Scotia" cluster, located in Alaska and northern British Columbia, and the "Great Lake" cluster in the Pacific Northwest. Previously it was believed that there had been only a single introduction, first reported near San Francisco in 1935 (Barbour & Rodman, 1970). Admixture between these two clusters is apparent in the western

North American introduced range. Our results also support the finding that *C. maritima* in Australia likely originated from at least two sources. Previously, it has been shown that *C. maritima* in south-eastern Australia originated from the Mediterranean (Ohadi et al., 2016; Rodman, 1986; Rosinger et al., 2021), and our analysis finds that the bulk of samples in this region cluster with the Mediterranean samples. We have previously identified a second source in Western Australia likely originating from Atlantic populations in Europe, as have others (Ohadi et al., 2016; Shaw et al., 2021). Rodman (1974) concluded that Western Australian samples are from the Baltic. Although we did not sample Western Australia in our whole-genome dataset, we did observe some admixed individuals in South Australia with Atlantic ancestry (red/blue cluster, Figure 3-2), consistent with a second introduction from Europe or gene flow from Western Australia (Ohadi et al., 2016). In western North American *C. maritima,* the closest genetic match in the home range can be found in the Mediterranean, although the invasions were genetically distinct from this putative source and despite our relatively extensive sampling of the native range in this study. These findings are again consistent with Rosinger et al., (2021) (Chapter 2), and potentially point to a founder effect or bottleneck during this invasion.

In general, self-compatible *C. edentula* was less genetically diverse than the self-incompatible *C. maritima* and hybrids (Figure 3-4). *Cakile edentula* had lower genome-wide nucleotide diversity, and a higher inbreeding coefficient. Australian *C. edentula* were less inbred than the home range individuals despite a reduction in Tajima's D. In contrast, *C. edentula* from western North America were much more inbred than the home range and showed much higher LD (Figure 3-S20). This is suggestive of a shift in the mating system to higher rates of self-pollination (Table 3-S19, Table 3-S20). However, there was a trend towards higher selfing in *C. edentula* in the invasive ranges, yet they were not significantly different (Figure 3-4). These *C. edentula* populations are found quite far north in British Columbia and Alaska and may experience more pollinator or mate limitation at this northern range margin. Although increased self-fertilization is typically associated with colonization, transitions in mating systems from outcrossing to selfing during invasion are rarely identified (Barrett, 2015; Hodgins et al., 2018). Pure *C. maritima* individuals from the invasive ranges showed similar nucleotide diversity and heterozygosity to the home range populations and hybrid individuals showed similar values to pure invasive *C. maritima*. This maintenance of genome-wide variation in the invasions may reflect multiple introductions of *C. maritima* (particularly in the case of Australia), large founding populations, or low levels of *C. edentula* ancestry in putatively pure parental *C.*

maritima samples. Future demographic modelling using our genomic data will provide greater insight into these alternative scenarios.

3.5.2 Hybridization in the invasive ranges

We identified hybrids in both invasive ranges; however, we found more hybrid ancestry in Australia than in western North America (Admixture: Australia 47% hybrids, western North America 23% hybrids) consistent with previous genetic analysis (Rosinger et al., 2021; Chapter 2). The higher number of hybrids in Australia could be due to several factors. First, the invasion of both species in Australia took place before the invasion in western North America, giving a longer time period for hybridization to take place. Second, the differences in hybridization rate between the ranges could reflect differences in reproductive barriers between the species. Importantly, there are many more allopatric populations of *C. edentula* in western North America, particularly in the far northern parts of the range. If we exclude areas where *C. edentula* has never been in western North America, the hybridization rate would increase slightly from 22 to 24%. Differences in climate adaptation might limit the northern range of western North American *C. maritima*, since southern Mediterranean genotypes were the likely source of this invasion.

Our results clearly demonstrate historic and ongoing hybridization between the two species, as we find early and advanced-generation hybrids in both ranges, which supports our previous analysis (Rosinger et al., 2021; Chapter 2). NewHybrid detected 14 (9%) early generation hybrids in Australia (11 BC_M, 3 F2) and 7 (5%) early generation hybrids in western North America (6 BC_M,1 F1). As before we identified biased backcrossing towards *C. maritima* (Figure 3-3), possibly explained by enhanced insect attraction of both hybrids and *C. maritima* and higher fitness of some backcrosses to *C. maritima* relative to other types of hybrids such as F2s (Li et al., 2019; Mesgaran et al., 2016).

3.5.3 Convergent and divergent patterns of trait evolution during invasion

Some theories predict that invasive species may evolve in similar ways when introduced to a new range e.g. comparisons across multiple introductions to the native range (particularly the introduction sources) gives greater capacity to test the generality of these theories, and if invasion is likely to induce parallel evolutionary change. Our study leveraged native-introduced comparisons across multiple invasions and species inhabiting very similar coastline

habitats worldwide to shed light on parallel evolutionary change during invasion. One widely-examined theory to explain the success of invasive species in the new range is the evolution of increased competitive ability (EICA) hypothesis (Blossey & Notzold, 1995), which predicts enhanced growth and reproductive output at the expense of investment in specialist herbivore defences, which may no longer be selected for if invasion facilitates an escape from these enemies. We found some support for increased insect damage and an enhancement of some performance related traits upon introduction, particularly increased damage by aphids and greater germination % in the introduced ranges of both species (Figure 3-8). However, our experiment was not designed to test EICA and the aphid damage was the result of a brief incidental outbreak. These aphids were not specialist herbivores (likely *Myzus persicae*) and were found within the Australian introduced range. *Myzus persicae* occurs worldwide and is known to occur on native *C. maritima* (Davy et al., 2006). We are not aware of any evidence of this species occurring on *C. edentula* in the native range, but a different species of aphids, *Hyadaphis erysimi*, can cause great damage to *C. edentula* (Payne & Maun, 1984) in the greenhouse and in the field. In Australia, these aphids have been observed on plants in the field near or in inflorescences (Li et al., 2019) and others have observed greater damage on *C. edentula* than *C. maritima* in the greenhouse (T. Jalali pers. comment). It should be noted that our pesticide treatment may have artificially truncated the negative impacts of the aphids, and it is possible that with a prolonged exposure *C. maritima* would have suffered greater damage, although few *C. maritima* were attacked or experienced any damage during the aphid exposure window. For germination % we also cannot exclude maternal environmental effects, as those are based on field-collected seeds and here the germination % may reflect the environments of the sampling location. Further, confounding factors preventing an appropriate test of this well-known hypothesis included invasive range-specific hybridization and the lack of a control group without herbivores. Nevertheless, these data are tantalizing, as they provide possible evidence of reduced herbivore defence during invasion across two species. Shifts in defence have been documented in many introductions (Felker-Quinn et al., 2013), but our data are relatively unique in identifying parallel evolutionary shifts in two introduced ranges and in two species, which may point to a parallel selective mechanism associated with introduction in both species.

As the native and introduced ranges of both species encompass broad climatic gradients, it is possible that trait changes that appear to be associated with introduction are not a reflection of adaptation to a general shift in the biotic community during invasion, but a response to climate

induced variation at a local scale. Latitudinal clines were apparent for *C. edentula* for aphid damage, with high latitude populations exhibiting greater damage, while this was not the case for *C. maritima* (Figure 3-10). It has been hypothesised that plants in higher latitudes invest less energy into defence mechanisms than plants from lower latitudes (Frenne et al., 2013), and this might have played a role in this pattern. Such clines may partly explain the divergence among the ranges in aphid damage for *C. edentula*, although Australia still exhibited greater damage even when accounting for latitude of origin. However, a future analysis using climate variables as covariates might be more appropriate given the differences in climate between the regions (Shaw et al., 2021) for a given latitude. Species differences and high levels of introgression in the introduced range might also contribute to the parallel patterns of damage identified in *C. maritima* introductions. *Cakile edentula* experienced significantly more damage by aphids compared to *C. maritima*. Further, greater *C. edentula* ancestry had a significant impact on the association with latitude and aphid damage, with greater aphid damage at higher latitudes in *C. maritima* accessions with greater *C. edentula* ancestry, reflecting the clines in *C. edentula* (Figure 3-10; Table 3-6). Consequently, the enhanced aphid damage observed in *C. maritima* in the introduced range may originate from introgression and its genetic basis in introduced *C. maritima* may be partly derived from interspecific gene flow. Future genome-wide association analyses combined with a phylogenetic analysis of candidate loci will confirm the origin of these convergent phenotypic patterns and if introgression is the likely cause.

In addition to shifts in defence, many invasive populations also experience enhanced growth relative to their native range (Felker-Quinn et al., 2013). Theory suggests that these patterns may reflect evolution along trade-offs in response to reduced environmental stressors in the introduced range, or heterosis if admixture is occurring (Blossey & Notzold, 1995; Karasov et al., 2017; Züst & Agrawal, 2017; Züst et al., 2015). In our experiment, evidence for enhanced performance of invasive populations was mixed. Biomass measures taken at the end of the experiment did not exhibit a range effect. However, it is possible this may be because of a greater attack rate on introduced individuals countering any enhanced growth due to the brief aphid outbreak. In *C. maritima*, biovolume at bud or flowering, which was measured earlier in the experiment and before the aphid outbreak (Table 3-S2), was enhanced in introduced populations, but this may reflect the delay in reproduction identified in these regions (Figure 3-7, Figure 3-S13, Figure 3-S15). Greater germination % in the introduced ranges appeared in

both species and were measured well in advance of the aphid outbreak. However, this effect may partly be attributed to latitudinal effects (Figure 3-9 vs Figure 3-S12, Figure 3-S15).

There were a number of traits that appeared to evolve in response to the specific environment of each introduced range but did so in similar ways for both species. This suggests that there were convergent evolutionary changes between species, but these evolutionary changes were not a function of the invasion process, but rather a response to the specific environment of each introduced range. For instance, SLA was much lower in Australia than the other ranges for both species, meaning that thicker/denser leaves were found in Australia than in western North America or the native ranges of either species, and this may reflect adaptation to drought conditions as it is associated with enhanced water use efficiency. However, SLA can also be influenced by factors like herbivory, salinity, and many other environmental factors (Poorter et al., 2009). Aspects of fruit (fruit PC1) and leaf shape (leaf PC1) were also differentiated among ranges but were not associated with invasion. These differences did not appear to reflect variation in species ancestry or latitude as these effects (Q value and latitude) were not significantly associated with these traits, but range differences were apparent between Australian and western North American *C. maritima*. Consequently, the convergent patterns between the species are unlikely simply a function of hybridization. We also identified species and range-specific effects for several traits, including individual fruit weight. Australian *C. edentula* produced smaller seeds than *C. maritima*, and when accounting for latitudinal effects, a reduction in fruit size and biovolume at bud was apparent in Australia compared to the native range. Although the cause is unclear, the large reduction in genome-wide variation (Figure 3-4) suggests a substantial invasion bottleneck in Australia (Table 3-S19), which could be contributing to inbreeding depression.

In addition to identifying convergent trait changes related to invasion across species, we also identified cases of divergent trait changes. In allopatric source populations we did not detect any significant differences between the species for the onset of bud, flowering or size estimates taken at these timepoints (Figure 3-9). However, large differences between the species were identified in introduced range populations, most of which had been recently (post invasion) or are currently sympatric. *Cakile edentula* flowered 25 days earlier than *C. maritima* on average in invasive ranges, and the shorter flowering period in *C. edentula* meant a substantial reduction in the flowering overlap between the species in the greenhouse. This pattern in flowering time and size was apparent even when accounting for (absolute) latitude of origin. Divergence in

reproductive traits of sympatric populations, compared to allopatric populations of closely related cross-compatible species, can be caused by selection to reduce costly interactions, including the production of low fitness hybrids or competition for pollinators. For these *Cakile* species fitness costs associated with cross-species pollination have been documented (Li et al., 2020).

It is possible the repeated evolution of divergent reproductive timing between the species following invasion is not due to ecological interactions between the *Cakile* species, but rather a divergent evolutionary response to other aspects of the environment experienced during invasion. For example, Allee effects during colonization may have resulted in increased rates of self-fertilization in the self-compatible *C. edentula* following invasion, although this is not evident in our population genomic data. By contrast, *C. maritima* is largely self-incompatible. Self-fertilization can be associated with the evolution of earlier flowering (Snell and Aarssen, 2005). Similarly, although we attempted to control for invasion history by only including putative source populations, founder effects and other non-adaptive processes could be contributing to trait divergence. Future field experiments examining selection on flowering time with and without conspecifics and a historical analysis of temporal changes in flowering time and species distributions using herbarium samples may be informative in this regard. Although it is widely understood that novel species interactions between congeners are increasing globally because of invasion, character displacement caused by novel species interactions are less frequently appreciated or studied, especially in plants (Beans, 2014).

3.5.4 The evolution of latitudinal trait clines

In a relatively short time span (140-160 years for *C. edentula* and 85-123 years for *C. maritima*) adaptation to local climates appears to have evolved in both invasive ranges (western North America and Australia) of each species. An earlier onset of reproduction (onset of bud and onset of seed) and decreased size (biovolume at bud, below ground biomass, seedling length) are apparent in plants from higher latitudes. Clines in phenology and size were also present in the native ranges of both species. Latitudinal clines in flowering time and size are frequent in widespread annual plants (e.g., Allard, 1945; Colautti et al., 2009; Leiblein-Wild & Tackenberg, 2014) and reflect variation in growing season (Colautti et al., 2009). In temperate environments, a decrease in season length at higher latitudes results in the evolution of earlier reproduction, which typically comes at a cost to size (Colautti et al., 2010), while delayed

flowering is frequently selected at lower latitudes with longer growing seasons, due enhanced fertility that is achieved through delayed maturation at a larger size. Although demographic changes that accompany invasion can contribute to trait clines that are non-adaptive (reviewed in (Colautti & Lau, 2015), the repeated evolution of clines in important life history traits across multiple species and ranges is unlikely to occur through stochastic processes alone, supporting climate adaptation as the predominant force in driving the latitudinal clines in phenology and size observed in our experiment.

Rapid climate adaptation has been identified in a growing number of widespread plant invaders, including species experiencing substantial invasion bottlenecks (van Boheemen et al., 2019). Indeed, in our common garden we identified strong latitudinal clinal patterns for several traits in Australian *C. edentula,* despite a substantial reduction in genome-wide SNP variation consistent with a single, bottlenecked introduction. Such rapid adaptation despite a loss of genetic variation during colonization may be aided by polygenic trait architectures that can buffer populations from the impacts of drift at individual loci (Dlugosch et al., 2015). Alternatively, introgression from *C. maritima* (Table 3-3, Table 3-6) may have contributed important adaptive genetic variation. However, introgression in this direction is not as apparent as the reverse (from *C. edentula* to *C. maritima*) (Chapter 2, 3).

Some expected clines (i.e., days until flowering, above-ground biomass) were not evident in all groups (*C. maritima* and the introduced ranges respectively). Both of these traits were associated with species ancestry in invasive *C. maritima* however, suggesting that latitudinal patterns, particularly in Australia where latitude and ancestry are not strongly correlated (Spearman's rho = -0.118, p = 0.734), might be mitigated by the impacts of introgression on these traits. Additionally, since *C. maritima* is occasionally biannual and experiences more indeterminate growth (Thrall et al., 2000), selection for early flowering at a smaller size at high latitudes may be weaker, especially in Australia where the winters are not as harsh as northern Europe or northwestern North America.

3.5.5 Species differences in traits and the replacement of *C. edentula*

Since its discovery, the cause of the recurrent pattern of the invasion of *C. edentula* followed by the invasion of *C. maritima* and corresponding replacement of *C. edentula* has puzzled ecologists. There have been a number of theories regarding the replacement of *C. edentula* by

C. maritima, including direct and indirect competition (Boyd & Barbour, 1993; Cody & Cody, 2004), differences in disease susceptibility (Bock, 2008; Cousens et al., 2013; Thrall et al., 2000), differential climate adaptation (Cousens et al., 2013) and hybridization (Cody & Cody, 2004; Mesgaran et al., 2016). Many of these theories are not mutually exclusive and our common garden data are consistent with several of them, as well as observations from previous research (Barbour, 1970; Mesgaran et al., 2016; Rodman, 1974). For most fitness-related traits such as biomass, seedling length, biovolume, germination %, and flower number, *C. maritima* appeared to outperform *C. edentula* in our common garden experiments, particularly in the introduced ranges, consistent with past observations (Barbour, 1970; Rodman, 1974, 1986). Although we did not directly assess competition in this experiment, the advantage afforded by these traits should give *C. maritima* a substantial edge in many circumstances. Differences in life history and mating system may contribute to the differences in size and reproductive output that are apparent in our experiment. Selfing species tend to have an earlier onset of reproduction, and flower at a smaller size when compared to their outcrossing relatives (Snell & Aarssen, 2005), a pattern which we also see in our species (*C. edentula*- self-compatible; *C. maritima*-self-incompatible). *Cakile maritima* is also biannual in some cases, while *C. edentula* is only known to be annual. Although we did not examine disease resistance, we did observe large differences between the species in herbivore susceptibility in the glasshouse, with the advantage, again, favouring *C. maritima*. An exception to this pattern was a trend towards reduced pollen viability in *C. maritima* in Australia, although this was not statistically significant and differences in pollen viability among hybrid classes has not been identified previously (Li et al., 2019).

Models examining the impact of hybridization on extinction frequently demonstrate that incomplete reproductive barriers contribute to the extinction of one or both co-occurring species (reviewed in Todesco et al., 2016). Our data support porous species boundaries in both introduced ranges as demonstrated by recent and advanced-generation hybrids. Pre-mating isolating mechanisms, such as selfing and differences in flowering time, are present but incomplete: The flowering period overlaps in sympatric zones in our common garden data (and in the field based on personal observations), and mixed mating appears to be common in *C. edentula* (Table 3-S19, Table 3-S20). Similarly, hybrid incompatibilities are also weak, especially for particular cross types (Li et al., 2019). This suggests that hybridization may be an important factor contributing to the local extinction of *C. edentula*. Simulations of *C. maritima*'s establishment and replacement of *C. edentula* have been conducted and

demonstrate a role of hybridization in overcoming Allee effects in SI *C. maritima* (Harrison & Darby, 1955; Li et al., 2019; Mesgaran et al., 2016). If mate limitation is overcome through hybridization, these simulations predict complete replacement of *C. edentula* through *C. maritima*'s enhanced fertility and survival within a matter of 11-16 years. This rapid and complete replacement does not match our high observed levels of ancestry across replacement zones in Australia and western North America (Figure 3-1). However, this model used a single locus for species identity and may have underestimated the degree of mixed ancestry retained in populations.

The simulations modelling the establishment and replacement were built to explore the demographic consequences of the species interactions during colonization and did not include the possibility of adaptive introgression which may enhance mixed-species ancestry. Particularly in western North America, *C. maritima* tends to extend into higher latitudes and cooler climates compared to the source populations (largely found around the Mediterranean and southern European Atlantic coast). Interestingly, for the onset of bud and the onset of flowering, a latitude by species ancestry (Q-value) interaction was observed in *C. maritima* invasive populations (Figure 3-10). In both cases, the slope between the trait and latitude became more negative with increasing *C. edentula* ancestry resulting in a stronger cline (particularly for days to bud). The geographic distribution of the source and invasive-range populations, and the interaction between species ancestry and latitude for traits critical for climate adaptation, suggests a potential role for introgression in facilitating local adaptation and the poleward range expansion of invasive *C. maritima*. Future analysis, including niche modelling alongside an examination of the genetic basis of climate adaptation in these species is required to assess this hypothesis. If introgression from *C. edentula* has been facilitating local adaptation of *C. maritima* at high latitudes, genes originating from the initial invader could, in fact, be contributing to its own local extinction. However, only time will tell if *C. edentula* and *C. maritima* will form a stable hybrid zone in these introduced ranges, or if the relentless advancement of *C. maritima* and their hybrids will continue, leading to the extinction of pure invasive *C. edentula*.

Author contributions

HSR conducted sampling, the greenhouse experiment and bioinformatic analysis. CL and JW conducted molecular laboratory work. KAH and PB helped with data analysis. AMG, KAH and RDC conducted sampling. LHR, RDC and KAH conceived and helped design study. HSR wrote the manuscript with contributions from PB, RDC and KAH.

3.6 Appendix II

Table 3-S1. Sampling locations

Population ID	Country	Range	Latitude	Longitude	Note
NSW10	Australia	AUS	-30.883926	153.044953	
NSW4	Australia	AUS	-28.338504	153.57597	
NSW6	Australia	AUS	-28.852774	153.599717	
NSW8	Australia	AUS	-30.107389	153.200778	
NSW9	Australia	AUS	-30.306468	153.139966	
QLD10	Australia	AUS	-27.415496	153.483564	
QLD11	Australia	AUS	-27.421813	153.516338	
SA2	Australia	AUS	-35.078028	138.496056	
SA4	Australia	AUS	-37.4766	140.020589	
SA6	Australia	AUS	-33.040345	137.588427	
TAS10	Australia	AUS	-42.822709	147.871184	
TAS12	Australia	AUS	-43.119061	147.735571	
TAS3	Australia	AUS	-43.459188	147.15439	
TAS4	Australia	AUS	-43.321809	147.241472	
TAS5	Australia	AUS	-43.35793	147.32689	
TAS8	Australia	AUS	-42.546766	147.886615	
TAS9	Australia	AUS	28.952669	-95.282137	
VIC1	Australia	AUS	-38.449781	145.219833	
VIC11	Australia	AUS	-37.576013	149.756789	
VIC6	Australia	AUS	-38.113639	144.654944	
VIC9	Australia	AUS	-38.392211	142.230022	
FL3	USA	eNA	29.70715	-81.229844	
GA1	USA	eNA	31.9896	-80.8531	
MA1	USA	eNA	42.419605	-70.907179	
TX2	USA	eNA	27.422259	-97.298383	outgroup
MD1	USA	eNA	38.385	-75.063	
ME2	USA	eNA	44.296944	-68.531667	
MI1	USA	eNA	43.125218	-86.275822	
NB1	Canada	eNA	46.164639	-63.826306	
NB2	Canada	eNA	45.725083	-64.670889	
NC1	USA	eNA	34.214	-77.789	
NS1	Canada	eNA	49.6915	-63.137444	
SC1	USA	eNA	32.7563	-79.842	
SC2	USA	eNA	33.574	-79.0005	
TX1	USA	eNA	28.952669	-95.282137	outgroup
VA1	USA	eNA	36.853	-75.975	
BUL3	Bulgaria	EU	42.581944	27.632222	not grown, sequenced
ESP1	Spain	EU	41.057069	1.032786	
EST(TLL)	Estonia	EU	59.491527	24.828227	
FIN1	Finland	EU	59.8241	22.9331	
FRA5	France	EU	49.286461	-0.215444	not grown, sequenced
FRA6	France	EU	43.5285652	3.9357383	
GRE13	Greece	EU	39.510766	20.222195	
GRE7	Greece	EU	40.3073	22.619	
ICE1	Iceland	EU	65	-18	
ITA1	Italy	EU	41.913805	15.691216	

ITA3	Italy	EU	43.835411	10.249181	
POR3	Portugal	EU	41.678	-8.83	
POR4	Portugal	EU	40.62301	-8.751189	
POR5	Portugal	EU	37.123213	-8.600583	
SWE1	Sweden	EU	55.603599	12.968092	
AK1	USA	wNA	59.63806	-151.54257	
BC11	Canada	wNA	48.928808	-125.5392	
BC14	Canada	wNA	48.408931	-123.47902	
BC16	Canada	wNA	53.5798723	-131.92984	
BC17	Canada	wNA	54.0741832	-131.79	
BC2	Canada	wNA	50.101694	-125.18772	
BC3	Canada	wNA	49.944195	-124.79847	
BC4	Canada	wNA	49.261411	-123.2618	
BC5	Canada	wNA	51.655723	-128.14149	
BC6	Canada	wNA	50.480051	-128.09545	
BC9	Canada	wNA	49.46588	-124.73559	
CA10	USA	wNA	37.893806	-122.63643	
CA17	USA	wNA	34.68244	-120.60634	
CA7	USA	wNA	39.303062	-123.79425	
CA9	USA	wNA	37.455171	-122.44463	
KodiakIsland	USA	wNA	57.78588	-152.40621	not grown, sequenced
MEX1	Mexico	wNA	31.7332	-116.6431	
ON1	USA	eNA	44.00426	-77.738124	
OR2	USA	wNA	45.110256	-123.98238	
OR3	USA	wNA	42.615317	-124.39889	
WA2	USA	wNA	47.006428	-124.17237	

Table 3-S2. Measurements of phenology and biovolume taken in relation to aphid outbreak (start 15/7/19- end 24/7/19). We classify before aphid measurements as a date before the 15/7/2019 and after aphid appearance as a date after the 15/7/2019. Total number of possible measurements for each trait is 373.

Trait	**Total number of measurements**	**Number of measurement before aphid appearance**	**Number of measurements after/ during aphid appearance**
Onset bud	360	337	23
Onset flower	363	261	102
Onset seed	361	146	215
Biovolume at onset bud	307	284	23
Biovolume at onset open flower	361	259	102

Table 3-S3. Traits included/excluded for trait summary PCA and the form of transformation if applicable.

Trait	**Analysis**		**Transformation of raw data**
	Traits summary all populations	Trait summary individuals base	
Germination %	included	included	/
Seedling size	included	included	log

Growth rate	included	included	sqr
SLA	included	included	/
Leaf shape PC1	included	included	/
Leaf shape PC2	included	included	/
Leaf shape PC3	included	included	/
Leaf shape PC4	included	included	/
Fruit weight	included	included	log
Fruit shape PC1	included	included	/
Fruit shape PC2	excluded	included	/
Fruit shape PC3	included	included	/
Fruit shape PC4	included	included	/
Onset branching	included	included	log
Onset bud	included	included	log
Onset open flower	excluded	included	log
Onset seed	excluded	included	log
Biovolume bud	excluded	included	log
Biovolume open flower	excluded	excluded	log
Above ground biomass	excluded	excluded	sqr
Below ground biomass	included	included	sqr
Total reproductive count	excluded	excluded	sqr
Pollen viability	included	included	asin
Aphid damage	included	included	/

Table 3-S4. Groups for trait summary. AUS= Australia, eNA= eastern North America, EU= Europe, wNA=western North America. E= *C. edentula (E e= C. edentula* subps*. edentula, C h= C. edentula subps. harperi)*, M= *C. maritima*, H=hybrids.

Group	Population_Species	Number of individuals
AUS_E	NSW10_E	7
	NSW4_E	9
	NSW6_E	10
	NSW9_E	10
	TAS12_E	5
	TAS8_E	9
AUS_H	NSW10_H	2
	NSW8_H	10
	QLD11_H	6
	TAS12_H	4
	VIC1_H	7
	VIC11_H	10
	VIC6_H	8
	VIC9_H	7
AUS_M	QLD11_M	4
	SA2_M	10
	SA4_M	10
	TAS12_E	5
	VIC6_M	2
	VIC9_M	3
eNA_E_e	ON1_E_e	5

	MA1_E_e	5
	MD1_E_e	5
	ME2_E_e	5
	MI1_E_e	5
	NB2_E_e	5
	NS1_E_e	5
	VA1_E_e	5
eNA_E_h	FL3_E_h	5
	GA1_E_h	5
EU_M	ESP1_M	5
	EST(TLL)_M	5
	FIN1_M	5
	FRA6_M	5
	GRE13_M	5
	ICE1_M	5
	ITA3_M	5
	POR3_M	5
	SWE1_M	3
wNA_E	AK1_E	5
	BC11_E	3
	BC16_E	3
	BC17_E	10
	BC4_E	10
	BC5_E	9
	BC9_E	10
	WA2_E	6
	OR2_E	3
wNA_H	BC11_H	6
	BC14_H	10
	BC16_H	5
	CA17_H	2
	WA2_H	4
	OR2_H	2
	OR3_H	2
wNA_M	BC16_M	2
	CA10_M	10
	CA17_M	7
	CA7_M	8
	MEX1_M	9
	OR2_M	5
	OR3_M	8

Table 3-S5. Groups for latitudinal clines. AUS= Australia, eNA= eastern North America, EU= Europe, wNA=western North America. E= *C. edentula*, HM= *C. maritima phenotypes*.

Group	Population_Range_Species	Number of individuals
AUS_E	NSW4_AUS_E	9
	NSW6_AUS_E	10
	NSW9_AUS_E	10
	NSW10_AUS_E	7

	TAS8_AUS_E	9
	TAS12_AUS_E	5
AUS_HM	QLD11_AUS_M	10
	NSW8_AUS_M	10
	NSW10_AUS_M	2
	SA2_AUS_M	10
	SA4_AUS_M	10
	VIC11_AUS_M	10
	VIC6_AUS_M	10
	VIC9_AUS_M	10
	VIC1_AUS_M	7
	TAS12_AUS_M	4
E_e	VA1_E_e	5
	MD1_E_e	5
	MA1_E_e	5
	MI1_E_e	5
	ON1_E_e	5
	ME2_E_e	5
	NB2_E_e	5
	NS1_E_e	5
EU_M	POR3_M_I	5
	GRE13_M_m	5
	ESP1_M_m	5
	FRA6_M_m	5
	ITA3_M_m	5
wNA_E	OR2_wNA_E	3
	WA2_wNA_E	6
	BC11_wNA_E	3
	BC4_wNA_E	10
	BC9_wNA_E	10
	BC5_wNA_E	9
	BC16_wNA_E	3
	BC17_wNA_E	10
	AK1_wNA_E	5
wNA_HM	MEX1_wNA_M	9
	CA17_wNA_M	9
	CA10_wNA_M	10
	CA7_wNA_M	9
	OR3_wNA_M	10
	OR2_wNA_M	7
	WA2_wNA_M	4
	BC14_wNA_M	10
	BC11_wNA_M	7
	BC16_wNA_M	7

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Table 3-S6. Mean and standard error for species range groups for Tajima's D, Nucleotide diversity pi and inbreeding coefficient F_{IS}. eNA= eastern North America, AUS= Australia, wNA= western North America, EU= Europe. E= *C. edentula*, M= *C. maritima*, H= hybrids. Values correspond to Figure 3-4.

	eNA_E	**AUS_E**	**wNA_E**	**EU_M**	**AUS_M**	**wNA_M**	**AUS_H**	**wNA_H**
Tajima's D	0.73	0.78	0.59	0.47	0.48	0.75	0.70	0.73
Tajima's D standard error	0.0064	0.0074	0.0076	0.0027	0.0030	0.0032	0.0032	0.0032
π	0.00032	0.00010	0.00026	0.006	0.0072	0.0071	0.0068	0.0072
π standard error	0.0000036	0.00000022	0.0000031	0.000029	0.000031	0.000030	0.000030	0.000029
Average number of individuals per population	5	8	8	5	10	8	8	7
H_O	0.0091	0.011	0.010	0.20	0.26	0.24	0.23	0.23
H_e	0.011	0.009	0.019	0.18	0.26	0.22	0.23	0.22
F_{IS}	0.083	-0.10	0.33	-0.12	0.0020	-0.061	-0.015	-0.061
F_{IS} standard error	0.19	0.036	0.12	0.040	0.031	0.022	0.051	0.022

Table 3-S7. Population statistics on the downsampled dataset for eastern North American populations for H_o, H_e, F_{IS} and the genome wide dataset for Tajima's D and nucleotide diversity (**π**).

Population	**MA1_E**	**ON1_E**	**VA1_E**	**MI1_E**	**GA1_E**	**FL3_E**	**NB2_E**	**ME2_E**	**NS1_E**	**MD1_E**
size	5	5	5	5	5	5	6	5	4	5
H_o	0.006	0.009	0.009	0.008	0.014	0.016	0.007	0.008	0.006	0.008
H_e	0.007	0.007	0.014	0.008	0.011	0.017	0.013	0.008	0.007	0.013
F_{IS}	0.13	-0.20	0.28	0.02	-0.18	0.038	0.36	-0.017	0.101	0.29
Tajima's D	0.93	0.67	0.83	0.96	0.76	1.05	0.50	0.48	0.40	0.67
Tajima's D standard error	0.0058	0.0083	0.0063	0.0063	0.0067	0.0058	0.0057	0.0050	0.0083	0.0061
π	0.00023	0.00015	0.00041	0.00019	0.00050	0.00072	0.00030	0.00020	0.00022	0.00034
π standard error	0.0000029	0.0000024	0.0000038	0.0000027	0.0000052	0.0000078	0.0000028	0.0000026	0.0000023	0.0000034

Table 3-S8. Population statistics on the downsampled dataset for European populations for H_o, H_e, F_{IS} and the genome wide dataset for Tajima's D and nucleotide diversity (**π**).

Population	**ICE1_M**	**EST(TLL)_M**	**ITA3_M**	**ESP1_M**	**FRA6_M**	**FIN1_M**	**FRA5_M**	**POR3_M**	**GRE13_M**
size	5	5	5	5	5	5	5	5	5
H_o	0.11	0.13	0.28	0.25	0.27	0.13	0.18	0.18	0.24
H_e	0.098	0.11	0.25	0.23	0.24	0.11	0.15	0.17	0.21
F_{IS}	-0.15	-0.17	-0.13	-0.077	-0.10	-0.17	-0.15	-0.059	-0.11
Tajima's D	0.64	0.72	0.22	0.30	0.23	0.81	0.53	0.43	0.34
Tajima's D standard error	0.0033	0.0030	0.0021	0.0022	0.0022	0.0031	0.0030	0.0026	0.0023
π	0.0047	0.0048	0.0079	0.0075	0.0080	0.0049	0.0063	0.0063	0.0078
π standard error	0.000023	0.000024	0.000034	0.000032	0.000033	0.000024	0.000028	0.000028	0.000033

Table 3-S9. Population statistics on the downsampled dataset for Australian *C. edentula* and *C. maritima* populations for H_o, H_e, F_{IS} and the genome wide dataset for Tajima's D and nucleotide diversity (**π**).

	AUS_E						**AUS_M**	
Population	**TAS8_E**	**TAS12_E**	**NSW4_E**	**NSW6_E**	**NSW9_E**	**NSW10_E**	**SA2_M**	**SA4_M**
size	9	5	9	10	10	7	10	10
H_o	0.008	0.01	0.01	0.008	0.015	0.012	0.27	0.24
H_e	0.007	0.008	0.008	0.007	0.014	0.01	0.27	0.25
F_{IS}	-0.087	-0.17	-0.091	-0.087	-0.069	-0.11	-0.02	0.024
Tajima'sD	0.62	0.70	0.41	0.42	-0.14	0.42	0.38	0.59
Tajima's D standard error	0.0062	0.0064	0.0080	0.0079	0.0084	0.0073	0.0029	0.0030
π	0.00011	0.00008	0.00011	0.00010	0.00011	0.00010	0.0084	0.0077
π standard error	0.0000023	0.0000021	0.0000023	0.0000022	0.0000022	0.0000022	0.000032	0.000030

Table 3-S10. Population statistics on the downsampled dataset for Australian hybrid populations for H_o, H_e, F_{IS} and the genome wide dataset for Tajima's D and nucleotide diversity (**π**).

	AUS_H							
Population	**SA6_H**	**VIC1_H**	**VIC6_H**	**VIC9_H**	**VIC11_H**	**NSW8_H**	**TAS12_H**	**QLD11_H**
size	10	10	8	7	10	10	5	6
H_o	0.26	0.26	0.25	0.25	0.23	0.24	0.17	0.21
H_e	0.25	0.25	0.24	0.25	0.24	0.22	0.20	0.20
F_{IS}	-0.02	-0.033	-0.042	-0.037	0.052	-0.041	0.075	-0.073
Tajima's D	0.52	0.65	0.54	0.49	0.48	0.75	0.57	1.06
Tajima's D standard error	0.0029	0.0030	0.0033	0.0028	0.0037	0.0035	0.0036	0.0028
π	0.0079	0.0082	0.0077	0.0078	0.0080	0.0072	0.0066	0.0059
π standard error	0.000031	0.000031	0.000030	0.000031	0.000030	0.000029	0.000029	0.000026

Table 3-S11. Populations statistic on the downsampled dataset for western North American *C. edentula* populations for H_o, H_e, F_{IS} and the genome wide dataset for Tajima's D and nucleotide diversity (**π**).

	wNA_E						
Population	**AK1_E**	**KodiakIsland_E**	**BC17_E**	**BC5_E**	**BC9_E**	**WA2_E**	**BC4_E**
size	5	5	10	9	10	6	10
H_o	0.006	0.006	0.007	0.012	0.015	0.013	0.009
H_e	0.009	0.009	0.023	0.032	0.024	0.021	0.016
F_{IS}	0.22	0.28	0.55	0.42	0.27	0.26	0.30
Tajima's D	1.025	0.69	0.51	0.73	-0.063	0.51	0.71
Tajima's D standard error	0.0057	0.0048	0.0089	0.0090	0.0085	0.0077	0.0087
π	0.00012	0.00013	0.00034	0.00043	0.00033	0.00024	0.00025
π standard error	0.0000024	0.0000023	0.0000037	0.0000037	0.0000032	0.0000030	0.0000031

Table 3-S12. Population statistic on the downsampled dataset for western North American *C. maritima* and hybrid populations for H_o, H_e, F_{IS} and the genome wide dataset for Tajima's D and nucleotide diversity (**π**).

	wNA_H			wNA_M					
Population	**BC11_H**	**BC14_H**	**BC16_H**	**CA7_M**	**OR2_M**	**CA17_M**	**CA10_M**	**OR3_M**	**MEX1_M**
size	6	10	5	8	5	7	10	8	9
H_o	0.24	0.23	0.24	0.24	0.24	0.24	0.26	0.24	0.23
H_e	0.21	0.23	0.22	0.218	0.22	0.23	0.24	0.23	0.21
F_{IS}	-0.099	-0.003	-0.081	-0.076	-0.092	-0.052	-0.067	-0.03	-0.051
Tajima's D	0.73	0.86	0.60	0.85	0.62	0.76	0.78	0.77	0.83
Tajima's D standard error	0.0031	0.0035	0.0030	0.0033	0.0028	0.0032	0.0032	0.0029	0.0038
π	0.0068	0.0077	0.0070	0.0069	0.0069	0.0072	0.0076	0.0069	0.0068
π standard error	0.000029	0.000029	0.000029	0.000029	0.000030	0.000030	0.000031	0.000029	0.000029

Table 3-S13. Hotelling's T2 test on groups of PCA of traits (Figure 3-S8), p-values are Bonferroni corrected.

Range	**Group1**	**Group2**	**Hotelling's *t*-squared statistic (t^2)**	**DF**	**p-value**
all ranges	*C. edentula*	*C. maritima*	104.61	2,43	1.30e-11
Subspecies comparisons					
eNA	*C. edentula* subsp. *edentula*	*C. edentula* subsp. *harperi*	24.49	2,7	0.0074
EU	*C. maritima* subsp. *maritima*	*C. maritima* subsp. *baltica*	28.94	2,4	0.065
EU	*C. maritima* subsp. *maritima*	*C. maritima* subsp. *integrifolia*	4.82	2,2	1
EU	*C. maritima* subsp. *baltica*	*C. maritima* subsp. *integrifolia*	41.41	2,1	0.64
Range comparisons *C. edentula*					
eNA, AUS	*C. edentula* subsp. *edentula*	Australian *C. edentula*	7.15	2,11	0.23
eNA, wNA	*C. edentula* subsp. *edentula*	western North American *C. edentula*	2.47	2,14	1
AUS, wNA	Australian *C. edentula*	Western North American *C. edentula*	2.08	2,12	1
Range comparisons *C. maritima*					
EU, AUS	*C. maritima* subsp. *maritima* and *integrifolia*	Australian *C. maritima*	3.70	2,7	0.79
EU, wNA	*C. maritima* subsp. *integrifolia* and *integrifolia*	Western North American *C. maritima*	10.68	2,9	0.11
AUS, wNA	Australian *C. maritima*	Western North American *C. maritima*	0.18	2,9	1

Table 3-S14. The results of linear (or generalized linear) mixed models for traits of native source (*C. edentula* subsp. *edentula*, *C. maritima* subsp. *maritima* and subsp. *integrifolia*) and introduced (western North America and Australia) populations of *Cakile maritima* and *Cakile edentula* measured in a common garden. The native range for *C. maritima* is Europe and *C. edentula* is eastern North America (*C. maritima* subsp. *maritima* and subsp. *integrifolia, C. edentula* subsp. *edentula)*. Each trait (response) was modelled as a function of species, range, and their interaction. Population was included as a random effect in the model. Type III tests and Kenward-Rogers degrees of freedom were used for the linear models. F-values (linear mixed models) or chi-squared values (generalized linear mixed models) with degrees of freedom as subscript and symbols specifying significance of effect are reported for the continuous traits. Trait descriptions are given in Table 3-1. Significant pairwise contrasts are also reported (FDR corrected) (M= *Cakile maritima*, E= *Cakile edentula*).

Trait	Model R^2	Species	Range	Species:Range	Species contrasts	Range contrasts
Days to flower	0.48	$80.46_{1,45.84}$***	$6.78_{2,43.65}$**	$4.70_{2,43.65}$*	E<M (AU, wNA)	Native < (wNA, AU) (M)
Days to seed set	0.57	$180.62_{1,45.17}$***	$6.51_{2,43.01}$**	$1.60_{2,43.06}$	E<M	AU > (native, wNA)
Days to branching	0.10	$7.89_{1,46.91}$**	$1.20_{2,43.56}$	$2.94_{2,43.56}$#	E>M	-
Seedling length	0.43	$32.89_{1,43.36}$***	$8.60_{2,42.45}$***	$1.89_{2,42.45}$	E<M	AU > wNA
Above ground biomass	0.61	$117.54_{1,43.30}$***	$0.94_{2,42.57}$	$0.48_{2,42.57}$	E<M	-
Below ground biomass	0.61	$94.60_{1,44.12}$***	$0.491_{2,42.84}$	$1.0_{2,42.84}$	E<M	-
Growth rate	0.25	$14.77_{1,44.64}$*	$1.94_{2,43.32}$	$3.2_{2,43.32}$(p=0.05)	M>E (native)	
Biovolume at flowering (apex)	0.55	$77.81_{1,43.98}$***	$2.63_{2,42.87}$#	$3.38_{2,42.87}$*	E<M (AU, wNA)	-
SLA	0.11	$0.20_{1,46.98}$	$7.43_{2,43.73}$**	$0.56_{2,43.73}$		AU< wNA
Leaf PC1	0.24	$21.65_{1,44.30}$	$3.43_{2,42.79}$*	$2.87_{2,42.79}$#	E>M	AU> (wNA, AU)
Leaf PC2	0.13	$28.20_{1,48.61}$***	$0.07_{2,43.61}$	$1.29_{2,43.61}$	E>M	-
Leaf PC3	0.36	$72.43_{1,45.43}$***	$0.19_{2,43.07}$	$2.52_{2,43.07}$#	E>M	-
Leaf PC4	0.10	$24.03_{1,48.23}$***	$0.23_{2,43.62}$	$0.03_{2,43.62}$	E<M	-
Fruit PC1	0.52	$37.95_{1,42.02}$***	$7.17_{2,41.22}$**	$1.00_{2,41.22}$	E<M	wNA> (native, AU)
Fruit PC2	0.10	$143.41_{1,42.62}$***	$8.46_{2,40.62}$***	$2.22_{2,40.62}$	E<M	Native > (wNA, AU)

Germination %	5.51_1*	11.86_2**	1.50_2	E<M	Native < (wNA, AU)
Pollen viability	4.44_1*	0.49_2	6.25_2*	M<E (AU#)	-
Aphid damage	11.28_1**	12.83_2**	2.61_2	E>M	Native<(wNA, AU)

ns p>0.1; # p<0.1; * p<0.05, ** p<0.01; *** p<0.001

Table 3- S15. Correlation analysis of latitude and worldclim bioclimatic variables. Cor= correlation, p and Bonferroni corrected p-value are presented.

row	column	cor	p	p_bonf
Latitude	Annual mean temperature	-0.80	3.02E-12	6.34E-10
Latitude	Mean temperature of coldest quarter	-0.79	5.58E-12	1.17E-09
Latitude	Min temperature of coldest month	-0.78	3.90E-11	8.20E-09
Latitude	Isothermality	-0.70	1.69E-08	3.55E-06
Latitude	Longitude	-0.66	1.43E-07	3.00E-05
Latitude	Mean temperature of driest quarter	-0.60	3.26E-06	6.84E-04
Latitude	Mean temperature of warmest quarter	-0.58	1.07E-05	2.25E-03
Latitude	Max. temperature of warmest month	-0.54	5.80E-05	1.22E-02
Latitude	Mean temperature of wettest quarter	-0.52	1.24E-04	2.60E-02
Latitude	Mean diurnal range	-0.48	3.59E-04	7.53E-02
Latitude	Precipitation seasonality	0.0068	9.63E-01	1.00E+00
Latitude	Precipitation of wettest month	0.16	2.75E-01	1.00E+00
Latitude	Precipitation of wettest quarter	0.16	2.60E-01	1.00E+00
Latitude	Precipitation of driest quarter	0.16	2.56E-01	1.00E+00
Latitude	Precipitation of coldest quarter	0.19	1.95E-01	1.00E+00
Latitude	Annual precipitation	0.19	1.92E-01	1.00E+00
Latitude	Precipitation of driest month	0.19	1.81E-01	1.00E+00
Latitude	Precipitation of warmest quarter	0.19	1.78E-01	1.00E+00
Latitude	Temperature annual range	0.31	2.70E-02	1.00E+00
Latitude	Temperature seasonality	0.61	2.88E-06	6.05E-04

Table 3-S16. The results of linear (or generalized linear) mixed models for traits of native source (*C. edentula* subsp. edentula, *C. maritima* subsp. *maritima* and subsp. *integrifolia*) and introduced (western North America and Australia) populations of *Cakile maritima* and *Cakile edentula* measured in a common garden. The native range for *C. maritima* is Europe and *C. edentula* is eastern North America (*C. maritima* subsp. *maritima* and subsp. *integrifolia*, *C. edentula* subsp. *edentula*). Each trait (response) was modeled as a function of species, range, and their interaction as well as latitude and all two and three way interactions with latitude, species and range (non-significant interactions with latitude were removed in a stepwise manner). Population was included as a random effect in the model. Type III tests and Kenward-Rogers degrees of freedom were used for the linear mixed models. F-values (linear mixed models) or chi-squared values (generalized linear mixed models) with degrees of freedom as subscript and symbols specifying significance of effect are reported for the continuous traits. Trait descriptions are given in Table 3-1. Significant pairwise contrasts are also reported (FDR corrected) (M= *Cakile maritima*, E=*Cakile edentula*).

Trait	R2	Species	Range	Species: Range	Latitude	Species: Latitude	Range: Latitude	Species: Range:Latitude	Species contrasts	Range contrasts
Days to flower	0.49	$1.64_{1,38.51}$	$7.11_{2,49.13}$**	$8.28_{2,44.62}$***	$21.71_{1,38.63}$***	$6.32_{1,38.63}$*	-	-	E<M (AU, wNA)	Native < (wNA, AU) (M) AU < wNA (E)
Days to seed set	0.56	$206.23_{1,47.36}$***	$2.05_{2,45.60}$	$1.16_{2,44.54}$	$17.89_{1,35.53}$***	-	-	-	E<M	
Below ground biomass	0.50	$125.55_{1,44.73}$***	$4.35_{2,45.89}$*	$0.65_{2,42.36}$	$34.47_{1,61.06}$***	-	$4.50_{1,46.55}$*	-	E<M	AU<Native (E)
Days to branching	0.11	$7.10_{1,48.94}$*	$1.31_{2,44.67}$	$3.56_{2,44.68}$*	$0.07_{1,48.57}$	$5.25_{1,48.57}$*	$1.53_{1,43.37}$	$3.32_{1,43.38}$*		
Seedling length	0.43	$34.26_{1,37.76}$***	$1.46_{2,44.48}$	$5.08_{2,43.86}$*	$28.33_{1,38.48}$***	-	-	-	E<M (AU, Native)	
Above ground biomass	0.61	$128.32_{1,41.76}$***	$3.76_{2,41.86}$	$0.01_{1,40.96}$	$20.12_{1,46.95}$***	-	$3.58_{1,42.21}$*	-	E<M	
Growth rate	0.26	$7.10_{1,48.94}$*	$1.31_{2,44.67}$	$3.56_{2,44.68}$*	$0.07_{1,48.57}$	$5.25_{1,48.57}$*	$1.53_{1,43.37}$	$3.32_{1,43.38}$*		

Biovolume flowering apex	0.53	$16.79_{1,38.12}$***	$6.22_{2,50.34}$**	$8.09_{2,50.34}$**	$36.54_{1,38.38}$***	$32.40_{1,38.28}$***	-	-	E<M	AU<(native, wNA)
Germination %		6.30_{1}*	12.19_{2}*	5.92_{2}# p=0.051	2.44_{1}	4.83_{1}*	12.39_{2}**			
Pollen viability		6.31_{1}*	6.79_{2}*	1.78_{2}	0.87_{1}	5.04_{1}*	6.62_{2}*			
Aphid damage		6.44_{1}*	18.18_{2}***	8.15_{2}*	7.38_{1}**	7.66_{1}**	-	-	E>M (AU)	AU > Native (E)

2631 ns p>0.1; # p<0.1; * p<0.05, ** p<0.01; *** p<0.001

Table 3-S17. Slope estimates for the relationship between the trait and latitude for groups involved in interactions with latitude. Slopes were estimated using the model in Table 3-S16 and the 95% confidence intervals are provided. Slopes significantly different from zero are bolded (M= *Cakile maritima*, E=*Cakile edentula*).

Trait (significant interaction)	Group	Slope	CI (lower, upper)
Days to flower (Species:Latitude)	E	**-0.017**	**-0.026, -0.001**
	M	-0.005	-0.012, 0.001
Below ground biomass (Range:Latitude)	AUS	**-0.015**	**-0.023, -0.007**
	Native	**-0.038**	**-0.057, -0.020**
	wNA	**-0.009**	**-0.016, -0.002**
Above ground biomass (Range:Latitude)	AUS	-0.035	-0.070, 0.0001
	Native	**-0.13**	**-0.20, -0.057**
	wNA	-0.026	-0.056, 0.005
Biovolume flowering (apex) (Species:Latitude)	E	**-0.12**	**-0.15, -0.087**
	M	-0.004	-0.029, 0.022
Fruit weight (Range:Latitude)	AUS	**-0.029**	**-0.056, -0.0022**
	Native	**-0.090**	**-0.14, -0.037**
	wNA	0.0018	-0.020, 0.04
Days to branching (Species:Range:Latitude)	E, AUS	-0.00073	-0.0052, 0.0038
	E, Native	-0.0013	-0.0092, 0.0066
	E, wNA	**-0.016**	**-0.023, -0.0090**
	M, AUS	0.002	-0.0031,0.0070
	M, Native	0.00089	-0.024,0.025
	M, wNA	-0.00028	-0.0035, 0.0030
Growth rate (Species:Range:Latitude)	E, AU	0.0069	-0.0025, 0.016
	E, Native	**0.015**	**0.00013, 0.029**
	E, wNA	0.010	-0.0025, 0.023
	M, AU	**0.011**	**0.0011, 0.022**
	M, Native	-0.037	-0.081, 0.0073
	M, wNA	-0.00028	-0.0070, 0.0065
Pollen viability (Species:Latitude)	E	-0.035	-0.13, 0.061
	M	**0.11**	**0.0072, 0.21**
Pollen viability (Range:Latitude)	AU	0.0015	-0.09, 0.09
	Native	0.19	-0.0062, 0.39
	wNA	**-0.087**	**-0.17, -0.0046**
Aphid damage (Species:Latitude)	E	**0.31**	**0.10, 0.53**
	M	-0.0028	-0.071, 0.065
Germination % (Species:Latitude)	E	0.014	-0.046, 0.074
	M	**-0.0910**	**-0.16, -0.017**
Germination % (Range:Latitude)	AU	**-0.12**	**-0.19, -0.055**
	Native	-0.041	-0.16, 0.078
	wNA	0.048	-0.014, 0.11

Table 3-S18. Contrast comparing differences between groups (range/species) for minimum and maximum values of latitude for those traits showing a significant two way interaction between range or species and latitude in 3-S16. Significant differences between groups are bolded (M= *Cakile maritima*, E=*Cakile edentula*).

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Trait (interaction)	Value tested	Contrast
Days to flower	min(E)	E-M -0.0870
	min(M)	E-M -0.076
	max(E)	**E-M -0.48**
	max(M)	**E-M -0.40**
Biovolume flowering	min(E)	E-M 0.21
	min(M)	E-M 0.31
	max(E)	**E-M -3.41**
	max(M)	**E-M -2.71**
Above ground biomass	min(AUS)	**AUS-Native -1.67**
		AUS-wNA -0.050
	min(Native)	**AUS-Native -0.79**
		Native-wNA 0.66
	min(wNA)	**Native-wNA 1.18**
		AUS-wNA -0.090
	max(AUS)	AUS-Native -0.21
		AUS-wNA -0.20
	max(Native)	AUS-Native 0.40
		AUS-wNA -0.26
	max(wNA)	**Native-wNA -1.67**
		AUS-wNA -0.35
Below ground biomass	min(AUS)	**AUS-Native -0.47**
		AUS-wNA -0.035
	min(Native)	**AUS-Native -0.25**
		Native-wNA 0.16
	min(wNA)	AUS-wNA -0.060
		Native-wNA 0.31
	max(AUS)	AUS-Native -0.10
		AUS-wNA -0.16
	max(Native)	AUS-Native 0.051
		Native-wNA -0.21
	max(wNA)	AUS-wNA -0.22
		Native-wNA -0.50
Fruit weight	min(AUS)	**AUS-Native -1.16**
		AUS-wNA 0.14
	min(Native)	**AUS-Native -0.59**
		Native-wNA 0.43
	min(wNA)	AUS-wNA 0.0049
		Native-wNA 0.90
	max(AUS)	AUS-Native -0.20
		AUS-wNA -0.55
	max(Native)	AUS-Native 0.20
		Native-wNA -0.75
	max(wNA)	**AUS-wNA -0.86**
		Native-wNA -1.67
Aphid damage	min(E)	E-M 0.067 (p=0.054)
	min(M)	E-M **0.051**
	max(E)	**E-M 1**
	max(M)	**E-M 1**
Pollen viability	min(E)	**E-M 0.94**
	min(M)	**E-M 0.95**
	max(E)	E-M 0.15
	max(M)	E-M 0.30.
Pollen viability	min(AUS)	AUS-Native 0.93
		AUS-wNA 0.12

	min(Native)	AUS-Native 0.69
		Native-wNA 0.12
	min(wNA)	AUS-wNA 0.17
		Native-wNA 0.032
	max(AUS)	AUS-Native 0.40
		AUS-wNA 0.35
	max(Native)	AUS-Native 0.16
		Native-wNA 0.83
	max(wNA)	AUS-wNA 0.70
		Native-wNA 0.99
Germination %	min(E)	**E-M 0.11**
	min(M)	**E-M 0.099**
	max(E)	E-M 0.76
	max(M)	E-M 0.63
Germination%	min(AUS)	AUS-Native 0.80
		AUS-wNA 0.91
	min(Native)	AUS-Native 0.80
		Native-wNA 0.72
	min(wNA)	AUS-wNA 0.68
		Native-wNA 0.53
	max(AUS)	AUS-Native 0.74
		AUS-wNA 0.83
	max(Native)	AUS-Native 0.53
		Native-wNA 0.39
	max(wNA)	**AUS-wNA 0.19**
		Native-wNA 0.26

Table 3-S19. Selfing estimates (sg2) of *C. edentula* and *C. maritima* per population are presented as well as number of individuals used for calculation.

Species	**Range**	**Population**	**Subspecies**	**n**	**sg2**
C. edentula	native	FL3	*C. edentula* subsp. *harperi*	5	0.15
	native	GA1	*C. edentula* subsp. *harperi*	5	0.15
	native	MA1	*C. edentula* subsp. *edentula*	5	0.20
	native	MD1	*C. edentula* subsp. *edentula*	5	0.16
	native	ME2	*C. edentula* subsp. *edentula*	5	0.085
	native	MI1	*C. edentula* subsp. *edentula*	5	0.19
	native	NB2	*C. edentula* subsp. *edentula*	5	0.38
	native	NS1	*C. edentula* subsp. *edentula*	5	0.35
	native	ON1	*C. edentula* subsp. *edentula*	5	0.16
	native	VA1	*C. edentula* subsp. *edentula*	5	0.023
	Australia	NSW10	Australian *C. edentula*	7	0.14
	Australia	NSW4	Australian *C. edentula*	9	0.39
	Australia	NSW6	Australian *C. edentula*	10	0.16
	Australia	NSW9	Australian *C. edentula*	10	0.51
	Australia	TAS12	Australian *C. edentula*	5	0.024
	Australia	TAS8	Australian *C. edentula*	9	0.23
	western North America	AK1	western North American *C. edentula*	5	0.088
	western North America	BC11	western North American *C. edentula*	3	0.23
	western North America	BC16	western North American *C. edentula*	3	0.11
	western North America	BC17	western North American *C. edentula*	10	0.36

	western North America	BC4	Wwestern North American *C. edentula*	10	0.28
	western North America	BC5	western North American *C. edentula*	9	0.33
	western North America	BC9	western North American *C. edentula*	10	0.48
	western North America	KodiakIsland	western North American *C. edentula*	5	0.19
	western North America	WA2	western North American *C. edentula*	6	0.39
	western North America	OR2	western North American *C. edentula*	3	0.16
C. maritima	native	BUL3	*C. maritima* subsp. *euxina*	3	0.0026
	native	ESP1	*C. maritima* subsp. *maritima*	5	0.00042
	native	EST1	*C. maritima* subsp. *baltica*	5	0.0036
	native	FIN1	*C. maritima* subsp. *baltica*	5	0.0021
	native	FRA5	*C. maritima* subsp. *integrifolia*	5	0.0013
	native	FRA6	*C. maritima* subsp. *maritima*	5	0.0008
	native	GRE13	*C. maritima* subsp. *maritima*	5	0.0014
	native	ICE1	*C. maritima* subsp. *islandica*	5	0.0011
	native	ITA3	*C. maritima* subsp. *maritima*	5	0.0013
	native	POR3	*C. maritima* subsp. *integrifolia*	5	0.0007
	native	SWE1	*C. maritima* subsp. *baltica*	3	0.0027
	Australia	NSW10	Australian *C. maritim*a/hybrid	2	0.084
	Australia	NSW8	Australian *C. maritim*a/hybrid	10	0.0037
	Australia	QLD11	Australian *C. maritim*a/hybrid	10	0.011
	Australia	SA6	Australian *C. maritim*a/hybrid	10	0.0071
	Australia	TAS12	Australian *C. maritim*a/hybrid	4	0.014
	Australia	VIC1	Australian *C. maritim*a/hybrid	10	0.0007
	Australia	VIC11	Australian *C. maritim*a/hybrid	10	0.024
	Australia	VIC6	Australian *C. maritim*a/hybrid	10	0.0031
	Australia	VIC9	Australian *C. maritim*a/hybrid	10	0.0028
	Australia	SA2	Australian *C. maritim*a/hybrid	10	0.0038
	Australia	SA4	Australian *C. maritim*a/hybrid	10	0.0029
	western North America	WA2	western North American *C. maritima*/hybrids	4	0.0009
	western North America	BC11	western North American *C. maritima*/hybrids	7	0.0040
	western North America	BC14	western North American *C. maritima*/hybrids	10	0.011
	western North America	BC16	western North American *C. maritima*/hybrids	7	0.0052
	western North America	CA17	western North American *C. maritima*/hybrids	9	0.0024
	western North America	CA7	western North American *C. maritima*/hybrids	9	0.0019
	western North America	OR2	western North American *C. maritima*/hybrids	7	0.00089
	western North America	OR3	western North American *C. maritima*/hybrids	10	0.0037
	western North America	CA10	western North American *C. maritima*/hybrids	10	0.0026
	western North America	MEX1	western North American *C. maritima*/hybrids	9	0.0036

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Table 3-S20. Pairwise Kruskal Wallis test on selfing data of source populations (*C. edentula* subsp. *edentula*, *C. maritima* subsp. *Maritima* and subsp. *integrifolia*) and invasive populations. Presented p-values are Bonferroni corrected. eNA= eastern North America, AUS= Australia, wNA= western North America, E= *C. edentula*, M= *C. maritima*.

	eNA_E	**AUS_E**	**wNA_E**	**EU_M**	**AUS_MH**
AUS_E	1	-	-	-	-
wNA_E	1	1	-	-	-
EU_M	0.0284	0.055	0.0174	-	-
AUS_M	0.0089	0.0265	0.0018	0.0298	-
wNA_M	0.0064	0.0196	0.0026	0.0802	1

Figure 3-S1. *Cakile* in their natural habitat. (a) *C. maritima* population in Tasmania along the beach, (b) *C. maritima* individual in Victoria and (c) *C. edentula* individual in New South Wales.

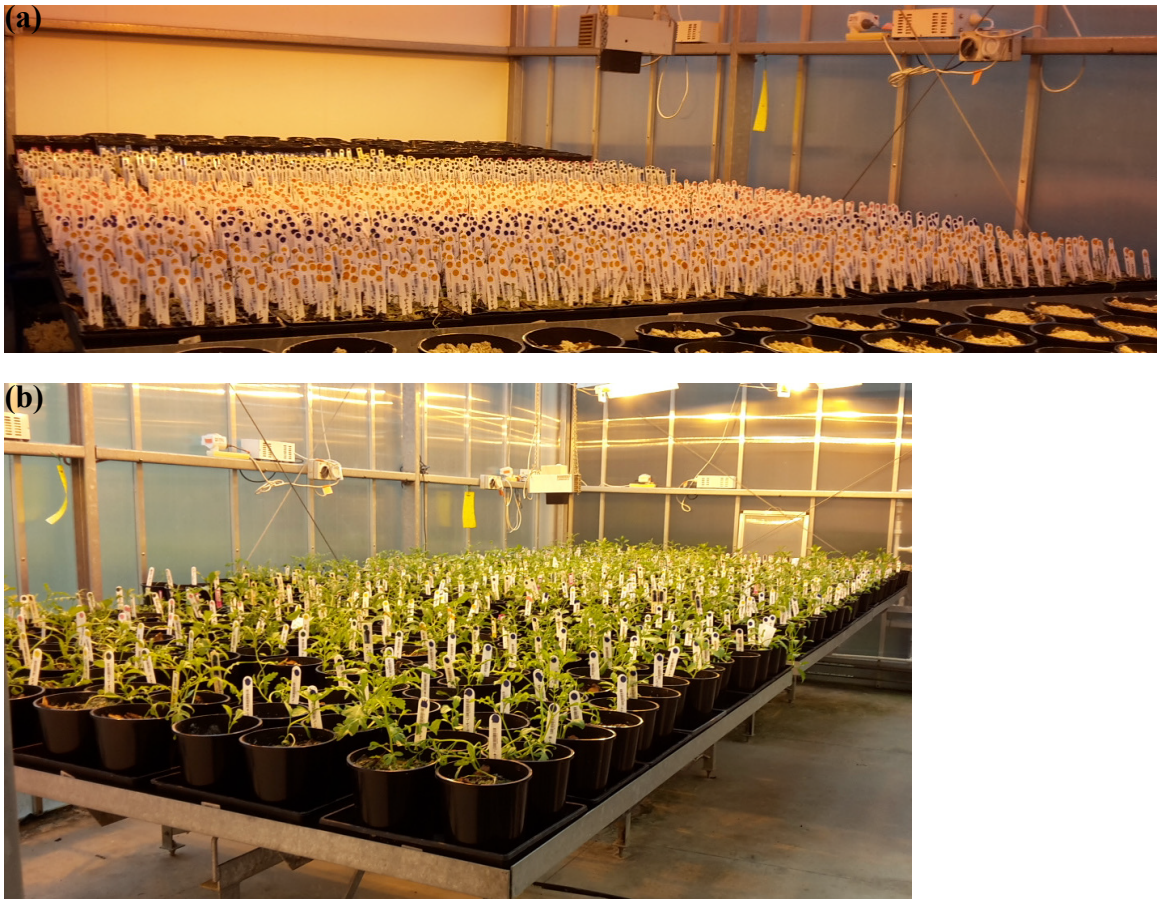


Figure 3-S2. Greenhouse set up. (a) Seedling trays with seedlings, (b) *Cakile* plants in big pots after spread out in greenhouse one.

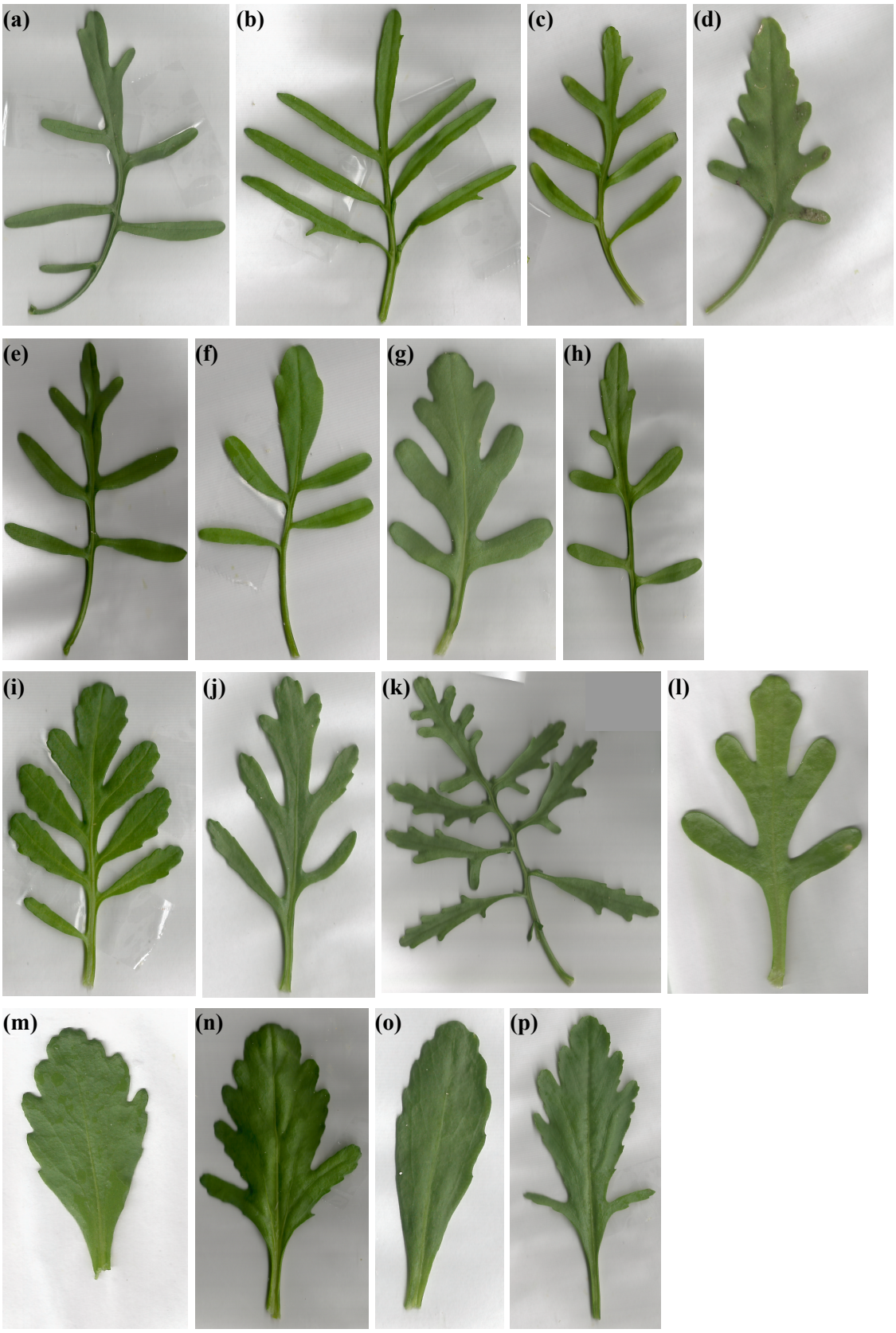


Figure 3-S3. Selected scans of leaves of native individuals. (a)-(l) *C. maritima* leaves, (m)-(p) *C. edentula* leaves.

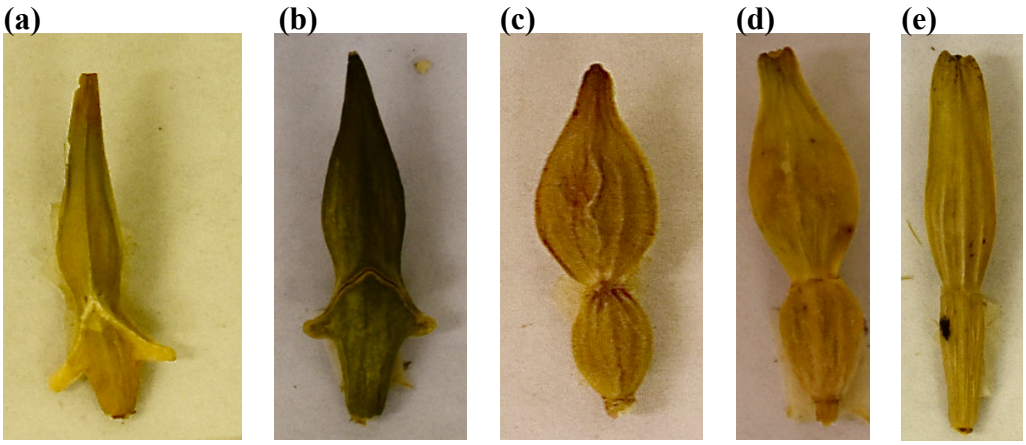


Figure 3-S4. Selected seeds of native individuals. (a) and (b) native *C. maritima* subsp. *maritima*, (c) and (d) native *C. edentula* subsp. *edentula*, (e) native *C. edentula* subsp. *harperi.*

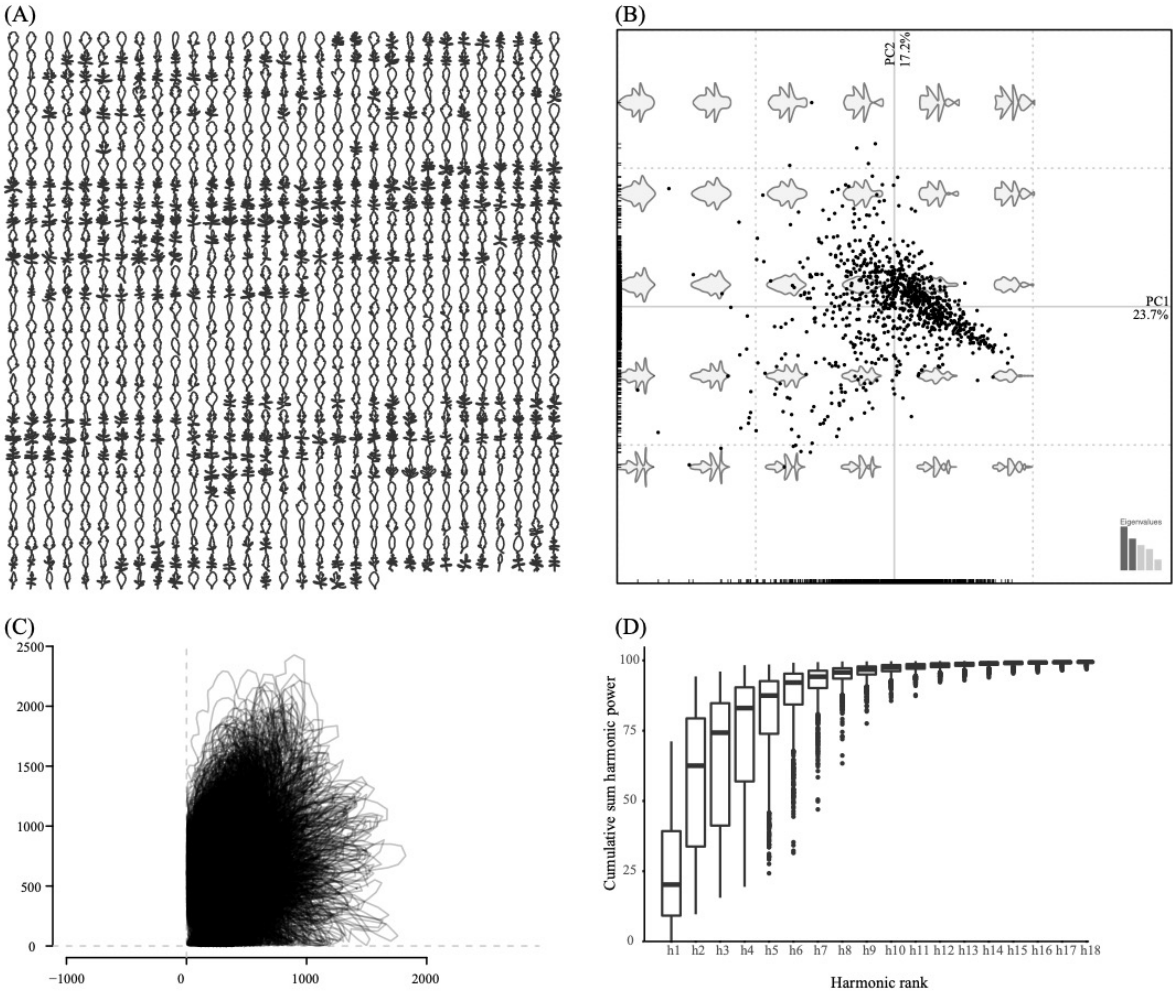


Figure 3-S5. Momocs leaf shape analysis. (A) Outlines of leaves, (B) PCA of outlines, (C) outline stack of all leaves, (D) harmonic power boxplots. Figures produced by Momocs package in R.

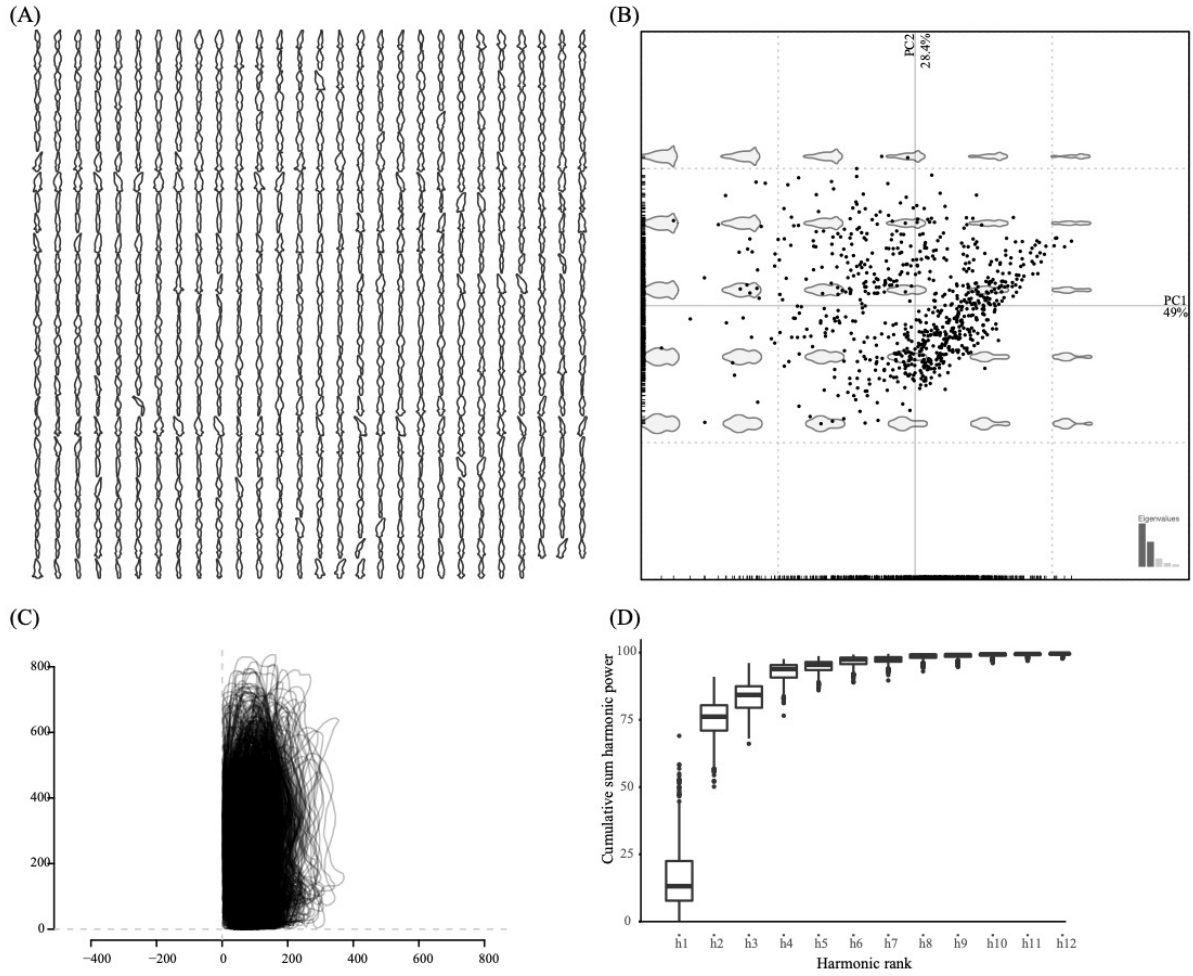


Figure 3-S6. Momocs seed shape analysis. (A) Outlines of seeds, (B) PCA of outlines, (C) outline stack of all seeds, (D) harmonic power boxplots. Figures produced by Momocs package in R.

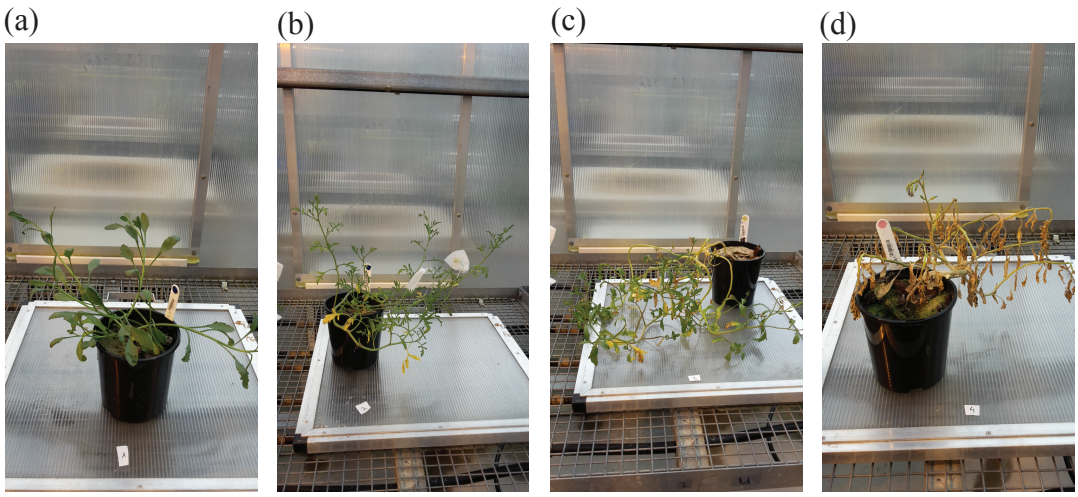


Figure 3-S7. Aphids suck on sap, resulting in a loss of vigour, and in some cases yellowing, stunting or distortion of plant parts. We developed a four-point scale to rate the level of aphid damage following the outbreak. (a) 1- No obvious or minor phenotypic effects. Plants are healthy, green and have no evidence of senescence. Few or no aphids observed. (b) 2- One or a few leaves yellowed, minor damage to buds and flowers and loss of plant vigour. (c) 3- More than a few yellowed leaves, high degree of damage to bud and flowers, substantial loss of plant vigour. (d) 4- Plant death. We also observed aphids on the majority of plants with damage (categories 2-4) with more aphids corresponding to the heavily damaged plants. No signs of senescence were observed on the plants prior to the outbreak. Further, the plants that senesced did so prematurely prior to the development of mature seeds.

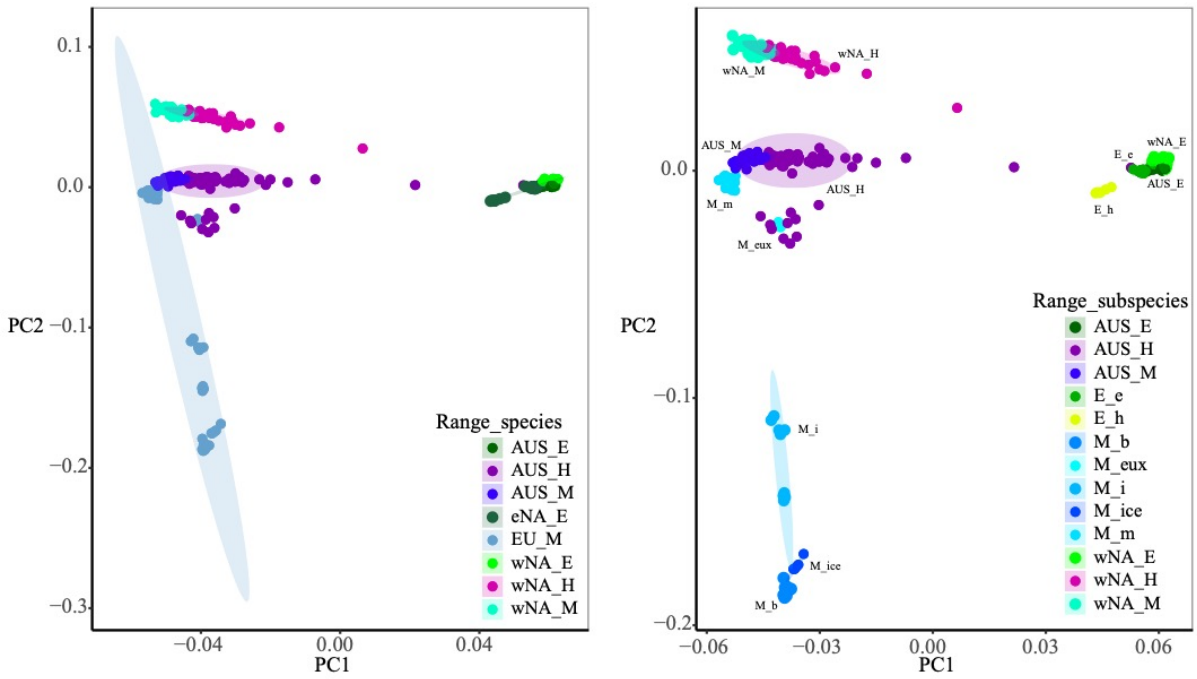


Figure 3-S8. PCA of the complete admixture dataset. Individuals coloured according to the range and species (left) and coloured according to the home range subspecies distribution (right). Ellipses indicate the 95% confidence range of the group (range_species, range_subspecies).

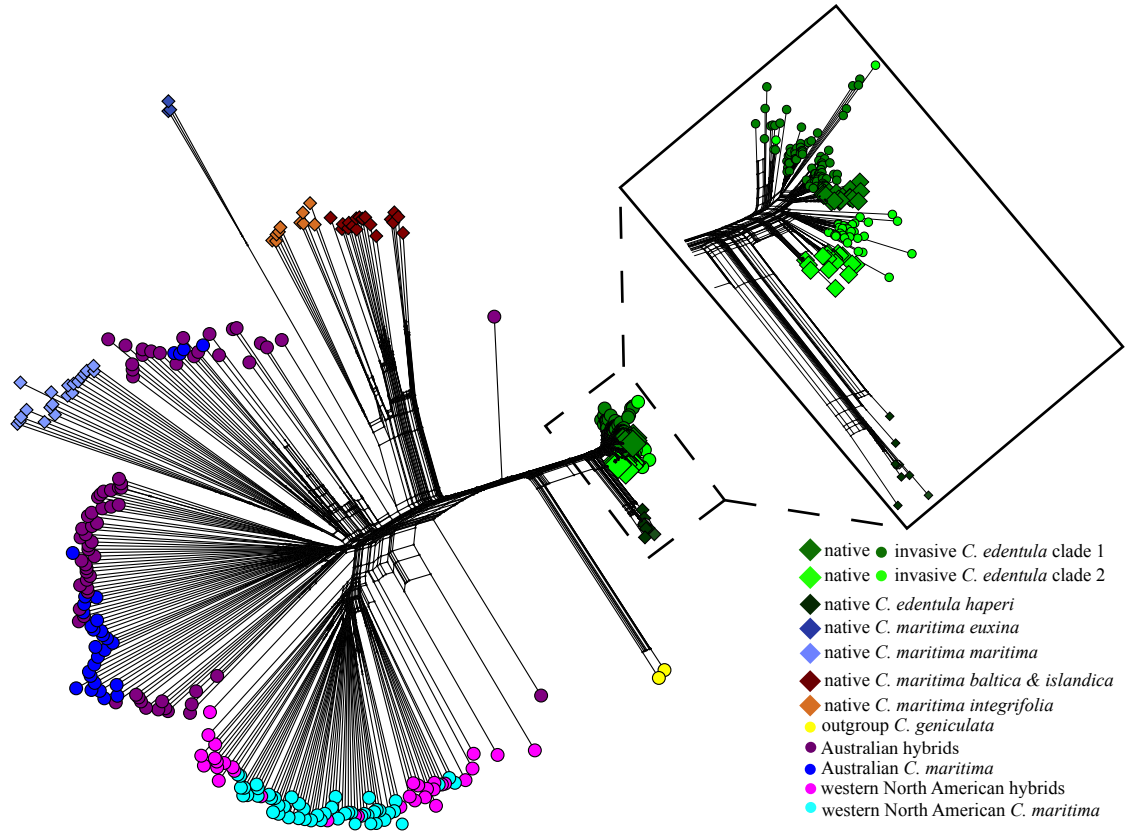


Figure 3-S9. Splitstree analysis. Produced with SplitsTree 5 and coloured according to their subspecies distribution of the home ranges and hybrids according to the hybrid admixture run.

Figure 3-S10. Linkage disequilibrium (LD) decay of groups. AUS= Australia, eNA= eastern North America, EU= Europe, wNA= western North America, E= *C. edentula*, M= *C. maritima*, H= hybrids.

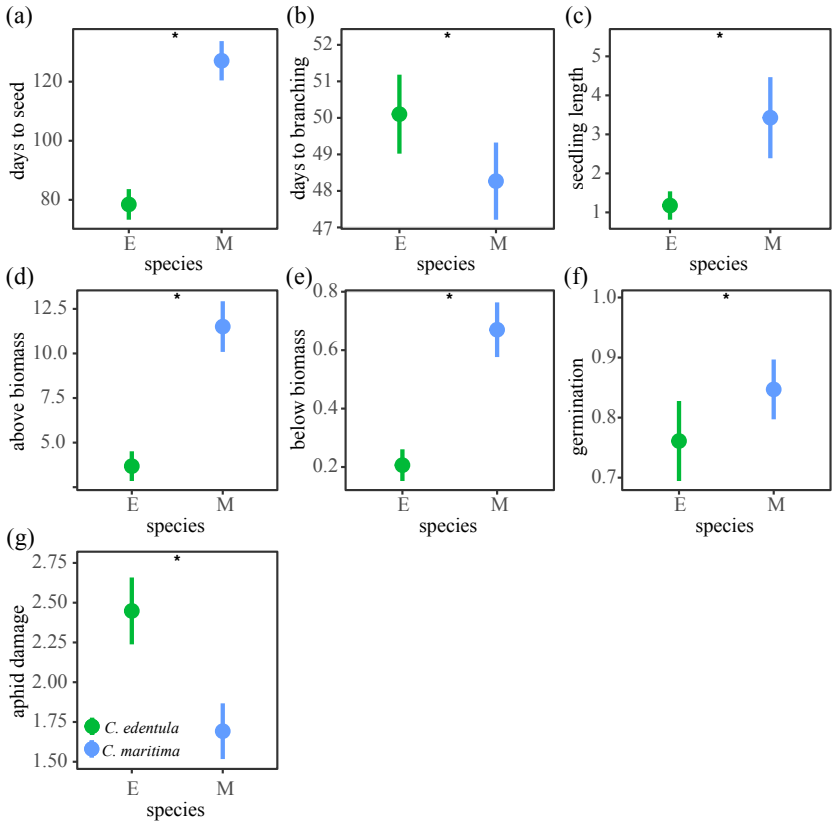


Figure 3-S11. The results of linear (or generalized linear) mixed models for traits of native source (*C. edentula* subsp. *edentula*, *C. maritima* subsp. *maritima* and subsp. *integrifolia*) and introduced (western North America and Australia) populations of *Cakile maritima* and *Cakile edentula* measured in a common garden. The native range for *C. maritima* is Europe and *C. edentula* is eastern North America. Each trait (response) was modelled as a function of species, range, and their interaction. Population was included as a random effect in the model. Trait descriptions are given in Table 3-1 and model results are in Table 3-S14. Significant pairwise contrasts are reported. Lsmeans and 95% confidence intervals are reported.

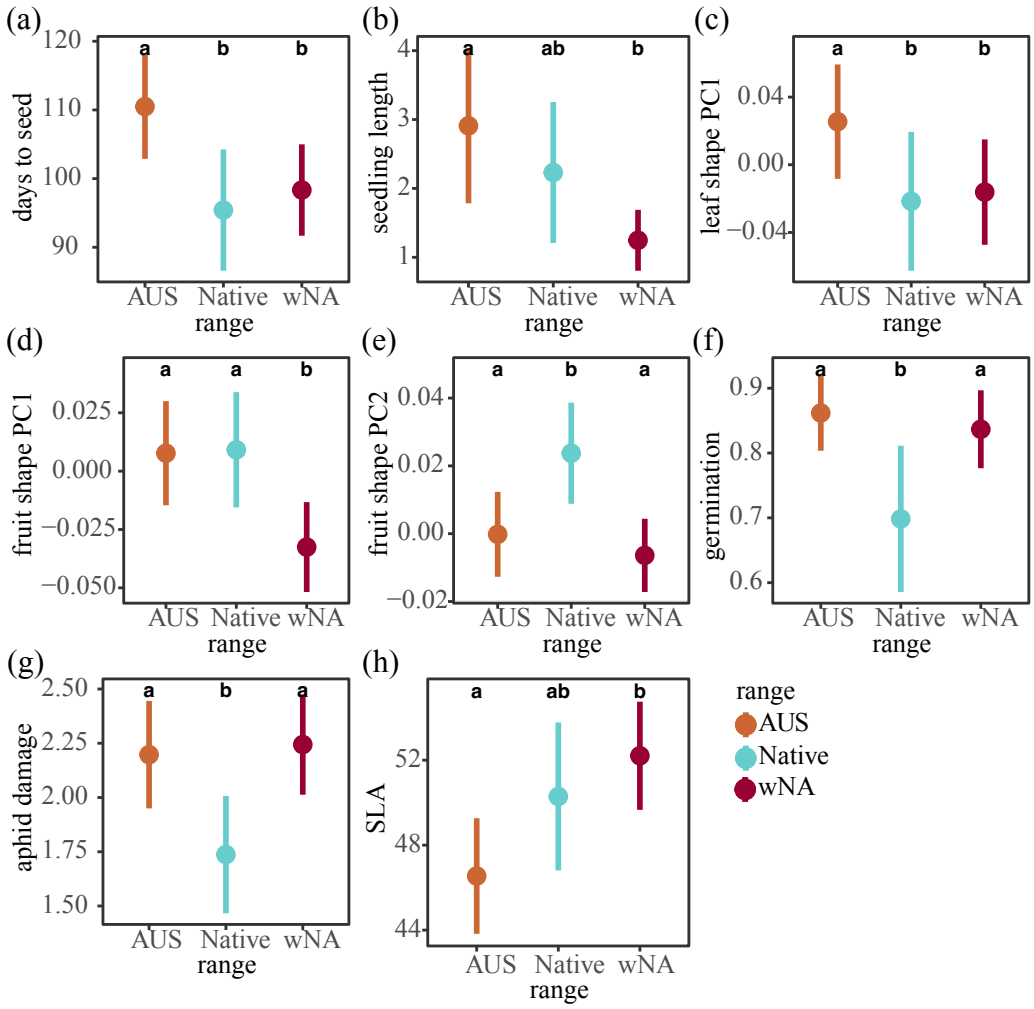


Figure 3-S12. The results of linear (or generalized linear) mixed models for traits of native source (*C. edentula* subsp. *edentula*, *C. maritima* subsp. *maritima* and subsp. *integrifolia*) and introduced (western North America and Australia) populations of *Cakile maritima* and *Cakile edentula* measured in a common garden. The native range for *C. maritima* is Europe and *C. edentula* is eastern North America. Each trait (response) was modelled as a function of species, range, and their interaction. Population was included as a random effect in the model. Trait descriptions are given in Table 3-1 and model results are in Table 3-S14. Significant pairwise contrasts are reported, where different letters denote significant differences between the ranges (groups with shared letters are not significantly different). Lsmeans and 95% confidence intervals are reported.

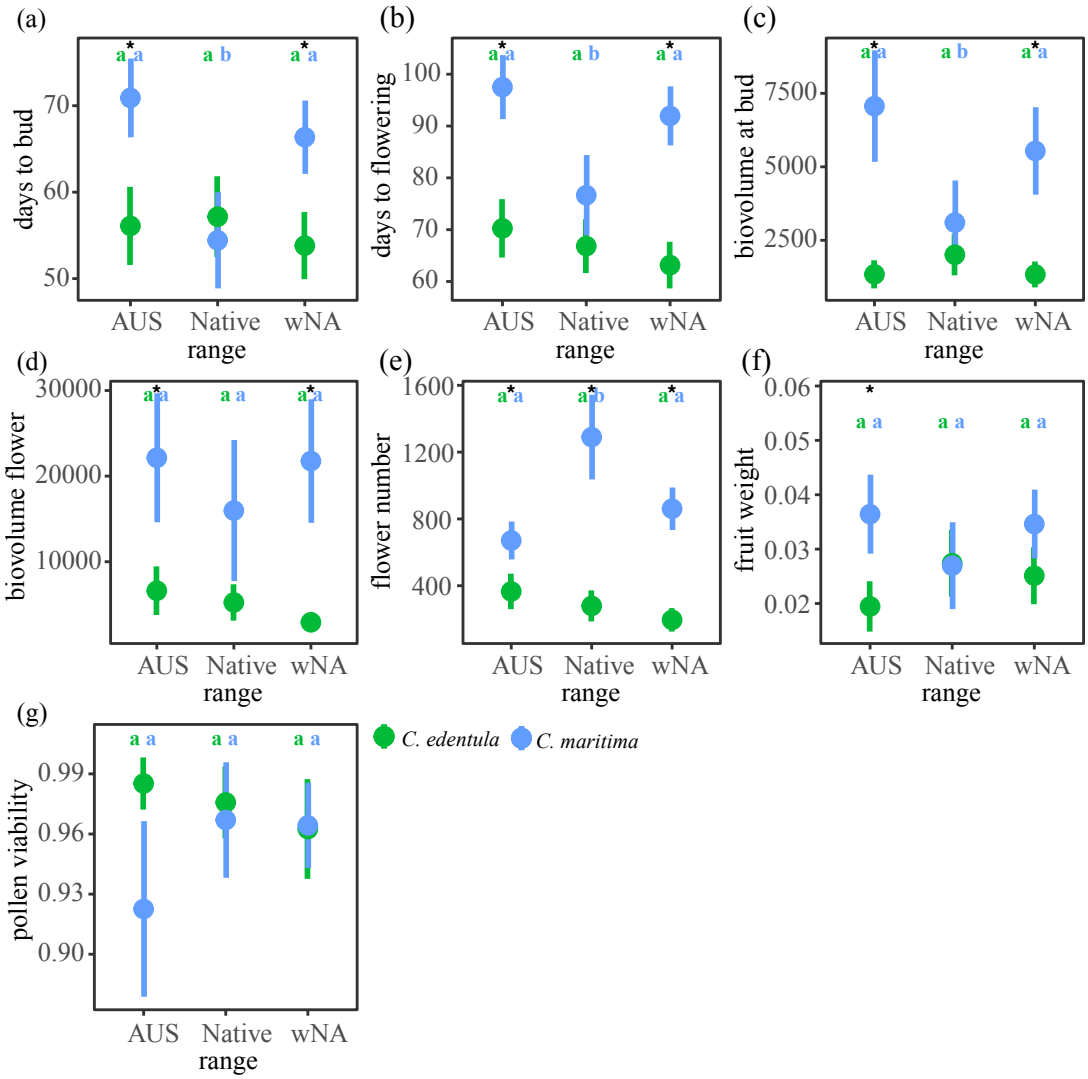
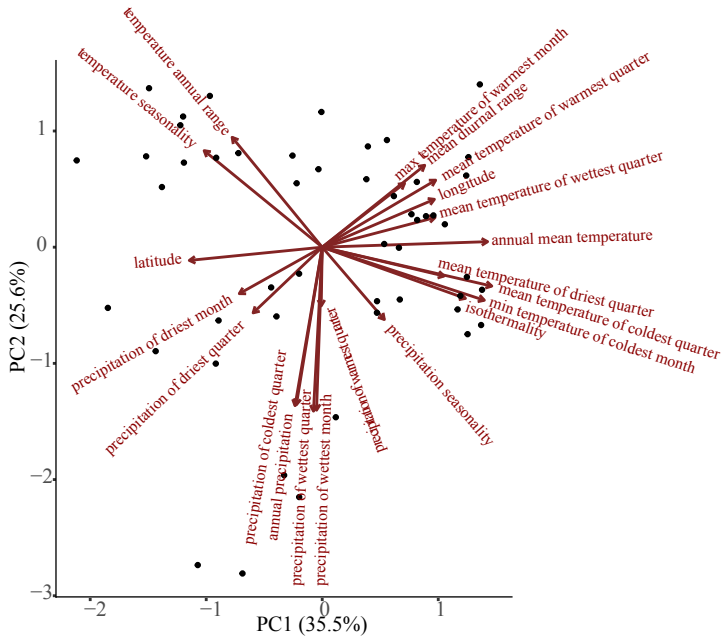


Figure 3-S13. The results of linear (or generalized linear) mixed models for traits of native source (*C. edentula* subsp. *edentula*, *C. maritima* subsp. *maritima* and subsp. *integrifolia*) and introduced (western North America and Australia) populations of *Cakile maritima* and *Cakile edentula* measured in a common garden. The native range for *C. maritima* is Europe and *C. edentula* is eastern North America. Each trait (response) was modelled as a function of species, range, and their interaction. Population was included as a random effect in the model. Trait descriptions are given in Table 3-1 and model results are in Table 3-4. Significant pairwise contrasts are reported, where different letters denote significant differences between the ranges within species (groups with shared letters are not significantly different). Lsmeans and 95% confidence intervals are reported.



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2742 Figure 3-S14. PCA of bioclimatic variables from worldclim.

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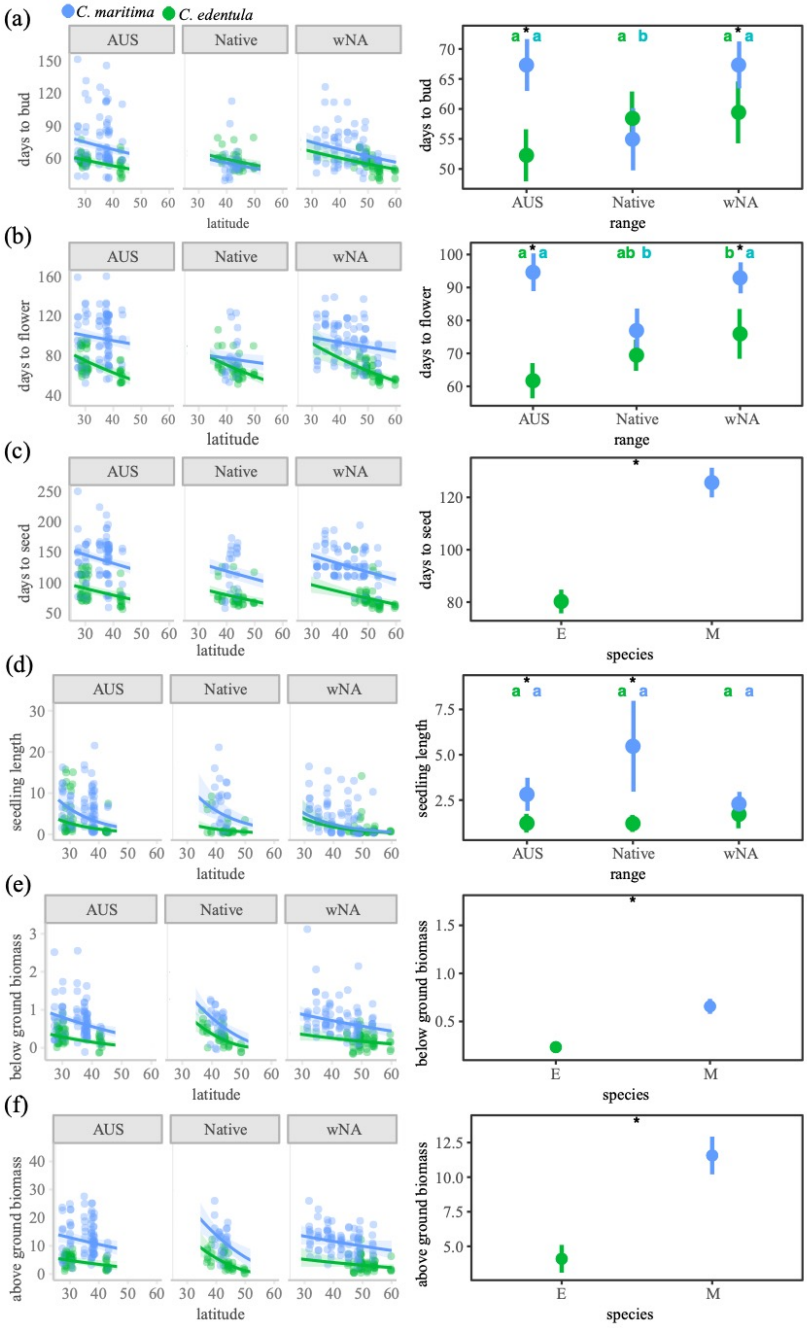
(a)
C. maritima
C. edentula
AUS
Native
wNA
days to bud
latitude
range
(b)
days to flower
(c)
days to seed
species
(d)
seedling length
(e)
below ground biomass
(f)
above ground biomass
E
M

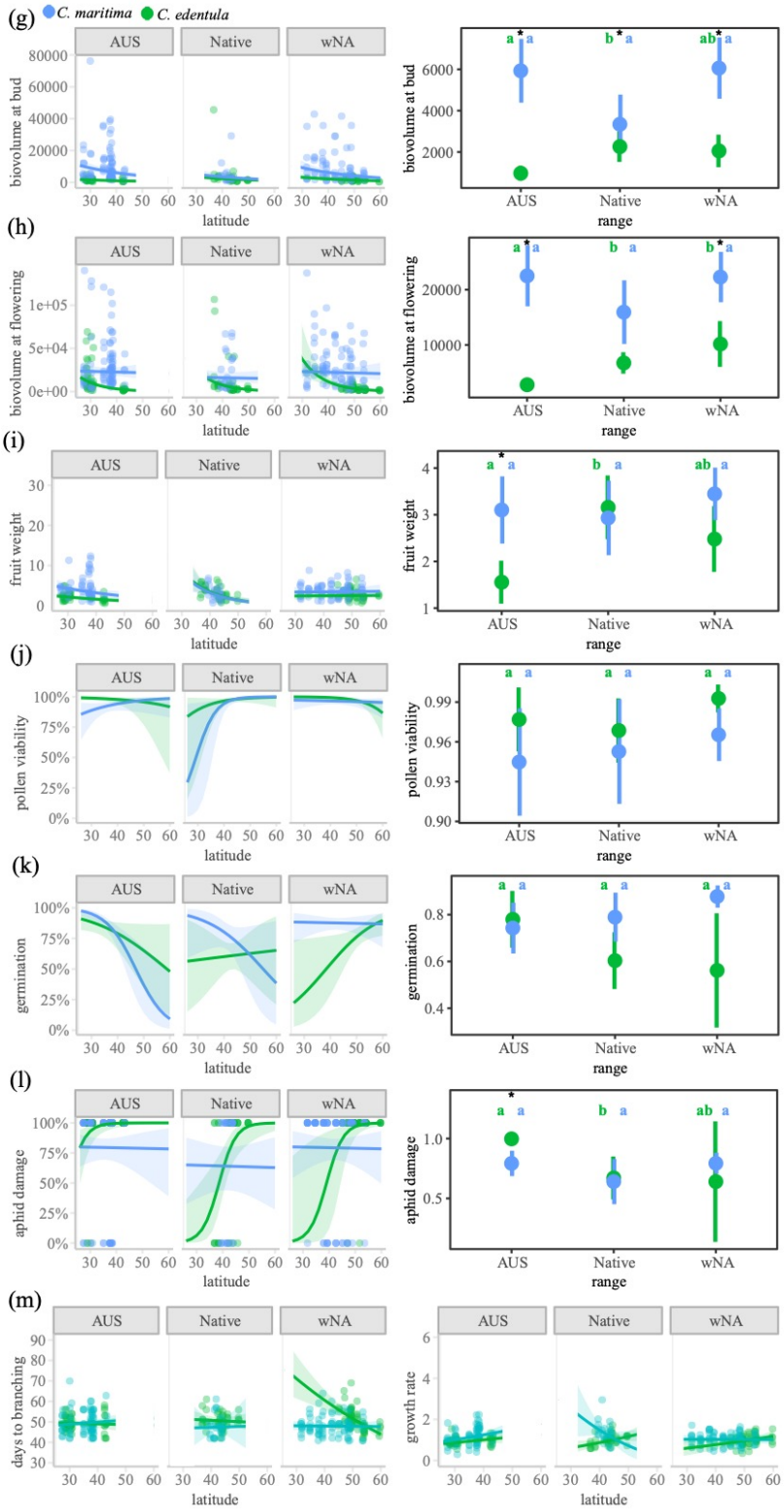

Figure 3-S15. The results of linear (or generalized linear) mixed models for traits of native source (*C. edentula* subsp. *edentula*, *C. maritima* subsp. *maritima* and subsp. *integrifolia*) and introduced (western North America and Australia) populations of *Cakile maritima* and *Cakile edentula* measured in a common garden. The native range for *C. maritima* is Europe and *C. edentula* is eastern North America. Each trait (response) was modelled as a function of species, range, their interaction, as well as latitude and significant interactions with latitude. Population was included as a random effect in the model. Trait descriptions are given in Table 3-1 and model results are in

Table 3-S16. The raw data, predicted values and CI intervals are reported in the left panel for significant relationships with latitude. Lsmeans and 95% confidence intervals for are reported in the right for significant categorical predictor variables. Significant pairwise contrasts are reported, where different letters denote significant differences between the ranges within species (groups with shared letters are not significantly different) and * denotes significant differences between the species.

Chapter 4 – Introgression contributes to parallel patterns of rapid adaptation in co-occurring global invaders

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4.1 Abstract

A global rise in invasion is increasing rates of hybridization among previously allopatric congeners due to shifts in species' distributions. While hybridization is often detrimental it can also lead to beneficial fitness effects, such as adaptive introgression. Invasion bottlenecks can limit the introduction of adaptive genetic variation, but hybridization can potentially replenish this loss thereby aiding range expansion. *Cakile edentula* and *Cakile maritima* are cross-compatible species, native to opposite sides of the Atlantic. Both are found in the same coastal habitat but have expanded their ranges across broad climatic gradients and invaded many of the same regions of the globe. Here, we combine traits measured in a common garden with whole-genome-resequencing of 398 individuals from *C. edentula* and *C. maritima*, and their hybrids to identify genomic signatures of selection across multiple invasions (Australia and western North America) and the native ranges (eastern North America and Europe). We then assessed the rate of convergent adaptation at the genetic level within and between species and identified signatures of adaptive introgression, which may contribute to this convergence. Using comparisons of the native range to the two introduced ranges in each species, we identified regions of the genome under climate mediated selection using associations with environmental and geographic variables and extreme divergence in allele frequency among populations. We found much higher levels of genomic parallelism of climate adaptation candidates among ranges within species (6-34%) compared to between species (3-9%). In the introduced ranges, where past hybridization has been documented, we discovered strong evidence that at least twelve of these candidate regions showed signals of introgression from *C. edentula*. For seven of these twelve regions the frequency of the introgressed haplotype was significantly correlated with latitude in both Australia and western North America (after accounting for population structure), with the *C. edentula* haplotype common at high latitudes. Strikingly, 33% of these windows also showed signatures of climate adaptation within *C. edentula* suggesting that these same regions are under climate mediated selection in this species as well. These twelve windows contained genes putatively involved in response to abiotic factors, including defence, salt tolerance, chilling response and circadian rhythm (based on homology with *Arabidopsis* genes). We also identified genes diverging parallel between the native and introduced ranges within each species. Some of these parallel invasion candidate adaptation genes had putative functions related to defence and flowering, and we also identified strong evidence of introgression being involved in *C. maritima* for seven of these regions. Our data support the hypothesis that adaptive introgression from *C. edentula* to *C. maritima*

contributed to its rapid and successful range expansion, allowing it to overcome limitations in adaptive genetic variation caused by founder effects.

4.2 Introduction

For an introduced species to be successful in a novel environment it can be critical for it to adapt to these new conditions (Colautti & Barrett, 2013). Changes in the abiotic and biotic environments from the source to the introduction are frequent (Colautti et al., 2004; Keane & Crawley, 2002) and can lead to the evolution of traits related to colonization, growth, reproduction and defence (Colautti & Lau, 2015; Felker-Quinn et al., 2013). Such evolutionary changes may even contribute to the invasion success of the species (Bock et al., 2015). For example, in some successful invasions reduced abiotic and biotic stressors have been implicated in the evolution of enhanced growth and reproductive success in the introduced range due to trade-offs between stress tolerance and enhanced performance (e.g., Blossey & Notzold, 1995; Felker-Quinn et al., 2013; Parker et al., 2013; Thébaud & Simberloff, 2001; see references in Bock et al. 2015). During invasion, if resource reallocation in response to reductions in environmental stressors commonly occurs and leads to the evolution of enhanced invasiveness, parallel evolutionary changes across multiple invasions within and between species might be expected (Hodgins et al., 2015). However, evidence of consistent evolutionary responses to invasion across diverse plant species has been limited (Bossdorf et al., 2005; Felker-Quinn et al., 2013; Hodgins et al., 2015).

Many invasive species are found across large geographic areas, both within their native and introduced ranges. This results in substantial environmental heterogeneity across populations, which can lead to local adaptation whereby local genotypes outperform those from elsewhere. Local adaptation can be rapid (< 50 years; Whitney & Gabler, 2008) and occurs frequently in plants (Hereford, 2009), including invaders (Oduor et al., 2016). In annual plants inhabiting broad climatic gradients, phenology and size are two traits that frequently adapt because of differences in season length and the optimal timing of reproduction (Colautti et al., 2010; Li et al., 2014). Similarly, traits related to abiotic stress tolerance, such as cold tolerance (Abbott et al., 2003) or drought response (Colomer-Ventura et al., 2015) can also evolve along temperature and precipitation gradients (e.g., Leiblein-Wild & Tackenberg, 2014). Similar types of environmental change can result in parallel changes in selective pressures and lead to similar phenotypic traits i.e., parallel evolution in closely related taxa (Conte et al., 2012;

Schluter et al., 2004; Stern & Orgogozo, 2009; Xie et al., 2019). When invaders expand across similar climatic gradients in their native and introduced ranges, parallel latitudinal clines in life history and physiological traits in each range have evolved in several instances, despite the recency of the introductions (Hodgins & Rieseberg, 2011; Leiblein-Wild & Tackenberg, 2014; Scalone et al., 2016; van Boheemen et al., 2019). For example, *Ambrosia artemisiifolia* latitudinal clines of phenology and size evolved in two invasive ranges that mirror patterns in the native range (van Boheemen et al., 2017; van Boheemen et al., 2019).

If parallel phenotypic changes evolve during invasion, are they as similar as they appear? Do changes at the phenotypic level also lead to parallel changes at the genetic level? The answer to this question is dependent on constraints and biases that might limit evolutionary changes to certain genes or genomic regions (Conte et al., 2012; Yeaman et al., 2018). Differences in fitness among genotypes can arise when mutations cause correlated effects on other traits that also impact fitness (i.e., pleiotropy). Independent of pleiotropy, architectures with different allele effect sizes and linkage relationships can have different fitness depending on the interaction between migration, selection, and drift. For instance, large effect loci (or clusters of like effect mutations that act as a large effect locus) are predicted to be favoured under divergent selection with migration, so conserved, large-effect loci are more likely to exhibit parallelism under these conditions (Yeaman et al., 2018; Yeaman & Whitlock, 2011). Biases can also drive repeatability in the genetic basis of adaptation (Conte et al., 2012). Repeatability will increase if populations experiencing a similar selective environment are seeded with the same beneficial variants, increasing the chances that these same variants will be selected in parallel (Conte et al., 2012; Yeaman et al., 2018). This could be achieved through adaptive introgression, as has been demonstrated in *Heliconius* butterflies (e.g., Enciso-Romero et al., 2017). However, although parallel evolutionary changes at the trait level have been identified within and between invasive species (e.g., Bhattarai et al., 2017; Keller et al., 2009; van Boheemen et al., 2019), few studies have examined the extent to which the same or different regions of the genome drive parallel evolutionary change using recent invasion as a study system (but see van Boheemen & Hodgins, 2020). This is despite replicate introductions occurring within the same species, facilitating tests of parallel evolution and the hypothesised importance of hybridisation to invasion.

Genetic variation is necessary for a response to selection, yet during invasion founder events and bottlenecks can reduce genetic variation, potentially limiting adaptive evolution (Estoup et

al., 2016; Lee, 2002). However, the loss of genetic variation expected during introduction can be ameliorated by large founding populations or multiple introductions and admixture (Bock et al., 2015; Bossdorf et al., 2005; Dlugosch & Parker, 2008; Ellstrand & Schierenbeck, 2000; Hedrick, 2013). In such instances, adaptive evolutionary change during invasion may be expected to be largely driven by pre-existing genetic variation introduced from the source populations, and lead to substantial parallelism (Morris et al., 2014). Adaptive genetic variation can also be introduced through hybridization. In this case, interspecific gene flow may even contribute to trans-species parallel genetic changes via adaptive introgression (Dasmahapatra et al., 2012). However, although hybridization is frequently cited as a possible driver of invasion (Ellstrand & Schierenbeck, 2000; references in Bock et al., 2015), clear instances of adaptive introgression during invasion are limited (but see Abbott et al., 2003; De La Torre et al., 2014; Owens et al., 2021). Instances of hybridization during invasion have shown that traits can be gained through hybridization such as temperature tolerance in *Rhododendron ponticum* (Milne & Abbott, 2000) or pollinator attractiveness in *Senecio vulgaris* var. *hibernicus* (Abbott et al., 2003). However, to our knowledge none have been implicated as causing parallel adaptation at the genetic level across multiple invasions. This might be expected since the same beneficial variants could be introduced through interspecific gene flow in each instance.

As invasions increase in frequency across the globe, novel species interactions between congeners are increasing opportunities for hybridization. Historically, studying hybridization has been difficult as hybrids can be hard to detect morphologically (Pfennig et al., 2016). Population-level whole-genome datasets are invaluable for identifying hybridization, quantifying its extent, and assessing its evolutionary significance (Chown et al., 2015). Hybridization can be detrimental or beneficial for both or one of the interacting species. The demographic costs of producing unfit hybrids can lead to species extinction (demographic swamping) or a loss of the pure parental types due incomplete reproductive barriers (i.e., genetic swamping) (Hodgins et al., 2018; Todesco et al., 2016). Alternatively, hybridization can facilitate genetic rescue, where fitness is recovered in small inbred populations (Conte et al., 2017; Ellstrand & Schierenbeck, 2000; Hodgins et al., 2018; Peischl et al., 2013), or demographic rescue, where hybridization provides compatible congeners to overcome Allee effects during colonization and establishment (Mesgaran et al., 2016). Evolutionary rescue, through adaptive introgression is another beneficial effect. Invasion offers an important opportunity to study these potential outcomes of hybridization, as visible hybrid zones might often be transient when allopatric species' ranges collide, and therefore rare in native species.

However, invasion creates opportunities for novel species interactions, hybridization and allows us to capture evolution "in action".

Cakile edentula and *C. maritima* are the two invasive species giving rise to hybrids in two isolated continents (Australia and western North America). Native to eastern North America, *C. edentula* is a self-compatible species, whereas *C. maritima*, native to Europe, possesses a self-incompatibility system. Both species are cross-compatible and in regions where they coexist hybrids are formed (Cousens et al., 2013; Rodman, 1974; Chapter 2, 3). Hybrids can be easily produced in greenhouses (Rodman, 1974), with both species acting as pollen donors although crosses are more successful when *C. maritima* is the pollen donor (Li et al., 2019; Mesgaran et al., 2016). On both continents the invasion history follows a similar pattern: 1) the invasion of *C. edentula* followed by an invasion of *C. maritima*; 2) the formation of hybrids; and 3) the apparent replacement of *C. edentula* by *C. maritima* (including many *C. maritima* with *C. edentula* ancestry) across much of the introduced range (Barbour & Rodman, 1970; Cousens et al., 2013; Rodman, 1986; Rosinger et al., 2021). We have previously shown with a genotype-by-sequencing dataset (Rosinger et al., 2021), as well as with a whole-genome-resequencing dataset, that the hybridization rate in Australia is higher than in western North America (Rosinger et al., 2021; Chapters 2 and 3). Furthermore, even though bi-directional backcrossing exists, there is a nuclear asymmetry in the hybrids with a bias towards *C. maritima* (Rosinger et al., 2021; Chapters 2 and 3). Greenhouse and field data (Li et al., 2019; Mesgaran et al., 2016) have shown that hybrids inherit the self-incompatible system from *C. maritima* and appear more similar in several other traits (e.g., flower number and size). These features aid in pollinator attraction and encourage further outcrossing between hybrids and *C. maritima.*

Repeated patterns of invasion, hybridization and replacement make the *C. maritima/edentula* species pair an excellent model to investigate the repeatability of adaptation within and between species, as well as the evolutionary consequences of hybridization during invasion. Our previous analysis of population structure has revealed that the northern regions of the *C. edentula* range are the likely sources of the invasions ("Nova Scotia" cluster and "Great Lake" cluster see Chapters 2 and 3), while the southern portions of the *C. maritima* distribution (Mediterranean and Atlantic coast of Europe) are the likely sources of the invasions in eastern Australia and western North America. Further, the *C. edentula* Australian populations have suffered from a substantial reduction in SNP diversity, likely caused by a bottleneck (Chapter

3); founder events experienced during introduction appear to have limited adaptive genetic variation from within the species. Despite this, using a common garden experiment we have found evidence of significant patterns of trait divergence within and between ranges and species consistent with parallel adaptation (Chapter 3). For instance, both days to bud and biovolume at (first) bud show parallel latitudinal clines within the native and introduced ranges of both species. Interestingly, days to bud shows a species ancestry (Q- value from Admixture) by latitude interaction, providing evidence that the evolution of the latitudinal pattern in *C. maritima* invasion may in fact be influenced by hybridization. However, we have not yet identified likely candidate regions involved in this parallel pattern of trait evolution within and between species or ascertained the role of introgression in driving parallel patterns at the genetic level.

We aimed to:

1) Identify the genomic basis of putatively adapting traits using genome-wide association studies in each species separately.
2) Identify signatures of selection across the genome both within (climate adaptation candidates) and between ranges (invasion adaptation candidates). We predicted that many of these candidate adaptation regions would be enriched for genes involved in flowering time, defence and other biological processes related to biotic and abiotic stress response.
3) Quantify the extent of parallel signatures of adaptation within and between species at the genetic level. In the absence of introgression, shared standing variation and similar genetic backgrounds might be expected to cause higher levels of parallel adaptation within species than between. However, adaptive introgression may elevate parallelism between species, while enhanced false positive rates caused by recent hybridization and range expansion may artificially inflate within-species parallelism.
4) Identify if these parallel adaptation candidate regions within species showed signatures of introgression consistent with parallel adaptive introgression.

Here, we present whole genome resequencing of 398 *Cakile* samples collected from the two invaded ranges (Australia, western North America) and both home ranges (Europe, eastern North America). We used this extensive dataset to map the genetic basis of the putatively adaptive traits described in Chapter 3 and implemented population-genomic tests of selection to assess the regions of the genome likely involved in local adaptation as well as the extent of

parallelism within and between species. We then ascertained whether any of these regions showed clear signatures of introgression in the invasive ranges.

4.3 Methods

4.3.1 Field collection and Experimental set-up

The data used in this Chapter were produced by a field collection followed by a greenhouse experiment. For details see Chapter 3 but in brief: During the years 2017 and 2018 field collections of *Cakile edentula*, *C. maritima* and their hybrids were carried out in both native ranges (eastern North America, Europe) and the two invasive ranges (southern and eastern Australia, western North America). In 2019 a greenhouse experiment was conducted at the Monash Clayton campus (Australia) and leaves of the plants were harvested for genomic analysis. In total, 400 individuals were selected for whole-genome-re-sequencing (54 populations, 16 Australia, 16 western North America, 10 eastern North America, 10 Europe, 2 outgroup populations, phenotypically 214 *C. maritima* individuals, 159 *C. edentula* individuals and 2 *C. geniculata* individuals). Most of the samples (375 individuals) were chosen from the greenhouse experiment, although in some cases we had to rely on field-collected leaf samples (25 individuals).

4.3.2 WGS and SNP data preparation

DNA extraction was performed on dried leaf material using the DNeasy Plant Mini Kit (QiaGEN) and whole-genome-resequencing (WGS) library preparation was carried out following the protocol of Carø et al., (2018). The WGS sequencing was performed using a NovaSeq (Genewiz) on seven lanes. Raw reads were aligned to the *C. edentula* reference using the Burrows wheeler aligner (BWA-MEM) (Li & Durbin, 2009). Indels were re-aligned using GATK (IndelTargetCreator and IndelRealigner) and duplicates were marked with Picard (http://picard.sourceforge.net). The GATK UnifiedGenotyper was used to call variants. Following this, the variants were filtered using hard filter recommendations (McKenna et al., 2010) (for details on SNP filtering see Chapter 3). Following filtering we imputed missing genotypes with Beagle (Browning & Browning, 2007) and filtered indels (see Chapter 3) which produced a vcf file we termed the *base file.*

4.3.3 Sample selection

To identify population structure and hybrids we relied on Admixture (Alexander et al., 2009; see Chapter 3 for details). In brief, population structuring was analysed with an unsupervised

Admixture run and the number of hybrids was determined by a supervised Admixture run, in which we set the home range individuals as reference individuals. *Cakile edentula* individuals from eastern North America, the native range of *C. edentula*, grouped into three clusters (Figure 4-1). In this Chapter however, we used only individuals representing the subspecies *C. edentula* subsp. *edentula* and excluded *C. edentula* subsp. *harperi* as they are genetically and phenotypically distinct. In Europe, the native range of *C. maritima*, several genetic clusters have been identified. Individuals were clustered into one cluster including subsp. *islandica* and subsp. *baltica*, a second cluster of subsp. *integrifolia*, a third cluster of subsp. *maritima* and subsp. *euxina.* In Australia, pure *C. edentula* (50 individuals), pure *C. maritima* (29 individuals) and 71 hybrids have been identified by Admixture. Western North American samples were divided into pure *C. edentula* ancestry (64 individuals), pure *C. maritima* (50 individuals) and further 33 hybrids. Recent hybrids were identified by the program NewHybrids (Anderson & Thompson, 2002) (see Chapter 3 for details) and F1, F2 and recent back-crosses to *C. edentula* were excluded from the analysis in this Chapter. This left us with 229 *C. maritima* (99 with some *C. edentula* ancestry) and 164 *C. edentula* samples across 52 populations. Of these, 209 *C. maritima* and 159 *C. edentula* were also phenotyped.

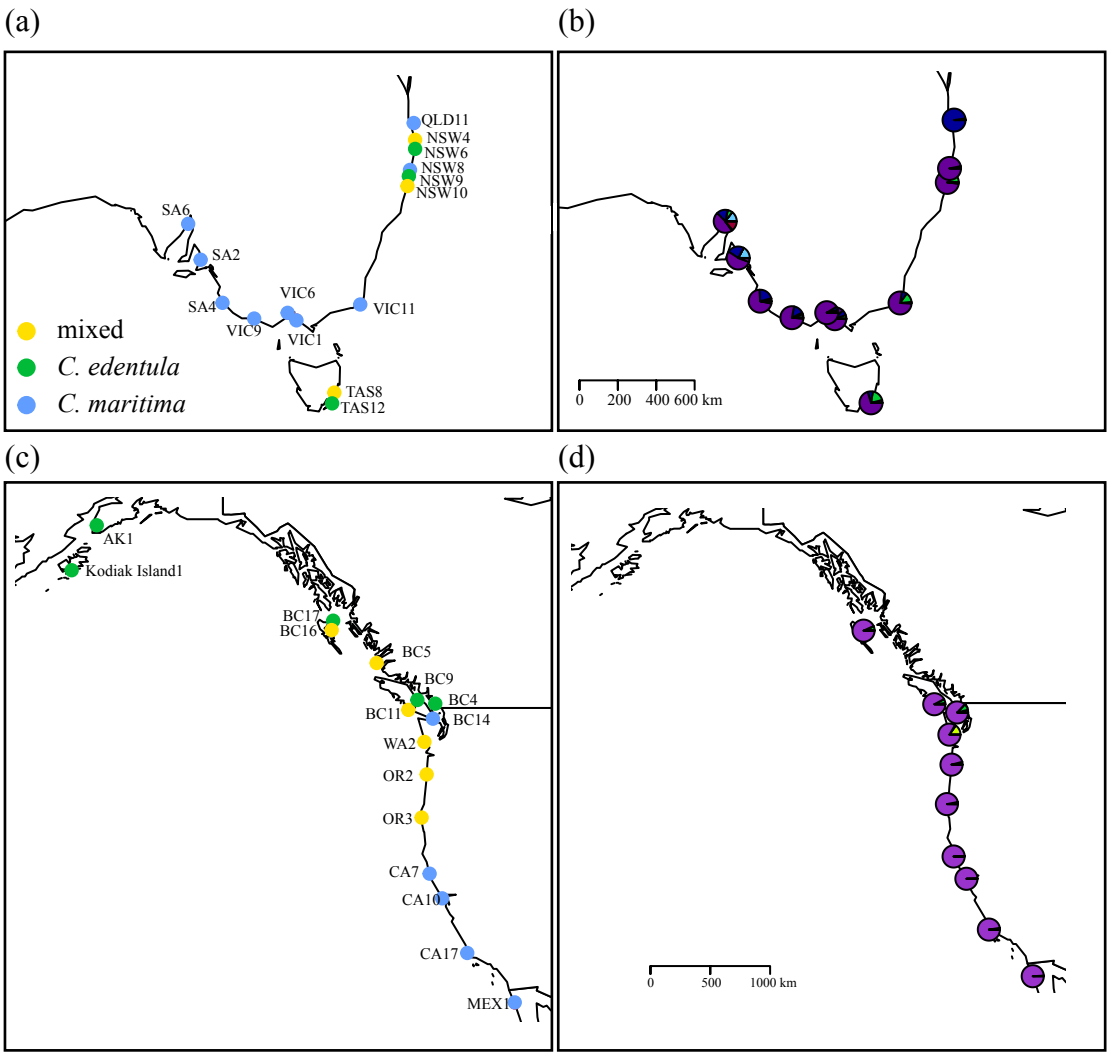

Figure 4- 1. (a) Australian and (c) western North American sampling locations of sequenced populations. Sampling locations are coloured according to species composition of supervised Admixture run (see Chapter 3), green = pure *C. edentula*, blue= *C. maritima* (phenotypic), orange = mixed populations. (b) Australian and (d) western North American population pie charts from Admixture run (K=8, see detail Chapter 3) for *C. maritima* individuals (recent hybrids and *C. edentula* excluded)

4.3.4 Data preparation

We prepared our data for the programs with the following criteria. For EMMAX (Kang et al., 2010) we grouped the 393 individuals identified above (sample selection) according to their species (*Cakile edentula* or *C. maritima*). However, we also excluded *C. maritima* subsp. *baltica* and *islandica* from the native ranges as those subspecies did not contribute to the invasions being studied and were phenotypically and genetically distinct. We then filtered for maf of 0.05 and heterozygosity (0.8) for each phenotype within the group. For BayPass v2.0 (Gautier, 2015) we divided our samples by range and species: Australian *C. edentula* (AUS_E), Australian *C. maritima* (AUS_M), eastern North American *C. edentula* (eNA_E), European *C. maritima* (EU_M, all subsp.), western North American *C. edentula* (wNA_E) and western

North American *C. maritima* (wNA_M). We then filtered our base file for a maf 0.05 per group. For Dsuite, we filtered the base file for maf 0.05. For H12 (Garud et al., 2015) we split the data into the invasive ranges and species and looked for sweeps within each range.

4.3.5 Patterns of introgression

The program Dsuite (Malinsky et al., 2021) was used to investigate the patterns of introgression across the genome and to calculate Patterson's D-statistic to detect introgression between the two parental species *C. edentula* and *C. maritima*. First, the Dsuite Dtrios function was used to estimate the D-statistic. To investigate introgression into Australian or western North American *C. maritima* we used the following tree topology (eNA,(AUS_M,EU),Outgroup) or (eNA,(wNA_M,EU),Outgroup). In contrast, to investigate possible introgression into *C. edentula* we used the tree files (EU,(AUS_E,eNA),Outgroup) and (EU,(wNA_E,eNA),Outgroup). The function (Dtrios) was run on Australian samples and western North American samples separately using the home range individuals (excluding *C. edentula* subsp. *harperi*) as pure parental individuals each time, and *C. geniculata* as the outgroup. To evaluate if a latitudinal pattern of introgression exists, we ran Dsuite separately on four different populations, representing the most northerly and southerly populations of (phenotypically) *C. maritima* in the invasive ranges, with the same parameters as previously.

4.3.6 Genome-wide association studies (GWAS)

For each of the traits measured in Chapter 3, we conducted GWAS in each species (*C. edentula* subsp. *edentula* or *C. maritima* subsp. *maritima* and *integrifolia*/hybrids; hereafter termed *C. edentula* and *C. maritima* respectively) separately. GWAS were performed in EMMAX (Kang et al., 2010) using an identity-by-state kinship matrix (generated in PLINK 1.9; Chang et al., 2015) to account for genetic structure among samples. The kinship matrix was produced by filtering the base file for the individuals in each group and phenotype followed by a LD (window size 50, step size 5, r2 0.5; Chang et al. 2015) pruning step. For each phenotype in each group we ran EMMAX by separating species from the base file (10,971,000 SNPs), filtered for missing phenotypic data for each phenotype and filtered for maf 0.05. As we allowed no missing data and excluded outliers, the amount of individuals and SNPs differed slightly between each phenotype of each group. We used all of the traits measured on genotyped individuals (Appendix III Table 4-S1) for GWAS analysis. P-values were corrected for lambda inflation if necessary (if lambda >1, Yang et al., 2011). GWAS results were

analysed with a weighted-Z analysis (WZA) (Booker et al., 2021) in 50,000 bp windows and the top 5% of windows for each trait were considered outliers.

4.3.7 Signatures of climate mediated selection within each range

We used the X^TX statistic (Bayesian approximation of F_{ST}) in BayPass v2.0 (Gautier, 2015) to scan the genome for signatures of selection. Genetic covariate matrices were estimated using LD-pruned (plink-indep pairwise 50 5 0.5; Chang et al. 2015), excluded genes and thinned to 5000 SNPs vcfs for each range and species, namely eastern North American *C. edentula*, European *C. maritima*, Australian *C. edentula*, Australian *C. maritima*, western North American *C. edentula* and western North American *C. maritima*. The results of BayPass were analysed with WZA (Booker et al., 2021) in 50,000 bp windows and the top 5% of X^TX were considered outliers). We also used BayPass v2.0 (Gautier, 2015) to perform an environment allele association analysis for each of the above groups, using latitude, longitude and 19 bioclimatic variables obtained from worldclim (Fick & Hijmans, 2017). All SNPs were tested for associations with each environmental variable and Manhattan plots were produced in R. We then used WZA to identify outlier windows in the same window size as before (50,000 bp windows and the top 5% of Bayes factors (BF) were considered outliers). We termed overlapping outlier windows of BayPass (X^TX and BF) for each species/range group candidate climate adaptation windows. We compared the outliers of each group to each other and concentrated on the overlaps of *C. maritima* because of low levels of diversity within *C. edentula*. We termed overlapping candidates climate adaptation windows among groups parallel climate adaptation candidate windows.

4.3.8 Signatures of selection during invasion

We ran multiple cross-range BayPass runs to identify outlier windows diverging between each introduced range and the native range for each species, potentially indicative of selection during invasion. Specifically, we compared: 1) Australian and European *C. maritima*; 2) western North American and European *C. maritima*; 3) Australian and eastern North American *C. edentula*; 4) western North American and eastern North American *C. edentula*. As, above, we analysed the results using the WZA statistic, in 50,000 bp windows and designated the top 5% as outliers. We termed these candidate invasion adaptation windows and overlaps within each species parallel candidate invasion adaptation windows. We also used the H12 statistic (Garud et al., 2015) to identify putative selective sweeps in each invasive range for each species. For

each range and species, SNP scans were run with a window size of 101 SNPs, a step size of one SNP, and a distance threshold of 0 between unique haplotypes (-w 100 -j 1 -d 0).

4.3.9 Repeated patterns of adaptation during invasion

To examine the repeatability of adaptation we compared outlier windows within and between species. First, we compared the climate adaptation candidate windows among groups to identify parallel climate adaptation candidate windows (Australian *C. edentula*, Australian *C. maritima*, eastern North America *C. edentula*, European *C. maritima*, western North American *C. edentula*, western North American *C. maritima*). Further, we also compared the candidate invasion adaptation X^TX outliers of the cross-range runs: Australian and eastern North America *C. edentula*, Australian and European *C. maritima*, western North American and eastern North America *C. edentula*, western North American and European *C. maritima*) to each other to identify parallel invasion adaptation candidate windows. After identifying the outliers and overlaps we checked, if the parallel windows were overrepresented with the phyper function in R, to determine if the overlaps are more likely than expected by a random sampling of windows across the genome using the hypergeometric distribution.

4.3.10 Identifying candidates for parallel adaptive introgression

First, we identified windows with parallel signals of climate adaptation in *C. maritima* between the invasive ranges (both BayPass X^TX and BF outlier windows within Australia and western North America). We did this because repeated patterns across multiple climate gradients is stronger evidence that these regions are involved in climate adaptation, as other evolutionary processes related to hybridization and range expansion are likely to produce false positives. We further reduced our parallel climate adaptation candidate set by linking the windows to putatively adapting traits (see Chapter 3) by only examining for introgression those windows that overlapped with *C. maritima* GWAS candidates. Genetic diversity and sample sizes limited a comparative analysis of climate adaptation among ranges within *C. edentula,* so we focused on *C. maritima*.

In addition to the climate adaptation analysis for *C. maritima*, we also compared our cross-range BayPass runs with the goal of identifying if repeated divergence among ranges was potentially caused by the introgression of regions from one species into another. We took the parallel invasion adaptation candidates for each species identified above using X^TX, and further

reduced our parallel candidate set by only including those windows that were also associated with traits in each species. Additionally, the top 1% of the H12 for each invasive range-species group was used to find an overlap of SNPs involved in local adaptation and signatures of selective sweeps.

For each parallel candidate identified above we examined the outlier windows for evidence of introgression by first using a local PCA. Here, we used the Splitstree file (see Chapter 3) split the vcf file into two separate vcfs: 1) Australia and home ranges; and 2) western North American individuals and home range individuals (eastern North American *C. edentula* subsp. *edentula*, European *C. maritima* subsp. *maritima*). The local PCA was implemented (snpgdsPCA function of SNPRelate ; Zheng et al., 2012) in R on each parallel candidate outlier window. We used kmeans clustering in R to cluster regions containing three visually distinct groups representing the two homozygotes with the heterozygotes clustered in between the two homozygous groups. We ensured that *C. edentula* and *C. maritima* from the home ranges were clearly segregated into the putatively homozygous clusters, since no introgression is expected in the native ranges. We determined if any of the introduced range samples clustered with the other species, indicative of samples with introgressed regions that were homozygous. We termed these windows candidates for parallel adaptive introgression. We estimated the frequency of the introgressed region in each population using the above clustering. We plotted haplotype frequency on maps to identify geographical patterns.

As most of our candidates for parallel adaptive introgression showed a striking geographic pattern, we statistically tested the connection of haplotype frequency and latitude using a generalized linear model in R. We also conducted PCA on 10,000 neutral sites, generated excluding genic and inversion regions using PLINK. Following this, we used generalized linear models (glm R) to assess how haplotype frequency of the introgressed regions (binomial response) changed over space. A count of each haplotype at a geographic location was the binomial response variable, range (southern and eastern Australia or western North America), latitude, and the interactions between these main effects were used as predictors. Non-significant interactions were removed. PC1 and PC2 were included as covariates to control for the effects of population structure on haplotype frequency. We tested the significance of the effects in our model using the Anova function (car package R; Fox et al., 2007) with type 3 tests. For interactions between range and latitude the significance and direction of the slopes were tested with the emtrends function using the emmeans R package (Lenth, 2016).

Once we identified evidence of parallel adaptive introgression using the local PCA and kmeans clustering, we further confirmed it using a tree-based approach. The programs RAxML-NG (Kozlov et al., 2019) and Figtree v.1.4.4 (Rambaut & Drummond, 2012) were used to construct maximum likelihood (ML) trees for each candidate window. We focused on windows where individuals classified as one species (both morphologically and using genome wide data - i.e., the majority assignment from a supervised Admixture run; Chapter 3) were grouped with the alternative species for that candidate window (putatively homozygous for the alternative species' haplotype). We removed all early generation hybrids prior to this analysis.

4.3.11 Functional annotations

Proteins from *C. edentula* gene models were blasted (BLAST+; Camacho et al., 2009) against the *Arabidopsis thaliana* annotations (TAIR 10 representative gene model proteins; Berardini et al., 2015). BLAST hits were filtered for e-values (dismissed hits with e-values > 1e-6e) and the top hit was retained. Genes found in outlier windows were categorized as candidate genes. We examined gene function for genes in the following outlier windows: (1) the outlier windows of GWAS (EMMAX) runs, whereby the outlier windows for all traits were grouped together for each species (i.e., all phenotype outliers for each species); (2) candidate climate adaptation windows; (3) parallel climate adaptation candidate windows; (4) invasion adaptation candidate windows; and (5) parallel adaptive introgression candidates. If > 100 genes were present then a functional gene enrichment analysis was conducted with topGo (Alexa et al., 2006). We compared our candidate genes with the flowering time genes of *Arabidopsis thaliana* (Bouché et al., 2016) and conducted an enrichment analysis with a Fisher's exact test in R.

4.4 Results

4.4.1 Patterns of introgression across the genome

We used Dsuite to confirm evidence of genome wide introgression using the D-statistic. We used the home range individuals as pure individuals and the hybrids identified by Admixture as the target population. Two separate runs were conducted, one for each invasive range. If the D-statistic is $\neq 0$ and the Z-score > 3, we determined that introgression occurred. The results show that in both invasive ranges introgression occurred (D-statistic and Z-score: Australia 0.23, 12.62; western North America 0.13, 6.29; Table 4-1). Furthermore, our separate runs using pure invasive *C. edentula* samples as target population also showed a genome wide signal

of introgression, although the signal was stronger in *C. maritima* in each range (D-statistic and Z-score: Australia 0.16, 6.21; western North America 0.08, 4.71, Table 4-1).

We found a latitudinal pattern in western North America in the genome wide signal of introgression. The two populations from the North (BC11, B16) have high D-statistics (0.20, 0.18) and Z-score (5.98, 7.62) in contrast to the two southerly populations (CA17, MEX1) for which the D-statistic and Z-scores are below the significance threshold (D-statistic: 0.09, 0.04; Z-score: 2.77, 1.47, Table 4-2). In Australia we find a similar pattern. In Queensland (QLD11) and New South Wales (NSW8) the D-statistic and p-values are high (D-statistic: 0.21, 0.24; Z-score: 6.12, 9.4), whereby in South Australia those statistics are low (D-statistic: 0.09; Z-score: 5.94; Table 4-2). Interestingly, in Victoria (VIC1) those statistics are not necessarily lower than in the higher latitudes (D-statistic: 0.25; Z-score: 13.61; Table 4-2), although *C. edentula* is no longer present here.

Table 4- 1. Results of Dsuite run per invasive range (Australia and western North America) are presented. D-statistic, Z-score, uncorrected p-value as well as f4-ration and the ABBA/BABA values. P1, P2, P3= Population 1, 2, 3. EU= native *C. maritima*, eNA= native *C. edentula*, AUS= Australia, wNA= western North America, M= *C. maritima*/hybrids, E= *C. edentula*.

P1	P2	P3	D-statistic	Z-score	p-value	f4-ratio	BBAA	ABBA	BABA
EU	AUS_M	eNA	0.23	12.62	0	0.11	480397	92616.9	58530.8
EU	wNA_M	eNA	0.13	6.29	1.59E-10	0.06	502541	81396	62596.3
eNA	AUS_E	EU	0.16	6.21	2.69E-10	0.01	322105	12546.6	9012.62
eNA	wNA_E	EU	0.08	4.71	1.22E-06	0.00	325345	10751.6	9138.6

Table 4- 2. Results of Dsuite run on selected hybrid populations in the invasive ranges (Australia and western North America) are presented. D-statistic, Z-score, uncorrected p-value as well as f4-ration and the ABBA/BABA values.
P1, P2, P3= Population 1, 2, 3. EU= native *C. maritima*, eNA= native *C. edentula*. Western North America: BC=British Columbia, CA= California, MEX= Mexico. Australia: QLD=Queensland, NSW= New South Wales, SA = South Australia, VIC= Victoria. Populations ordered in descending latitudinal order.

Range	P1	P2	P3	D-statistic	Z-score	p-value	f4-ratio	BBAA	ABBA	BABA
wNA	EU	BC16	eNA	0.18	7.62	1.23E-14	0.08	495444	86408.4	60459.7
	EU	BC11	eNA	0.20	5.98	1.09E-09	0.09	489225	89542.2	59949.2
	EU	CA17	eNA	0.09	2.77	0.003	0.04	507682	77393.2	64214
	EU	MEX1	eNA	0.05	1.47	0.07	0.02	520028	71939.9	65295.3
AUS	EU	QLD11	eNA	0.21	6.12	4.70E-10	0.10	478749	91738.2	59383.8
	EU	NSW8	eNA	0.24	9.40	0	0.12	476005	95114.5	57828.6
	EU	SA4	eNA	0.09	5.94	1.46E-09	0.04	513091	75781.7	62813.5
	EU	VIC1	eNA	0.25	13.61	0	0.12	474421	96478	57694.7

4.4.2 Genome wide associations with diverging traits

We investigated the genetic basis of diverging traits using GWAS within each species (*C. edentula* and *C. maritima*/hybrids) followed by a windowed Z analysis. For each of the 23 phenotypes, we identified a slightly higher number of outlier windows (top 5%) in the *C. maritima*/hybrid group than in the *C. edentula* group (523-524 vs. 444-473) due to differences in the number of windows excluded due to low SNP number. In total, we identified 5626 unique trait-associated windows for *C. maritima,* and 4071 unique trait associated windows for *C. edentula*. We conducted a GO enrichment analysis for all unique windows associated with traits in each species (Table 4-S2, Table 4-S3). We found a number of GO terms over-represented including positive regulation of circadian rhythm, response to heat, suberin biosynthetic process, defence response by callose deposition in cell wall, floral organ abscission regulation of flower development and positive regulation of growth for *C. edentula*. For the *C. maritima* individuals we found GO terms enriched for leaf development and senescence, regulation of seed germination, flower development, regulation of vegetative phase change, regulation of growth and regulation of brassinosteroid mediated signaling pathway. Between the species 5-49 windows per phenotype were shared (Table 4-S4).

4.4.3 Signatures of climate-mediated selection within each range

We used BayPass to examine the signatures of climate-mediated selection across the genome. We first identified windows that were climate adaptation candidates in each group (Australian *C. edentula*, Australian *C. maritima*, western North America *C. edentula*, western North American *C. maritima*, eastern North American *C. edentula* and European *C. maritima*). Outlier windows were identified as being in the top 5% WZA windows for each group and we considered a window a climate adaptation candidate if it was both an X^TX and BF outlier (Figure 4-2). For each species-range group our analysis showed a range of climate adaptation candidates (Table 4-3). For *C. edentula* 21-41% of X^TX outlier windows were also associated with one or more environmental variables. In *C. maritima* the overlap was much higher with 82-91% of X^TX outlier windows also showing associations with one or more environmental variables. However, due to the low number of SNPs in the *C. edentula* Australian populations, we removed this group from all remaining climate adaptation analysis. For each group 5-72 candidate windows for climate-mediated selection also overlapped with trait associated windows (Table 4-3).

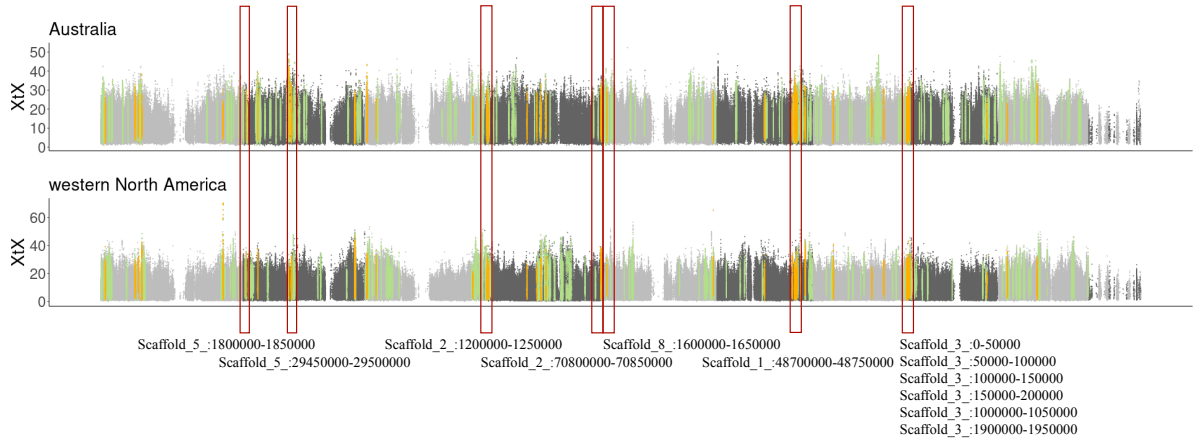

Figure 4- 2. Manhattan plots of overlapping climate adaptation candidate outliers of BayPass runs for Australian and western North American *C. maritima*.
Green= overlap of X^TX and BF (climate adaptation candidates) of the respective range (Australia, western North America), orange= overlapping BayPass result (X^TX and BF) between invasive ranges (parallel climate adaptation candidates), red boxes = parallel adaptation candidates examined for introgression (trait associations and highly differentiated between the species using allopatric native range samples). Scaffolds ordered by size.

We conducted a GO term analysis to look for signals regarding the biological function of the climate adaptation candidates for each species and range. We found multiple genes linked to defence in each group (Table 4-S5). Genes were enriched for functions related to defence response to bacteria, fungus, oomycetes, initiation of immune response-activating signalling pathway. Further, we also identified genes enriched for functions potentially related to climate adaptation, such as temperature compensation of the circadian rhythm, circadian rhythm (eastern North America *C. edentula*, western North American *C. edentula*), response to cold (Australian *C. maritima*) or photoperiodism. Genes were also enriched for abiotic stress-tolerance functions including salt stress, nitric oxide, oxidative stress, cellular response to carbon dioxide. We did not observe an enrichment of *Arabidopsis* flowering time pathway genes in any of these results (Table 4-S6, 4-S7).

Table 4- 3. Results of BayPass followed by a WZA analysis for 50,000 bp windows for each range and species group. The top 5% were taken as outliers.
The number of X^TX and BF outlier windows for at least one environmental variable and the number of overlapping windows is presented. We considered X^TX and BF outlier windows climate adaptation candidates for each species and range.

Range	X^TX outlier	EAA (BF outlier)	Number of overlapping windows of X^TX and BF overlap (percentages show the number of X^TX outliers that are also EAA outliers) Climate	Trait associated windows	Overlap climate adaptation candidates and trait associated	Associated traits

			adaptation candidates		windows	
C. edentula						
Native (eNA)	507	706	209 (41%)	4071	200 (96%)	Aphid damage, above-ground biomass, below-ground biomass, biovolume at bud onset, biovolume at open flower onset, onset of branching, onset of bud, onset of open flowers, onset of seed, total number of flowers, pollen viability, SLA, growth rate, seedling size, fruit weight, fruit shape PC1-PC4, leaf shape PC1-PC4
AUS	69	69	15 (21%)		9 (60%)	Aphid damage, above-ground biomass, below-ground biomass, biovolume at bud onset, biovolume at open flower onset, onset branching, onset of bud, onset of open flowers, total number of flowers, SLA, seedling size, fruit shape PC1-PC4, leaf shape PC1 and PC4
wNA	395	452	118 (30%)		117 (99%)	Aphid damage, above-ground biomass, below-ground biomass, biovolume at bud onset, biovolume at open flower onset, onset of branching, onset of bud, onset of open flowers, onset seed, total number of flowers, pollen viability, SLA, growth rate, seedling size, fruit weight, fruit shape PC1-PC4, leaf shape PC1-PC4
Total number of unique windows	876	1077	308 (35%)			
C. maritima						
Native (EU)	530	2897	487 (91%)	5626	231 (47%)	All traits
AUS	522	2756	458 (88%)		242 (53%)	All traits
wNA	521	2277	428 (82%)		210 (49%)	All traits
Total number of unique windows	1403	5434	1241 (88%)			

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4.4.4 Parallel genomic signals for climate-mediated selection

We examined the extent to which climate adaptation candidates were shared between the ranges and species (Table 4-4). Pairwise comparisons within species, but between ranges, identified between 30-65 (6-34%) shared outlier windows between the ranges, with all overlaps significantly greater than expected by chance ($p < 0.01$; Table 4-4). The greatest overlap was between the *C. edentula* native range and western North America, while the second highest level of parallelism was between the two introduced *C. maritima* ranges. By contrast, between-species comparisons had far fewer overlapping candidate windows (2-3%, 2-9%), and two comparisons (both involving native *C. edentula*) were not significant at the 0.05 threshold (Australian *C. maritima* and European *C. maritima* versus eastern North American *C. edentula*).

GO analyses for parallel climate adaptation candidates within *C. maritima* (65 windows, 155 genes, Australian, western North American and European *C. maritima*) genes enriched functions related to gibberellin biosynthetic process, positive regulation of hydrogen peroxide biosynthetic process and responses to iron ion among others (Table 4-S8). For native and western North American *C. edentula* genes were enriched for temperature compensation of the circadian clock, photoperiodism (flowering) and for responses to glucose, carbon and phosphate starvation.

Table 4- 4. Parallel candidate windows for climate adaptation among ranges and species.
The number of overlapping WZA windows for each comparison is presented using results from BayPass (both BF and X^TX outliers - top 5%) species range run. A hypergeometric test (p-value) to assess if the overlap between each comparison was significantly greater than expected by chance is presented. The overlap with Australian *C. edentula* was not examined due to limited SNP sample sizes.

Comparison	Number of overlaps (Percentage of overlaps for adaptation candidates in each range)	p-value
C. edentula		
wNA vs eNA	34 (34% wNA, 16% eNA)	4.52E-44
C. maritima		
AUS vs wNA	65 (14% AUS, 15% wNA)	1.42E-21
AUS vs EU	45 (10% AUS, 10% EU)	6.95E-08
wNA vs EU	30 (6% wNA, 6% EU)	0.0032
Total (number of unique pairwise overlaps)	65	
***C. maritima* vs *C. edentula* (eNA)**		
AUS	11 (2% AUS, 5% eNA)	0.1434
EU	13 (2% EU, 5% eNA)	0.0688
wNA	13 (3% wNA, 5% eNA)	0.0281
Total (number of unique pairwise overlaps)	32	

***C. maritima* vs *C. edentula* (wNA)**		
AUS	15 (2% AUS, 9% wNA)	2.1417e-05
EU	10 (2% EU, 9% wNA)	0.0129
wNA	9 (3% wNA- *C. maritima*, 9% wNA-*C. edentula*)	0.0141
Total (number of unique pairwise overlaps)	24	

4.4.5 Signals of introgression for candidate adaptation windows

As we identified signals of hybridization between the species in the introduced ranges, (particularly for *C. maritima*) we sought to identify if parallel candidate windows for climate adaptation in the introduced ranges were caused by introgression. Since signals of parallel climate-mediated selection during invasion were not apparent in *C. edentula* due to SNP density leading to power limitations in Australia, and patterns of introgression were most apparent in the introduced ranges of *C. maritima* (Table 4-1,4-2), we restricted our analysis to Australian and western North American *C. maritima* (Figure 4-2; Table 4-4). Out of the 65 parallel candidates for climate adaptation between these ranges, we identified 42 that were also associated with one or more traits (Table 4-5 for a breakdown of trait associations). Associations with latitude were identified for many of these traits in *C. maritima* (Chapter 3). GO analysis revealed that these windows were enriched for genes involved in plastoquinone biosynthetic process, cellular response to cold and fruit development as well as response to osmotic stress, regulation of nitrate assimilation and response to ozon (Table 4-S9, Table 4-S10 for annotation and enrichment details).

Table 4- 5. Number of overlapping outlier windows of the invasive *C. maritima* groups (Australia and western North America).
Each window is an outlier for one or more traits (GWAS), and a parallel climate adaptation candidate (BayPass X^TX and BF outlier) for the group using a 50,000 bp window and the top 5% WZA windows.

Phenotype	Number of outlier window
Above ground biomass	4
Aphid damage	13
Below - ground biomass	6
Biovolume at onset of bud	6
Biovolume at onset of open flowers	5
Onset branching	4
Onset bud	4
Number of flowers (total reproductive count)	4
Fruit weight	12
Growth rate	4
Onset open flower	7
Pollen viability	3
Onset seed	3
Seedling size	10

To identify haplotype segregation indicative of introgression in these regions, we first ran a local PCA on the 42 windows. Only 16 of the 42 outlier windows showed clear genetic differentiation in the PCA between the species using allopatric samples from the home ranges. Of these, 12 showed three segregating groups, indicative of heterozygous intermediate samples in both invasive ranges. In all of these, some *C. maritima* samples clustered in the intermediate (heterozygote) or *C. edentula* group, consistent with introgression in this genomic region in the introduced range. All twelve showed *C. maritima* samples that were homozygous for the *C. edentula* haplotype in both invasive ranges, indicative of a relatively high frequency of the *C. edentula* allele in the *C. maritima* samples. For these twelve windows we plotted the haplotype frequencies in both invasive ranges of the *C. maritima* samples (e.g., Figure 4-3, Figure 4-4). The haplotype frequency maps revealed a repeated geographic pattern for most of the windows. In western North America a north-south pattern emerged, in which *C. edentula* haplotypes dominated the north and were replaced by the *C. maritima* haplotypes to the south. In Australia, a U-shape pattern to north-south could be observed along the south east coast. In lower latitudes *C. maritima* haplotypes dominated (South Australia, New South Wales, Queensland), whereas in more high latitude locations (Victoria, Tasmania) the *C. edentula* haplotypes were at higher frequency (Figures 4-3, Figure 4-4). We then assessed if the latitude of origin was correlated with haplotype frequency in each range using a generalized linear model and found a significant correlation for eight of the windows in Australia and all twelve windows tested in western North America (Table 4-6, Table 4-7). Interestingly, four of these 12 windows were also climate adaptation candidates for western North American *C. edentula* (outliers for X^TX and BF).

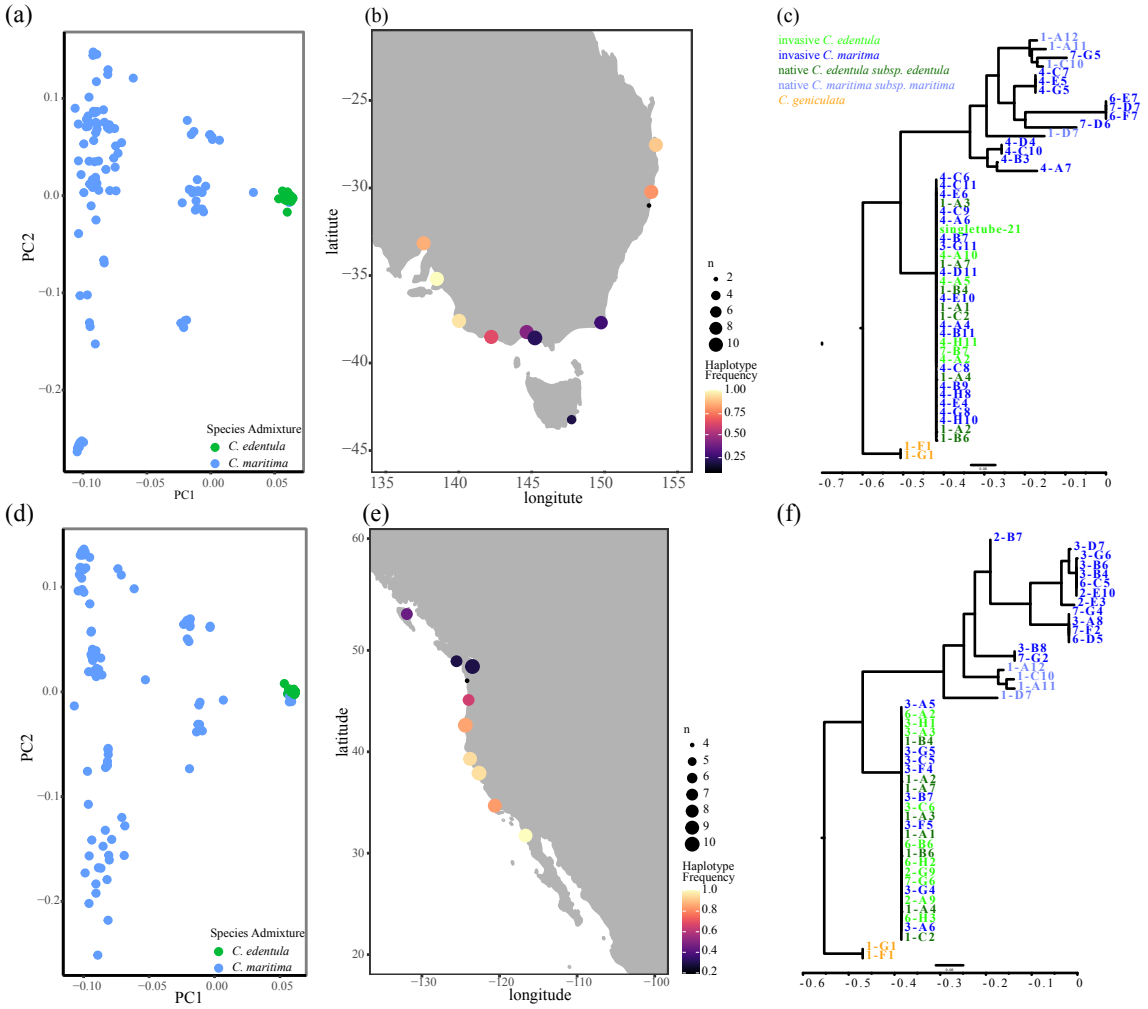

Figure 4- 3. (a) & (d) Principal component analysis for the window Scaffold_3_1000000-1050000. (b) & (e) Map of haplotype frequencies of *C. maritima*. (c) and (f) RaxML tree for homozygous individuals in native and invasive ranges. (a)-(c) for Australia, (d)-(f) for western North America.

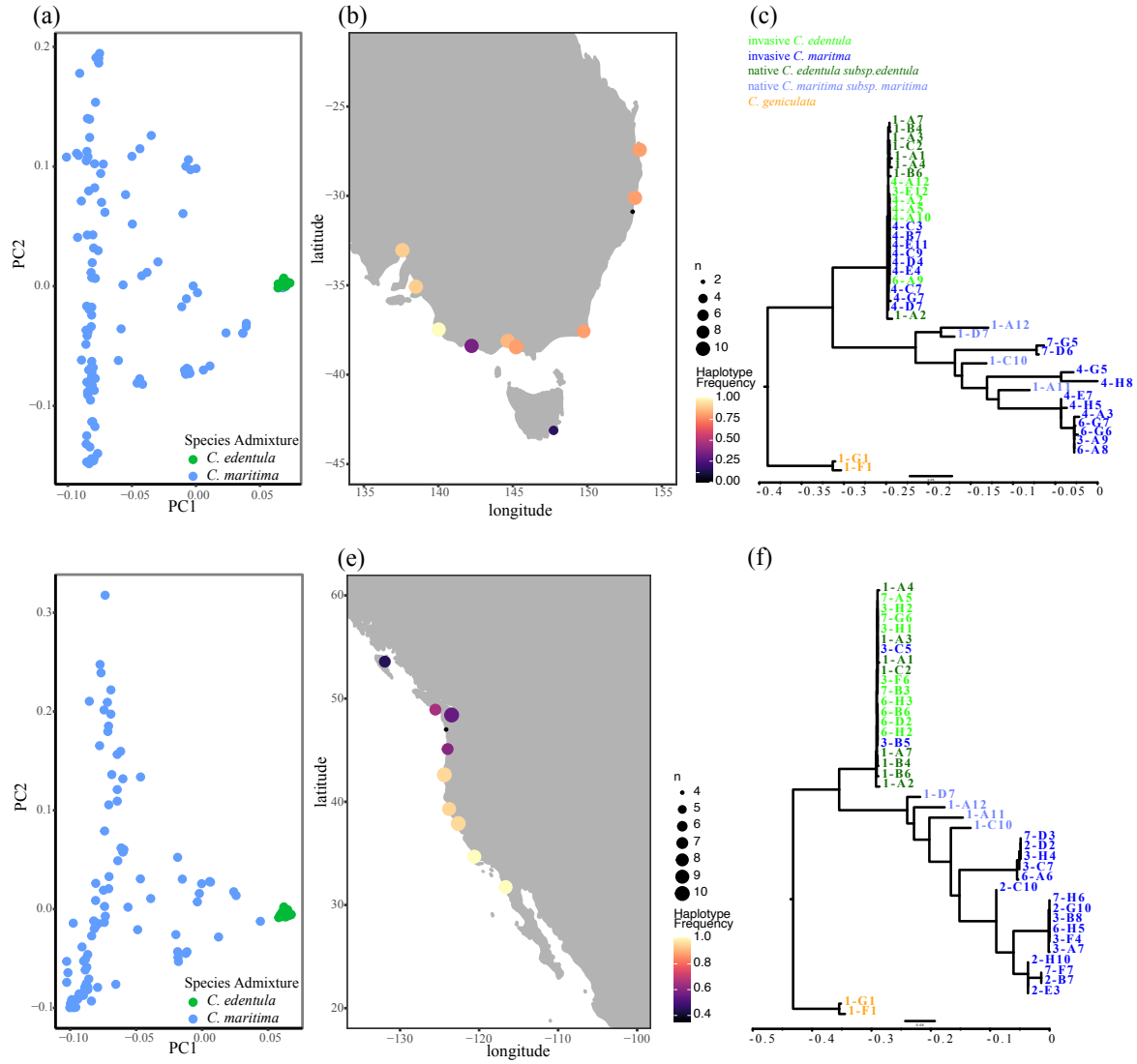

Figure 4- 4. (a) & (d) Principal component analysis for the window Scaffold_8_1600000-165000. (b) & (e) Map of haplotype frequencies of *C. maritima*. (c) and (f) RaxML tree for homozygous individuals in native and invasive ranges. (a)-(c) for Australia, (d)-(f) for western North America.

Table 4- 6. Reported F-values, degrees of freedom and p-values of generalized linear models comparing outlier windows with introgression haplotype frequency to latitude, range and significant interactions between range and latitude.

window	PC1		PC2		Range(AUS/wNA)		Absolute latitude		Range * absolute latitude	
	F_{DF}	p	F_{DF}	p	F_{DF}	p	F_{DF}	p	F_{DF}	p
Scaffold_1_48700000-48750000	80.47_1	4.757e-16***	2.4791_1	0.117193	8.2568_1	0.004569**	5.2981_1	0.02254*	/	/
Scaffold_2_1200000-1250000	48.40_1	6.976e-11***	5.3795_1	0.02155*	22.0895_1	5.310e-16***	42.6757_1	7.029e1-***	7.5161_1	0.006762**
Scaffold_2_70800000-70850000	70.89_1	1.428e-14***	2.2921_1	0.131866	20.083_1	1.349e-05 ***	7.4727_1	0.006919**	8.4236_1	3.031e-7 ***
Scaffold_3_0-50000	64.10_1	1.693e-13***	2.9986_1	0.08513	23.5523_1	2.712e-06***	1.314_1	0.25327	18.317_1	3.100e-05***
Scaffold_3_100000-150000	57.89_1	1.748e-12***	3.5932_1	5.612e-05***	13.6674_1	0.0002932***	0.9484_1	0.3314908	17.071_1	5.612e-5***
Scaffold_3_1000000_1050000	168.40_1	< 2.2e-16***	12.669_1	0.0004801***	40.809_1	1.499e-09***	175.504_1	< 2.2e-16***	/	/
Scaffold_3_150000-200000	68.12_1	3.886e-14***	6.2471_1	0.0133*	27.8414_1	3.924e-07***	0.9383_1	0.33409	20.58_1	1.069e-05***
Scaffold_3_1900000-1950000	65.74_1	9.280e-14***	17.742_1	4.074e-05***	2.1884_1	0.1409	86.5264_1	< 2.2e-16***	19.209_1	2.034e-05***
Scaffold_3_50000-100000	57.88_1	1.748e-12***	3.5932_1	0.0596924	13.6674_1	0.0002932***	0.9484_1	0.3314908	17.071_1	5.612e-05***
Scaffold_5_1800000-1850000	222.45_1	< 2.2e-16***	55.077_1	5.107e-12***	37.1633_1	6.943e-09***	1.0286_1	0.3119173	12.824_1	0.0004452***
Scaffold_5_29450000-29500000	60.72_1	5.969e-13***	1.1398_1	0.2872	6.5984_1	0.01106*	3.7727_1	0.05373	6.755_1	0.01016*
Scaffold_8_1600000-1650000	35.63_1	1.33e-08***	6.5609_1	0.01128*	25.9417_1	9.180e-07***	7.2052_1	0.00798**	16.806_1	6.372e-05***

Table 4- 7. Emtrends reported slopes, standard errors, lower and upper confidence limits for haplotype frequency and latitude for the outlier windows. Significant slopes are bolded.

window	type	absolute latitude trend	SE	LCL	UCL	contrast estimate	contrast SE	contrast p-value
Scaffold_1_48700000-48750000	AUS wNA	**0.038**	**0.0082**	**0.022**	**0.054**	0	0	NA

Scaffold_2_1200000-1250000	AUS	**0.13**	**0.013**	**0.10**	**0.15**	-0.089	0.022	<.0001	
	wNA	**0.21**	**0.017**	**0.18**	**0.245**				
Scaffold_2_70800000-70850000	AUS	**-0.064**	**0.012**	**-0.088**	**-0.040**	-0.17	0.017	<.0001	
	wNA	**0.11**	**0.012**	**0.082**	**0.13**				
Scaffold_3_0-50000	AUS	0.023	0.012	-0.00022	0.047	-0.12	0.017	<.0001	Consecutive window on Scaffold 3
	wNA	**0.14**	**0.011**	**0.12**	**0.16**				
Scaffold_3_50000-100000	AUS	0.020	0.012	-0.0034	0.044	-0.12	0.016	<.0001	Consecutive window on Scaffold 3
	wNA	**0.14**	**0.011**	**0.11**	**0.16**				
Scaffold_3_150000-200000	AUS	0.02	0.012	-0.0040	0.043	-0.13	0.017	<.0001	Consecutive window on Scaffold 3
	wNA	**0.15**	**0.011**	**0.12**	**0.17**				
Scaffold_3_100000-150000	AUS	0.020	0.012	-0.0034	0.044	-0.12	0.016	<.0001	Consecutive window on Scaffold 3
	wNA	**0.14**	**0.011**	**0.11**	**0.16**				
Scaffold_3_1000000_1050000	AUS	**0.17**	**0.0098**	**0.15**	**0.19**	0	0	NA	
	wNA								
Scaffold_3_1900000-1950000	AUS	**0.26**	**0.021**	**0.22**	**0.31**	0.17	0.024	<.0001	
	wNA	**0.098**	**0.011**	**0.076**	**0.12**				
Scaffold_5_1800000-1850000	AUS	0.018	0.013	-0.0082	0.044	-0.086	0.018	<.0001	
	wNA	**0.10**	**0.013**	**0.079**	**0.13**				
Scaffold_5_29450000-29500000	AUS	**0.05**	**0.017**	**0.019**	**0.085**	-0.093	0.022	<.0001	
	wNA	**0.15**	**0.014**	**0.12**	**0.17**				
Scaffold_8_1600000-1650000	AUS	**0.06**	**0.014**	**0.034**	**0.088**	-0.14	0.021	<.0001	
	wNA	**0.20**	**0.015**	**0.17**	**0.23**				

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To confirm introgression for our parallel climate adaptation candidates we constructed RAxML trees (Figure 4-3, Figure 4-4). We focused on the twelve parallel climate adaptation candidate windows that were also candidates for introgression using the PCA method and contained *C. maritima* samples homozygous for the *C. edentula* haplotype in both ranges. We only included homozygous individuals (individuals from the extreme clusters in the PCA) to avoid complications involving phasing the haplotypes for heterozygotes. The construction of ML trees confirmed the PCA findings as *C. maritima* samples clustered with *C. edentula* in the PCA also grouped with *C. edentula* samples in the ML trees, providing further evidence that *C. edentula* haplotypes were introgressed in these regions. Annotation of the twelve windows can be found in Table 4-S11.

4.4.6 Parallel patterns of adaptive divergence during invasion

We identified genomic windows with unusually high divergence between the native and each introduced range (candidate invasion adaptation windows) using X^TX from BayPass (Table 4-8). The enrichment analysis (Table 4-S12) for *C. edentula* invasion adaptation candidates identified genes involved in defence, flower and fruit development and plant hormones important for stress response. For *C. maritima* the invasion adaptation candidates were also enriched for a number of functions linked to defence response, stress response and phenology (Table 4-S12).

Table 4- 8. Number of invasion adaptation candidates (X^TX outliers of cross-range BayPass runs) and number of parallel invasion adaptation candidates are presented. AUS= Australia, eNA= eastern North America, EU= Europe, wNA= western North America.

BayPass run	Number of X^TX outliers	Number of overlapping X^TX and H12 outliers
C. edentula		
AUS vs eNA	490	6
wNA vs eNA	472	8
C. maritima		
AUS vs EU	527	88
wNA vs EU	528	117

To complement our signatures of selection analysis with the X^TX from BayPass we used the H12 statistic to identify selective sweeps in each invasive range for each species and identified the top 1% of H12 values. As the next step we compared the top 1% of the H12 statistic of each range to the cross-range BayPass outliers to see if a sweep signal was apparent in the introduced range for regions with high X^TX between native and introduced range (Figure 4-5). Here, we identified six windows for Australian *C. edentula*, eight windows for western North American

C. edentula, 117 windows for Australian *C. maritima* and 88 windows for western North American *C. maritima*.

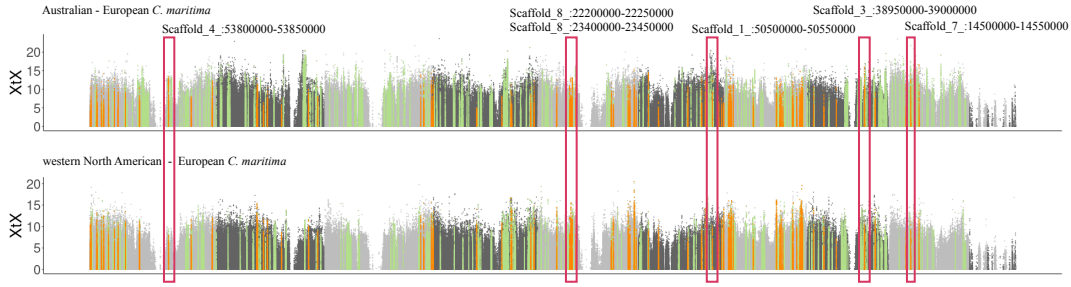

Figure 4- 5. Manhattan plot of BayPass X^TX of the cross range runs. Native (Europe) and invasive ranges (Australia, western North America) for *C. maritima*. Green= X^TX outlier, orange = X^TX and H12 outlier, red boxes= overlapping H12 and X^TX outliers of cross-range *C. maritima* runs. Scaffolds ordered by size.

We compared invasion adaptation candidates within each species group to determine if there was a parallel pattern across multiple invasions within and between species. We first examined overlap between X^TX outliers alone. We identified 135 overlapping X^TX outlier windows for *C. maritima* and 198 outlier windows for *C. edentula* (Table 4-9). We then compared overlap among X^TX and H12 outliers. We did not find any overlap between the *C. edentula* groups for X^TX and H12 outliers. We only identified a single window (Scaffold_6_2050000-2100000) that was a X^TX outlier (Australian *C. edentula*, western North American *C. edentula*) and an H12 outlier for western North American *C. edentula*. In contrast, we found six overlapping windows for the *C. maritima* groups (which were also overlaps of GWAS and BayPass; Figures 4-S1).

Table 4- 9. Parallel candidate windows for adaptive divergence during invasion.
The number of overlapping WZA windows for each comparison is presented using results from BayPass (X^TX outliers for native and introduced range comparisons - top 5%). The native and two introduced ranges are compared separately for each species. A hypergeometric test (p-value) to assess if the overlap between each group was significantly greater than expected by chance is presented.

Comparison	Number of X^TX overlaps	p-value	X^TX and H12 (top1 overlap)	p-value
Within species between ranges				
***C. edentula* (AUS vs wNA)**	198 (40% AUS, 42% wNA)	1.22E-152	0	/
***C. maritima* (AUS vs wNA)**	135 (26% AUS, 26% wNA)	2.57E-66	6	3.6072e-05
Between species within ranges				
***C. edentula vs C. maritima* (AUS)**	46 (9% *C. edentula*,	2.03E-06	0	/

	9% *C. maritima*)			
***C. edentula vs C. maritima* (wNA)**	29 (6% *C. edentula*, 5% *C. maritima*)	0.015	0	/
Between species & introduced range				
***C. edentula* (AUS) *vs C. maritima* (wNA)**	15 (3% *C. edentula*, 3% *C. maritima*)	0.954	0	/
***C. edentula* (wNA) *vs C. maritima* (AUS)**	44 (9% *C. edentula*, 8% *C. maritima*)	5.19E-07	1	0.0016

We then looked for signals of introgression for the X^TX outlier window from the cross-range BayPass runs. As above we limited our analysis to candidates showing parallel patterns within each species and associations with one or more traits (Table 4-S13, flowering genes in Table 4-S14). For the cross range runs for *C. maritima*, this left 97 out of the 135 parallel invasion candidates in western North America and Australia. However, only 18 windows showed a clear differentiation between the native range individuals in the local PCA, and only seven windows were identified as having *C. maritima* samples clustering with *C. edentula* in both invasive ranges (Figure 4-S1; Table 4-S15, 4-S16, 4-S17). The windows did not show a geographic pattern but intriguingly revealed sometimes high frequency of *C. edentula* haplotypes in regions the species has not seen in decades (e.g., Mexico). For the cross range runs of *C. edentula*, we found 192 unique windows, although here only 65 showed a clear differentiation between home range individuals. However, examination of haplotype frequencies in the introduced ranges and ML trees did not reveal significant levels of introgression at these loci.

4.5 Discussion

We set out to investigate repeatability in adaptation during invasion on two continents and across two species. These cross-compatible species, allopatric in their native ranges, experienced sympatry during the historic invasion in both Australia and western North America. Hence, our second goal was to identify if introgression might aid adaptation during invasion. We utilized whole genome re-sequencing data, phenotypic data as well as environmental variables to investigate the genomic basis of local adaptation and the role of hybridization during invasion in these *Cakile* species. We identified highly significant patterns of repeatability at the genetic level within species among ranges for climate adaptation candidates, while parallelism between species was much lower. This points to the importance of standing variation for rapid adaptation during invasion, as well as the impacts of genetic background on adaptive trajectories. We identified several strong candidates for parallel adaptive introgression within *C. maritima* introductions (western North America and Australia). These candidate windows were putatively involved in climate adaptation and

contained several genes with functions related to defence as well as candidates related to abiotic stress response including salt tolerance, cold response and nutrient stress. In most cases the *C. edentula* haplotype was at a high frequency in the high absolute latitude environments in both introduced ranges, and this pattern was not explained by gradients in genome wide species ancestry. The benefit of the *C. edentula* alleles at high latitudes in *C. maritima* might be explained by the impacts of founder effects limiting adaptive variation within the species. *Cakile maritima* is largely sourced from the Mediterranean, while *C. edentula* is largely sourced from the north eastern coast of Canada and the US, which has a much colder climate. However, non-adaptive explanations also need to be explored. Additionally, we find genes connected to defence and flowering in regions with signatures of divergent selection in comparisons of native and invasive ranges, and some of these regions also show strong signals of introgression in the introduced range. We have previously shown (Chapter 3) that invasive individuals experienced lower herbivory than the native individuals and that the onset of flowering has also diverged among the ranges. Our results reveal some evidence of repeated selection and adaptation at both the phenotypic and genetic level, with repeatability both within and between species, and a potential role for adaptive introgression in the poleward spread of one species at the expense of its donor.

4.5.1 Signatures of climate adaptation within species

We found evidence of climate-mediated selection in several genomic regions in both *C. edentula* and *C. maritima*. The climate adaptation candidates in *C. edentula* (those windows showing signatures of climate-mediated selection) were also associated with traits within this species (Table 4-3). Even in Australian accessions where genome-wide SNP variation was substantially limited, nine out of 18 windows were trait-associated). Many of these traits, such as aphid damage, biovolume of bud, onset of bud, seedling size, showed latitudinal clines in this species, consistent with climate adaptation shaping among-population variation in these traits (Chapter 3). In addition, several over-represented GO terms for genes found in these regions were associated with responses to the environment or traits that were found to be divergent among populations (Table 4-S12), including defence response in Australian accessions (e.g., defence response to bacterium; defence response to fungus; immune response-activating signal transduction), phenology in western and eastern North American accessions (both: temperature compensation of the circadian clock; western North American circadian rhythm cellular response; circadian rhythm cellular response eastern North American) was well

as nutrient stress (response to iron ion). Similarly, in *C. maritima* a smaller, but still substantial fraction of climate adaptation candidates were also trait-associated outlier windows. All traits were associated with one or more climate adaptation candidate windows, including many traits that showed associations with latitude in the common garden (Chapter 3). As with *C. edentula*, several over-represented GO terms for genes found in these regions were associated with responses to the environment or traits that were found to be divergent among populations, including defence (e.g., Australian: innate immune response; European: regulation of defence response to bacterium; defence response to oomycetes, defence response to bacterium; western North American: regulation of defence response), cold response (Australian: cellular response to cold), reproduction (Australian: fruit development; Australian & western North American: seed germination; European: photoperiodism, flowering), and salinity (Australian: salinity response). Consequently, in both species there are biological signals in the function of the genes found in the candidate windows that support their involvement with climate adaptation.

We identified many more climate adaptation candidates in *C. maritima*, which were both X^TX outliers and associated with environmental variables. Several factors could be contributing to this, including differences in the importance of climate adaptation between the species. However non-adaptive explanations must also be considered that may impact the detectability of loci involved in climate adaptation, including differences in SNP density across the genome (especially in *C. edentula*), differences in sample size, extended linkage disequilibrium caused by mating system (*C. edentula* is a mixed mater; Chapter 3) or recent admixture within and between species in the introduced ranges, and the confounding effects of population structure. Some of these factors have the effect of reducing power, resulting in false negatives, including using population structure corrections in the tests of selection (Hodgins & Yeaman, 2019; Lotterhos & Whitlock, 2014; Whitlock & Lotterhos, 2015; Yeaman et al., 2016), while others have the effect of creating false positives, such as underlying population structure (Lotterhos & Whitlock, 2014; Whitlock & Lotterhos, 2015). A geographic gradient in admixture, as seen in *C. maritima*, will likely enhance false positive rates as it can generate high levels of divergence among populations, which might even be correlated with environmental variables. In some cases, it might result in false negatives if there is an overcorrection for population structure because of a covariance between population structure and the environmental variables driving selection as seen in other species such as interior spruce (Yeaman et al., 2016).

4.5.2 Parallel patterns of climate adaptation on two continents and the role of introgression

We identified several regions of the genome that were highly divergent within each range and correlated with environmental variables in parallel across the ranges within a species. In *C. edentula*, we identified the strongest signal of parallelism (p = 4.52e-44) between western North America and eastern North America. In fact, 34% of climate adaptation candidates in western North American were also candidates in the native ranges, suggestive of substantial gene reuse during local adaptation in western North American *C. edentula*. This could be related to the multiple introductions we identified (Chapter 3) and the import of adaptive variation to western North American from eastern North American. We also identified genes that appeared to have important biological functions for local adaptation, and over-represented GO terms including functions related to flowering time (e.g., photoperiodism) and nutrient stress (e.g., phosphate starvation) and defence (negative regulation of defence response to oomycetes). Interestingly, both flowering time and aphid damage showed a clear latitudinal cline in *C. edentula* in both of these ranges.

In *C. maritima* the signal of parallelism was highly significant, but the percent overlap was lower than *C. edentula*, and ranged from 6-15% of climate adaptation candidates. The lower percentage of parallel candidates might reflect the impacts of hybridization and the strong population structure evident in Europe (Chapter 3) on the detectability of adapting loci. Overlap was lowest in western North American versus Europe, perhaps reflecting the single introduction source into western North America (Chapter 3), which could have limited the adaptive genetic variation introduced into this region. By contrast, evidence is consistent with multiple introductions into eastern Australia, and more parallelism was identified between this region and the native range of Europe. Similarities in climate between the source and introduced range may also play a role. The greatest level of parallelism in *C. maritima* was identified between the introduced ranges, likely reflecting the impact of introgression. Many of these parallel climate adaptation candidates were associated with traits that exhibited parallel latitudinal clines, some of them, such as days to bud, strengthened with increased *C. edentula* ancestry (Chapter 3). These genomic regions were also enriched for genes with putative biological functions related to climate adaptation, such as cellular response to cold and fruit development. None of our groups of climate candidates or parallel climate candidates identified

an over-representation of flowering time genes using the FLOR-ID database (Bouché et al., 2016), but there were many flowering time genes present in outlier windows. While maladaptive flowering times likely have strong fitness consequences (as evidenced by the re-emergence of latitudinal clines across three different regions in the two different *Cakile* species), flowering time is also a highly polygenic trait (Monnahan & Kelly, 2017; Zan & Carlborg, 2019), which may reduce the strength of selection at most individual genes to below what we can detect given our sample sizes.

Parallel patterns between the species were weak and frequently not significant and a random selection of windows would have provided a similar level of overlap in these cases. This is despite the hypothesized importance of introgression for climate adaptation in *C. maritima*, which might be expected to elevate between-species parallelism. Since the main source of introductions for this species appears to be the Mediterranean, and so the introductions originated from a region with much less climate heterogeneity than the entire native range of *C. maritima*, the injection of adaptive variation from *C. edentula* might be expected drive a significant parallelism between species for these candidates, but this was not the case.

Using convergent patterns across multiple bouts of adaptation can aid in identifying true positives, especially when population structure is correlated with the environmental variables driving adaptation (Yeaman et al., 2016). This is because it is very unlikely to get the same false positive across multiple independent bouts of selection by chance alone. However, when the lineages are closely related, or have experienced gene flow, the probability of shared false positives increases because of the non-independence of allele frequencies among the groups. However, strong correlations between allele frequency and the environment in the same direction is added evidence that such patterns are not due to drift, especially when these regions are associated with traits important for local adaptation. Occurred the case of *Cakile*, identifying parallel adaptation candidates may help reduce the identification of false positives caused by admixture, however the covariance between admixture levels and the main axis of temperature variation is confounded, particularly in western North America. In western North America Q value and latitude are strongly associated (Spearman's rho = 0.818, p = 0.007), although this is not the case in Australia (Spearman's rho = -0.118, p = 0.734). Moreover, following hybridization, differential selection against strongly deleterious and more weakly deleterious or neutral regions of the genome might contribute to parallel signatures of climate adaptation among the ranges. This is because highly deleterious regions of the genome (e.g.,

those with excess genetic load or incompatibilities) will be quickly removed from the genome, while more neutral regions may linger, and may even experience 'allele surfing' during range expansion (Currat et al., 2008). In such circumstances, even parallel signals might occur through this interaction between drift, heterogeneity in negative selection across the genome and range expansion, and lead to false positive parallel climate adaptation candidates. In maize, parallel adaptative introgression along altitudinal clines that were also confounded by genome-wide species ancestry were identified by creating empirically informed null distributions of ancestry (Calfee et al., 2021) and this approach may be fruitful for *Cakile* as well. Additionally, estimates of selection on these genomic regions using outdoor common gardens of artificial hybrids are underway in Vancouver. This experiment will be key in assessing the adaptive value of candidate regions. Finally, recombination rate heterogeneity across the genome can also contribute to reduced detectability of adaptive loci (Booker et al., 2021) and impact levels of introgression across the genome (Brandvain et al., 2014). In the future, it will be important to develop a recombination rate map for *Cakile* and include this in analysis of both selection and introgression across the genome.

Surviving the climate in the new range is key for the establishment of an invasive species (Lee, 2002) as otherwise the invasion will fail. Hybridization can transfer locally adapted genes from a (resident) species to another species and can assist the newcomer to adapt to its new environment (Milne & Abbott, 2000; Pfennig et al., 2016). For invasive freshwater snails, it has been shown that *Pomacea maculata* was able to expand into colder areas by hybridization with the invasive species *Pomacea canaliculata* (Matsukura et al., 2016). In plants, *Rhododendron catawbiense* introgression into *Rhododendron ponticum* improved the cold tolerance of the species in Britain (Milne & Abbott, 2000). Our main goal was to establish if a parallel pattern of adaptive introgression following invasion exists in the two *Cakile* species. We have some support that hybridization of *C. edentula* and *C. maritima* aids in adaptation of *C. maritima* to high latitude locations in both ranges. Haplotypes of *C. edentula* are prominent in *C. maritima* phenotypes in Victoria, in Australia and British Columbia in Canada. Especially Victoria, a considerable time has passed since the hybridization of the two species and phenotypically the appearance of *C. edentula* has long disappeared.

Keeping in mind the caveats above, we identified twelve loci that were strong candidates for introgression as well as climate adaptation in *C. maritima*. It is likely that if only ancestry-informed sites are used in the PCA approach we will find even more evidence of introgression,

as clear differentiation of the species prevented haplotype assignment in many cases. Four of these windows were consecutive and had very similar associations with traits and climate (mainly precipitation variables). It is likely that these regions are in high LD and could represent a single region of low recombination. Once we exclude recent hybrids, our genetic data shows low levels of genome-wide ancestry of *C. edentula* in introduced *C. maritima* (population mean Australian = 0.086; range 0.004-0.197; population mean western North American = 0.05; range 0.00001-0.155), however, for these twelve genomic regions, *C. edentula* haplotypes are at very high frequency in some populations (e.g., Scaffold_8_1600000-16500: western North American high latitude populations (BC16, BC11)=0.35-0.55; Australian high latitude populations (VIC1, VIC11)=0.2-0.2; Scaffold_3_1000000_10500: western North American high latitude populations=0.55-0.7; Australian high latitude populations=0.7-0.75). For all twelve of these regions, we identified strong associations of the *C. edentula* haplotype frequency with latitude, even when accounting for population structure using the first two PCs as covariates in western North America. For Australian accessions, no associations with latitude were identified in the large introgressed region on Scaffold 3 (0bp- 200kbp), nor for one region on Scaffold 5 (1,800,000-1,850,000bp) (Table 4-7). The reverse association was also identified for Scaffold_2_70800000-70850000 where in Australia a negative correlation with latitude occurred and in western North America a positive one. This might reflect differences in the relationship of the climate variables with latitude in western North America versus Australia. For example, Scaffold 3 (0bp- 200kbp) in particular was correlated with precipitation rather than temperature variables in Australia. Future analyses of haplotype frequency should examine associations with these specific climate variables. Another piece of evidence in support of their involvement in climate adaptation is that 33% of these regions are also climate adaptation candidates in *C. edentula,* a far greater overlap than would be expected by chance (hypergeometric test: p = 8.58e-08). This pattern also suggests that the regions involved in adaptation within *Cakile* species are more likely to be involved in adaptive introgression between species.

There were several interesting genes in the candidate windows for adaptive introgression that have putative functions potentially related to local adaptation (Table 4-S11). We outline several examples below. Scaffold2_:1200000-1250000, associated with aphid damage, contained three genes involved in defence: GOX3 involved in nonhost resistance; CYCT1 with roles in infection with Cauliflower Mosaic virus (CaMV); and AT4G19510, a disease resistant protein. Scaffold_5_:29450000-29500000 also contained a defence related gene, EMB2789.

Scaffold_3_:100000-150000 contained ATAIRP2, a gene involved in ABA signaling and the response to high salt in *Arabidopsis*. Both species are considered halophytes (see https://www.sussex.ac.uk/affiliates/halophytes) and there is interest in understanding the genetic basis of this trait. It would be interesting to see if variation in salt tolerance was associated with this introgressed region. Scaffold_3_:1000000-1050000 contained a gene involved in chilling response (ALA1). Finally, Scaffold_8_:1600000-1650000, associated with aphid damage, leaf shape and days to branching, contained Senescence Associated Gene2, which encodes a senescence-associated thiol protease and ATSIZ1. This final gene is a main controller of Pi starvation-dependent responses. It is also involved in immunity in *Arabidopsis* as well as the regulation of plant growth, drought responses and freezing tolerance as well as leaf cell division and expansion.

Even after over 100 years of invasion, *C. maritima* has not succeeded in invading areas north of British Columbia, even though its spread southward has replaced all of *C. edentula*. In addition, both species have coexisted in Washington, Oregon and British Columbia for many years (62-79), despite the rapid replacement of *C. edentula* in other regions such as California or Victoria (34-64 years). Genome-wide analysis of species ancestry in western North America compliments this pattern as southern populations show limited signals of introgression (D statistic: 0.093, 0.048; Table 4-2), whereas northern *C. maritima* populations show a higher level of introgression, perhaps reflecting the continued presence of *C. edentula* in this region (D statistic:0.177, 0.198). However, in Australia, a different geographic pattern of genome-wide ancestry is observed (Chapter 2). First, more *C. edentula* ancestry is present in *C. maritima,* even when removing populations that historically were never sympatric in western North America. Second, a latitudinal pattern in species ancestry is absent in Australia, but present in western North America. Almost all populations in east Australia show some low-level hybridization even after several generations of phenotypic replacement whereas southern populations in western North America (in warmer regions) do not show this. For instance, the D-statistic reveals the strongest signals of introgression in Victoria although *C. edentula* has not been seen there for decades, whereas mixed ancestry is not apparent in southern California. These differences in the genome wide pattern of ancestry present a puzzle that does not seem to be explained by pre-adaptation to climate of the source populations, since the climate of south east Australia is more similar to the Mediterranean (*C. maritima* source) than north eastern Canada and the US (*C. edentula* source). Nor does it seem to be explained solely by differences in the range of *C. edentula* prior to invasion of *C. maritima*, as this species is

recorded as far south as the US-Mexican border. The complex interaction between range expansion, hybridization and selection is likely responsible for ancestry patterns we observe. The evidence we present suggests that there are differences in the rates of purging or retention of the *C. edentula* genome from *C. maritima* in the two ranges, and latitude or variables correlated with latitude in western North America might contribute to these differences.

4.5.3 Signatures of adaptation during invasion

We used X^TX outliers to identify windows diverging between the native source populations and their corresponding introductions to identify regions of the genome potentially experiencing selection during invasion. Strikingly, top GO terms enriched in all four pairwise comparisons contained biological processes related to defence (Table 4-S12); e.g., Australian *C. edentula*, defence response to other organism; western North American *C. edentula*, defence response to bacterium; Australian *C. maritima*, defence response to insect, defence response by callose deposition in cell wall, defence response to bacterium; western North American *C. maritima*, defence response to nematode). Introduction to novel ranges allows many species to escape natural enemies found in their native ranges (Blossey & Notzold, 1995; Chun et al., 2010; Hill & Kotanen, 2009; Liu & Stiling, 2006), alternatively novel pests and pathogens might be encountered (Colautti et al., 2004; Hodgins et al., 2018). Several hypotheses have been developed to predict how changes in defence might evolve during invasion in response to shifts in the biotic environment. For example, EICA proposes that reduction in specialist herbivores should lead to the evolution of enhanced performance through resource reallocation. However, there has been limited empirical support for this hypothesis (Colautti et al., 2009; Felker-Quinn et al., 2013) but see (Uesugi & Kessler, 2013). The shifting defence hypothesis proposes that specialist targeted defences should decline while generalist targeted defences should be maintained or even increase in response to invasion (Doorduin & Vrieling, 2011; Joshi & Vrieling, 2005; Müller-Schärer & Steinger, 2004). While others have proposed reductions in constitutive defences and a corresponding increase in induced defences (Koricheva et al., 2004). In meta-analyses of common garden experiments comparing native and introduced populations, defence related traits were broadly found to diverge during invasion, but support for specific theories was limited (Bossdorf et al., 2005; Colautti et al., 2009; Felker-Quinn et al., 2013). Our genomic analysis is consistent with selection on defence related genes during invasion and may reflect a shift in the composition of enemies experienced during invasion in each range. As further evidence for the evolution of defence related traits

occurring repeatedly during introduction, we found parallel patterns of divergence for aphid damage in our common garden data (Chapter 3), which could be driven by changes in plant defence traits. Experiments are underway to investigate the glucosinolate composition of these samples, an important class of secondary metabolite in the family (Benett and Wallsgrove, 1994, Tsunoda et al., 2017) to further characterise the evolution of defence related traits.

In addition to divergence in aphid damage, we also identified divergence in germination, flowering time and size at flowering among the ranges (Chapter 3). Although flowering time genes were not over-represented, we did identify many flowering time genes in these parallel windows (Table 4-S14). Twelve were identified in *C. edentula*, including a homolog of TERMINAL FLOWER 1 (TFL1) in *C. edentula* a key repressor of flowering time in *Arabidopsis* that is also involved in the degree of indeterminate/determinate growth (Moraes et al 2019), Vernalization independence 4 (VIP4), which regulates flowering through the vernalization pathway, and FLC a major repressor of flowering in *Arabidopsis* (Deng et al., 2011). In *C. maritima* we identified 3 flowering time genes in outlier windows, including ESD4 and FCA, which both regulate the major flowering time repressor FLC. The window with FCA also showed evidence of a sweep in both introduced ranges and was associated with the onset of seed. Finally, several other terms related to biotic and abiotic stress, including response to salt, jasmonic acid metabolic process, salicylic acid metabolic process, two key plant hormones involved in stress response (Khan et al., 2015; Raza et al., 2021) were identified in our invasion adaptation candidate windows, potentially reflecting adaptation to environmental change experienced during invasion.

4.5.4 Future directions

The case study of *Cakile* has great promise to both provide insight in the evolutionary significance of hybridization generally, and its importance, more specifically, during invasion. We have pointed out above several future avenues for analysis of our rich whole genome resequencing data, as well as new research that could be key to addressing questions raised from our current analysis. However, there are many more avenues that this research can take to shed light on the impact of hybridization during the invasion. Theory suggests that regions of the genome that are not introgressed will harbor incompatibilities or a high number of additive deleterious alleles in the introgressing species (Harris & Nielsen, 2016; Juric et al., 2016). Therefore, in addition to regions of low recombination, it is likely that genic regions of

the genome will generally show a depletion of introgressions in older hybrids, as adaptive introgression should influence only a small portion of the genome (Racimo et al., 2015). The results of our GO enrichment tests point to both defence and edaphic factors as important contributors to adaptive divergence. In the future it could be important to include edaphic factors in our environmental data (e.g., salinity, nutrient concentrations) and target traits and common garden environments to investigate the ecological and evolutionary relevance of these patterns. Additionally, metagenomic analysis of field collected samples (both historic and modern), have been fruitful in identifying the spatial and temporal changes in pathogens in ragweed (Bieker et al., 2022) and this approach might also be applied to *Cakile*. A population genomic analysis of herbarium samples will also be informative in characterizing hybridization rates over time and identifying genomic regions under selection. Finally, field experiments using artificial hybrids will be critical for disentangling the impacts of range expansion, the history of hybridization and selection across the genome.

Author contributions

HSR conducted sampling, the greenhouse experiment and bioinformatic analysis. CL and JW conducted molecular laboratory work. KAH and PB helped with data analysis. JW annotated the reference genome. AMG, KHC and RDC conducted sampling. LHR, RDC and KAH conceived and helped design study. HSR wrote the manuscript with contributions from PB, RDC and KAH.

4.6 Appendix III

Table 4-S1. Traits used in genome wide association study, program EMMAX.

Trait	Description
Individual Seedling size	Measured in [cm] before planting
Growth rate	Stem length measured in [cm] for first 8 weeks after planting
SLA	Specific Leaf Area [leaf area/leaf mass]
Leaf shape PC1-PC4	Leaf shape Principal components values by the R package MOMOCS
Fruit weight	Average weight of 3 fruits (were applicable) per plant in [g]
Fruit shape PC1-PC4	Fruit shape Principal components values by the R package MOMOCS
Onset branching	Date of the first branching
Onset bud	Date of the first bud development
Onset open flower	Date of the first open flower
Onset seed	Date of the first seed onset
Biovolume bud	Biovolume at onset of bud
Biovolume open flower	Biovolume at onset of open flower
Above-ground biomass	Above-ground biomass [g] at harvest
Below-ground biomass	Below-ground biomass [g] at harvest
Total reproductive count	Count of pedicals, buds, flowers and seed at harvest
Pollen viability	Count of viable pollen
Aphid damage	Categorisation of aphid damage. 1- light damage, 2-medium damage ,3- severe damage, 4- death

3792 Table 4-S2. TopGO enrichment analysis of GWAS *C. edentula* outlier windows (all unique EMMAX *C. edentula*).

3793

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0030026	cellular manganese ion homeostasis	21	18	10.26	0.00052
GO:0042538	hyperosmotic salinity response	104	67	50.82	0.00055
GO:0010074	maintenance of meristem identity	93	46	45.45	0.0016
GO:0090110	cargo loading into COPII-coated vesicle	19	16	9.29	0.0016
GO:0010114	response to red light	100	62	48.87	0.0029
GO:0006355	regulation of transcription, DNA-template	3091	1602	1510.57	0.0038
GO:0009624	response to nematode	139	84	67.93	0.0040
GO:0006457	protein folding	329	168	160.78	0.0047
GO:0042753	positive regulation of circadian rhythm	14	12	6.84	0.0051
GO:0010584	pollen exine formation	52	32	25.41	0.0056
GO:1902977	mitotic DNA replication preinitiation complex assembly	7	7	3.42	0.0067
GO:1900087	positive regulation of G/S transition of mitotic cell cycle	7	7	3.42	0.0067
GO:0006279	premeiotic DNA replication	7	7	3.42	0.0067
GO:0031938	regulation of chromatin silencing at telomere	7	7	3.42	0.0067
GO:0010042	response to manganese ion	12	8	5.86	0.0067
GO:0019253	reductive pentose-phosphate cycle	29	22	14.17	0.0069
GO:0048280	vesicle fusion with Golgi apparatus	19	15	9.29	0.0074
GO:0006949	syncytium formation	26	20	12.71	0.0084
GO:0002238	response to molecule of fungal origin	34	25	16.62	0.0087
GO:0010022	meristem determinacy	53	37	25.9	0.0089
GO:0006972	hyperosmotic response	118	78	57.67	0.0090
GO:0032786	positive regulation of DNA-templated transcription, elongation	66	36	32.25	0.0091
GO:0010345	suberin biosynthetic process	36	25	17.59	0.010
GO:0006401	RNA catabolic process	221	115	108	0.012
GO:0000380	alternative mRNA splicing, via spliceosome	80	46	39.1	0.012
GO:0006415	translational termination	29	21	14.17	0.012
GO:0032876	negative regulation of DNA endoreduplication	18	14	8.8	0.012
GO:0045040	protein import into mitochondrial outer membrane	18	14	8.8	0.012
GO:0010227	floral organ abscission	34	21	16.62	0.013
GO:1902464	regulation of histone H3-K27 trimethylation	8	8	3.91	0.014

GO:1900871	chloroplast mRNA modification	6	6	2.93	0.014
GO:0009102	biotin biosynthetic process	6	6	2.93	0.014
GO:0009700	indole phytoalexin biosynthetic process	32	15	15.64	0.014
GO:0052544	defence response by callose deposition in cell wall	35	24	17.1	0.015
GO:0009911	positive regulation of flower development	65	41	31.77	0.015
GO:0035304	regulation of protein dephosphorylation	47	31	22.97	0.016
GO:0034389	lipid droplet organization	17	13	8.31	0.016
GO:0035436	triose phosphate transmembrane transport	9	8	4.4	0.017
GO:0043328	protein transport to vacuole involved in ubiquitin-dependent protein catabolic process via the multivesicular body sorting pathway	9	8	4.4	0.017
GO:0009723	response to ethylene	262	143	128.04	0.017
GO:0009828	plant-type cell wall loosening	64	40	31.28	0.019
GO:0009938	negative regulation of gibberellic acid mediated signaling pathway	17	13	8.31	0.020
GO:0000162	tryptophan biosynthetic process	22	16	10.75	0.020
GO:0045927	positive regulation of growth	67	35	32.74	0.020
GO:0009567	double fertilization forming a zygote and endosperm	75	41	36.65	0.021
GO:0010582	floral meristem determinacy	43	28	21.01	0.023
GO:0010117	photoprotection	14	11	6.84	0.024
GO:0006828	manganese ion transport	31	19	15.15	0.024
GO:0071249	cellular response to nitrate	19	14	9.29	0.025
GO:0090158	endoplasmic reticulum membrane organization	19	14	9.29	0.025
GO:0009646	response to absence of light	73	43	35.68	0.026
GO:0048527	lateral root development	206	105	100.67	0.027
GO:1900865	chloroplast RNA modification	17	15	8.31	0.028
GO:0055073	cadmium ion homeostasis	6	6	2.93	0.028
GO:0010422	regulation of brassinosteroid biosynthetic process	6	6	2.93	0.028
GO:1901601	strigolactone biosynthetic process	5	5	2.44	0.028
GO:0048838	release of seed from dormancy	5	5	2.44	0.028
GO:0033353	S-adenosylmethionine cycle	5	5	2.44	0.028
GO:0050891	multicellular organismal water homeostasis	5	5	2.44	0.028
GO:0042761	very long-chain fatty acid biosynthetic process	33	22	16.13	0.03
GO:0002237	response to molecule of bacterial origin	89	54	43.49	0.030
GO:0071585	detoxification of cadmium ion	12	10	5.86	0.030
GO:0006723	cuticle hydrocarbon biosynthetic process	8	7	3.91	0.030

GO:0010434	bract formation	8	7	3.91	0.030
GO:0030187	melatonin biosynthetic process	8	7	3.91	0.030
GO:0009555	pollen development	580	316	283.45	0.031
GO:0048229	gametophyte development	780	418	381.19	0.033
GO:0009733	response to auxin	545	284	266.34	0.034
GO:0031936	obsolete negative regulation of chromatin silencing	23	16	11.24	0.037
GO:0048278	vesicle docking	82	49	40.07	0.037
GO:2000031	regulation of salicylic acid mediated signaling pathway	42	25	20.53	0.038
GO:0006887	exocytosis	99	59	48.38	0.039
GO:0010218	response to far red light	67	42	32.74	0.039
GO:0015743	malate transport	24	17	11.73	0.039
GO:0080119	ER body organization	13	10	6.35	0.039
GO:0034497	protein localization to phagophore assembly site	13	10	6.35	0.039
GO:0090615	mitochondrial mRNA processing	21	14	10.26	0.039
GO:0006906	vesicle fusion	80	52	39.1	0.042
GO:0010583	response to cyclopentenone	61	37	29.81	0.043
GO:0009910	negative regulation of flower development	109	61	53.27	0.043
GO:0032502	developmental process	5363	2691	2620.9	0.043
GO:0048443	stamen development	165	86	80.64	0.043
GO:0030968	endoplasmic reticulum unfolded protein response	45	26	21.99	0.046
GO:0043067	regulation of programmed cell death	113	68	55.22	0.047
GO:0061817	endoplasmic reticulum-plasma membrane tethering	20	14	9.77	0.047
GO:0034059	response to anoxia	12	10	5.86	0.047
GO:0006750	glutathione biosynthetic process	10	8	4.89	0.047
GO:0007033	vacuole organization	133	73	65	0.048
GO:0006261	DNA-dependent DNA replication	254	134	124.13	0.048
GO:0009408	response to heat	428	216	209.16	0.048
GO:0015693	magnesium ion transport	26	19	12.71	0.049
GO:0010088	phloem development	20	14	9.77	0.050
GO:0006880	intracellular sequestering of iron ion	15	11	7.33	0.050

3794

3795 Table 4-S3. TopGo enrichment analysis of *C. maritima* GWAS outlier windows (all unique *C. maritima* outliers).

3796

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0048366	leaf development	701	454	430.88	0.00038
GO:0045324	late endosome to vacuole transport	61	49	37.49	0.00057
GO:0019438	aromatic compound biosynthetic process	4424	2724	2719.3	0.0011
GO:1901002	positive regulation of response to salt stress	13	13	7.99	0.0018
GO:0016567	protein ubiquitination	953	614	585.78	0.0042
GO:0033617	mitochondrial cytochrome c oxidase assembly	16	15	9.83	0.0046
GO:0016558	protein import into peroxisome matrix	20	15	12.29	0.0047
GO:0009736	cytokinin-activated signaling pathway	98	69	60.24	0.0068
GO:0045787	positive regulation of cell cycle	86	60	52.86	0.0077
GO:0006260	DNA replication	279	179	171.49	0.011
GO:1900055	regulation of leaf senescence	67	48	41.18	0.011
GO:0010052	guard cell differentiation	30	24	18.44	0.011
GO:0019915	lipid storage	42	33	25.82	0.012
GO:0043967	histone H4 acetylation	16	14	9.83	0.013
GO:0006729	tetrahydrobiopterin biosynthetic process	9	9	5.53	0.013
GO:0006814	sodium ion transport	23	18	14.14	0.016
GO:0009880	embryonic pattern specification	34	26	20.9	0.017
GO:0000373	Group II intron splicing	31	25	19.05	0.019
GO:0010187	negative regulation of seed germination	44	34	27.05	0.020
GO:0032979	protein insertion into mitochondrial inner membrane from matrix	8	8	4.92	0.020
GO:0031648	protein destabilization	8	8	4.92	0.020
GO:0006955	immune response	638	374	392.16	0.021
GO:0010087	phloem or xylem histogenesis	167	114	102.65	0.021
GO:0010608	posttranscriptional regulation of gene expression	381	248	234.19	0.022
GO:2000070	regulation of response to water deprivation	60	46	36.88	0.022
GO:0071277	cellular response to calcium ion	16	14	9.83	0.024
GO:0033356	UDP-L-arabinose metabolic process	23	19	14.14	0.025
GO:0006531	aspartate metabolic process	13	12	7.99	0.025
GO:0006103	2-oxoglutarate metabolic process	12	11	7.38	0.025
GO:0046685	response to arsenic-containing substance	24	20	14.75	0.026
GO:0000302	response to reactive oxygen species	268	166	164.73	0.029
GO:0010628	positive regulation of gene expression	938	609	576.56	0.029

GO:0051301	cell division	466	291	286.44	0.033
GO:1902977	mitotic DNA replication preinitiation complex assembly	7	7	4.3	0.033
GO:1900087	positive regulation of G1/S transition of mitotic cell cycle	7	7	4.3	0.033
GO:0044154	histone H3-K14 acetylation	7	7	4.3	0.033
GO:0060145	RNAi-mediated antiviral immune response	7	7	4.3	0.033
GO:0006279	premeiotic DNA replication	7	7	4.3	0.033
GO:0090436	leaf pavement cell development	7	7	4.3	0.033
GO:0031938	obsolete regulation of chromatin silencing at telomere	7	7	4.3	0.033
GO:0010113	negative regulation of systemic acquired resistance	7	7	4.3	0.033
GO:0010265	SCF complex assembly	7	7	4.3	0.033
GO:0015804	neutral amino acid transport	17	12	10.45	0.033
GO:0009832	plant-type cell wall biogenesis	296	197	181.94	0.034
GO:0009910	negative regulation of flower development	109	77	67	0.035
GO:0010411	xyloglucan metabolic process	70	49	43.03	0.036
GO:0048268	clathrin coat assembly	22	18	13.52	0.036
GO:0000162	tryptophan biosynthetic process	22	18	13.52	0.036
GO:0006796	phosphate-containing compound metabolic process	3083	1828	1895.03	0.037
GO:1900865	chloroplast RNA modification	17	15	10.45	0.037
GO:0006048	UDP-N-acetylglucosamine biosynthetic process	11	10	6.76	0.037
GO:0043433	negative regulation of DNA-binding transcription factor activity	11	10	6.76	0.037
GO:0006654	phosphatidic acid biosynthetic process	11	10	6.76	0.037
GO:0010321	regulation of vegetative phase change	11	10	6.76	0.037
GO:1900457	regulation of brassinosteroid mediated signaling pathway	34	24	20.9	0.037
GO:0010337	regulation of salicylic acid metabolic process	34	24	20.9	0.037
GO:0061408	positive regulation of transcription from RNA polymerase II promoter in response to heat stress	38	29	23.36	0.040
GO:0006075	(1->3)-beta-D-glucan biosynthetic process	27	21	16.6	0.041
GO:0010104	regulation of ethylene-activated signaling pathway	44	34	27.05	0.043
GO:0006906	vesicle fusion	80	59	49.17	0.044
GO:0009911	positive regulation of flower development	65	47	39.95	0.045
GO:0010200	response to chitin	170	115	104.49	0.046
GO:0010215	cellulose microfibril organization	31	24	19.05	0.047
GO:0045927	positive regulation of growth	67	48	41.18	0.049
GO:0030968	endoplasmic reticulum unfolded protein response	45	33	27.66	0.049

Table 4-S4. Comparison of outlier windows between species (*C. edentula* vs *C. maritima*) of GWAS results.

Phenotype	Number of overlapping windows
Above-ground biomass	19
Aphid damage	24
Below-ground biomass	36
Biovolume at bud onset	15
Biovolume at open flower onset	24
Onset of branching	26
Onset of bud	49
Total number of flowers	20
Fruit shape PC1	33
Fruit shape PC2	33
Fruit shape PC3	35
Fruit shape PC4	5
Fruit weight	35
Growth rate	30
Leaf shape PC1	27
Leaf shape PC2	21
Leaf shape PC3	46
Leaf shape PC4	20
Onset of open flower	31
Pollen viability	33
Onset of seeds	20
Seedling size	21
SLA	21

Table 4-S5. TopGo enrichment analysis for outliers of climate adaptation candidates (BayPass species range runs (X^TX and BF)). Run, GO.ID term for the Biological process annotation, annotation description and p-value are presented. AUS= Australia, wNA= western North America, EU= Europe, eNA= eastern North America, E= *C. edentul*a, M= *C. maritima*.

Run	GO.ID	Term	Annotated	Significant	Expected	p-value
AUS_E	GO:0010942	positive regulation of cell death	44	2	0.04	0.00087
	GO:0009816	defence response to bacterium	87	2	0.09	0.0034
	GO:0000461	endonucleolytic cleavage to generate mature 3'-end of SSU-rRNA from (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	4	1	0	0.0040
	GO:0009727	detection of ethylene stimulus	5	1	0	0.0049
	GO:0009626	plant-type hypersensitive response	107	2	0.11	0.0050
	GO:0009817	defence response to fungus	123	2	0.12	0.0066
	GO:0000447	endonucleolytic cleavage in ITS1 to separate SSU-rRNA from 5.8S rRNA and LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	8	1	0.01	0.0079
	GO:0010222	stem vascular tissue pattern formation	12	1	0.01	0.012
	GO:0009854	oxidative photosynthetic carbon pathway	14	1	0.01	0.014
	GO:0001709	cell fate determination	16	1	0.02	0.016
	GO:0050665	hydrogen peroxide biosynthetic process	17	1	0.02	0.017
	GO:0000290	deadenylation-dependent decapping of nuclear-transcribed mRNA	19	1	0.02	0.019
	GO:0019827	stem cell population maintenance	116	2	0.11	0.022
	GO:0010078	maintenance of root meristem identity	23	1	0.02	0.023
	GO:0033962	P-body assembly	26	1	0.03	0.025
	GO:0019685	photosynthesis, dark reaction	30	1	0.03	0.029
	GO:0006364	rRNA processing	415	3	0.41	0.030
	GO:0000373	Group II intron splicing	31	1	0.03	0.030
	GO:0010215	cellulose microfibril organization	31	1	0.03	0.030
	GO:0050829	defence response to Gram-negative bacterium	38	1	0.04	0.037
	GO:0002758	innate immune response-activating signalling pathway	45	1	0.04	0.044
eNA_E	GO:0043328	protein transport to vacuole involved in ubiquitin-dependent protein catabolic process via the multivesicular body sorting pathway	9	4	0.3	0.00014
	GO:0010039	response to iron ion	35	7	1.18	0.00014
	GO:1904222	positive regulation of serine C-palmitoyltransferase activity	2	2	0.07	0.0011
	GO:0010345	suberin biosynthetic process	36	6	1.21	0.0012

GO:0035336	long-chain fatty-acyl-CoA metabolic process	16	4	0.54	0.0017
GO:0045842	positive regulation of mitotic metaphase/anaphase transition	9	3	0.3	0.0028
GO:0051665	membrane raft localization	3	2	0.1	0.0033
GO:0002939	tRNA N1-guanine methylation	4	2	0.13	0.0065
GO:0002757	immune response-activating signal transduction	49	3	1.65	0.0065
GO:0000725	recombinational repair	177	7	5.97	0.0065
GO:0010233	phloem transport	32	5	1.08	0.0085
GO:0016127	sterol catabolic process	5	2	0.17	0.011
GO:1902479	positive regulation of defence response to bacterium	5	2	0.17	0.011
GO:0034434	sterol esterification	5	2	0.17	0.011
GO:0006075	(1->3)-beta-D-glucan biosynthetic process	27	4	0.91	0.012
GO:0009651	response to salt stress	748	39	25.24	0.013
GO:0052324	plant-type cell wall cellulose biosynthetic process	28	4	0.94	0.015
GO:0006621	protein retention in ER lumen	16	3	0.54	0.015
GO:0007188	adenylate cyclase-modulating G protein-coupled receptor signaling pathway	8	3	0.27	0.016
GO:0080165	callose deposition in phloem sieve plate	6	2	0.2	0.016
GO:0090549	response to carbon starvation	6	2	0.2	0.016
GO:0009687	abscisic acid metabolic process	55	4	1.86	0.021
GO:0048533	sporocyte differentiation	7	2	0.24	0.021
GO:0090153	regulation of sphingolipid biosynthetic process	7	2	0.24	0.021
GO:0034551	mitochondrial respiratory chain complex III assembly	7	2	0.24	0.021
GO:0006891	intra-Golgi vesicle-mediated transport	84	7	2.83	0.023
GO:0042546	cell wall biogenesis	386	17	13.02	0.025
GO:0018345	protein palmitoylation	51	5	1.72	0.028
GO:1902289	negative regulation of defence response to oomycetes	8	2	0.27	0.028
GO:0000492	box C/D snoRNP assembly	8	2	0.27	0.028
GO:0009414	response to water deprivation	606	29	20.45	0.031
GO:0071731	response to nitric oxide	11	2	0.37	0.034
GO:0044648	histone H3-K4 dimethylation	1	1	0.03	0.034
GO:0010378	temperature compensation of the circadian clock	1	1	0.03	0.034
GO:0031535	plus-end directed microtubule sliding	1	1	0.03	0.034
GO:0047496	vesicle transport along microtubule	1	1	0.03	0.034
GO:0010094	specification of carpel identity	1	1	0.03	0.034
GO:2000604	negative regulation of secondary growth	1	1	0.03	0.034

	GO:0080114	positive regulation of glycine hydroxymethyltransferase activity	1	1	0.03	0.034
	GO:0019216	regulation of lipid metabolic process	139	7	4.69	0.035
	GO:0010375	stomatal complex patterning	23	3	0.78	0.035
	GO:2000582	obsolete positive regulation of microtubule motor activity, plus-end-directed	9	2	0.3	0.035
	GO:0070979	protein K11-linked ubiquitination	9	2	0.3	0.035
	GO:0006572	tyrosine catabolic process	9	2	0.3	0.035
	GO:0006122	mitochondrial electron transport, ubiquinol to cytochrome c	22	3	0.74	0.037
	GO:0009639	response to red or far red light	333	13	11.23	0.037
	GO:0070534	protein K63-linked ubiquitination	23	3	0.78	0.041
	GO:0016117	carotenoid biosynthetic process	39	4	1.32	0.042
	GO:0000919	cell plate assembly	27	3	0.91	0.043
	GO:0010236	plastoquinone biosynthetic process	10	2	0.34	0.043
	GO:0080144	amino acid homeostasis	10	2	0.34	0.043
	GO:0042147	retrograde transport, endosome to Golgi	57	5	1.92	0.043
	GO:2000067	regulation of root morphogenesis	24	3	0.81	0.046
	GO:0006559	L-phenylalanine catabolic process	24	3	0.81	0.046
	GO:0045892	negative regulation of transcription, DNA-templated	466	22	15.72	0.048
	GO:0007623	circadian rhythm	246	15	8.3	0.049
wNA_E	GO:0034497	protein localization to phagophore assembly site	13	3	0.24	0.0015
	GO:0010106	cellular response to iron ion starvation	15	3	0.27	0.0023
	GO:0046856	phosphatidylinositol dephosphorylation	42	4	0.77	0.0071
	GO:0071712	ER-associated misfolded protein catabolic process	22	3	0.4	0.0072
	GO:0045943	positive regulation of transcription by RNA polymerase I	8	2	0.15	0.0087
	GO:0030187	melatonin biosynthetic process	8	2	0.15	0.0087
	GO:0006888	ER to Golgi vesicle-mediated transport	164	9	3	0.011
	GO:0048026	positive regulation of mRNA splicing, via spliceosome	10	2	0.18	0.014
	GO:0016024	CDP-diacylglycerol biosynthetic process	28	3	0.51	0.014
	GO:2000008	regulation of protein localization to cell surface	14	3	0.26	0.016
	GO:0071731	response to nitric oxide	11	2	0.2	0.018
	GO:0015741	fumarate transport	1	1	0.02	0.018
	GO:0010378	temperature compensation of the circadian clock	1	1	0.02	0.018
	GO:0009609	response to symbiotic bacterium	1	1	0.02	0.018
	GO:0035444	nickel cation transmembrane transport	1	1	0.02	0.018

GO:0032780	negative regulation of ATPase activity	1	1	0.02	0.018
GO:0031535	plus-end directed microtubule sliding	1	1	0.02	0.018
GO:0055068	cobalt ion homeostasis	1	1	0.02	0.018
GO:0006876	cellular cadmium ion homeostasis	1	1	0.02	0.018
GO:1900186	negative regulation of clathrin-dependent endocytosis	1	1	0.02	0.018
GO:0018215	protein phosphopantetheinylation	1	1	0.02	0.018
GO:0047496	vesicle transport along microtubule	1	1	0.02	0.018
GO:0046855	inositol phosphate dephosphorylation	31	3	0.57	0.019
GO:0042325	regulation of phosphorylation	183	7	3.35	0.019
GO:0009939	positive regulation of gibberellic acid mediated signaling pathway	12	2	0.22	0.020
GO:0045926	negative regulation of growth	45	4	0.82	0.023
GO:0010039	response to iron ion	35	3	0.64	0.026
GO:0010117	photoprotection	14	2	0.26	0.026
GO:0042759	long-chain fatty acid biosynthetic process	14	2	0.26	0.026
GO:0010311	lateral root formation	92	5	1.68	0.027
GO:0010345	suberin biosynthetic process	36	3	0.66	0.028
GO:0009738	abscisic acid-activated signaling pathway	339	14	6.2	0.030
GO:0018377	protein myristoylation	95	5	1.74	0.036
GO:0010335	response to non-ionic osmotic stress	2	1	0.04	0.036
GO:1902009	positive regulation of toxin transport	2	1	0.04	0.036
GO:0006982	response to lipid hydroperoxide	2	1	0.04	0.036
GO:0097510	base-excision repair, AP site formation via deaminated base removal	2	1	0.04	0.036
GO:2000035	regulation of stem cell division	2	1	0.04	0.036
GO:0070994	detection of oxidative stress	2	1	0.04	0.036
GO:0061388	regulation of rate of cell growth	2	1	0.04	0.036
GO:2000694	regulation of phragmoplast microtubule organization	2	1	0.04	0.036
GO:0071629	cytoplasm protein quality control by the ubiquitin-proteasome system	2	1	0.04	0.036
GO:0032467	positive regulation of cytokinesis	2	1	0.04	0.036
GO:1903648	positive regulation of chlorophyll catabolic process	2	1	0.04	0.036
GO:0000389	mRNA 3'-splice site recognition	2	1	0.04	0.036
GO:0043484	regulation of RNA splicing	95	6	1.74	0.038
GO:0006605	protein targeting	503	11	9.2	0.039
GO:0080155	regulation of double fertilization forming a zygote and endosperm	18	2	0.33	0.042
GO:0018230	peptidyl-L-cysteine S-palmitoylation	43	3	0.79	0.044

	GO:0060866	leaf abscission	19	2	0.35	0.047
	GO:0090158	endoplasmic reticulum membrane organization	19	2	0.35	0.047
	GO:0090110	cargo loading into COPII-coated vesicle	19	2	0.35	0.047
	GO:0010030	positive regulation of seed germination	45	3	0.82	0.049
AUS_M	GO:0035067	negative regulation of histone acetylation	14	6	1	0.00096
	GO:0045492	xylan biosynthetic process	49	8	3.5	0.0024
	GO:0044262	cellular carbohydrate metabolic process	620	51	44.31	0.0033
	GO:0009727	detection of ethylene stimulus	5	3	0.36	0.0033
	GO:1901651	regulation of mitotic chromosome decondensation	5	3	0.36	0.0033
	GO:0071158	positive regulation of cell cycle arrest	5	3	0.36	0.0033
	GO:0010154	fruit development	1312	101	93.77	0.0033
	GO:0071244	cellular response to carbon dioxide	10	4	0.71	0.0039
	GO:0071763	nuclear membrane organization	6	3	0.43	0.0062
	GO:0045717	negative regulation of fatty acid biosynthetic process	6	3	0.43	0.0062
	GO:0007154	cell communication	2716	181	194.12	0.0063
	GO:0009845	seed germination	285	26	20.37	0.0066
	GO:0043970	histone H3-K9 acetylation	11	5	0.79	0.010
	GO:0051645	Golgi localization	7	3	0.5	0.010
	GO:0009052	pentose-phosphate shunt, non-oxidative branch	7	3	0.5	0.010
	GO:0070417	cellular response to cold	43	8	3.07	0.010
	GO:0048868	pollen tube development	373	33	26.66	0.013
	GO:0009416	response to light stimulus	1236	99	88.34	0.015
	GO:0007091	metaphase/anaphase transition of mitotic cell cycle	35	5	2.5	0.015
	GO:1904143	positive regulation of carotenoid biosynthetic process	3	2	0.21	0.015
	GO:0010602	regulation of 1-aminocyclopropane-1-carboxylate metabolic process	3	2	0.21	0.015
	GO:0045604	regulation of epidermal cell differentiation	8	3	0.57	0.016
	GO:0000226	microtubule cytoskeleton organization	269	27	19.23	0.016
	GO:2000032	regulation of secondary shoot formation	22	5	1.57	0.018
	GO:0030001	metal ion transport	400	35	28.59	0.021
	GO:0009556	microsporogenesis	58	9	4.15	0.021
	GO:0035066	positive regulation of histone acetylation	11	4	0.79	0.022
	GO:0070979	protein K11-linked ubiquitination	9	3	0.64	0.022
	GO:0010230	alternative respiration	9	3	0.64	0.022

GO:0045962	positive regulation of development, heterochronic	9	3	0.64	0.022
GO:0046939	nucleotide phosphorylation	134	11	9.58	0.022
GO:0009742	brassinosteroid mediated signaling pathway	133	16	9.51	0.024
GO:0045087	innate immune response	618	44	44.17	0.026
GO:0051301	cell division	466	44	33.31	0.026
GO:0009826	unidimensional cell growth	623	59	44.53	0.027
GO:0042814	monopolar cell growth	13	3	0.93	0.028
GO:0009769	photosynthesis, light harvesting in photosystem II	4	2	0.29	0.028
GO:0002240	response to molecule of oomycetes origin	4	2	0.29	0.028
GO:0006370	7-methylguanosine mRNA capping	4	2	0.29	0.028
GO:0060151	peroxisome localization	4	2	0.29	0.028
GO:0032025	response to cobalt ion	4	2	0.29	0.028
GO:0006283	transcription-coupled nucleotide-excision repair	4	2	0.29	0.028
GO:0036292	DNA rewinding	4	2	0.29	0.028
GO:0009423	chorismate biosynthetic process	17	4	1.22	0.029
GO:0030050	vesicle transport along actin filament	25	5	1.79	0.030
GO:0010091	trichome branching	52	8	3.72	0.030
GO:0009808	lignin metabolic process	130	18	9.29	0.031
GO:2000652	regulation of secondary cell wall biogenesis	41	6	2.93	0.035
GO:0030154	cell differentiation	1063	89	75.97	0.036
GO:0042274	ribosomal small subunit biogenesis	135	15	9.65	0.039
GO:0042549	photosystem II stabilization	11	3	0.79	0.039
GO:0006879	cellular iron ion homeostasis	52	9	3.72	0.042
GO:0048658	anther wall tapetum development	30	5	2.14	0.042
GO:0090158	endoplasmic reticulum membrane organization	19	4	1.36	0.042
GO:0071215	cellular response to abscisic acid stimulus	385	30	27.52	0.044
GO:0000303	response to superoxide	34	6	2.43	0.044
GO:0048478	replication fork protection	5	2	0.36	0.044
GO:0007142	male meiosis II	5	2	0.36	0.044
GO:0009729	detection of brassinosteroid stimulus	5	2	0.36	0.044
GO:0010226	response to lithium ion	5	2	0.36	0.044
GO:0071475	cellular hyperosmotic salinity response	5	2	0.36	0.044
GO:0042593	glucose homeostasis	48	4	3.43	0.044

EU_M	GO:0001682	tRNA 5'-leader removal	8	4	0.34	0.0002
	GO:0000388	spliceosome conformational change to release U4 (or U4atac) and U1 (or U11)	4	3	0.17	0.0003
	GO:0008380	RNA splicing	428	32	18.31	0.0012
	GO:0006606	protein import into nucleus	86	10	3.68	0.0015
	GO:0043144	snoRNA processing	19	4	0.81	0.0018
	GO:0009653	anatomical structure morphogenesis	1774	77	75.91	0.0024
	GO:0048283	indeterminate inflorescence morphogenesis	3	2	0.13	0.0053
	GO:0019676	ammonia assimilation cycle	9	3	0.39	0.0054
	GO:0048026	positive regulation of mRNA splicing, via spliceosome	10	3	0.43	0.0075
	GO:0010023	proanthocyanidin biosynthetic process	10	3	0.43	0.0075
	GO:0051762	sesquiterpene biosynthetic process	19	4	0.81	0.0077
	GO:0006342	chromatin silencing	143	10	6.12	0.0088
	GO:0010942	positive regulation of cell death	44	5	1.88	0.0099
	GO:0009769	photosynthesis, light harvesting in photosystem II	4	2	0.17	0.010
	GO:0033194	response to hydroperoxide	4	2	0.17	0.010
	GO:0046719	regulation by virus of viral protein levels in host cell	4	2	0.17	0.010
	GO:0034968	histone lysine methylation	105	8	4.49	0.013
	GO:1900424	regulation of defence response to bacterium	87	6	3.72	0.013
	GO:0080188	RNA-directed DNA methylation	35	5	1.5	0.016
	GO:0010623	programmed cell death involved in cell development	13	3	0.56	0.016
	GO:2000630	positive regulation of miRNA metabolic process	5	2	0.21	0.017
	GO:0033353	S-adenosylmethionine cycle	5	2	0.21	0.017
	GO:0009641	shade avoidance	34	6	1.45	0.018
	GO:0046685	response to arsenic-containing substance	24	4	1.03	0.018
	GO:0006333	chromatin assembly or disassembly	100	5	4.28	0.018
	GO:0009834	plant-type secondary cell wall biogenesis	121	12	5.18	0.021
	GO:0010218	response to far red light	67	7	2.87	0.024
	GO:0006303	double-strand break repair via nonhomologous end joining	15	3	0.64	0.024
	GO:0034090	maintenance of meiotic sister chromatid cohesion	10	3	0.43	0.024
	GO:0048573	photoperiodism, flowering	212	10	9.07	0.024
	GO:0000132	establishment of mitotic spindle orientation	6	2	0.26	0.025
	GO:2000636	positive regulation of primary miRNA processing	6	2	0.26	0.025
	GO:0080165	callose deposition in phloem sieve plate	6	2	0.26	0.025
	GO:1990481	mRNA pseudouridine synthesis	6	2	0.26	0.025

GO:0010114	response to red light	100	9	4.28	0.028
GO:0002229	defence response to oomycetes	92	8	3.94	0.028
GO:0010225	response to UV-C	16	3	0.68	0.029
GO:0017148	negative regulation of translation	76	8	3.25	0.029
GO:0045893	positive regulation of transcription, DNA-templated	811	37	34.7	0.030
GO:0071472	cellular response to salt stress	56	6	2.4	0.032
GO:0046856	phosphatidylinositol dephosphorylation	42	5	1.8	0.033
GO:0009816	defence response to bacterium	87	8	3.72	0.033
GO:0019079	viral genome replication	15	3	0.64	0.033
GO:0010387	COP9 signalosome assembly	7	2	0.3	0.033
GO:0019419	sulfate reduction	7	2	0.3	0.033
GO:0006349	regulation of gene expression by genetic imprinting	29	4	1.24	0.034
GO:0018230	peptidyl-L-cysteine S-palmitoylation	43	5	1.84	0.036
GO:0006612	protein targeting to membrane	124	9	5.31	0.036
GO:0030154	cell differentiation	1063	51	45.49	0.037
GO:0032876	negative regulation of DNA endoreduplication	18	3	0.77	0.040
GO:0006006	glucose metabolic process	85	6	3.64	0.042
GO:0044648	histone H3-K4 dimethylation	1	1	0.04	0.043
GO:0046833	positive regulation of RNA export from nucleus	1	1	0.04	0.043
GO:0060733	GCN2-mediated signaling	1	1	0.04	0.043
GO:0097552	mitochondrial double-strand break repair via homologous recombination	1	1	0.04	0.043
GO:0070544	histone H3-K36 dimethylation	1	1	0.04	0.043
GO:0080003	thalianol metabolic process	1	1	0.04	0.043
GO:0060567	negative regulation of DNA-templated transcription, termination	1	1	0.04	0.043
GO:0042794	plastid rRNA transcription	1	1	0.04	0.043
GO:0018215	protein phosphopantetheinylation	1	1	0.04	0.043
GO:1903646	positive regulation of chaperone-mediated protein folding	1	1	0.04	0.043
GO:0000495	box H/ACA RNA 3'-end processing	1	1	0.04	0.043
GO:1900091	regulation of raffinose biosynthetic process	1	1	0.04	0.043
GO:0016576	histone dephosphorylation	1	1	0.04	0.043
GO:1900088	regulation of inositol biosynthetic process	1	1	0.04	0.043
GO:0071291	cellular response to selenium ion	1	1	0.04	0.043
GO:0071264	positive regulation of translational initiation in response to starvation	1	1	0.04	0.043
GO:0035513	oxidative RNA demethylation	1	1	0.04	0.043

	GO:1901177	lycopene biosynthetic process	1	1	0.04	0.043
	GO:0052889	9,9'-di-cis-zeta-carotene desaturation to 7,9,7',9'-tetra-cis-lycopene	1	1	0.04	0.043
	GO:2000605	positive regulation of secondary growth	1	1	0.04	0.043
	GO:0080114	positive regulation of glycine hydroxymethyltransferase activity	1	1	0.04	0.043
	GO:0015680	protein maturation by copper ion transfer	1	1	0.04	0.043
	GO:0016480	negative regulation of transcription by RNA polymerase III	1	1	0.04	0.043
	GO:0090677	reversible differentiation	1	1	0.04	0.043
	GO:1902448	positive regulation of shade avoidance	8	2	0.34	0.043
	GO:0007095	mitotic G2 DNA damage checkpoint signaling	8	2	0.34	0.043
	GO:0010581	regulation of starch biosynthetic process	8	2	0.34	0.043
	GO:0046244	salicylic acid catabolic process	8	2	0.34	0.043
	GO:0000103	sulfate assimilation	32	4	1.37	0.046
wNA_M	GO:0070898	RNA polymerase III preinitiation complex assembly	3	3	0.2	0.00028
	GO:0000381	regulation of alternative mRNA splicing, via spliceosome	62	12	4.04	0.00058
	GO:0019276	UDP-N-acetylgalactosamine metabolic process	4	3	0.26	0.0011
	GO:0010236	plastoquinone biosynthetic process	10	4	0.65	0.0028
	GO:0048507	meristem development	39	32.21	0.00292	
	GO:0006048	UDP-N-acetylglucosamine biosynthetic process	11	4	0.72	0.0041
	GO:1904222	positive regulation of serine C-palmitoyltransferase activity	2	2	0.13	0.0043
	GO:0034462	small-subunit processome assembly	2	2	0.13	0.0043
	GO:0010569	regulation of double-strand break repair via homologous recombination	12	4	0.78	0.0058
	GO:0052544	defence response by callose deposition in cell wall	35	7	2.28	0.0066
	GO:0043086	negative regulation of catalytic activity	121	13	7.89	0.0078
	GO:0055047	generative cell mitosis	7	3	0.46	0.0079
	GO:0042147	retrograde transport, endosome to Golgi	57	9	3.72	0.011
	GO:0045292	mRNA cis splicing, via spliceosome	56	9	3.65	0.012
	GO:2000306	positive regulation of photomorphogenesis	8	3	0.52	0.012
	GO:0033499	galactose catabolic process via UDP-galactose	8	3	0.52	0.012
	GO:1902448	positive regulation of shade avoidance	8	3	0.52	0.012
	GO:0019216	regulation of lipid metabolic process	139	12	9.06	0.012
	GO:0006968	cellular defence response	13	3	0.85	0.012
	GO:0097577	sequestering of iron ion	18	3	1.17	0.012

GO:0034971	histone H3-R17 methylation	3	2	0.2	0.012
GO:0000902	cell morphogenesis	810	56	52.82	0.014
GO:1902290	positive regulation of defence response to oomycetes	15	4	0.98	0.014
GO:0042325	regulation of phosphorylation	183	16	11.93	0.017
GO:0006417	regulation of translation	180	15	11.74	0.017
GO:0071108	protein K48-linked deubiquitination	16	4	1.04	0.017
GO:0071277	cellular response to calcium ion	16	4	1.04	0.017
GO:0009099	valine biosynthetic process	17	4	1.11	0.022
GO:0009555	pollen development	580	49	37.82	0.023
GO:0045931	positive regulation of mitotic cell cycle	34	6	2.22	0.023
GO:0070483	detection of hypoxia	4	2	0.26	0.023
GO:0006275	regulation of DNA replication	84	8	5.48	0.023
GO:0032456	endocytic recycling	10	3	0.65	0.023
GO:0009682	induced systemic resistance	47	9	3.06	0.024
GO:0006406	mRNA export from nucleus	49	8	3.2	0.028
GO:0010200	response to chitin	170	18	11.09	0.029
GO:0010239	chloroplast mRNA processing	11	3	0.72	0.031
GO:0007030	Golgi organization	57	8	3.72	0.031
GO:0030163	protein catabolic process	895	81	58.36	0.032
GO:0002758	innate immune response-activating signal transduction	45	6	2.93	0.032
GO:0010161	red light signaling pathway	19	4	1.24	0.032
GO:0090110	cargo loading into COPII-coated vesicle	19	4	1.24	0.032
GO:0009845	seed germination	285	30	18.59	0.032
GO:0030433	ubiquitin-dependent ERAD pathway	68	9	4.43	0.032
GO:0016998	cell wall macromolecule catabolic process	28	5	1.83	0.033
GO:0000469	cleavage involved in rRNA processing	41	7	2.67	0.037
GO:0000480	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	5	2	0.33	0.037
GO:0000472	endonucleolytic cleavage to generate mature 5'-end of SSU-rRNA from (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	5	2	0.33	0.037
GO:0010254	nectary development	5	2	0.33	0.037
GO:0016255	attachment of GPI anchor to protein	5	2	0.33	0.037
GO:0071457	cellular response to ozone	5	2	0.33	0.037
GO:2000022	regulation of jasmonic acid mediated signaling pathway	81	10	5.28	0.038

	GO:0006730	one-carbon metabolic process	47	7	3.06	0.038
	GO:0051123	RNA polymerase II preinitiation complex assembly	25	4	1.63	0.039
	GO:0006564	L-serine biosynthetic process	12	3	0.78	0.039
	GO:0070536	protein K63-linked deubiquitination	12	3	0.78	0.039
	GO:0006826	iron ion transport	37	6	2.41	0.043
	GO:0006357	regulation of transcription by RNA polymerase II	575	47	37.5	0.044
	GO:0009097	isoleucine biosynthetic process	21	4	1.37	0.044
	GO:0031347	regulation of defence response	429	39	27.98	0.046
	GO:0007015	actin filament organization	184	16	12	0.046
	GO:0006904	vesicle docking involved in exocytosis	31	5	2.02	0.048
	GO:0080119	ER body organization	13	3	0.85	0.048
	GO:0019264	glycine biosynthetic process from serine	13	3	0.85	0.048
	GO:0006565	L-serine catabolic process	13	3	0.85	0.048
	GO:1904482	cellular response to tetrahydrofolate	13	3	0.85	0.048
	GO:0019464	glycine decarboxylation via glycine cleavage system	13	3	0.85	0.048

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3806 Table 4-S6. Flowering time genes in climate adaptation candidates.

3807

Group	TAIR10	alternative names	Annotation
AUS_E	/		
wNA_E	AT1G22770	Protein GIGANTEA	gigantea protein (GI)
	AT2G44680	/	casein kinase II beta subunit 4
	AT3G18990	REDUCED VERNALIZATION RESPONSE 1	AP2/B3-like transcriptional factor family protein
	AT5G03840	TERMINAL FLOWER 1, TFL-1, TFL1	PEBP (phosphatidylethanolamine-binding protein) family protein
	AT5G10140	AGAMOUS-LIKE 25, AGL25, FLC, FLF, FLOWERING LOCUS C, FLOWERING LOCUS F, REDUCED STEM BRANCHING 6, RSB6	K-box region and MADS-box transcription factor family protein
	AT5G64610	HAM1	histone acetyltransferase of the MYST family 1
	AT1G05830	ARABIDOPSIS TRITHORAX 2	trithorax-like protein 2
	AT1G22770	Protein GIGANTEA	gigantea protein (GI)
	AT1G35460	ATCFL1 ASSOCIATED PROTEIN 2	basic helix-loop-helix (bHLH) DNA-binding superfamily protein
	AT1G79460	Ent-kaur-16-ene synthase, chloroplastic	Terpenoid cyclases/Protein prenyltransferases superfamily

			protein
	AT2G18790	OUT OF PHASE 1	phytochrome B
	AT2G44680	/	casein kinase II beta subunit 4
	AT2G44950	HISTONE MONO-UBIQUITINATION 1	histone mono-ubiquitination 1
	AT3G28730	ATHMG, HIGH MOBILITY GROUP	high mobility group
	AT3G63010	ATGID1B, GA INSENSITIVE DWARF1B	alpha/beta-Hydrolases superfamily protein
	AT3G63070	HUA2 LIKE 3, HULK3, SL4	Tudor/PWWP/MBT domain-containing protein
	AT4G29830	VIP3	Transducin/WD40 repeat-like superfamily protein
	AT5G10140	AGAMOUS-LIKE 25, AGL25, FLC, FLF, FLOWERING LOCUS C, FLOWERING LOCUS F, REDUCED STEM BRANCHING 6, RSB6	K-box region and MADS-box transcription factor family protein
	AT5G11530	Protein EMBRYONIC FLOWER 1	embryonic flower 1 (EMF1)
	AT5G20320	ATDCL4, DCL4, DICER-LIKE 4	dicer-like 4
	AT5G60910	Floral homeotic protein AGL8	AGAMOUS-like 8
	AT5G61060	ATHDA5, HDA05, HDA5, HISTONE DEACETYLASE 5	histone deacetylase 5
	AT5G61150	VERNALIZATION INDEPENDENCE 4, VIP4	leo1-like family protein
eNA_E	AT1G05830	ARABIDOPSIS TRITHORAX 2	trithorax-like protein 2
	AT1G22770	Protein GIGANTEA	gigantea protein (GI)
	AT1G35460	ATCFL1 ASSOCIATED PROTEIN 2	basic helix-loop-helix (bHLH) DNA-binding superfamily protein
	AT1G79460	Ent-kaur-16-ene synthase, chloroplastic	Terpenoid cyclases/Protein prenyltransferases superfamily protein
	AT2G18790	OUT OF PHASE 1	phytochrome B
	AT2G44680	/	casein kinase II beta subunit 4
	AT2G44950	HISTONE MONO-UBIQUITINATION 1	histone mono-ubiquitination 1
	AT3G28730	ATHMG, HIGH MOBILITY GROUP	high mobility group
	AT3G63010	ATGID1B, GA INSENSITIVE DWARF1B	alpha/beta-Hydrolases superfamily protein
	AT3G63070	HUA2 LIKE 3, HULK3, SL4	Tudor/PWWP/MBT domain-containing protein
	AT4G29830	VIP3	Transducin/WD40 repeat-like superfamily protein
	AT5G10140	AGAMOUS-LIKE 25, AGL25, FLC, FLF, FLOWERING LOCUS C, FLOWERING LOCUS F, REDUCED STEM BRANCHING 6, RSB6	K-box region and MADS-box transcription factor family protein
	AT5G11530	Protein EMBRYONIC FLOWER 1	embryonic flower 1 (EMF1)
	AT5G20320	ATDCL4, DCL4, DICER-LIKE 4	dicer-like 4
	AT5G60910	Floral homeotic protein AGL8	AGAMOUS-like 8
	AT5G61060	ATHDA5, HDA05, HDA5, HISTONE DEACETYLASE 5	histone deacetylase 5
	AT5G61150	VERNALIZATION INDEPENDENCE 4, VIP4	leo1-like family protein

AUS_M	AT1G10120	ACTIVATOR FOR CELL ELONGATION 2	basic helix-loop-helix (bHLH) DNA-binding superfamily protein
	AT1G15550	ATGA3OX1, GA REQUIRING 4, GA3OX1, GA4	gibberellin 3-oxidase 1
	AT1G19330	AFR2, SAP30 FUNCTION-RELATED 2	
	AT1G78580	Alpha,alpha-trehalose-phosphate synthase (UDP-forming)	trehalose-6-phosphate synthase
	AT2G17290	AtCDPK3	Calcium-dependent protein kinase family protein
	AT2G28550	RAP2.7	related to AP2.7
	AT2G31650	ARABIDOPSIS TRITHORAX 1	homologue of trithorax
	AT2G42200	ATSPL9, SPL9	squamosa promoter binding protein-like 9
	AT2G44150	ASHH3	histone-lysine N-methyltransferase ASHH3
	AT3G01090	Non-specific serine/threonine protein kinase	SNF1 kinase homolog 10
	AT3G22380	/	time for coffee
	AT3G57300	Chromatin-remodeling ATPase INO80	INO80 ortholog
	AT3G57390	AGAMOUS-LIKE 18, AGL18	AGAMOUS-like 18
	AT3G57390	AGAMOUS-LIKE 18, AGL18	AGAMOUS-like 18
	AT4G16280	FCA, FLOWERING CONTROL LOCUS A	RNA binding;abscisic acid binding
	AT4G20400	Probable lysine-specific demethylase JMJ14	JUMONJI 14
	AT4G21200	GA 2-oxidase 8	gibberellin 2-oxidase 8
	AT4G21200	GA 2-oxidase 8	gibberellin 2-oxidase 8
	AT4G22140	hypothetical protein	PHD finger family protein / bromo-adjacent homology (BAH) domain-containing protein
	AT4G22140	hypothetical protein	PHD finger family protein / bromo-adjacent homology (BAH) domain-containing protein
	AT4G22950	Agamous-like MADS-box protein AGL19	AGAMOUS-like 19
	AT4G38960	B-BOX DOMAIN PROTEIN 19, BBX19	B-box type zinc finger family protein
	AT5G03840	TERMINAL FLOWER 1, TFL-1, TFL1	PEBP (phosphatidylethanolamine-binding protein) family protein
	AT5G09740	HAC11, HAG05, HAG5, HAM2, HISTONE ACETYLTRANSFERASE OF THE CBP FAMILY 11, HISTONE ACETYLTRANSFERASE OF THE GNAT/MYST SUPERFAMILY 5	histone acetyltransferase of the MYST family 2
	AT5G10140	AGAMOUS-LIKE 25, AGL25, FLC, FLF, FLOWERING LOCUS C, FLOWERING LOCUS F, REDUCED STEM BRANCHING 6, RSB6	K-box region and MADS-box transcription factor family protein
	AT5G17490	GRAS family protein 27	RGA-like protein 3
	AT5G17690	TERMINAL FLOWER 2	like heterochromatin protein (LHP1)
	AT5G40490	RNA-binding (RRM/RBD/RNP motifs) family protein	RNA-binding (RRM/RBD/RNP motifs) family protein
	AT5G60410	ATSIZ1, SAP AND MIZ1 DOMAIN- CONTAINING LIGASE1, SIZ1	DNA-binding protein with MIZ/SP-RING zinc finger, PHD-

			finger and SAP domain
	AT5G61060	ATHDA5, HDA05, HDA5, HISTONE DEACETYLASE 5	histone deacetylase 5
	AT5G61380	APRR1, ATTOC1, PRR1	CCT motif -containing response regulator protein
	AT5G61380	APRR1, ATTOC1, PRR1	CCT motif -containing response regulator protein
	AT5G61920	FLOWERING LOCUS C EXPRESSOR-LIKE 4	
	AT5G62040	BROTHER OF FT AND TFL1	PEBP (phosphatidylethanolamine-binding protein) family protein
	AT5G63960	EMBRYO DEFECTIVE 2780	DNA binding;nucleotide binding;nucleic acid binding;DNA-directed DNA polymerases;DNA-directed DNA polymerases
	AT5G64170	Night light-inducible and clock-regulated 1	dentin sialophosphoprotein-related
wNA_M	AT1G04400	AT-PHH1, ATCRY2, FHA, PHH1	cryptochrome 2
	AT1G05830	ARABIDOPSIS TRITHORAX 2	trithorax-like protein 2
	AT1G06040	B-BOX DOMAIN PROTEIN 24	B-box zinc finger family protein
	AT1G06040	B-BOX DOMAIN PROTEIN 24	B-box zinc finger family protein
	AT1G08970	HEME ACTIVATED PROTEIN 5C, NF-YC9	nuclear factor Y, subunit C9
	AT1G15550	ATGA3OX1, GA REQUIRING 4, GA3OX1, GA4	gibberellin 3-oxidase 1
	AT1G18450	ARP4	actin-related protein 4
	AT1G30970	Protein SUPPRESSOR OF FRI 4	zinc finger (C2H2 type) family protein
	AT1G78580	Alpha,alpha-trehalose-phosphate synthase (UDP-forming)	trehalose-6-phosphate synthase
	AT1G80070	ABNORMAL SUSPENSOR 2	Pre-mRNA-processing-splicing factor
	AT2G13540	ABA-hypersensitive protein 1	ARM repeat superfamily protein
	AT2G18790	OUT OF PHASE 1	phytochrome B
	AT3G20740	Protein FERTILIZATION-INDEPENDENT SEED 3	Transducin/WD40 repeat-like superfamily protein
	AT3G20810	/	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
	AT3G48430	Lysine-specific histone demethylase REF6	relative of early flowering 6
	AT3G49600	AtUBP26	ubiquitin-specific protease 26
	AT3G54560	H2A.Z, HISTONE H2A 11	histone H2A 11
	AT3G57230	AGL16	AGAMOUS-like 16
	AT3G57920	SPL15	squamosa promoter binding protein-like 15
	AT3G63070	HUA2 LIKE 3, HULK3, SL4	Tudor/PWWP/MBT domain-containing protein
	AT4G21200	GA 2-oxidase 8	gibberellin 2-oxidase 8
	AT4G23100	ROOT MERISTEMLESS 1	glutamate-cysteine ligase
	AT4G39100	SHORT LIFE	PHD finger family protein / bromo-adjacent homology (BAH) domain-containing protein

	AT5G03840	TERMINAL FLOWER 1, TFL-1, TFL1	PEBP (phosphatidylethanolamine-binding protein) family protein
	AT5G10140	AGAMOUS-LIKE 25, AGL25, FLC, FLF, FLOWERING LOCUS C, FLOWERING LOCUS F, REDUCED STEM BRANCHING 6, RSB6	K-box region and MADS-box transcription factor family protein
	AT5G13790	AGL15	AGAMOUS-like 15
	AT5G17690	TERMINAL FLOWER 2	like heterochromatin protein (LHP1)
	AT5G18240		myb-related protein 1
	AT5G23150	HUA2	Tudor/PWWP/MBT domain-containing protein
	AT5G60410	ATSIZ1, SAP AND MIZ1 DOMAIN- CONTAINING LIGASE1, SIZ1	DNA-binding protein with MIZ/SP-RING zinc finger, PHD-finger and SAP domain
	AT5G61380	APRR1, ATTOC1, PRR1	CCT motif -containing response regulator protein
	AT5G63470	NF-YC4, NUCLEAR FACTOR Y, SUBUNIT C4	nuclear factor Y, subunit C4
	AT5G64960	Cdc2-like protein kinase	cyclin dependent kinase group C2
EU_M	AT1G06040	B-BOX DOMAIN PROTEIN 24	B-box zinc finger family protein
	AT1G15550	ATGA3OX1, GA REQUIRING 4, GA3OX1, GA4	gibberellin 3-oxidase 1
	AT1G30040	ATGA2OX2, GA2OX2	gibberellin 2-oxidase
	AT2G30140	UDP-GLUCOSYL TRANSFERASE 87A2, UGT87A2	UDP-Glycosyltransferase superfamily protein
	AT2G46340	SPA1, SUPPRESSOR OF PHYA-105 1	SPA (suppressor of phyA-105) protein family
	AT3G20740	Protein FERTILIZATION-INDEPENDENT SEED 3	Transducin/WD40 repeat-like superfamily protein
	AT3G20810	/	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
	AT3G49660	ATWDR5A	Transducin/WD40 repeat-like superfamily protein
	AT4G00450	CRYPTIC PRECOCIOUS	RNA polymerase II transcription mediators
	AT4G20370	TSF, TWIN SISTER OF FT	PEBP (phosphatidylethanolamine-binding protein) family protein
	AT4G21200	GA 2-oxidase 8	gibberellin 2-oxidase 8
	AT4G23100	ROOT MERISTEMLESS 1	glutamate-cysteine ligase
	AT4G34530	CIB1	cryptochrome-interacting basic-helix-loop-helix 1
	AT5G03840	TERMINAL FLOWER 1	PEBP (phosphatidylethanolamine-binding protein) family protein
	AT5G04240	ELF6	Zinc finger (C2H2 type) family protein / transcription factor jumonji (jmj) family protein
	AT5G06600	ATUBP12	ubiquitin-specific protease 12
	AT5G09740	HAC11, HAG05, HAG5, HAM2, HISTONE ACETYLTRANSFERASE OF THE CBP FAMILY 11, HISTONE ACETYLTRANSFERASE OF THE GNAT/MYST SUPERFAMILY 5	histone acetyltransferase of the MYST family 2

AT5G23150	HUA2	Tudor/PWWP/MBT domain-containing protein
AT5G23730	EARLY FLOWERING BY OVEREXPRESSION 2	Transducin/WD40 repeat-like superfamily protein
AT5G37055	SERRATED LEAVES AND EARLY FLOWERING (SEF)	HIT-type Zinc finger family protein
AT5G42400	ARABIDOPSIS TRITHORAX-RELATED7	SET domain protein 25
AT5G64960	Cdc2-like protein kinase	cyclin dependent kinase group C2
AT5G67180	AP2-like ethylene-responsive transcription factor TOE3	target of early activation tagged (EAT) 3

Table 4-S7. Flowering time enrichment analysis.

Run	Group	Number of total genes in outlier windows	Number of flowering genes	p-value
Species range BayPass (X^TX and BF)	AUS_M	2348	36	0.72
	EU_M	1410	24	0.43
	wNA_M	2151	33	0.78
	AUS_E	34	0	/
	eNA_E	1168	17	1
	wNA_E	607	6	0.40
Cross range BayPass (X^TX)	AUS_M	1183	24	0.11
	wNA_M	1568	21	0.39
	AUS_E	1576	25	0.67
	wNA_E	1562	27	0.83
Cross range BayPass (X^TX) and H12	AUS_M	355	9	0.11
	wNA_M	575	7	0.86
	AUS_E	6	0	/
	wNA_E	52	0	/

Table 4-S8. Gene enrichment analysis for outliers for parallel climate adaptation candidates (Baypass X^TX and BF) within a species. Comparison (*C. maritima* in Australia, western North America and Europe; *C. edentula* in western and eastern North America; Australian *C. edentula* was excluded due to the low number of windows), GO.ID term for the biological process annotation, term, number of annotated, significant and expected genes and p-values are presented.

Comparison	GO.ID	Term	Annotated	Significant	Expected	p-value

C. maritima **(Australia, western North America, Europe)**	GO:0009686	gibberellin biosynthetic process	63	2	0.09	0.0036
	GO:0071158	positive regulation of cell cycle arrest	5	1	0.01	0.0071
	GO:0010729	positive regulation of hydrogen peroxide biosynthetic process	5	1	0.01	0.0071
	GO:0045717	negative regulation of fatty acid biosynthetic process	6	1	0.01	0.0085
	GO:0071480	cellular response to gamma radiation	6	1	0.01	0.0085
	GO:0046786	viral replication complex formation and maintenance	8	1	0.01	0.011
	GO:0070979	protein K11-linked ubiquitination	9	1	0.01	0.013
	GO:0000056	ribosomal small subunit export from nucleus	9	1	0.01	0.013
	GO:0035066	positive regulation of histone acetylation	11	1	0.02	0.016
	GO:0018258	protein O-linked glycosylation via hydroxyproline	13	1	0.02	0.018
	GO:0035067	negative regulation of histone acetylation	14	1	0.02	0.020
	GO:0000055	ribosomal large subunit export from nucleus	14	1	0.02	0.020
	GO:0010405	arabinogalactan protein metabolic process	15	1	0.02	0.021
	GO:0006880	intracellular sequestering of iron ion	15	1	0.02	0.021
	GO:0000724	double-strand break repair via homologous recombination	173	2	0.24	0.025
	GO:0017157	regulation of exocytosis	21	1	0.03	0.029
	GO:0000162	tryptophan biosynthetic process	22	1	0.03	0.031
	GO:0006620	posttranslational protein targeting to endoplasmic reticulum membrane	23	1	0.03	0.032
	GO:0002221	pattern recognition receptor signaling pathway	26	1	0.04	0.036
	GO:0031145	anaphase-promoting complex-dependent catabolic	27	1	0.04	0.038

	45	process				
	GO:0009913	epidermal cell differentiation	34	1	0.05	0.047
	GO:0007091	metaphase/anaphase transition of mitotic cell cycle	35	1	0.05	0.048
	GO:0010039	response to iron ion	35	1	0.05	0.048
***C. edentula* (eastern North America, western North America)**	**GO.ID**	**Term**	**Annotated**	**Significant**	**Expected**	**p-value**
	GO:2000008	regulation of protein localization to cell surface	14	2	0.06	0.0018
	GO:0080155	regulation of double fertilization forming a zygote and endosperm	18	2	0.08	0.0029
	GO:0090158	endoplasmic reticulum membrane organization	19	2	0.09	0.0033
	GO:0061817	endoplasmic reticulum-plasma membrane tethering	20	2	0.09	0.0036
	GO:0071731	response to nitric oxide	11	2	0.05	0.0045
	GO:0010378	temperature compensation of the circadian clock	1	1	0	0.0045
	GO:0047496	vesicle transport along microtubule	1	1	0	0.0045
	GO:0031535	plus-end directed microtubule sliding	1	1	0	0.0045
	GO:0006891	intra-Golgi vesicle-mediated transport	84	3	0.38	0.0065
	GO:0018377	protein myristoylation	95	2	0.43	0.0089
	GO:0097510	base-excision repair, AP site formation via deaminated base removal	2	1	0.01	0.009
	GO:1902009	positive regulation of toxin transport	2	1	0.01	0.009
	GO:0032258	protein localization by the Cvt pathway	3	1	0.01	0.013
	GO:0072657	protein localization to membrane	228	4	1.02	0.015

	GO:00182 30	peptidyl-L-cysteine S-palmitoylation	43	2	0.19	0.016
	GO:20003 78	negative regulation of reactive oxygen species metabolic process	4	1	0.02	0.018
	GO:00709 81	L-asparagine biosynthetic process	4	1	0.02	0.018
	GO:00001 83	chromatin silencing at rDNA	4	1	0.02	0.018
	GO:00800 40	positive regulation of cellular response to phosphate starvation	5	1	0.02	0.022
	GO:00509 92	dimethylallyl diphosphate biosynthetic process	5	1	0.02	0.022
	GO:19909 18	double-strand break repair involved in meiotic recombination	5	1	0.02	0.022
	GO:20000 12	regulation of auxin polar transport	52	2	0.23	0.023
	GO:00905 49	response to carbon starvation	6	1	0.03	0.027
	GO:00315 73	intra-S DNA damage checkpoint	6	1	0.03	0.027
	GO:00421 47	retrograde transport, endosome to Golgi	57	2	0.26	0.027
	GO:00062 25	UDP biosynthetic process	7	1	0.03	0.031
	GO:00302 42	autophagy of peroxisome	7	1	0.03	0.031
	GO:19010 06	ubiquinone-6 biosynthetic process	7	1	0.03	0.031
	GO:00183 45	protein palmitoylation	51	3	0.23	0.035
	GO:00197 45	pentacyclic triterpenoid biosynthetic process	8	1	0.04	0.035
	GO:00301 87	melatonin biosynthetic process	8	1	0.04	0.035
	GO:19022 89	negative regulation of defence response to oomycetes	8	1	0.04	0.035
	GO:00421 49	cellular response to glucose starvation	8	1	0.04	0.035
	GO:19900	ER to chloroplast lipid transport	8	1	0.04	0.035

	52					
	GO:0006890	retrograde vesicle-mediated transport, Golgi to endoplasmic reticulum	68	2	0.31	0.038
	GO:2000582	positive regulation of microtubule motor activity, plus-end-directed	9	1	0.04	0.040
	GO:0071732	cellular response to nitric oxide	10	1	0.04	0.044
	GO:0048573	photoperiodism, flowering	212	3	0.95	0.047

Table 4-S9. Gene annotation of parallel invasion candidates of *C. maritima*.

window	***Arabidopsis thaliana* gene**	***C.edentula* gene name**	**Scaffold**	**start**	**end**	**percentage identity**	**e-value**	**Description**	**GO-term**
Scaffold_6_15600000_15650000	AT1G48210	edentula10818-RA	Scaffold_6_	15641091	15643665	87.637	0	Protein kinase superfamily protein	GO:0005634;GO:0005886;GO:0006468
	AT1G48230	edentula10816-RA	Scaffold_6_	15598548	15601148	94.58	0	nodulin MtN21 /EamA-like transporter family protein	GO:0015297;GO:0008514;GO:0005768;GO:0005802;GO:0005886;GO:0005794;GO:0022857
Scaffold_9_14950000_15000000	AT3G42870	edentula25096-RA	Scaffold_9_	14970906	14973467	49.194	4.11E-33	/	GO:0003674;GO:0005739;GO:0008150
	AT5G44790	edentula25097-RA	Scaffold_9_	14983814	14989261	83.201	0	copper-exporting ATPase / responsive-to-antagonist 1 / copper-transporting ATPase (RAN1)	GO:0009723;GO:0009873;GO:0005794;GO:0043682;GO:0005507;GO:0005768;GO:0015662;GO:0005802;GO:0010119;GO:0005375
	AT5G44800	edentula25095-RA	Scaffold_9_	14950287	14960109	79.344	0	chromatin remodeling 4	GO:0042735;GO:0005515;GO:0005634;GO:0009506
Scaffold_7_29100000_29150000	AT3G51460	edentula33703-RA	Scaffold_7_	29116449	29118527	52.846	1.71E-30	Phosphoinositide phosphatase	GO:0005886;GO:0035619;GO:0048768;GO:00315

								family protein	20;GO:0046856;GO:0005783;GO:0090404;GO:0009611;GO:0005829;GO:0043812;GO:0009932;GO:0005739;GO:0009506
	AT4G14746	edentula33702-RA	Scaffold_7_	29100174	29102005	76.744	7.39E-101	/	GO:0031225;GO:0008150
Scaffold_7_30300000_30350000	AT4G14510	edentula33721-RA	Scaffold_7_	30327601	30331399	73.098	0	CRM family member 3B	GO:0048316;GO:0009507;GO:0000373;GO:0003729
Scaffold_7_51850000_51900000	AT4G13550	edentula34235-RA	Scaffold_7_	51868449	51874120	75.989	0	triglyceride lipases;triglyceride lipases	GO:0006629;GO:0016042;GO:0009507;GO:0004620;GO:0005576;GO:0016298;GO:0009408
Scaffold_9_17100000_17150000	/								
Scaffold_6_46700000_46750000	/								

Table 4-S10. TopGO enrichment analysis of the 42 parallel climate adaptation candidate windows in invasive *C. maritima* (BayPass and GWAS overlap).

GO.ID	Term	Annotated	Significant	Expected	p-value
GO:0010236	plastoquinone biosynthetic process	10	2	0.07	0.0022
GO:0046713	borate transport	15	2	0.11	0.0051
GO:0015741	fumarate transport	1	1	0.01	0.0072
GO:0015887	pantothenate transmembrane transport	1	1	0.01	0.0072
GO:1904183	regulation of pyruvate dehydrogenase activity	1	1	0.01	0.0072
GO:0098717	pantothenate import across plasma membrane	1	1	0.01	0.0072
GO:0000278	mitotic cell cycle	560	9	4.04	0.0087
GO:0051301	cell division	466	8	3.36	0.012
GO:0010335	response to non-ionic osmotic stress	2	1	0.01	0.014
GO:0034462	small-subunit processome assembly	2	1	0.01	0.014
GO:0006982	response to lipid hydroperoxide	2	1	0.01	0.014
GO:0015742	alpha-ketoglutarate transport	3	1	0.02	0.022
GO:0015744	succinate transport	3	1	0.02	0.022
GO:1990575	mitochondrial L-ornithine transmembrane	3	1	0.02	0.022

GO:0090352	regulation of nitrate assimilation	3	1	0.02	0.022
GO:0048451	petal formation	3	1	0.02	0.022
GO:0070898	RNA polymerase III preinitiation complex assembly	3	1	0.02	0.022
GO:0010247	detection of phosphate ion	3	1	0.02	0.022
GO:0008285	negative regulation of cell population proliferation	34	2	0.25	0.025
GO:0010039	response to iron ion	35	2	0.25	0.026
GO:0080156	mitochondrial mRNA modification	35	2	0.25	0.026
GO:0006351	transcription, DNA-templated	3307	31	23.86	0.028
GO:0046506	sulfolipid biosynthetic process	4	1	0.03	0.029
GO:1901347	negative regulation of secondary cell wall biogenesis	4	1	0.03	0.029
GO:0060151	peroxisome localization	4	1	0.03	0.029
GO:0006370	7-methylguanosine mRNA capping	4	1	0.03	0.029
GO:0001944	vasculature development	4	1	0.03	0.029
GO:0030418	nicotianamine biosynthetic process	4	1	0.03	0.029
GO:0007010	cytoskeleton organization	500	5	3.61	0.031
GO:0000480	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	5	1	0.04	0.036
GO:0000472	endonucleolytic cleavage to generate mature 5'-end of SSU-rRNA from (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	5	1	0.04	0.036
GO:0010493	Lewis a epitope biosynthetic process	5	1	0.04	0.036
GO:0071457	cellular response to ozone	5	1	0.04	0.036
GO:1901651	regulation of mitotic chromosome decondensation	5	1	0.04	0.036
GO:0008615	pyridoxine biosynthetic process	5	1	0.04	0.036
GO:0016192	vesicle-mediated transport	835	9	6.02	0.036
GO:0070417	cellular response to cold	43	2	0.31	0.039
GO:0010154	fruit development	1312	11	9.47	0.042
GO:0015994	chlorophyll metabolic process	131	3	0.95	0.042
GO:0033468	CMP-keto-3-deoxy-D-manno-octulosonic aciacid biosynthetic process	6	1	0.04	0.043
GO:0032889	regulation of vacuole fusion, non-autophagic	6	1	0.04	0.043
GO:0006435	threonyl-tRNA aminoacylation	6	1	0.04	0.043
GO:0009304	tRNA transcription	6	1	0.04	0.043
GO:0006896	Golgi to vacuole transport	49	2	0.35	0.049
GO:0045492	xylan biosynthetic process	49	2	0.35	0.049
GO:0006406	mRNA export from nucleus	49	2	0.35	0.049

GO:0006636	unsaturated fatty acid biosynthetic process	49	2	0.35	0.049
GO:0015746	citrate transport	7	1	0.05	0.049
GO:0051645	Golgi localization	7	1	0.05	0.049
GO:0010113	negative regulation of systemic acquired resistance	7	1	0.05	0.049
GO:0090436	leaf pavement cell development	7	1	0.05	0.049

Table 4-S11. Gene annotation of 12 candidates for parallel adaptive introgression. Window, *Arabidopsis thaliana* and *C. edentula* gene name, Scaffold, start and end as well as percentage identity, e-value, description and GO term are presented.

Window	**Trait association *C. edentula***	**Trait association *C. maritima***	**Environmental variables (BF) in each range group**	**gene name**	**Tair ID**	**GO terms**	**Tair gene description**
Scaffold_1_:48700000-48750000		Biovolume bud, fruit weight, aphid damage, onset bud, onset seed	AUS_MH-bio12,14,17,18	GOX3	AT4G18360	GO:0042742;GO:0050665;GO:0052854;GO:0052852;GO:0005777;GO:0010181;GO:0052853;GO:0008891;GO:0009854;GO:0055114;GO:0016491	Encodes a glycolate oxidase that modulates reactive oxygen species-mediated signal transduction during nonhost resistance.
			EU-bio15	NA	AT4G18372	GO:0031417;GO:0005634;GO:0003674;GO:0008150	Small nuclear ribonucleoprotein family protein;(source:Araport11)
			wNA_MH-bio7				
Scaffold_2_:1200000-1250000	Fruit shape PC3, fruit weight	Fruit weight, SLA	AUS_MH-bio1-18	NA	AT4G19450	GO:0003674;GO:0008150;GO:0016020;GO:0005739	Major facilitator superfamily protein;(source:Araport11)
			wNA_MH-bio101-6,8-11,14,15,17,18	NA	AT4G19460	GO:0009507;GO:0016757	UDP-Glycosyltransferase superfamily protein;(source:Araport11)
				ATVPS54	AT4G19490	GO:0000938;GO:0006896;GO:0000139;GO:0042147;GO:0005794;GO:0019905;GO:0005515	Putative homolog of yeast Vps54. Thought to associate with POK and ATVPS53 in a plant GARP-like complex involved in the membrane trafficking system.

				NA	AT4G19510	GO:0009507;GO:0043531;GO:0005737;GO:0050135;GO:0061809;GO:0007165	Disease resistance protein (TIR-NBS-LRR class);(source:Araport11)
				CYCT1;4	AT4G19600	GO:0008024;GO:0016538;GO:0032786;GO:0009615;GO:0061575;GO:0048366;GO:0005634;GO:0010090;GO:0006357;GO:0042025	Encodes a cyclin T partner CYCT1;4. Plays important roles in infection with Cauliflower mosaic virus (CaMV). The mRNA is cell-to-cell mobile.
Scaffold_2_:70800000-70850000		Aphid damage, fruit shape PC3	AUS_MH-bio1,3,8,10,12,14,15,17,18	no annotation	/	/	
			wNA_MH-bio1,2,5,7-11	no annotation	/	/	
Scaffold_3_:0-50000	Biovolume bud, Biovolume open flower, Above ground biomass, below ground biomass	Fruit weight, seedling size, below ground biomass, aphid damage, fruit shape PC1, leaf shape PC1	AUS_MH-bio2,3,12,14,15,17,18	NA	AT3G63050		hypothetical protein;(source:Araport11)
			wNA_E-bio19	NA	AT5G01010	GO:0005739	retinal-binding protein;(source:Araport11)
			wNA_MH-bio1-11,14,15,17,18	RAE1	AT5G01720	GO:0019005;GO:0006511;GO:0031146;GO:0005634;GO:0016567	RAE1 is an F-box protein component of a SCF-type E3 ligase complex. It is part of an alumium induced regulatory loop: its activity is induced by STOP1 and it in turn ubiquitinates STOP1 which is then targeted for degradation.
				NA	AT5G01750	GO:0005737;GO:0005886;GO:0003674;GO:0005829	LURP-one-like protein (DUF567);(source:Araport11)

				TOL7	AT5G01760	GO:0015031;GO:0006886	Encodes a member of the Arabidopsis TOL (TOM1-LIKE) family of ubiquitin binding proteins that acts redundantly in the recognition and further endocytic sorting of a PIN-FORMED (PIN)-type auxin carrier protein at the plasma membrane, modulating dynamic auxin distribution and associated growth responses.
				NA	AT5G01920	GO:0042549;GO:0004672;GO:0009507;GO:0009579;GO:0005634;GO:0006468	Chloroplast thylakoid protein kinase STN8 is specific in phosphorylation of N-terminal threonine residues in D1, D2 and CP43 proteins, and Thr-4 in PsbH protein of photosystem II. Phosphorylation of Thr-4 in the wild type required both light and prior phosphorylation at Thr-2.
				ATMAN6	AT5G01930	GO:0016985;GO:0009845;GO:0005975;GO:0005576	Encodes a endo-beta-mannanase involved in seed germination.
				NA	AT5G01950	GO:0004672;GO:0005886;GO:0006468;GO:0016020;GO:0005515;GO:0016301;GO:0005524;GO:0009507	Leucine-rich repeat protein kinase family protein;(source:Araport11)
				AML4	AT5G07290	GO:0045836;GO:0000398;GO:0003729;GO:0048507;GO:0045927;GO:0003676;GO:0005634	AML4 A member of mei2-like gene family, predominantly plant-based family of genes encoding RNA binding proteins with characteristic presence of a highly conserved RNA binding motif first described in the mei2 gene of the fission yeast S. pombe. In silico analyses reveal nine mei2 -like genes in *A. thaliana*. They were grouped into four distinct clades, based on overall sequence similarity and subfamily-specific sequence elements. AML4 is a member of two sister clades of mei2-like gene family, AML1 through AML5, and belongs to the clade named ALM14. AML4 is expressed during embryo development (heart and torpedo stage) and in vegetative and floral apices.
Scaffold_3_:500	Biovolume	Fruit	AUS_MH-	FERR	AT5G01	GO:0009570;GO:000550	Encodes a ferretin protein that is targeted to the

00-100000	bud, Biovolume open flower, above ground biomass, below ground biomass	weight, seedling size, above ground biomass, below ground, aphid damage , fruit shape PC1,leafshape_PC1	bio3,12,14,15,17,18	ETIN 1	600	6;GO:0006880;GO:0009617;GO:0006826;GO:0015979;GO:0048366;GO:0009507;GO:0008198;GO:0009535;GO:0042542;GO:0010043;GO:0005739;GO:0055072;GO:0008199;GO:0009908;GO:0005737;GO:0000302;GO:0009409;GO:0005886;GO:0009579;GO:0004322;GO:0010039	chloroplast. Member of a Ferritin gene family. Gene expression is induced in response to iron overload and by nitric oxide. Expression of the gene is downregulated in the presence of paraquat, an inducer of photoxidative stress.
			EU-bio10 1,2,5,6,8,12-19	NA	AT5G01610	GO:0008150	hypothetical protein (Protein of unknown function, DUF538);(source:Araport11)
			wNA_E-bio19	NA	AT5G01620	GO:0045492;GO:0016413;GO:0005794;GO:1990538	Encodes a member of the TBL (TRICHOME BIREFRINGENCE-LIKE) gene family containing a plant-specific DUF231 (domain of unknown function) domain. TBL gene family has 46 members, two of which (TBR/AT5G06700 and TBL3/AT5G01360) have been shown to be involved in the synthesis and deposition of secondary wall cellulose, presumably by influencing the esterification state of pectic polymers. A nomenclature for this gene family has been proposed (Volker Bischoff & Wolf Scheible, 2010, personal communication).TBL35 are required only for xylan 3-O-monoacetylation and 2,3-di-O-acetylation. The biochemical phenotype can be observed in tbl35 esk1, double mutant and tbl34 tbl35 esk1 triple mutants.
			wNA_MH-bio1-11,14,15,17,18	ATBRCA2(V	AT5G01630	GO:0005515;GO:0006355;GO:0051321;GO:0005634;GO:0000724;GO:0005739	Ortholog of breast cancer susceptibility protein 2. Essential at meiosis. Interacts with with both Rad51 and Dss1(I) or both Dmc1 and Dss1(I) in a tripartite complex.
				PRA1.B5	AT5G01640	GO:0016192;GO:0005515;GO:0005794;GO:0005783;GO:0016020	prenylated RAB acceptor 1.B5;(source:Araport11)

				MIF/D-DT-LIKE 2	AT5G01650	GO:0009507;GO:0005829;GO:0003674	Chemokine-like MDL protein; modulate flowering time and innate immunity in plants.
				NA	AT5G01670	GO:0016491;GO:0055114;GO:0005737;GO:0005829;GO:0008106;GO:0004032	NAD(P)-linked oxidoreductase superfamily protein;(source:Araport11)
				ATCHX27	AT5G01690	GO:0012505;GO:0006885;GO:0006812;GO:0005451;GO:0015672;GO:0015385	member of Putative Na+/H+ antiporter family
				NA	AT5G01700	GO:0005634;GO:0006470;GO:0005829	Protein phosphatase 2C family protein;(source:Araport11)
				NA	AT5G01710	GO:0000138	methyltransferase;(source:Araport11)
				RAE1	AT5G01720	GO:0019005;GO:0006511;GO:0031146;GO:0005634;GO:0016567	RAE1 is an F-box protein component of a SCF-type E3 ligase complex. It is part of an alumium induced regulatory loop: its activity is induced by STOP1 and it in turn ubiquitinates STOP1 which is then targeted for degradation.
Scaffold_3_:100000-150000	Biovolume bud, Biovolume open flower, above ground biomass, below ground biomass	Fruit weight, seedling size, aphid damage, fruit shape PC1,leaf shape PC1	AUS_MH-bio3,14,15,17	RUS5	AT5G01510	GO:0009507;GO:0003674	root UVB sensitive protein (Protein of unknown function, DUF647);(source:Araport11)
			EU-bio5,10,13-17	ATAIRP2	AT5G01520	GO:0006511;GO:0004842;GO:0061630;GO:0005515;GO:0009737;GO:0009789;GO:0009651;GO:0005829;GO:0016567;GO:0005634	Encodes a cytosolic RING-type E3 ubiquitin (Ub) ligase that is critical for ABA and high salinity responses during germination. AtAIRP2 and SDIR1 likely play a combinatory role in ABA signaling and the response to high salt in Arabidopsis
			wNA_E-bio19	LECTIN	AT5G01540	GO:0004675;GO:0005515;GO:0042742;GO:0009	Encodes LecRKA4.1, a member of the lectin receptor kinase subfamily A4 (LecRKA4.1

				RECEPTOR KINASE A4.1		737;GO:0009738;GO:0002229;GO:0009845;GO:0006468;GO:0005886;GO:0005576;GO:0006952;GO:0002221;GO:0016301	At5g01540; LecRKA4.2 At5g01550; LecRKA4.3 At5g01560). Together with other members of the subfamily, functions redundantly in the negative regulation of ABA response in seed germination. Positively regulates pattern-triggered immunity.
			wNA_MH-bio1-11,14,15,17,18	OSH1	AT5G01580	GO:0016491;GO:0005576;GO:0008150	thiol reductase in OAS metabolism
				TIC56	AT5G01590	GO:0005515;GO:0009941;GO:0045037;GO:0009706;GO:0005634;GO:0009507;GO:0009536;GO:0008320;GO:0005622;GO:0005886	histone-lysine N-methyltransferase ATXR3-like protein;(source:Araport11)
Scaffold_3_:150000-200000	Biovolume bud, Biovolume open flower, above ground biomass, below ground biomass	Fruit weight, seedling size, aphid damage, fruit shape PC1	AUS_MH-bio3,12,14,15,17	NA	AT5G01110	GO:0005739	Tetratricopeptide repeat (TPR)-like superfamily protein;(source:Araport11)
			eNA-bio12,13,16	NA	AT5G01150	GO:0003674;GO:0008150;GO:0005575	hypothetical protein (DUF674);(source:Araport11)
			wNA_E-bio19	SQD2	AT5G01220	GO:0046506;GO:0008194;GO:0016036;GO:0009941;GO:0046510;GO:0005886;GO:0009507;GO:0009247;GO:0016757;GO:0009536	Encodes a UDP-sulfoquinovose:DAG sulfoquinovosyltransferase that is involved in sulfolipid biosynthesis and whose expression is responsive to both phosphate (Pi) and phosphite (Phi) in both roots and shoots.
			wNA_MH-bio101-6,8-11,14,15,17,18	NA	AT5G01260	GO:0005576;GO:2001070	Carbohydrate-binding-like fold;(source:Araport11)
				ATCPL2	AT5G01270	GO:0070940;GO:0006970;GO:0008420;GO:0005515;GO:0005634;GO:00	Encodes CPL2, a carboxyl-terminal domain (CTD) phosphatase that dephosphorylates CTD Ser5-PO4 of the RNA polymerase II complex.

			04647;GO:0010025;GO:0009733;GO:0045893;GO:0005737;GO:0016791;GO:0009734;GO:0048589	Regulates plant growth, stress and auxin responses.
	NA	AT5G01290	GO:0042025;GO:0008138;GO:0005634;GO:0004725;GO:0004651;GO:0006370;GO:0004484	mRNA capping enzyme family protein;(source:Araport11)
	ATMSFC1	AT5G01340	GO:0015744;GO:0031966;GO:0015746;GO:0015741;GO:0015141;GO:0006810;GO:0015742;GO:0005739;GO:0015743	Transports citrate, isocitrate and aconitate, succinate and fumarate. Catalyzes a fast counter-exchange transport as well as a low uniport of substrates, exhibits a higher transport affinity for tricarboxylates than dicarboxylates. Might be involved in storage oil mobilization 78 at early stages of seedling growth and in nitrogen assimilation in root tissue by 79 catalyzing citrate/isocitrate or citrate/succinate exchanges.
	TBL3	AT5G01360	GO:0045489;GO:0005794;GO:0045492;GO:0030244;GO:1990538;GO:0009827;GO:0016413;GO:0005634;GO:0005886	Encodes a member of the TBL (TRICHOME BIREFRINGENCE-LIKE) gene family containing a plant-specific DUF231 (domain of unknown function) domain. TBL gene family has 46 members, two of which (TBR/AT5G06700 and TBL3/AT5G01360) have been shown to be involved in the synthesis and deposition of secondary wall cellulose, presumably by influencing the esterification state of pectic polymers. A nomenclature for this gene family has been proposed (Volker Bischoff & Wolf Scheible, 2010, personal communication).The dwarf phenotype can only be seen in tbl3 tbl31 esk1 triple mutant. tbl3 and tbl31 are specifically involved in 3-O-monoacetylation of xylan.
	NA	AT5G01380	GO:0005515;GO:0006355;GO:0043565;GO:0005634;GO:0003700;GO:0042802	Homeodomain-like superfamily protein;(source:Araport11)

				ATPDX1	AT5G01410	GO:0010224;GO:0042823;GO:0008615;GO:0046982;GO:0010335;GO:0005886;GO:0005737;GO:0015994;GO:0042819;GO:0036381;GO:0005515;GO:0006520;GO:0042538;GO:0005829;GO:0042803;GO:0012505;GO:0009651;GO:0006982;GO:0006979;GO:0016843;GO:0009646	Encodes a protein predicted to function in tandem with PDX2 to form glutamine amidotransferase complex with involved in vitamin B6 biosynthesis.
				NA	AT5G01430	GO:0005886;GO:0016192;GO:0003674	Got1/Sft2-like vescicle transport protein family;(source:Araport11)
				APD2	AT5G01450	GO:0005515;GO:0009705;GO:0004842;GO:0016567;GO:0009555;GO:0005768;GO:0000278	RING/U-box superfamily protein;(source:Araport11)
				TAAC	AT5G01500	GO:0042651;GO:0010206;GO:0010117;GO:0055085;GO:0009941;GO:0005347;GO:0009526;GO:0009536;GO:0009507;GO:0005886;GO:0005739	encodes an ATP/ADP carrier that is located to the thylakoid membrane involved in providing ATP during thylakoid biogenesis and turnover The mRNA is cell-to-cell mobile.
				RUS5	AT5G01510	GO:0009507;GO:0003674	root UVB sensitive protein (Protein of unknown function, DUF647);(source:Araport11)
				IDL3	AT5G09805	GO:0010227;GO:0048046;GO:0005739	Similar to Inflorescence deficient in abscission (IDA). Involved in floral organ abscission.
Scaffold_3_:19000000-1950000	Biovolume bud, Onset seed, growth rate, leaf shape PC3	Fruit weight	AUS_MH-bio2,4,5,7	IDL3	AT5G09805	GO:0010227;GO:0048046;GO:0005739	Similar to Inflorescence deficient in abscission (IDA). Involved in floral organ abscission.
			EU-bio9	ACT7	AT5G09810	GO:0005829;GO:0009733;GO:0009506;GO:0005200;GO:0048767;GO:0005730;GO:0005739;GO:0048364;GO:0010053;G	Member of Actin gene family.Mutants are defective in germination and root growth. The mRNA is cell-to-cell mobile.

				O:0005856;GO:0005618;GO:0005886;GO:0009611;GO:0005515;GO:0009416;GO:0005737;GO:0051301;GO:0009941;GO:0009570;GO:0007010;GO:0009845	
	wNA_MH-bio8,12-19	FBN5	AT5G09820	GO:0009507;GO:0010236;GO:0005515;GO:0009570;GO:0008150	Encodes fibrillin 5 (FBN5). Located in chloroplast stroma. Essential for plastoquinone-9 biosynthesis. Stimulates enzymatic activity of solanesyl diphosphate synthases (SPS) 1 and 2 through binding to solanesyl moiety. Two splicing variants, named FBN5-A shorter one and FBN5-B longer one. FBN5-B is the protein detected in chloroplast stroma. Involved in plastoquinone biosynthesis.
		MED26C	AT5G09850	GO:0005634;GO:0005829	Transcription elongation factor (TFIIS) family protein;(source:Araport11)
		CESA5	AT5G09870	GO:0030244;GO:0005886;GO:0016759;GO:0005802;GO:0010192;GO:0016757;GO:0005794;GO:0009832;GO:0009833;GO:0016760;GO:0010583	Encodes a cellulose synthase CESA5 that produces seed mucilage cellulose.Mutants are defective in seed coat mucilage.Involved in the regulation of mucilage composition and/or mucilage synthesis.
		NA	AT5G09880	GO:0006397;GO:0005634	Splicing factor, CC1-like protein;(source:Araport11)
		NA	AT5G09940	GO:0003674;GO:0005739;GO:0008150	hypothetical protein (DUF1635);(source:Araport11)
		HEAT INTOLERANT 4	AT5G10010	GO:0060969;GO:0010286;GO:1901651;GO:1900034;GO:0009408;GO:0003674;GO:0005730;GO:0005737;GO:0010369	myosin-H heavy protein;(source:Araport11)
		SIRK1	AT5G10020	GO:0005886;GO:0006468;GO:0004672;GO:0005515;GO:0005829	Leucine-rich receptor-like protein kinase family protein;(source:Araport11)
		RPN5B	AT5G64760	GO:0005634;GO:0003674;GO:0008541;GO:0007275;GO:0030163;GO:00	Encodes one of two isoforms for the 26S proteasome regulatory protein (RN) subunit RPN5. For many functions it acts redundantly

					06511;GO:0043161;GO:0031595;GO:0000502;GO:0005737	with the paralogous genes RPN5a.
Scaffold_3_:10000000-1050000	Seedling size, leaf shape_PC2	AUS_MH-bio1-3,5-8,10,11,14,15,17	VPS60.1	AT3G10640	GO:0016192;GO:0005515;GO:0007034;GO:0032511;GO:0006900;GO:0005771;GO:0005737	SNF7 family protein;(source:Araport11)
		wNA_MH-bio1-3,5,6,8,9-11,14,15	AR2	AT4G30210	GO:0005783;GO:0009507;GO:0003958;GO:0050660;GO:0005886;GO:0009698;GO:0010181;GO:0005829;GO:0016709;GO:0016491	Encodes NADPH-cytochrome P450 reductase that catalyzes the first oxidative step of the phenylpropanoid general pathway. The mRNA is cell-to-cell mobile.
			ATCPK1	AT5G04870	GO:0005516;GO:0005778;GO:0004674;GO:0010941;GO:0005886;GO:0046777;GO:0005509;GO:0010857;GO:0006468;GO:0018105;GO:0016020;GO:1900055;GO:0004683;GO:0005515;GO:0005777;GO:0004672;GO:0005634;GO:0009931;GO:0005737;GO:0035556	A calcium-dependent protein kinase that can phosphorylate phenylalanine ammonia lyase (PAL), a key enzyme in pathogen defence.Phosphorylates, in vivo, the transcription factor ORE1, a master regulator of senescence.
			BGLC3	AT5G04885	GO:0009969;GO:0005886;GO:0008422;GO:0009251;GO:0031225	Encodes a beta-glucosidase involved in xyloglucan metabolism.
			NOL	AT5G04900	GO:0015996;GO:0005886;GO:0009507;GO:0005515;GO:0010304;GO:0034256	Encodes a chlorophyll b reducatase involved in the degradation of chlorophyll b and LHCII (light harvesting complex II).
			ALA1	AT5G04930	GO:0045332;GO:0016020;GO:0005886;GO:0015662;GO:0005515;GO:0140326;GO:0000287	Encodes a putative aminophospholipid translocase (p-type ATPase) involved in chilling response. It is targeted to the plasma membrane following association in the endoplasmic reticulum with an ALIS protein beta-subunit. The mRNA is cell-to-cell mobile.
			SUVH1	AT5G04940	GO:0005515;GO:0005634;GO:0031490;GO:0034	Encodes a SU(VAR)3-9 homolog, a SET domain protein. Known SET domain proteins

					968;GO:0001228;GO:0042054;GO:0040029;GO:0010385;GO:0008270	are involved in epigenetic control of gene expression and act as histone methyltransferases. There are 10 SUVH genes in Arabidopsis and members of this subfamily of the SET proteins have an additional conserved SRA domain. SUVH1 has been shown to have a preference for binding methylated DNA.
			ATNAS1	AT5G04950	GO:0009860;GO:0010233;GO:0009555;GO:0030418;GO:0030410	Encodes a nicotianamide synthase.
Scaffold_5_:1800000-1850000	SLA	AUS_MH-bio142,3,5,7,14,15,17	ADS1	AT1G06080	GO:0042761;GO:0016717;GO:0006636;GO:0005789;GO:0009979;GO:0005739	Encodes a protein homologous to delta 9 acyl-lipid desaturases of cyanobacteria and acyl-CoA desaturases of yeast and mammals. expression down-regulated by cold temperature. It is involved in the desaturation of VLCFAs to make monounsaturated VLCFAs.
		wNA_MH-bio2-4,14,15,17,18	MEF3	AT1G06140	GO:0080156;GO:0005739	Encodes MEF3 (mitochondrial editing factor 3), a PPR (pentatricopeptide repeat) protein of the E domain subclass. Functions in mitochondrial RNA editing.
			EMB1444	AT1G06150	GO:0006355;GO:0005634;GO:0003700;GO:0048364;GO:0046983	Encodes a LHW-like protein with 79% amino acid identity to LHW.
			ERF59	AT1G06160	GO:0006355;GO:0009753;GO:0009723;GO:0009861;GO:0009873;GO:0005634;GO:0003700;GO:0005622;GO:0003677	encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/AP2 transcription factor family. The protein contains one AP2 domain. There are 18 members in this subfamily including ATERF-1, ATERF-2, AND ATERF-5.
			BHLH089	AT1G06170	GO:0052543;GO:0048658;GO:0006355;GO:0009555;GO:0003700;GO:0046983;GO:0005634	Encodes a bHLH transcription factor that together with bHLH010 and bHLH091 is important for the normal transcriptome of the developing Arabidopsis anther, possibly by forming a feed-forward loop with DYT1. Recognizes the TCATGTGC box to activate the expression of target genes, including ATA20, EXL4, and MEE48.
			ATMY	AT1G06	GO:0009909;GO:000563	member of MYB3R- and R2R3- type MYB-

				B13	180	4;GO:0003700	encoding genes
				RHON 1	AT1G06 190	GO:0006353;GO:000950 7;GO:0009737;GO:1901 259;GO:0010239;GO:00 03729;GO:0005576;GO: 0043621;GO:0019843;G O:0005634;GO:0005515	Encodes a novel ribonucleic acid-binding protein that interacts with the endonuclease RNase E and supports its function in processing plastid ribonucleic acids.
				TOL2	AT1G06 210	GO:0043130;GO:000688 6;GO:0005794;GO:0005 737	Encodes a member of the Arabidopsis TOL (TOM1-LIKE) family of ubiquitin binding proteins that acts redundantly in the recognition and further endocytic sorting of a PIN-FORMED (PIN)-type auxin carrier protein at the plasma membrane, modulating dynamic auxin distribution and associated growth responses.
				CLE3	AT1G06 225	GO:0033612;GO:004804 6;GO:0045168;GO:0005 739	Member of a large family of putative ligands homologous to the Clavata3 gene. Consists of a single exon.
				GTE4	AT1G06 230	GO:0048364;GO:000563 4;GO:0045931;GO:0009 294	This gene is predicted to encode a bromodomain-containing protein. Plant lines expressing RNAi constructs targeted against GTE4 show some resistance to agrobacterium-mediated root transformation.
				NA	AT1G06 250	GO:0008970;GO:000573 7;GO:0006629;GO:0005 576	alpha/beta-Hydrolases superfamily protein;(source:Araport11)
				NA	AT3G24 610	GO:0003674;GO:000815 0;GO:0005634	Galactose oxidase/kelch repeat superfamily protein;(source:Araport11)
Scaffold_5_:294 50000-29500000	Fruit shape PC1, leaf shape PC3 & PC4	Fruit shape PC4, leaf shape PC3, onset open flower	AUS_MH-bio4,15,19	PAI1	AT1G07 780	GO:0006568;GO:000464 0;GO:0000162;GO:0005 634;GO:0009507	Encodes phosphoribosylanthranilate isomerase which catalyzes the third step of the tryptophan biosynthetic pathway. Member of gene family. The mRNA is cell-to-cell mobile.
			EU-bio8,10	APC1	AT5G05 560	GO:0005680;GO:003114 5;GO:0009553;GO:0010 252;GO:0009793;GO:00 06511;GO:0048481;GO:	Encodes a subunit of the Arabidopsis thaliana E3 ubiquitin ligase complex that plays a synergistic role with APC4 both in female gametogenesis and in embryogenesis.

					0000151;GO:0005634;GO:0007091;GO:0070979;GO:0060090	
		wNA_MH-bio1,3,4,6,7,11-13,15-19	TMS	AT5G05570	GO:0005634;GO:0005886;GO:0032259;GO:0016192;GO:0005096;GO:0016021;GO:0005737;GO:0008168;GO:0017157;GO:0003676;GO:0045159;GO:0017137;GO:0019905	transducin family protein / WD-40 repeat family protein;(source:Araport11)
			ATFAD8	AT5G05580	GO:0006636;GO:0009507;GO:0009941;GO:0005886;GO:0016717;GO:0009266;GO:0006633;GO:0042389;GO:0006629;GO:0055114	Encodes a temperature sensitive plastidic fatty acid desaturase.
			JAO2	AT5G05600	GO:2000022;GO:0120091;GO:0005737;GO:0080167;GO:0005829;GO:0097237;GO:0051213	Encodes a protein with similarity to flavonol synthases that is involved in the detoxifcation polycyclic aromatic hydrocarbons.One of 4 paralogs encoding a 2-oxoglutarate/Fe(II)-dependent oxygenases that hydroxylates JA to 12-OH-JA.
			EMB2789	AT5G05680	GO:0006606;GO:0006611;GO:0000055;GO:0017056;GO:0005643;GO:0000056;GO:0045087;GO:0009627;GO:0006406;GO:0005634;GO:0005635;GO:0005515	Encodes MOS7 (Modifier of snc1,7), homologous to human and Drosophila melanogaster nucleoporin Nup88. Resides at the nuclear envelope. Modulates the nuclear concentrations of certain defence proteins regulates defence outputs.
Scaffold_8_:16000000-1650000	Aphid damage , leaf shape PC3,branching onset	AUS_MH-bio2-5,7,9,14,15,17	DOF5.3	AT5G60200	GO:0000976;GO:0090057;GO:0006355;GO:0003700;GO:0001944;GO:0005634;GO:0048364	Encodes a Dof-type transcription factor. PEAR protein involved in the formation of a short-range concentration gradient that peaks at protophloem sieve elements, and activates gene expression that promotes radial growth. Locally promotes transcription of inhibitory HD-ZIP III genes, and thereby establishes a negative-feedback loop that forms a robust boundary that demarks the zone of cell division.

	wNA_MH-bio1-3,5-11,14,15,17,18	RIP5	AT5G60210	GO:0005576;GO:0005886	Encodes RIP5 (ROP interactive partner 5), a putative Rho protein effector, interacting specifically with the active form of ROPs (Rho proteins of plants).
		LECRK-I.7	AT5G60270	GO:0002229;GO:0004675;GO:0042742;GO:0005886;GO:0016301;GO:0006952;GO:0006468;GO:0005576	Concanavalin A-like lectin protein kinase family protein;(source:Araport11)
		NA	AT5G60330	/	
		SENESCENCE ASSOCIATED GENE 2	AT5G60360	GO:0051603;GO:0004197;GO:0008234;GO:0009723;GO:0005576;GO:0005764;GO:0006508;GO:0009536;GO:0007568;GO:0005773;GO:0099503;GO:0005615	Encodes a senescence-associated thiol protease. The mRNA is cell-to-cell mobile.
		EXONUCLEASE V-LIKE, EXOVL	AT5G60370	GO:0045145;GO:0005634;GO:0009507;GO:0036297	exonuclease V-like protein;(source:Araport11)
		NA	AT5G60390	GO:0048471;GO:0006412;GO:0006414;GO:0005886;GO:0005739;GO:0005634;GO:0003729;GO:0005773;GO:0009506;GO:0005515;GO:0005737;GO:0003746;GO:0003924	GTP binding Elongation factor Tu family protein;(source:Araport11)
		NA	AT5G60400	GO:0003674;GO:0008150;GO:0009507	hypothetical protein;(source:Araport11)
		ATSIZ1	AT5G60410	GO:0010183;GO:0040008;GO:0009910;GO:0019789;GO:0005634;GO:00	Encodes a plant small ubiquitin-like modifier (SUMO) E3 ligase that is a focal controller of Pi starvation-dependent responses. Also required

			10113;GO:0009787;GO:0009826;GO:0048481;GO:0090352;GO:0016036;GO:0010247;GO:0008270;GO:0010286;GO:0009870;GO:0009553;GO:0016925;GO:0009414;GO:0005515;GO:0010337;GO:2000070;GO:0050826;GO:0048589;GO:0051301	for SA and PAD4-mediated R gene signalling, which in turn confers innate immunity in Arabidopsis. Also involved in the regulation of plant growth, drought responses and freezing tolerance. This latter effect is most likely due to SIZ1 dependent ABI5 sumoylation. Regulates leaf cell division and expansion through salicylic acid accumulation. signaling
	AGAMOUS-LIKE 62	AT5G60440	GO:0003700;GO:0005634;GO:0000977;GO:2000012;GO:0005515;GO:0045944;GO:0009960;GO:0043565;GO:0000976;GO:0046983;GO:0008134;GO:0000981	AGL62 encodes a Type I MADS domain protein that likely functions as a transcription factor. It is expressed AGL62 is expressed exclusively in the endosperm. AGL62 supresses suppresses cellularization during the syncytial phase of endosperm development.
	AGAMOUS-LIKE 62	AT5G60440	GO:0003700;GO:0005634;GO:0000977;GO:2000012;GO:0005515;GO:0045944;GO:0009960;GO:0043565;GO:0000976;GO:0046983;GO:0008134;GO:0000981	AGL62 encodes a Type I MADS domain protein that likely functions as a transcription factor. It is expressed AGL62 is expressed exclusively in the endosperm. AGL62 supresses suppresses cellularization during the syncytial phase of endosperm development.

Table 4-S12. TopGo enrichment analysis for invasive adaptation candidates, GO.ID term for biological process, annotation and p-value are presented.

Run	GO.ID	Term	Annotated	Significant	Expected	p-value
Australia & eastern North America *C. edentula*	GO:0010182	sugar mediated signaling pathway	72	12	3.43	0.00015
	GO:0043201	response to leucine	4	3	0.19	0.00042
	GO:0080052	response to histidine	4	3	0.19	0.00042
	GO:0080053	response to phenylalanine	4	3	0.19	0.00042
	GO:0010118	stomatal movement	294	22	14.03	0.00051

	GO:0098542	defence response to other organism	1262	54	60.21	0.0034
	GO:0019745	pentacyclic triterpenoid biosynthetic process	8	3	0.38	0.0051
	GO:0006261	DNA-dependent DNA replication	254	20	12.12	0.0057
	GO:0016139	glycoside catabolic process	3	2	0.14	0.0066
	GO:0033234	negative regulation of protein sumoylation	3	2	0.14	0.0066
	GO:0071294	cellular response to zinc ion	3	2	0.14	0.0066
	GO:1904216	positive regulation of protein import in chloroplast stroma	3	2	0.14	0.0066
	GO:0044262	cellular carbohydrate metabolic process	620	33	29.58	0.0073
	GO:0048530	fruit morphogenesis	9	3	0.43	0.0073
	GO:0006401	RNA catabolic process	221	19	10.54	0.0091
	GO:0090359	negative regulation of abscisic acid biosynthetic process	4	2	0.19	0.013
	GO:0006747	FAD biosynthetic process	4	2	0.19	0.013
	GO:0042938	dipeptide transport	11	3	0.52	0.013
	GO:0071493	cellular response to UV-B	11	3	0.52	0.013
	GO:0043928	exonucleolytic catabolism of deadenylated mRNA	20	4	0.95	0.014
	GO:0010215	cellulose microfibril organization	31	5	1.48	0.015
	GO:2000032	regulation of secondary shoot formation	22	4	1.05	0.019
	GO:0009867	jasmonic acid mediated signaling pathway	192	13	9.16	0.020
	GO:0016127	sterol catabolic process	5	2	0.24	0.021
	GO:1990569	UDP-N-acetylglucosamine transmembrane transport	5	2	0.24	0.021
	GO:0036071	N-glycan fucosylation	5	2	0.24	0.021
	GO:0034434	sterol esterification	5	2	0.24	0.021
	GO:1990918	double-strand break repair involved in meiotic recombination	5	2	0.24	0.021
	GO:0042939	tripeptide transport	13	3	0.62	0.022
	GO:0010154	fruit development	1312	61	62.59	0.025
	GO:0009102	biotin biosynthetic process	6	2	0.29	0.030
	GO:0045717	negative regulation of fatty acid biosynthetic process	6	2	0.29	0.030
	GO:0033386	geranylgeranyl diphosphate biosynthetic process	6	2	0.29	0.030
	GO:0043100	pyrimidine nucleobase salvage	6	2	0.29	0.030
	GO:0032544	plastid translation	26	4	1.24	0.033
	GO:0006457	protein folding	329	17	15.7	0.037
	GO:0044255	cellular lipid metabolic process	1219	69	58.15	0.037
	GO:0010440	stomatal lineage progression	42	6	2	0.038

	GO:0009687	abscisic acid metabolic process	55	6	2.62	0.041
	GO:1901006	ubiquinone- biosynthetic process	7	2	0.33	0.041
	GO:0033615	mitochondrial proton-transporting ATP synthase complex assembly	7	2	0.33	0.041
	GO:0010617	circadian regulation of calcium ion oscillation	7	2	0.33	0.041
	GO:0009052	pentose-phosphate shunt, non-oxidative branch	7	2	0.33	0.041
	GO:0090693	plant organ senescence	257	13	12.26	0.041
	GO:0009863	salicylic acid mediated signaling pathway	88	5	4.2	0.043
	GO:0010629	negative regulation of gene expression	888	40	42.36	0.043
	GO:0080187	floral organ senescence	17	3	0.81	0.045
	GO:0042538	hyperosmotic salinity response	104	10	4.96	0.047
	GO:1903508	positive regulation of nucleic acid-templated transcription	812	44	38.74	0.048
	GO:0071731	response to nitric oxide	11	3	0.52	0.048
	GO:0006449	regulation of translational termination	6	2	0.29	0.048
	GO:2000488	positive regulation of brassinosteroid biosynthetic process	1	1	0.05	0.048
	GO:0051562	negative regulation of mitochondrial calcium ion concentration	1	1	0.05	0.048
	GO:0035444	nickel cation transmembrane transport	1	1	0.05	0.048
	GO:0006337	nucleosome disassembly	1	1	0.05	0.048
	GO:1904975	response to bleomycin	1	1	0.05	0.048
	GO:0071951	conversion of methionyl-tRNA to N-formyl-methionyl-tRNA	1	1	0.05	0.048
	GO:0042246	tissue regeneration	1	1	0.05	0.048
	GO:0006178	guanine salvage	1	1	0.05	0.048
	GO:0032263	GMP salvage	1	1	0.05	0.048
	GO:0007060	male meiosis chromosome segregation	1	1	0.05	0.048
	GO:0000448	cleavage in ITS2 between 5.8S rRNA and LSU-rRNA of tricistronic rRNA transcript (SSU-rRNA, 5.8rRNA, LSU-rRNA)	1	1	0.05	0.048
	GO:0032780	negative regulation of ATPase activity	1	1	0.05	0.048
	GO:2000685	positive regulation of cellular response to X-ray	1	1	0.05	0.048
	GO:2000604	negative regulation of secondary growth	1	1	0.05	0.048
	GO:0055068	cobalt ion homeostasis	1	1	0.05	0.048
	GO:0015843	methylammonium transport	1	1	0.05	0.048
	GO:0080127	fruit septum development	1	1	0.05	0.048

	GO:0046100	hypoxanthine metabolic process	1	1	0.05	0.048
	GO:0009609	response to symbiotic bacterium	1	1	0.05	0.048
	GO:1900186	negative regulation of clathrin-dependent endocytosis	1	1	0.05	0.048
	GO:0023052	signaling	2330	107	111.16	0.048
western & eastern North America *C. edentula*	GO:0010114	response to red light	100	14	4.64	0.00021
	GO:0043201	response to leucine	4	3	0.19	0.00038
	GO:0080052	response to histidine	4	3	0.19	0.00038
	GO:0080053	response to phenylalanine	4	3	0.19	0.00038
	GO:0010186	positive regulation of cellular defence response	5	3	0.23	0.00093
	GO:0010182	sugar mediated signaling pathway	72	10	3.34	0.0012
	GO:0042938	dipeptide transport	11	4	0.51	0.0012
	GO:0032968	positive regulation of transcription elongation from RNA polymerase II promoter	53	8	2.46	0.0029
	GO:0042939	tripeptide transport	13	4	0.6	0.0047
	GO:0006265	DNA topological change	25	5	1.16	0.0052
	GO:0019240	citrulline biosynthetic process	3	2	0.14	0.0062
	GO:0033234	negative regulation of protein sumoylation	3	2	0.14	0.0062
	GO:0090428	perianth development	3	2	0.14	0.0062
	GO:0048498	establishment of petal orientation	3	2	0.14	0.0062
	GO:0009638	phototropism	38	6	1.76	0.0076
	GO:0010236	plastoquinone biosynthetic process	10	3	0.46	0.0093
	GO:0009408	response to heat	428	34	19.84	0.011
	GO:0072387	flavin adenine dinucleotide metabolic process	10	4	0.46	0.012
	GO:0090359	negative regulation of abscisic acid biosynthetic process	4	2	0.19	0.012
	GO:0042789	mRNA transcription by RNA polymerase II	4	2	0.19	0.012
	GO:0006747	FAD biosynthetic process	4	2	0.19	0.012
	GO:0009058	biosynthetic process	7912	369	366.82	0.012
	GO:0010119	regulation of stomatal movement	184	15	8.53	0.012
	GO:0010218	response to far red light	67	8	3.11	0.016
	GO:0010244	response to low fluence blue light stimulus by blue low-fluence system	12	3	0.56	0.016
	GO:0009640	photomorphogenesis	165	14	7.65	0.017
	GO:0009751	response to salicylic acid	238	17	11.03	0.019

	GO:0018008	N-terminal peptidyl-glycine N-myristoylation	5	2	0.23	0.020
	GO:0048437	floral organ development	402	22	18.64	0.020
	GO:0006814	sodium ion transport	23	4	1.07	0.020
	GO:0006627	protein processing involved in protein targeting to mitochondria	13	3	0.6	0.020
	GO:0030001	metal ion transport	400	26	18.55	0.022
	GO:0042538	hyperosmotic salinity response	104	10	4.82	0.023
	GO:0043068	positive regulation of programmed cell death	33	5	1.53	0.023
	GO:0042742	defence response to bacterium	769	47	35.65	0.024
	GO:0000381	regulation of alternative mRNA splicing, via spliceosome	62	7	2.87	0.024
	GO:1902074	response to salt	36	5	1.67	0.024
	GO:0046294	formaldehyde catabolic process	14	3	0.65	0.025
	GO:0006468	protein phosphorylation	1582	91	73.35	0.025
	GO:0051170	import into nucleus	92	6	4.27	0.028
	GO:0042450	arginine biosynthetic process via ornithine	6	2	0.28	0.028
	GO:0070827	chromatin maintenance	6	2	0.28	0.028
	GO:0009102	biotin biosynthetic process	6	2	0.28	0.028
	GO:0016045	detection of bacteria	6	2	0.28	0.028
	GO:0031117	positive regulation of microtubule depolymerization	6	2	0.28	0.028
	GO:0006813	potassium ion transport	92	10	4.27	0.030
	GO:0045839	negative regulation of mitotic nuclear division	35	5	1.62	0.030
	GO:0010106	cellular response to iron ion starvation	15	3	0.7	0.030
	GO:0071805	potassium ion transmembrane transport	63	7	2.92	0.030
	GO:0061408	positive regulation of transcription from RNA polymerase II promoter in response to heat stress	38	5	1.76	0.030
	GO:0009737	response to abscisic acid	928	53	43.02	0.032
	GO:0071577	zinc ion transmembrane transport	27	4	1.25	0.034
	GO:0009753	response to jasmonic acid	339	19	15.72	0.036
	GO:0010584	pollen exine formation	52	6	2.41	0.036
	GO:0046621	negative regulation of organ growth	7	2	0.32	0.039
	GO:0090153	regulation of sphingolipid biosynthetic process	7	2	0.32	0.039
	GO:0010617	circadian regulation of calcium ion oscillation	7	2	0.32	0.039
	GO:1901141	regulation of lignin biosynthetic process	20	4	0.93	0.042
	GO:0070816	phosphorylation of RNA polymerase II C-terminal domain	42	5	1.95	0.044

	GO:0009749	response to glucose	112	11	5.19	0.046
	GO:0090696	post-embryonic plant organ development	307	18	14.23	0.046
	GO:0030835	negative regulation of actin filament depolymerization	12	2	0.56	0.046
	GO:0042371	vitamin K biosynthetic process	1	1	0.05	0.046
	GO:2000488	positive regulation of brassinosteroid biosynthetic process	1	1	0.05	0.046
	GO:0071000	response to magnetism	1	1	0.05	0.046
	GO:0035444	nickel cation transmembrane transport	1	1	0.05	0.046
	GO:0032194	ubiquinone biosynthetic process via 3,4-dihydroxy-5-polyprenyl benzoate	1	1	0.05	0.046
	GO:0022417	protein maturation by protein folding	1	1	0.05	0.046
	GO:0071951	conversion of methionyl-tRNA to N-formyl-methionyl-tRNA	1	1	0.05	0.046
	GO:0006178	guanine salvage	1	1	0.05	0.046
	GO:0090414	molybdate ion export from vacuole	1	1	0.05	0.046
	GO:0032263	GMP salvage	1	1	0.05	0.046
	GO:0007039	protein catabolic process in the vacuole	1	1	0.05	0.046
	GO:0032780	negative regulation of ATPase activity	1	1	0.05	0.046
	GO:0044794	positive regulation by host of viral process	1	1	0.05	0.046
	GO:0045041	protein import into mitochondrial intermembrane space	1	1	0.05	0.046
	GO:0000237	leptotene	1	1	0.05	0.046
	GO:0055068	cobalt ion homeostasis	1	1	0.05	0.046
	GO:0046100	hypoxanthine metabolic process	1	1	0.05	0.046
	GO:0080114	hydroxymethyltransferase activity	1	1	0.05	0.046
	GO:0009609	response to symbiotic bacterium	1	1	0.05	0.046
	GO:0048453	sepal formation	1	1	0.05	0.046
	GO:1900186	negative regulation of clathrin-dependent endocytosis	1	1	0.05	0.046
	GO:0015851	nucleobase transport	27	2	1.25	0.046
	GO:0015706	nitrate transport	51	7	2.36	0.047
	GO:0045040	protein import into mitochondrial outer membrane	18	3	0.83	0.048
	GO:0007019	microtubule depolymerization	16	4	0.74	0.050
	GO:1902347	response to strigolactone	8	2	0.37	0.050
	GO:1902025	nitrate import	8	2	0.37	0.050
Australian & European *C. maritima*	GO:0000266	mitochondrial fission	37	7	1.35	0.00033

	GO:0009700	indole phytoalexin biosynthetic process	32	4	1.17	0.00089
	GO:0006592	ornithine biosynthetic process	6	3	0.22	0.00089
	GO:0006526	arginine biosynthetic process	22	5	0.8	0.001
	GO:0018283	iron incorporation into metallo-sulfur cluster	2	2	0.07	0.0013
	GO:0019722	calcium-mediated signaling	77	9	2.81	0.0019
	GO:0007035	vacuolar acidification	17	4	0.62	0.0029
	GO:0015970	guanosine tetraphosphate biosynthetic process	3	2	0.11	0.0039
	GO:0006750	glutathione biosynthetic process	10	3	0.36	0.0048
	GO:0015770	sucrose transport	35	6	1.28	0.0049
	GO:1902334	fructose export from vacuole to cytoplasm	4	2	0.15	0.0076
	GO:0009755	hormone-mediated signaling pathway	1033	48	37.66	0.0092
	GO:2000377	regulation of reactive oxygen species metabolic process	85	6	3.1	0.011
	GO:0006556	S-adenosylmethionine biosynthetic process	5	2	0.18	0.012
	GO:0044375	regulation of peroxisome size	14	3	0.51	0.013
	GO:0007076	mitotic chromosome condensation	14	3	0.51	0.013
	GO:0009561	megagametogenesis	107	9	3.9	0.015
	GO:0046686	response to cadmium ion	643	35	23.44	0.015
	GO:0009791	post-embryonic development	2816	124	102.67	0.015
	GO:0007030	Golgi organization	57	6	2.08	0.017
	GO:2001141	regulation of RNA biosynthetic process	3097	118	112.92	0.018
	GO:0048825	cotyledon development	93	8	3.39	0.019
	GO:0009738	abscisic acid-activated signaling pathway	339	19	12.36	0.019
	GO:0070940	dephosphorylation of RNA polymerase II C-terminal domain	17	3	0.62	0.022
	GO:0007021	tubulin complex assembly	7	2	0.26	0.025
	GO:0032876	negative regulation of DNA endoreduplication	18	3	0.66	0.026
	GO:0002188	translation reinitiation	18	3	0.66	0.026
	GO:0010431	seed maturation	112	8	4.08	0.028
	GO:0019288	isopentenyl diphosphate biosynthetic process, methylerythritol 4-phosphate pathway	19	3	0.69	0.030
	GO:0009793	embryo development ending in seed dormancy	958	51	34.93	0.031
	GO:0006651	diacylglycerol biosynthetic process	8	2	0.29	0.032
	GO:0002213	defence response to insect	45	5	1.64	0.034
	GO:0071230	cellular response to amino acid stimulus	34	4	1.24	0.034

	GO:1901141	regulation of lignin biosynthetic process	20	3	0.73	0.035
	GO:0009051	pentose-phosphate shunt, oxidative branch	20	3	0.73	0.035
	GO:0009416	response to light stimulus	1236	53	45.07	0.035
	GO:0032970	regulation of actin filament-based process	104	6	3.79	0.036
	GO:2000369	regulation of clathrin-dependent endocytosis	2	2	0.07	0.036
	GO:1902979	mitotic DNA replication termination	1	1	0.04	0.036
	GO:1903730	regulation of phosphatidate phosphatase activity	1	1	0.04	0.036
	GO:1901038	cyanidin 3-O-glucoside metabolic process	1	1	0.04	0.036
	GO:0001193	maintenance of transcriptional fidelity during DNA-templated transcription elongation from RNA polymerase II promoter	1	1	0.04	0.036
	GO:0000372	Group I intron splicing	1	1	0.04	0.036
	GO:1903646	positive regulation of chaperone-mediated protein folding	1	1	0.04	0.036
	GO:0030574	collagen catabolic process	1	1	0.04	0.036
	GO:0071171	site-specific DNA replication termination at RTS1 barrier	1	1	0.04	0.036
	GO:0035511	oxidative DNA demethylation	1	1	0.04	0.036
	GO:1903329	regulation of iron-sulfur cluster assembly	1	1	0.04	0.036
	GO:1905639	positive regulation of mitochondrial mRNA catabolic process	1	1	0.04	0.036
	GO:0015843	methylammonium transport	1	1	0.04	0.036
	GO:0033494	ferulate metabolic process	1	1	0.04	0.036
	GO:0090677	reversible differentiation	1	1	0.04	0.036
	GO:1900186	negative regulation of clathrin-dependent endocytosis	1	1	0.04	0.036
	GO:0030198	extracellular matrix organization	32	2	1.17	0.036
	GO:0010393	galacturonan metabolic process	265	7	9.66	0.037
	GO:0051260	protein homooligomerization	42	5	1.53	0.037
	GO:0080188	RNA-directed DNA methylation	35	4	1.28	0.038
	GO:0052544	defence response by callose deposition in cell wall	35	4	1.28	0.038
	GO:0045892	negative regulation of transcription, DNA-templated	466	26	16.99	0.039
	GO:0031930	mitochondria-nucleus signaling pathway	21	3	0.77	0.039
	GO:0009816	defence response to bacterium	87	7	3.17	0.040
	GO:0010074	maintenance of meristem identity	93	6	3.39	0.040
	GO:0006883	cellular sodium ion homeostasis	9	2	0.33	0.040
	GO:0010032	meiotic chromosome condensation	9	2	0.33	0.040
	GO:0006572	tyrosine catabolic process	9	2	0.33	0.040

	GO:0009875	pollen-pistil interaction	95	5	3.46	0.040
	GO:0009933	meristem structural organization	132	11	4.81	0.041
	GO:0030422	production of siRNA involved in RNA interference	64	6	2.33	0.041
	GO:0010345	suberin biosynthetic process	36	4	1.31	0.041
	GO:0042631	cellular response to water deprivation	70	6	2.55	0.042
	GO:0070370	cellular heat acclimation	22	3	0.8	0.044
	GO:0006607	NLS-bearing protein import into nucleus	22	3	0.8	0.044
	GO:0010073	meristem maintenance	251	15	9.15	0.048
	GO:0001174	transcriptional start site selection at RNA polymerase II promoter	10	2	0.36	0.049
	GO:0010023	proanthocyanidin biosynthetic process	10	2	0.36	0.049
	GO:0019827	stem cell population maintenance	116	9	4.23	0.049
western North American & European *C. maritima*	GO:0019563	glycerol catabolic process	12	5	0.57	0.00014
	GO:0060969	negative regulation of gene silencing	41	6	1.93	0.00082
	GO:0042026	protein refolding	67	10	3.16	0.0011
	GO:0009694	jasmonic acid metabolic process	75	8	3.54	0.0014
	GO:0090173	regulation of synaptonemal complex assembly	2	2	0.09	0.0022
	GO:0000769	syncytium formation by mitosis without cytokinesis	2	2	0.09	0.0022
	GO:0002215	defence response to nematode	2	2	0.09	0.0022
	GO:0009696	salicylic acid metabolic process	81	8	3.82	0.0024
	GO:0070919	production of siRNA involved in gene silencing by small RNA	16	5	0.75	0.0034
	GO:0080188	RNA-directed DNA methylation	35	6	1.65	0.0054
	GO:0006725	cellular aromatic compound metabolic process	7015	377	330.69	0.0059
	GO:0046838	phosphorylated carbohydrate dephosphorylation	34	4	1.6	0.0065
	GO:0015970	guanosine tetraphosphate biosynthetic process	3	2	0.14	0.0065
	GO:0006127	glycerophosphate shuttle	3	2	0.14	0.0065
	GO:0019748	secondary metabolic process	589	32	27.77	0.0078
	GO:0010082	regulation of root meristem growth	38	6	1.79	0.0082
	GO:0010114	response to red light	100	10	4.71	0.0086
	GO:0032776	DNA methylation on cytosine	17	4	0.8	0.0098
	GO:0071555	cell wall organization	469	23	22.11	0.012
	GO:1902334	fructose export from vacuole to cytoplasm	4	2	0.19	0.013

	GO:0045128	negative regulation of reciprocal meiotic recombination	4	2	0.19	0.013
	GO:0046167	glycerol--phosphate biosynthetic process	4	2	0.19	0.013
	GO:0000706	meiotic DNA double-strand break processing	4	2	0.19	0.013
	GO:0010390	histone monoubiquitination	4	2	0.19	0.013
	GO:0033523	histone H2B ubiquitination	12	4	0.57	0.013
	GO:1905157	positive regulation of photosynthesis	11	3	0.52	0.013
	GO:0010148	transpiration	11	3	0.52	0.013
	GO:0010244	response to low fluence blue light stimulus by blue low-fluence system	12	3	0.57	0.017
	GO:0070370	cellular heat acclimation	22	4	1.04	0.018
	GO:0051085	chaperone cofactor-dependent protein refolding	85	9	4.01	0.019
	GO:0009727	detection of ethylene stimulus	5	2	0.24	0.020
	GO:0033353	S-adenosylmethionine cycle	5	2	0.24	0.020
	GO:0033306	phytol metabolic process	13	3	0.61	0.021
	GO:0033169	histone H3-K9 demethylation	24	4	1.13	0.025
	GO:0009933	meristem structural organization	132	15	6.22	0.025
	GO:0008219	cell death	306	17	14.43	0.026
	GO:0045490	pectin catabolic process	151	13	7.12	0.027
	GO:0040014	regulation of multicellular organism growth	6	2	0.28	0.029
	GO:0031117	positive regulation of microtubule depolymerization	6	2	0.28	0.029
	GO:0051782	negative regulation of cell division	15	3	0.71	0.031
	GO:0080036	regulation of cytokinin-activated signaling pathway	27	5	1.27	0.032
	GO:0051707	response to other organism	1706	81	80.42	0.032
	GO:0007017	microtubule-based process	356	21	16.78	0.036
	GO:0009759	indole glucosinolate biosynthetic process	16	3	0.75	0.037
	GO:0010090	trichome morphogenesis	124	11	5.85	0.038
	GO:0043489	RNA stabilization	16	3	0.75	0.040
	GO:0006641	triglyceride metabolic process	66	5	3.11	0.040
	GO:0010258	NADH dehydrogenase complex (plastoquinone) assembly	7	2	0.33	0.040
	GO:0006225	UDP biosynthetic process	7	2	0.33	0.040
	GO:0010617	circadian regulation of calcium ion oscillation	7	2	0.33	0.040
	GO:0016998	cell wall macromolecule catabolic process	28	4	1.32	0.041
	GO:0009657	plastid organization	447	26	21.07	0.043
	GO:0006207	'de novo' pyrimidine nucleobase biosynthetic process	17	3	0.8	0.043

	GO:0006139	nucleobase-containing compound metabolic process	6321	336	297.98	0.045
	GO:0010497	plasmodesmata-mediated intercellular transport	29	4	1.37	0.046
	GO:0006083	acetate metabolic process	2	2	0.09	0.047
	GO:0010070	zygote asymmetric cell division	7	2	0.33	0.047
	GO:0010398	xylogalacturonan metabolic process	1	1	0.05	0.047
	GO:0060250	germ-line stem-cell niche homeostasis	1	1	0.05	0.047
	GO:1903775	regulation of DNA double-strand break processing	1	1	0.05	0.047
	GO:0051572	negative regulation of histone H3-K4 methylation	1	1	0.05	0.047
	GO:0030245	cellulose catabolic process	1	1	0.05	0.047
	GO:0001193	maintenance of transcriptional fidelity during DNA-templated transcription elongation from RNA polymerase II promoter	1	1	0.05	0.047
	GO:0080038	positive regulation of cytokinin-activated signaling pathway	1	1	0.05	0.047
	GO:0043619	regulation of transcription from RNA polymerase II promoter in response to oxidative stress	1	1	0.05	0.047
	GO:0010792	DNA double-strand break processing involved in repair via single-strand annealing	1	1	0.05	0.047
	GO:1903647	negative regulation of chlorophyll catabolic process	1	1	0.05	0.047
	GO:1900091	regulation of raffinose biosynthetic process	1	1	0.05	0.047
	GO:0030574	collagen catabolic process	1	1	0.05	0.047
	GO:1900088	regulation of inositol biosynthetic process	1	1	0.05	0.047
	GO:1901918	negative regulation of exoribonuclease activity	1	1	0.05	0.047
	GO:0046103	inosine biosynthetic process	1	1	0.05	0.047
	GO:2001173	regulation of histone H2B conserved C-terminal lysine ubiquitination	1	1	0.05	0.047
	GO:0019427	acetyl-CoA biosynthetic process from acetate	1	1	0.05	0.047
	GO:0019428	allantoin biosynthetic process	1	1	0.05	0.047
	GO:0071578	zinc ion import across plasma membrane	1	1	0.05	0.047
	GO:0090677	reversible differentiation	1	1	0.05	0.047
	GO:0030198	extracellular matrix organization	32	2	1.51	0.047
	GO:0009900	dehiscence	46	2	2.17	0.047
	GO:0032970	regulation of actin filament-based process	104	4	4.9	0.047
	GO:0090333	regulation of stomatal closure	56	6	2.64	0.048

Table 4-S13. Number of overlapping windows of invasion adaptation candidates (X^TX outliers of cross-range BayPass runs) with GWAS phenotypes.

GWAS phenotype	Number of overlapping windows X^TX and GWAS *C. maritima* (Australia, Europe, western North America)	Number of overlapping windows X^TX and GWAS *C. edentula* (Australia, eastern North America, western North America)
Onset branching	6	5
Onset bud	9	6
Onset open flower	10	8
Onset seed	7	10
Above-ground biomass	14	24
Below-ground biomass	10	15
Biovolume at onset bud	15	77
Biovolume at onset open flower	13	26
Seedling size	15	12
Growth rate	11	45
Flower number	17	10
Fruit weight	4	14
Pollen viability	15	16
Aphid damage	12	17
SLA	9	9
Fruit shape PC1	4	15
Fruit shape PC2	8	12
Fruit shape PC3	7	35
Fruit shape PC4	3	59
Leaf shape PC1	11	40
Leaf shape PC2	11	33
Leaf shape PC3	16	5
Leaf shape PC4	16	13

Table 4-S14. Flowering genes identified in invasive adaptation candidates (cross range BayPass runs). TAIR10 ID, annotation and alternative names and in which group identified are presented.

BayPass *C. edentula* cross range runs (X^TX and GWAS overlaps)		
TAIR10 ID	**Annotation**	**Alternative names**
AT5G60910	AGAMOUS-like 8	Floral homeotic protein AGL8
AT1G04400	cryptochrome 2	AT-PHH1, ATCRY2, FHA, PHH1
AT5G63960	DNA binding;nucleotide binding;nucleic acid binding;DNA-directed DNA polymerases;DNA-directed DNA polymerases	EMBRYO DEFECTIVE 2780
AT5G07200	gibberellin 20-oxidase 3	ATGA20OX3, GA20OX3, YAP169
AT1G15550	gibberellin 3-oxidase 1	ATGA3OX1, GA REQUIRING 4, GA3OX1, GA4
AT5G09740	histone acetyltransferase of the MYST family 2	HAC11, HAG05, HAG5, HAM2, HISTONE ACETYLTRANSFERASE OF THE CBP FAMILY 11, HISTONE ACETYLTRANSFERASE OF THE GNAT/MYST SUPERFAMILY 5
AT5G61060	histone deacetylase 5	ATHDA5, HDA05, HDA5, HISTONE DEACETYLASE 5
AT5G10140	K-box region and MADS-box transcription factor family protein	AGAMOUS-LIKE 25, AGL25, FLC, FLF, FLOWERING LOCUS C, FLOWERING LOCUS F, REDUCED STEM BRANCHING 6, RSB6
AT5G61150	leo1-like family protein	VERNALIZATION INDEPENDENCE 4, VIP4
AT3G28910	myb domain protein 30	ATMYB30, MYB DOMAIN PROTEIN 30, MYB30
AT5G03840	PEBP (phosphatidylethanolamine-binding protein) family protein	TERMINAL FLOWER 1, TFL-1, TFL1
AT1G25540	phytochrome and flowering time regulatory protein (PFT1)	GLH1, MED25, MEDIATOR 25, PFT1, PHYTOCHROME AND FLOWERING TIME 1
BayPass *C. maritima* cross range runs (X^TX and GWAS overlaps)		
AT2G30140	UDP-Glycosyltransferase superfamily protein	UDP-GLUCOSYL TRANSFERASE 87A2, UGT87A2
AT4G15880	Cysteine proteinases superfamily protein	ATESD4, EARLY IN SHORT DAYS 4, ESD4
AT4G16280	RNA binding;abscisic acid binding	FCA, FLOWERING CONTROL LOCUS A

Table 4-S15. Description of worldclim variables.

Worldclim	Description
BIO1	Annual Mean Temperature

BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) (×100)
BIO4	Temperature Seasonality (standard deviation ×100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

Table 4-S16. Annotation of overlapping invasion adaptation candidates and selective sweep (H12) candidate windows of invasive *C. maritima*. Worldclim bio variables description see Table 4-S15.

window	***Arabidopsis thaliana* gene**	***ID***	**Description**	**GO terms**
Scaffold_1_50500000-50550000	AT2G28500	LOB DOMAIN-CONTAINING PROTEIN 11	LOB domain-containing protein 11	GO:0008150;GO:0005634
	AT2G28510	DOF PROTEIN 2.1, DOF2.1	DOF transcription factor with a conserved zinc finger (ZF) DNA-binding domain.	GO:0005634;GO:0003700;GO:0008270;GO:0006355;GO:0005730
Scaffold_3_38950000-39000000	AT4G15880	EARLY IN SHORT DAYS 4	EARLY IN SHORT DAYS 4 Arabidopsis mutant shows extreme early flowering and alterations in shoot development. It encodes a SUMO protease, located predominantly at the periphery of the nucleus. Accelerates	GO:0009909;GO:0005634;GO:0070139;GO:0009911;GO:0008234;GO:0016926;GO:0006508;GO:0019900;GO:0016929

			the transition from vegetative growth to flowering. Probably acts in the same pathway as NUA in affecting flowering time, vegetative and inflorescence development. The mRNA is cell-to-cell mobile.	
	AT4G15890	CONDENSIN	CAP-3D is a subunit of condensin. It a target of MMD1 regulation and also involved in meiotic chromosome condensation. Mutants have reduced fertility. Required for the correct spatial relationship between centromeres and rDNA arrays.	GO:0003682;GO:0007076;GO:0010032;GO:0009556;GO:0005634;GO:0051304;GO:0000799;GO:0098653;GO:0042393;GO:0000779
	AT4G15900	PLEIOTROPIC REGULATORY LOCUS 1	Mutations confer hypersensitivity to glucose and sucrose and augments sensitivity to cytokinin, ethylene, ABA and auxin. Encodes a nuclear WD40 protein that is imported into the nucleus. Essential for plant innate immunity. Interacts with MOS4 and AtCDC5. It is also predicted to have two DWD motifs. It can bind to DDB1a in Y2H assays, and DDB1b in co-IP assays, and may be involved in the formation of a CUL4-based E3 ubiquitin ligase, and may affect the stability of AKIN10.	GO:0005515;GO:0050832;GO:0005634;GO:0048825;GO:0045892;GO:0009870;GO:0009755;GO:0010154;GO:0048364;GO:0048366;GO:0016567;GO:0080008;GO:0010182;GO:0006508;GO:0000398;GO:0071013;GO:0005662;GO:0000974;GO:0005829;GO:0042742;GO:0009749
	AT5G46290	3-KETOACYL-ACYL CARRIER PROTEIN SYNTHASE I	Encodes beta-ketoacyl-[acyl carrier protein] synthase I (KASI). Crucial for fatty acid synthesis. Plays a role in chloroplast division and embryo development.	GO:0009570;GO:0009507;GO:0009793;GO:0016747;GO:0006633;GO:0009941;GO:0009536;GO:0010020;GO:0004315
Scaffold_4_53800000-53850000	AT2G19770	PROFILIN 5	Encodes profilin 5, originally named profilin 4 (PRO4/PFN4). Low-molecular weight, actin monomer-binding protein that regulates the organization of actin cytoskeleton. Pollen-specific plant profilin present predominantly in mature pollen and growing pollen tubes.	GO:0005737;GO:0003785;GO:0030036;GO:0005938;GO:0042989;GO:0005634;GO:0009524;GO:0005739
Scaffold_7_14500000-14550000	AT3G17000	UBIQUITIN-CONJUGATING ENZYME 32	Group XIV ubiquitin-conjugating enzyme that functions negative regulation of drought stress.	GO:0042631;GO:1902457;GO:0005783;GO:0005515;GO:0016020;GO:0000209;GO:0061631;GO:0006511;GO:0004842;GO:0005634;GO:0016567;GO:0048471
	AT4G16155	/	dihydrolipoamide dehydrogenase	GO:0050660;GO:0009941;GO:0009507;GO:0004148;GO:0009570;GO:0045454;GO:0055114;GO:0005829;GO:0046685
	AT4G16160	ATOEP16-2, ATOEP16-S	Homologous to pea OEP16 and barley pPORA (OEP16), a member of Arabidopsis OEP16 family. Two OEP16	GO:0031359;GO:0009527;GO:0015171;GO:0042803;GO:0034

			genes are closely related to each other and are conserved in all land plants, OEP16-2, also named OEP16-S, and OEP16-1 (renamed OEP16-L) are result of the gene duplication event that occurred prior to divergence of bryophytes and seed plants. Predominantly expressed in seed and is not inducible by cold treatment. atOEP16-S gained an additional exon. The promoter region of atOEP16-S (but not atOEP16-L) contains multiple G-box ABA-responsive elements. The atOEP16-S promoter conferred developmentally regulated seed- and pollen-specific GUS expression in tobacco.	220
	AT4G16180	/	transmembrane protein	GO:0005783;GO:0008150;GO:0005886;GO:0003674;GO:0005829
	AT4G16190	/	Papain family cysteine protease	GO:0000323;GO:0005576;GO:0005764;GO:0004197;GO:0005773;GO:0051603;GO:0005615
	AT4G16260	/	Encodes a putative beta-1,3-endoglucanase that interacts with the 30C02 cyst nematode effector. May play a role in host defence.	GO:0004553;GO:0009817;GO:0002215;GO:0005618;GO:0099503;GO:0005774;GO:0005975;GO:0046658;GO:0042973;GO:0005739
	AT4G16265	NRPD9B	RNA polymerases M/15 Kd subunit	GO:0005665;GO:0006283;GO:0000419;GO:0001193;GO:0006367;GO:0006379;GO:0000418;GO:0080188;GO:0005634;GO:0003676;GO:0008270
	AT4G16270	PEROXIDASE40, PRX40	Encodes a class III peroxidase that is genetically redundant with PRX9, expressed in the tapetum, and essential for proper anther and pollen development.	GO:0048658;GO:0005576;GO:0020037;GO:0006979;GO:0004601
	AT4G16280	FCA, FLOWERING CONTROL LOCUS A	Involved in the promotion of the transition of the vegetative meristem to reproductive development. Four forms of the protein (alpha, beta, delta and gamma) are produced by alternative splicing. Involved in RNA-mediated chromatin silencing. At one point it was believed to act as an abscisic acid receptor but the paper describing that function was retracted.	GO:0000785;GO:0003729;GO:0005634;GO:0009793;GO:0009553;GO:1990904;GO:0031048;GO:0009909;GO:0003723;GO:0005515;GO:0005737
Scaffold_8_22200000-22250000	AT1G32375	/	F-box/RNI-like/FBD-like domains-containing protein	GO:0005575;GO:0003674;GO:0008150

	AT5G38660	ACCLIMATION OF PHOTOSYNTHESIS TO ENVIRONMENT, APE1	mutant has Altered acclimation responses	GO:0009535;GO:0009534;GO:0005739;GO:0009507;GO:0009941;GO:0003729;GO:0009536
Scaffold_8_23400000-23450000	/			

3845

3846 Table 4-S17. Annotation of the seven introgressed parallel invasion adaptation windows in *C. maritima*.

3847

window	***Arabidopsis thaliana* gene**	***ID***	**Description**	**GO terms**
Scaffold_6_15600000_15650000	AT1G48210	NA	Protein kinase superfamily protein;(source:Araport11)	GO:0005634;GO:0005886;GO:0006468
	AT1G48230	NA	Nucleotide/sugar transporter family protein	GO:0015297;GO:0008514;GO:0005768;GO:0005802;GO:0005886;GO:0005794;GO:0022857
Scaffold_6_46700000_46750000	/			
Scaffold_7_29100000_29150000	AT3G51460	RHD4	Encodes RHD4 (ROOT HAIR DEFECTIVE4), a phosphatidylinositol-4-phosphate phosphatase required for root hair development. The mRNA is cell-to-cell mobile.	GO:0005886;GO:0035619;GO:0048768;GO:0031520;GO:0046856;GO:0005783;GO:0090404;GO:0009611;GO:0005829;GO:0043812;GO:0009932;GO:0005739;GO:0009506
	AT4G14746	NA	neurogenic locus notch-like protein;(source:Araport11)	GO:0031225;GO:0008150
Scaffold_7_30300000_30350000	AT4G14510	ATCFM3B	Encodes a CRM domain protein CFM3b. Homolog of CFM3a (AT3G23070). CFM3a is shown to function in the splicing of group IIB introns in chloroplasts.	GO:0048316;GO:0009507;GO:0000373;GO:0003729
Scaffold_7_51850000_51900000	AT4G13550	HIL1	Heat stress inducible plastid monogalactosyldiacylglycerol lipase.	GO:0006629;GO:0016042;GO:0009507;GO:0004620;GO:0005576;GO:0016298;GO:0009408
Scaffold_9_14950000_15000000	AT3G42870	NA	heat shock protein;(source:Araport11)	GO:0003674;GO:0005739;GO:0008150
	AT5G44790	ATHMP51	ATP dependent copper transporter vital for ethylene response pathway	GO:0009723;GO:0009873;GO:0005794;GO:0043682;GO:0005507;GO:0005768;GO:0015662;GO:0005802;GO:0010119;GO:0005375
	AT5G44800	CHR4	Interacts with transcription factors involved in floral	GO:0042735;GO:0005515;GO:0005634;G

	meristem identity and affects the expression of key floral regulators. Affects H3K27me3 and H3K4me3 levels at a subset of loci in the genome.	O:0009506
Scaffold_9_17100000_17150000 /		

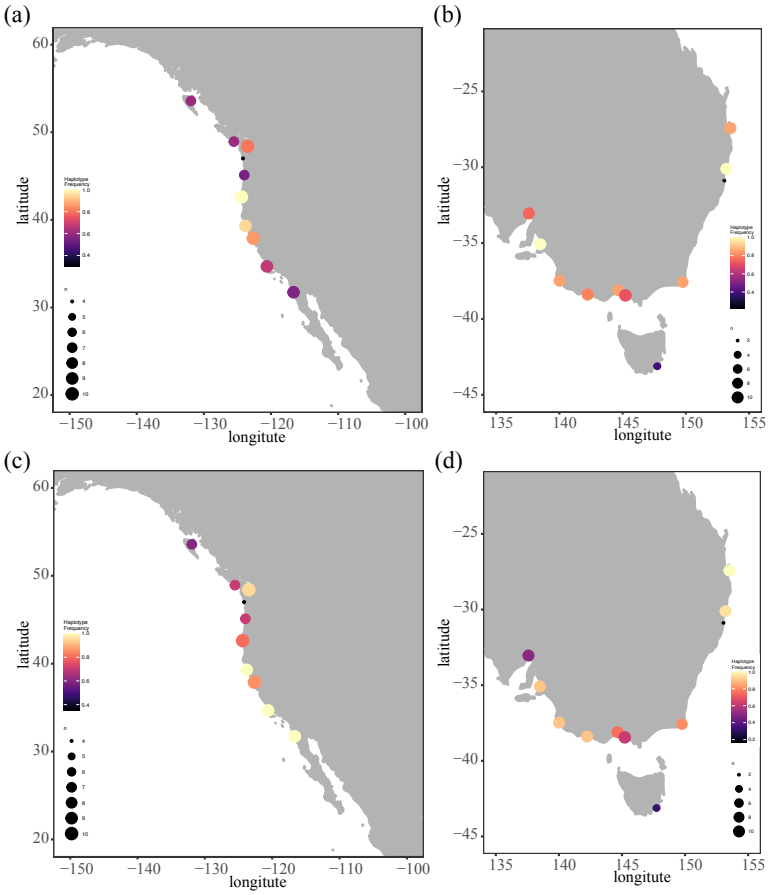

Figure 4-S1. Haplotype frequency pattern of *C. maritima* on two candidate windows of parallel invasion adaption candidates. Scaffold_6_15600000_15650000 (a) and (b). Scaffold_9_17100000_17150000 (c) and (d).

Chapter 5 - Discussion and future directions

5.1 Overview

In Australia and western North America, seemingly common beaches are more than meets the eye, as they harbour natural experiments that allow evolution to be observed on a contemporary time scale. Here, two alien plant species originating from two separate continents coexist, until finally one appears to replace the other. The invasive plant species are *Cakile edentula* and *C. maritima* and were the focus of this thesis. The main goal of this thesis was to investigate the extent of hybridization between these species, and the role hybridization might play in adaptive evolution during the course of their invasion. To achieve this aim, I used wide-ranging samples from four ranges- the species' native ranges (eastern North America, Europe) and two ranges that they have both invaded (Australia, western North America). I developed and analysed two large-scale, independent genetic datasets. Furthermore, I collected and analysed extensive phenotypic data from a greenhouse common garden experiment. And, by leveraging a suite of bioinformatics approaches, I quantified the extent of hybridization and examined the invasion history on both continents.

The research in this thesis has improved the understanding of the repeatability of adaptation within and between species on two isolated continents. Understanding such instances of parallel evolution, and their driving factors, are major unanswered questions in the field of evolutionary biology (Conte et al., 2012; Martin & Orgogozo, 2013; Smith & Rausher, 2011). Here, I not only investigated the repeatability of adaptation, but also the contribution of hybridization to repeatability on a genomic and phenotypic level. Adaptation relies on genetic variation (Bock et al., 2015; Ellstrand & Schierenbeck, 2000), which is reduced by genetic bottlenecks often associated with invasion. Yet, this loss can be ameliorated by hybridization with another species. Moreover, the mechanism known as adaptive introgression can take place (Milne & Abbott, 2000; Pfennig et al., 2016), where locally adapted genes from a resident species can be introduced to an invading one.

For this thesis, I used the *Cakile maritima*/*edentula* pair to identify source populations of the invasions, to shed light on the population structuring in native and invasive ranges, and to estimate the extent of hybridization between species during the invasions. Moreover, a common garden experiment enabled me to identify extensive parallelism in the evolution of traits during invasion, not only within but also between the species. An extensive genomic

analysis identified high levels of parallel signatures of adaptation within species as well as a smaller level between species. Further, I identified some evidence consistent with parallel patterns of adaptive introgression facilitating climate adaptation during invasion. Interestingly, many of these introgressed regions were also involved in adaptation in the donor species.

Many studies that examine adaptation during invasion (e.g., Barrett et al., 2008; Gaskin, 2017; Hernández et al., 2019; van Boheemen et al., 2019; Whitney & Gabler, 2008) demonstrate that adaptation to the local environment can be rapid, and furthermore that adaptive introgression can help a newcomer to establish (Hedrick, 2013; Milne & Abbott, 2000; Pfennig et al., 2016; Suarez-Gonzalez et al., 2018). The importance of the work presented in this thesis is the remarkable similarity in invasion histories of the same species on two continents. To the best of my knowledge this study is unique in investigating and identifying parallel adaptation within and between species in concurrent, naturally occurring, replicated invasions. In this last chapter, I will discuss the implication of the results of Chapters 2, 3 and 4, as well as suggest some future research directions.

5.2 Discussion and further directions

5.2.1 Source populations of the invasions and their significance for adaptation

In Chapters 2 and 3, I used two different genetic datasets to investigate the invasion sources for both species on two continents. Identifying the source population of an invasion is important, as it allows the comparison of genetic differentiation between introductions to their source. For example, without accounting for this, trait differences in common gardens might reflect invasion history; adaptive hypotheses would therefore be difficult to address (Shaw et al., 2021). Both independent datasets have shown that in Australia, *Cakile maritima* originated from multiple native areas, but only one source region was identified for *C. edentula*. By contrast, in western North America a single introduction of *C. maritima* took place and it appears that multiple invasions of *C. edentula* occurred. I was able to identify recent and older-generation hybrids in both invaded ranges, although I observed a much higher rate of hybridization in Australia. I provided the first genetic evidence of hybrids in western North America (Chapter 2), which had previously only been hypothesised based on the morphological identification of rare samples. Hybridization is predicted to have occurred in both ranges by the demographic rescue hypothesis, whereby the presence of a cross-compatible species can aid the establishment of an invader through overcoming Allee effects (Mesgaran et al., 2016).

In support of this, I identified the gradient in species ancestry that would be expected with demographic rescue (Chapter 2).

The fate of an invasive species may be decided by the speed at which it is able to adapt to a new environment (Bock et al., 2015). Leveraging standing genetic variation, species can adapt more rapidly than waiting for new mutations. Hence, alien species whose new range is similar to the native range might be more successful (Bock et al., 2015), and if the difference between the two ranges is too substantial, the spread of the alien species might be limited. However, it has been shown that these *Cakile* species are capable of colonizing broad climatic ranges, even those beyond the climate niches of the native populations (Shaw et al., 2021). This is despite the limited number of source regions I identified; whose realized climatic niches are but a subset of the native range environmental breadth. In *C. edentula*, both introductions are sourced from a cold climate zone, while in *C. maritim*a source populations are likely from the Mediterranean climate (temperate climate zone; Koeppen-Geiger classification, Beck et al., 2018). Furthermore, not only are these species present across broad gradients, they also display replicated patterns of trait divergence within and between species during invasion, consistent with local adaptation during range expansion.

Another source of genetic variation can be hybridization (Ellstrand & Schierenbeck, 2000), which allows the transfer of adaptive variants to the newcomer via adaptive introgression (Milne & Abbott, 2000; Pfennig et al., 2016). I identified evidence consistent with parallel adaptive introgression into *Cakile maritima* in both invaded ranges. Consequently, hybridization may not only aid the *C. maritima* invasion by overcoming Allee effects through the supply of suitable mates (Mesgaran et al., 2016), but also by transferring genes possibly linked to adaptation to colder climates (colder than the source populations). Although alternative possibilities beside climate adaptation may explain these parallel patterns of introgression (such as neutral introgression, allele surfing, or the variable effects of genetic load across the genome), these data provide intriguing candidate loci for further examination. In a broader context, my research highlights the fact that management efforts should be focused on preventing hybridization of closely-related invasive species, especially those adapted to different environments, to prevent the formation of a highly adaptable hybrid invader.

5.2.2 Repeated adaptation on a phenotypic and genotypic level

Invasive species provide us with the possibility to observe ecological and evolutionary processes operating in natural environments over contemporary timescales (Bock et al., 2015). One of those processes is local adaptation. In Chapters 3 and 4, I explored an extensive set of phenotypic data in combination with a whole-genome-re-sequencing dataset with the goal to examine the repeatability of local adaptation, and furthermore ascertain whether hybridization aids adaptation during invasion. In the greenhouse, traits related to phenology, defence, performance, physiology and morphology were measured. A suite of genomic analyses was employed to adaptive loci. These included genome scans for extreme divergence in population allele frequencies, relationships between population allele frequencies and environmental variables, signatures of selective sweeps, and associations between genetic variation and fitness-related phenotypes. My results yielded parallel signals of climate adaptation in traits within and between species, and this parallelism extended to the genetic level, where many of the same genomic regions were implicated in climate adaptation within species.

In all ranges, a latitudinal cline for phenology and biomass was apparent in both species. The repeated evolution of these clines in important life history traits across four ranges and two different species, is unlikely to be the result of neutral processes. I propose that they instead reflect the classical trade-off between flowering time and size observed in adaptation to latitudinal differences in season length and local environmental cues (e.g., Leiblein-Wild & Tackenberg, 2014). The clines in the introduced ranges evolved to mirror the native range patterns in only 100-150 generations following the range expansion in Australia and western North America, and likely reflect rapid local adaptation. Similar patterns have been observed in *Arabidopsis thaliana* (Samis et al., 2012) and *Ambrosia artemisiifolia* (van Boheemen & Hodgins, 2020; van Boheemen et al., 2017), and it has been concluded in those cases that rapid, local adaptation is responsible for the evolution of those clines. *Arabidopsis thaliana* was introduced to North America 150-200 years ago, and since then longitudinal clines evolved despite a loss of genetic variation (Samis et al., 2012). *Ambrosia artemisiifolia*'s invasive ranges in Europe and Australia mirror the native North American range's pattern of phenology and size. This pattern evolved rapidly, over ~80 generations and regardless of the large difference in the invasion history between the introduced ranges (van Boheemen & Hodgins, 2020; van Boheemen et al., 2017).

When comparing the native range to the two introduced ranges to uncover regions involved in climate adaptation, I found high levels of parallelism, higher within (6-34%) than between (2-

9%) species. Although the effects of population structure and hybridization on false positive and false negative rates must be investigated further, this could reflect a real biological signal. For example, greater parallelism within species might be expected due to shared standing variation, and similar genetic backgrounds influencing evolutionary trajectories in similar ways (Conte et al., 2012). However, I found twelve candidate climate adaptation windows in invasive *C. maritima* in both ranges with signals of introgression from *C. edentula*. Genes within those windows were linked (via homology to *A. thaliana* gene annotations) to cold tolerance (chilling response), but also to circadian rhythm. Seven of these windows experienced a latitudinal cline in both invasive ranges, even when accounting for population structure. Moreover, 33% of these candidates for adaptive climate introgression showed signals of adaptive divergence with climate in *C. edentula*. Consequently, although I did not see a strong signal of convergence in general between the species, I did see a strong signal of gene reuse for introgression candidates.

In addition to parallel signals of climate adaptation, I also discovered evidence for convergent and divergent patterns of adaptation in response to invasion within and between species at both the trait level and genetic level. In both invaded ranges, I uncovered a convergent pattern of germination rate and aphid damage for both species. Here, the alien populations experience greater herbivore damage, especially in *C. edentula,* but at the same time enhanced germination. Defence and performance-related traits are frequently found to evolve during introduction (Bossdorf et al., 2005; Felker-Quinn et al., 2013), and reduced specialist pressure may allow for greater competitive ability (Blossey & Notzhold, 1995). Nevertheless, the evidence in support of that idea is mixed (Bossdorf et al., 2005; Colautti et al., 2009). Similar to other common garden studies, I found evidence for a shift in a defence related trait in *Cakile* (Chapter 3), although, my study was not designed to test the evolution of specialist defence. In line with the phenotypic patterns for aphid damage, and theories about the evolution of defence related traits during invasion (e.g. Blossey & Notzold, 1995; Bossdorf et al., 2005; Felker-Quinn et al., 2013) I also identified an over-representation of defence related gene ontology terms in regions of the genome diverging among the ranges.

Somewhat unexpectedly, I also identified the evolution of divergent patterns of trait evolution between the species that coincided with introduction. Specifically, *C. maritima* evolved a much later flowering onset in both introductions relative to the source populations of both species where flowering time overlaps. One possibility is that reinforcement contributed to reproductive character displacement (Alexander & Levine, 2019; Comeault & Matute, 2016),

in recent or currently sympatric invasions, such that delayed flowering was favoured to avoid hybridization with *C. edentula.* However, other explanations are possible for this pattern. Fitness reductions in certain classes of experimental hybrids between these species have been identified, and therefore a fitness cost of overlapping flowering in the field is likely (Li et al., 2020). These results also suggest that for establishment of the outcrosser, hybridization might be initially useful, but is subsequently costly, and the species may evolve to minimize opportunities for mating (Pfennig et al., 2016). Consequently, it is likely that there are both beneficial and detrimental effects of hybridization at different times and acting in different ways for *C. maritima*.

5.2.3 Hybridization

Investigating hybridization is now more important than ever, as rising invasion rates also increase the interaction of closely-related species, which were previously geographically isolated (Ellstrand & Schierenbeck, 2000; Hovick & Whitney, 2014; Ward et al., 2008). Additionally, in response to climate change, shifts in community composition are expected to enhance hybridization events (Pfennig et al., 2016). Some evidence exist that hybridization preceded invasiveness in 16 plant families (Schierenbeck & Ellstrand, 2009), and eleven percent of all plant species are thought to originate from hybrids (Ellstrand et al., 1996). Genomic data is invaluable in detecting hybrids, as older generations of hybrids are especially hard to detect morphologically; repeated back-crossing with one parental line often hides their hybrid status (Ohadi et al., 2016).

In both ranges, I have demonstrated that early and late-generation hybrids exist, and furthermore that bi-directional gene flow occurs between the species (Chapter 2, 3, 4; Ohadi et al., 2016). However, my data reflect biased backcrossing towards *C. maritima* (Chapter 2, 3, 4). A strong nuclear asymmetry exists where *C. maritima* ancestry dominates: *C. edentula* ancestry is in the minority in almost all hybrids, including new generation hybrids (Chapter 3, 4). The hybridization of both species is still ongoing in both alien ranges where the species co-exist, although at relatively low levels (New South Wales, Queensland and Tasmania in Australia; Washington, Oregon and British Columbia in western North America).

The question of whether hybridization between *C. edentula* and *C. maritima* facilitates range expansion is still unanswered. I have shown that founder effects during invasion have occurred,

limiting genetic variation in the introduced ranges to a subset of that in the native range. I have also shown that hybridization exists in the introduced ranges, providing a novel source of genetic variation. My data also suggest that some of the introgressed regions in *C. maritima* are involved in climate adaptation in both species, consistent with parallel adaptive introgression in multiple ranges. Given the fact that I have demonstrated that *C. edentula's* source region is a cold climate zone region and *C. maritima's* source region is a temperate climate zone (Koeppen-Geiger classification, Beck et al., 2018), introgression from *C. edentula* may facilitate rapid adaptation of *C. maritima* to the more colder climate zone regions of both introduced ranges.

Hybridization can supply beneficial alleles from a resident species to a newcomer (Milne & Abbott, 2000; Pfennig et al., 2016), yet disadvantageous impacts are also possible, as hybridization can also introduce deleterious genetic variation (Brandvain et al., 2014). As *C. edentula* is a self-compatible species (Rodman, 1974), and selfing species typically harbour more weakly deleterious alleles (Brandvain et al., 2014), which might be amplified by recent range expansion (Excoffier et al., 2009); consequently, hybridisation with *C. edentula* has the possibility to introduce those deleterious alleles into *C. maritima*. On the other hand, introgression from *C. maritima* into *C. edentula* might alleviate the burden of a high genetic load in the self-compatible species (Brandvain et al., 2014). However, I found limited evidence of substantial introgression from *C. maritima* into *C. edentula*.

Although the invasion and hybridization history in both alien ranges are similar, it seems that the details of the replacement of *C. edentula* by *C. maritima* might differ between ranges. The areas where the both species are in sympatry and/ or hybrids occur have stagnated in western North America since Barbour and Rodman (1970) published their saga of the West Coast sea-rockets. Yet, in Australia replacement seems to be completed in some states (e.g., Victoria), and *C. maritima* is still invading new areas (e.g., Queensland, Tasmania). Is this replacement in part facilitated by hybridization (Todesco et al., 2016)? Is *C. edentula*'s rapid local extinction being hastened by genetic or demographic swamping caused the invasion of *C. maritima*? Or are ecological factors such as lottery competition i.e., the substantial greater reproductive outcome of *C. maritima*, the only driver? Another possibility stems from the fact that phenotypically *C. maritima* samples are in most cases hybrids, especially in Australia. It is therefore plausible that both invasive species will be replaced by hybrids, which will be phenotypically *C. maritima* with integrated regions of the *C. edentula* genome. Is it really "The

rise of *C. maritima* and the fall of *C. edentula*" as Barbour and Rodman (1970) suggested? Or is it more accurate to term it "The doom of the pure species and the rise of hybrids"?

5.3 Future directions

Although in this thesis I have identified many exciting patterns in the genomes of these two invaders, I have only just scratched the surface of what this system has to offer to our understanding of hybridization and adaptation, and my work has identified many intriguing patterns that warrant further explanation.

Cakile maritima invaded South America (Shaw et al., 2021) without the presence of *C. edentula*, demonstrating that this is not a requirement for a successful invasion. Yet, this presents the opportunity to compare the co-invasions of two species versus a single invasion of one species. I might predict, for example, that flowering onset will not yet have diverged from the source populations, if sympatry with *C. edentula* influences this trait's evolution as I hypothesized. Similarly, depending on the source of *C. maritima* in this region, I might expect a more restricted climatic range than in western North America or Australia, or a weaker signal of climate adaptation, if adaptive introgression has indeed played a role in *C. maritima*'s rapid climate adaptation in western North America and Australia.

I have identified phenotypic evidence of enhanced herbivore damage within the invaded ranges. Yet, this was a coincidental finding. Designing an experiment to test if invasion and a subsequent release of natural enemies contributed to the evolution of reduced defence appears to be a promising new direction. For example, we are currently testing the amount and composition of glucosinolates in the *Cakile* individuals we sequenced. Glucosinolates can contribute to plant defence (Bennett & Wallsgrove, 1994; Tsunoda et al., 2017) and changing levels or composition might hint towards a change of defence allocation. Further, a controlled greenhouse experiment with herbivore treatment and a control treatment of native and invasive populations (and perhaps a competition treatment as well) will shed more light on this topic (but see Bossdorf et al., 2005).

Another future analysis should quantify the genetic load in both parental species and their hybrids and examine its impact on introgression patterns. Here, the presented phenotypic and genotypic dataset as well as forward in time simulations (Gilbert et al., 2017; Haller & Messer,

2017; Liu et al., 2017; Peischl et al., 2013) can be used to provide needed insight into consequences of hybridization between a self-compatible and an outcrossing species during range expansion. The self-compatible *C. edentula* may be expected to harbour more deleterious alleles than the outcrosser *C. maritima*, particularly following range expansion. Further, it is yet to be determined if hybridization in this system leads to a reduction of genetic load in hybrids (Conte et al., 2017; Ellstrand & Schierenbeck, 2000) or if genetic incompatibilities appear (Moran et al., 2021).

One of my most intriguing findings is the potential reproductive character displacement in both invasive ranges, in which *C. maritima* evolved to flower later in the invasive ranges than *C. edentula*. More research is needed to determine if reproductive character displacement occurs in field conditions. One such test could include tracking flowering time of the two invasive species via herbarium records. I would expect that *C. maritima,* when it first invaded, had a more similar flowering time to *C. edentula* and subsequently evolved a later flowering season. Moreover, testing flowering overlap in the field, in natural populations which are allopatric and sympatric, on the invasion core and invasion front may provide more insight into this question. Finally, measuring selection on flowering time in the field using collections from sympatric and allopatric populations and in mixed and single species treatments could reveal if divergence in flowering time exists under field conditions for sympatric populations and if divergent flowering times are favoured by selection in sympatry.

My population-genetic structure analyses provided important insight into the source of the invasions and the impact of introduction on genetic diversity. However, an improved analysis of my data would potentially provide even greater insight into the invasion history of these species. Approximate Bayesian computation (Fraimout et al., 2017; Pudlo et al., 2016) has been used to identify the number and origin of invasions, as well as the timing and extent of any bottlenecks in several invasive species (e.g., van Boheemen et al., 2017). Such analyses would be helpful in addressing several yet unanswered questions in this system, such as the likely cause of the genetic divergence of western North American *C. maritima* and the extent of the bottleneck in Australian *C. edentula.*

Another methodological approach that could be improved is the identification of species ancestry in my resequenced genomes. Currently, I identified outlier regions of the genome and then accessed their ancestry using local PCAs and phylogenetic trees. However, ancestry across

the genome can be assigned using ancestry-informative markers and Hidden Markov Models, such as the one implemented in Ancestry_HMM (Corbett-Detig & Nielsen, 2017). Once the genome-wide assessment of ancestry within each *C. maritima* individual is completed, admixture mapping of traits could be performed which would improve the power of the trait association mapping. In addition, an empirical null distribution of ancestry against latitude or other climate variables could be obtained, providing a better assessment of whether the candidate regions I identified are true outliers.

Here, I have concentrated on the parallelism on two continents along climatic gradients, focusing on latitudinal variation in particular (but not exclusively). However, although I see clear parallel latitudinal patterns of traits in common gardens, the climatic characteristics of the different continents and regions within those continents might be quite divergent and actually produce very different patterns of divergent selection on traits and across the genome, thereby reducing parallelism. Consequently, an analysis of climate adaptation within each continent, though reciprocal transplants for example, would be important in addressing this issue.

How is it that *C. maritima* has not yet reached north-western North America (i.e., Alaska), hybridized, and replaced *C. edentula* there after all those years? The area where the both species are in sympatry (and/or hybrids occur) appears to have stabilized since Barbour and Rodman (1970) reported it (Washington, Oregon, British Columbia). Did the two invasions of *C. edentula* into western North America play an important role in this context, e.g., by providing pre-adapted genotypes well suited to these northern climates? Or did the colder climate prevent the success of the Mediterranean climate-adapted *C. maritima* (Cousens et al., 2013)? Certainly, in the native range of *C. maritima*, the subspecies appear to occupy different ecological niches, and a more careful examination of the climatic distributions of the different subspecies through ecological niche modelling in the context of invasion might yield important insights. Similarly, niche modelling of the different clusters identified by Admixture in both species would be a promising path to shed light on this mystery. At the same time, this system would be an excellent test case to better integrate genomic information into species distribution modelling, due to the clear presence of founder effects and multiple independent invasions across the globe.

My final chapter raised the possibility that adaptive introgression has aided climate adaptation in *C. maritima* during its range expansion. This raises the question of whether such

introgression is required for the establishment of high-latitude *C. maritima* in each introduced range. A possible test could include demographic analysis of pure versus introgressed experimental lines for the candidate loci to provide estimates of population growth rates in the field. This is something that would be challenging to do, since beach experiments are frequently subject to foul weather and human disturbance. A more straightforward test of selection on these regions in F2s in outdoor trials (not on a beach) has already been conducted at the University of British Columbia, but even these experiments have been subject to extensive loss by herbivory.

5.4 Conclusion

As the Anthropocene continues to upend species' ranges and shift climatic conditions, understanding the genetic basis of rapid adaptation becomes ever more important. In this thesis I sought to broaden this understanding using two species that have mounted parallel, human-mediated invasions. This thesis investigated the repeatability of adaptation within and between species on two continents and the role of hybridization within. Using the unique replicated invasion history of *Cakile spp.,* I identified probable source populations and past and present hybridization. Moreover, I identified phenotypic evidence for the evolution of clinal patterns for phenology and size, and parallel evolutionary shifts in flowering time and herbivore damage during introduction. On a genomic level, parallel signatures of adaptation within and between species exist, and while these parallel patterns shed light on genetic bases of adaptation during range expansion, the most exciting findings identified hybridization and signals of adaptive introgression between species during their invasions. It appears hybridization has influenced the success of *C. maritima*, and this underscores the importance of interspecific interactions in the ever-increasing wave of biological invasions.

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