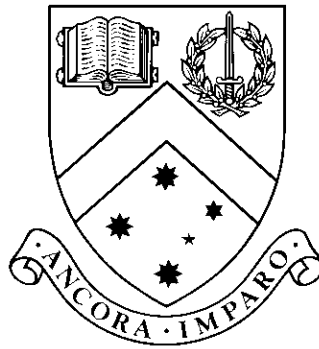# Infrastructure investment in leaf-cutter ants *Atta*

by

Thomas Bochynek, Dipl.-Biol.

Thesis

Submitted by Thomas Bochynek

for fulfillment of the Requirements for the Degree of

Doctor of Philosophy (0190)

Supervisor: Prof. Dr. Bernd Meyer

Associate Supervisor: A/Prof. Martin Burd

Caulfield School of Information Technology
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Monash University, 2017

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Abstract

Much like humans, social insects construct a diverse range of infrastructure, including bridges, rafts, tunnels, and highways. The construction of such infrastructure needs to be considered from an economic perspective: while allowing for reduced energetic costs during locomotion and resource transport, their construction can be expensive both in energetic measures and in work time. I investigate one of the most outstanding examples of infrastructure construction in the animal world: that of cleared trails in leaf-cutter ants *Atta*, which form networks that can extend over several kilometres.

In my thesis, I focus on three different aspects:

- the energetics of foraging on cleared trails,
- the mechanism of trail clearing,
- the progressive evolution of clearing.

I construct a comprehensive cost/benefit model of foraging on cleared and uncleared trails that incorporates energetic costs of clearing as well as the previously unexplored cost of providing an additional clearer workforce. With it, I show that these trails provide energetic benefits only for a range of plausible foraging scenarios and that workforce size and level of trail obstruction are limiting factors.

Not all trails within a colony are necessarily cleared, and uncleared trails can persist for extended periods. While the cost/benefit model

provides a possible energetic explanation, the mechanism of trail construction has not been explored. Based on behavioural experiments, I develop a simulation of the trail clearing behaviour. I find that the individual behaviour underlying trail construction is relatively simple, involves worker/obstruction interactions only, and requires no communication between workers.

Trail clearing influences the territorial dynamics of leaf-cutter ant colonies, and the localisation of foraging and scouting workers to existing trails leads to an effective reduction of territory size and neighbourhood interactions. In an evolutionary game setting, I investigate the influence of these effects on the evolutionary origin of trail clearing. Surprisingly, the reduction of territory and neighbourhood size propagates the evolution of trail clearing; a mechanism that has not been considered in the expansive work on evolution of collectively optimal strategies.

Infrastructure investment in leaf-cutter ants *Atta*

Declaration

I declare that this thesis is my own work and has not been submitted
in any form for another degree or diploma at any university or other
institute of tertiary education. Information derived from the published
and unpublished work of others has been acknowledged in the text and
a list of references is given.

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March 30, 2017

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Foremost, I am grateful to my supervisors Prof. Bernd Meyer and A. Prof. Martin Burd for their unfaltering support (and, where needed, patience) during my Ph.D. project. They have greatly expanded my understanding about biological complexity and approaches to identify underlying dynamics. I could not have asked for more.

The Faculty of Information Technology created an environment that encouraged my curiosity and led to countless interesting discussions across diverse fields. I have yet to find a faculty with comparable interest in – and investment into – their student body. This is the work of both academic and administrative staff, to which I am indebted.

I further thank the FIT Graduate Research Committee for the opportunity to partake in their work as student representative. It has been an exciting glance into the workings behind research administration.

I am further grateful for the financial support provided by the Faculty of Information Technology, Monash Graduate Education, and NICTA/ CSIRO DATA61, in the form of received scholarships, awards, and additional travel funding, which allowed me to visit a conference in the Netherlands and a work group in the United Kingdom.

During my project, I was given the opportunity to perform field research in Panama, to conduct experiments at Konstanz University, and to visit a work group at the University of Exeter. I am thankful for the friendly support provided by the Smitsonian Tropical Research Institute (STRI) and the interesting discussions and new methods learned in Exeter with the help of Dr. Ana Duarte. I explicitly want to thank Prof. Christoph Kleineidam and his work group for their heart-felt welcome and support during my visits. Their culture of thorough discussion was inspiring, and I have greatly benefited from it.

Above all I am grateful to my fellow Ph.D. students, who turned the office space into a place of stimulating debates. Whether in spontaneous

late-evening whiteboard talks or in everyday discussions, I have benefited greatly from their company.

<div align="right">Thomas Bochynek</div>

Monash University
June 2017

Chapter 1

Introduction

The fitness of any living organism is inextricably connected to its capacity of securing energy. In foraging organisms, the act of locating and collecting resources itself requires the expense of energy. The balance of energetic intake versus the cost of foraging is frequently described via economic cost/benefit models; the net benefit (i.e. *Benefit - Cost*) controls the organism's ability to maintain physiological functions, to grow, and to proliferate (e.g. Stephens and Krebs; 1986). Foraging strategies should therefore aim to minimise costs and to maximise the benefit.

The use of transport infrastructure, e.g. in the form of roads or highways, allows an organism to drastically reduce the cost of locomotion, directly influencing the net benefits it draws from foraging. Trail construction is known across many different animal groups, including elephants (Blake and Inkamba-Nkulu; 2004) and rodents (Gauthier and Bider; 1986) (see Perna and Latty; 2014, for a review).

Two groups stand out in the diversity of structures and dimensions of collaboration: humans and social insects. Transport infrastructure is ubiquitous in human cities, in the shape of highways, bridges, and tunnels, the benefits of which are likely intuitively evident to the reader. Analogues exist in social insects: weaver- and army ants assemble into bridges to cover gaps in their environment (e.g. Anderson and McShea; 2001b; Bochynek and Robson; 2014; Reid et al.; 2015); ants and termites construct roofed tunnels (e.g. Anderson and McShea; 2001a; Tschinkel; 2010), and some ant species build cleared highways to foraging sites or to connect sub-nests (Lanan; 2014).

While analogues between human and insect construction might suggest immediate comparability, the method of organisation underlying collective efforts in each is fundamentally different: human societies are hierarchical "top-down" constructs, in which decisions are made by a minority of informed individuals that delegate relevant tasks to the lower tiers. In contrast, social insects are self-organised: decisions are made collectively (or, from the "bottom-up"), and there exist no hierarchical structures (Bonabeau et al.; 1997). Decision-making and mechanisms of the construction process are fundamentally different between these two groups.

1.1   Self-organisation in social insects

The collective behaviours of social insects have fascinated philosophers and scientists throughout history, as reflected by numerous poetic references. This is in part due to an apparent conundrum: individual ants, termites, and bees appear to be cognitively limited, but as a leaderless collective perform feats of remarkable complexity.

This complexity is the result of self-organised processes. Self-organisation refers to the spontaneous emergence of order in an unordered system. Spontaneous, in this context, means that no order-giving or organising entity is involved; rather, order arises from the interaction of the non-organised elements of the system via positive feedback mechanisms. These processes were first described in non-living systems, for example in the formation of convection cells in heated layers of oil, in which individual molecules following a local density gradient organise into so called Bénard convection cells (Whitesides and Grzybowski; 2002; Getling; 1998). They have since been used in explaining phenomena in many scientific disciplines, from chemistry and physics (Taylor and Tinsley; 2009) to physiology (Vendruscolo et al.; 2003), biology (Camazine; 2003), and astrophysics (Fridman and Gorkavyi; 1999), and have inspired the design of algorithms (Mamei et al.; 2006) and technological applications (Brambilla et al.; 2013).

Self-organisation has developed into the framework for investigating collective behaviour in social insects (Bonabeau et al.; 1997). Individual workers have access to and can respond to local information only. This

information can be an environmental gradient (e.g. of temperature, humidity, light intensity or pheromones) or a worker-created signal or cue (e.g. via the laying of trail pheromones).

Individual responses can be based on templates, where specific cues elicit behaviours (Theraulaz et al.; 2003; Camazine; 2003): CO_2 gradients influence brood sorting (Cox and Blanchard; 2000), and elevated levels trigger excavation behaviour to enhance nest ventilation (Hangartner; 1969). In the termite *Macrotermes subhyalinus*, workers construct walls of a royal chamber in a fixed distance to their queen, as estimated by the diffusion gradient of a queen pheromone (Bonabeau et al.; 1998).

The reaction to such stimuli in some cases is governed via behavioural thresholds: workers will perform a task (e.g. the deposition of building material) if a stimulus (e.g. a pheromone or CO_2 concentration) exceeds the individual response threshold for that behaviour (Theraulaz et al.; 1998; Bonabeau et al.; 1998).

These templates can be adaptive: termite chambers are extended as the queen grows and the intensity of the pheromone gradient increases, and the ant *Leptothorax tuberointerruptus* uses the number of workers and brood in the colony to assess and adapt the size of their nest chambers (Franks and Deneubourg; 1997).

At the same time, worker behaviour can produce new stimuli. If workers respond to these by repetition of the behaviour, they create a positive feedback loop. Such behaviour, in which worker action is coordinated via indirect information transfer, is termed stigmergy (Theraulaz and Bonabeau; 1999). Classic examples are the trail following behaviour in ants and nest construction in termites: ants following pheromone trails will reinforce the existing trail (Czaczkes et al.; 2015), and both ants and termites will preferentially extend nest walls that have recently been constructed (Petersen et al.; 2015; Khuong et al.; 2016).

As the process continues, the nature of the initial stimulus can change and elicit a different behaviour, leading to a multi-stage construction process (Smith; 1978; Theraulaz et al.; 2003). Such stigmergic processes are limited by negative feedback, which prevents an infinite repetition, either via environmental feedback, e.g. by pheromone evaporation (Deneubourg and Franks; 1995) or absence of spatial cues (Theraulaz et al.; 2002), or by modulation of worker behaviour: workers on crowded trails in the ant

Lasius niger deposit less trail pheromones to limit overcrowding (Czaczkes et al.; 2013), and honey bees inhibit foraging to unproductive sites by interfering with recruitment behaviour of nest mates (Seeley et al.; 2012). The interplay of these mechanisms shapes the colony-level behaviour. In Chapter 3 – Mechanism of trail clearing, I review examples of how these processes shape the nest construction behaviour.

In my thesis, I investigated the trail clearing behaviour in the leaf-cutter ants *Atta*. Below, I review what is known about trail clearing in ants in general; then, I will introduce the study species.

1.2 Cleared trails in ants

Ants are successful in large part because of their ability to cooperate in groups, accomplishing collectively far more than individuals could achieve alone. While most ant species rely on pheromone trails to guide their collective movements (Hölldobler and Wilson; 1990; Czaczkes et al.; 2015), a small number of ant species additionally build tangible trails cleared of undergrowth and organic debris, sometimes down to bare soil – outstanding features of order in an otherwise unstructured environment. In the literature, such trails are often referred to as "trunk trails", but the term is at times also applied to persistent, non-cleared trails and underground tunnels (see Lanan; 2014). Here, I used the term "cleared trails" to avoid confusion.

Cleared trails have been reported for species of *Atta* and *Acromymrex* (Gamboa; 1975; Wetterer; 1995; Hölldobler and Wilson; 1990) *Formica* (Savolainen; 1990), *Lasius* (Quinet et al.; 1997), *Pogonomyrmex* (Hölldobler and Wilson; 1990), *Messor* (Acosta et al.; 1993; Plowes et al.; 2013), *Camponotus* (Marlin; 1971), and *Iridomyrmex* (Greaves and Hughes; 1974) (but see supplementary material in Lanan (2014) for additional, sometimes ambiguous, literature mentions).

They are either used to connect individual nests in polydomous species, or lead to foraging sites (Lanan; 2014), and can persist for several years (e.g. Howard; 2001; Wirth et al.; 2009; Lanan; 2014).

Even in absence of pheromone trails, they serve as physical guide to resource sites (Hölldobler; 1976; Shepherd; 1982). They function as territory markers (Fowler and Stiles; 1980; Hölldobler and Lumsden; 1980) as well as foraging memory (Shepherd; 1982; Wirth et al.; 2003a;

Elizalde and Farji-Brener; 2012; Farji-Brener and Sierra; 2016). Scouts use trunk trails as base for new exploration, thereby localising colony activity (Fowler and Stiles; 1980; Shepherd; 1982; Kost et al.; 2005; Farji-Brener and Sierra; 2016), and serving as "anchor" for future trail expansions (Kost et al.; 2005).

In a review of foraging methods and foraged resources across ant species, Lanan (2014) finds cleared trails are associated with foraging behaviour on leaves, seeds, and honeydew, which are patchy, persistent and regenerating resources. Colonies are known to switch between trunk trails (Gordon; 1991), a behaviour that has been interpreted as conservative resource management (Fowler and Stiles; 1980).

Benefits derived from trail use depend on the ecology of the trail-clearing species. Ants on these trails move at greatly increased speed (Rockwood and Hubbell; 1987; Fewell; 1988). In harvester ants *Pogonomyrmex*, the resulting higher resource rate intake might be the main benefit (Fewell; 1988): seeds are stored underground and do not readily spoil, and fast exploitation of a contested resource is essential.

1.3 Cleared trails in the leaf-cutter ants *Atta*

Most prominent amongst trail clearers are the New World leaf-cutter ants in the genus *Atta*. Their trails can extend to lengths of up to 250 m (Lewis et al.; 1974; Shepherd; 1982; Wirth et al.; 2003b), and a single *Atta* colony may clear thousands of meters of trails over a year (an average of 2730 m yr^{-1} for colonies of *Atta colombica* in the rainforest of Barro Colorado Island (BCI), Panama (Howard; 2001)). Figure 1.1 and Figure 1.2 show an overview and a close-up of these trails. Such cleared trails connect the monodomous nests housing up to several million individuals (Villesen et al.; 1999) to persistent feeding sites, from which foragers retrieve leaf fragments and other plant material. These serve as substrate for their symbiotic fungus, which provides the main source of nutrients to the colony (Hölldobler and Wilson; 1990, 2010).

Costs and benefits derived by trail use in leaf-cutter ants are not well described. Benefits for leaf-cutter ants have only been given in terms of movement speed increase (Rockwood and Hubbell; 1987); energetic costs have been considered conceptionally (Lugo et al.; 1973; Shepherd; 1982),

Figure 1.1: *Atta* sp. trail in Costa Rica. Picture courtesy of Dmitri Logunov, Manchester Museum; used with permission

Figure 1.2: Close-up of a cleared trail. Trail width (outlined) measured approximately 8 cm. Picture taken on Barro Colorado Island, Panama

and quantified in a case study (Howard; 2001). I defer a detailed analysis of known literature to Chapter 2.

Further benefits might be associated with the adaptivity of trail clearing, which I review in Chapter 3.

1.4 Leaf-cutter ant biology

The model organism for my research is the ant genus *Atta*. The genera *Atta* and *Acromyrmex* (located in the tribe Attini, subfamily Myrmicinae, family Formicidae, order Hymenoptera) are communally referred to as leaf-cutter ants. The name reflects their unique diet (Schultz and Brady; 2008): they harvest plant material, which serves as a substrate for the cultivation of the symbiotic fungus *Leucoagaricus gongylophorus* (Hölldobler and Wilson; 1990, 2010). The ability to convert an abundant but low-quality resource like plant material into a viable food resource has enabled leaf-cutter ants to become the dominant herbivore in the New World tropics (Hölldobler and Wilson; 1990), with colony sizes of up to 8 million individuals (Hölldobler and Wilson; 2010). Estimates of biomass collected each year in the form of plant material by leaf-cutter ants range as high as 939.6 kg per colony in *Atta cephalotes*, or about 2% of the annual leaf production in a study surveying a 100 ha forest patch (Herz et al.; 2007). Assuming an average fragment dry weight of

4.5 mg, this approximates **210** million fragments harvested (Fowler and Robinson; 1979). In addition to their environmental impact, the species causes several hundred billion dollars of damage annually to commercial plantations in South America (Capinera; 2008).

1.5   Thesis scope

In recent decades, the self-organised construction behaviour of social insects has been in the focus of researchers from diverse academic fields. It has predominantly focussed on aspects of nest construction (e.g. in Petersen et al.; 2015; Buhl et al.; 2004; Bonabeau et al.; 1998; Khuong et al.; 2016; Camazine et al.; 1990; Franks et al.; 1992; Theraulaz and Bonabeau; 1995) and the digging of tunnels (e.g. Su and Lee; 2009; Buhl et al.; 2004; Bardunias and Su; 2010; Buhl et al.; 2006; Lee et al.; 2008). At the same time, the construction of cleared trails has received comparatively little attention.

In my thesis, I used a threefold approach to investigate trail clearing in *Atta*, focussing on trail energetics, construction mechanism, and the evolution of the behaviour.

In absence of an analysis of energetic and ecological benefits derived from their use, one cannot assess the ultimate function of such trails. While some pioneering work exists in the quantification of construction (in *Atta*, Howard; 2001) and foraging costs (in *Pogonomyrmex*, Fewell; 1988), it provides only a snapshot of trail energetics. In Chapter 2 – Energetics of trail clearing, I developed a comprehensive model of costs in *Atta* that takes into account previously unrecognised workforce requirements, and proposed a method for the quantification of trail benefits.

Not all trails in nature are cleared, which suggests the possible existence of regulatory mechanisms for trail clearing. In Chapter 3 – Mechanism of trail clearing, I quantified individual trail clearing behaviours and worker/trail obstruction interactions. Based on these, I proposed a model for the control mechanisms underlying *Atta* trail construction.

A number of studies found that cleared trails bind foraging workforce and as scouts to their vicinity, which results in a reduction of the colony territory and reduces rate of resource discovery (e.g. Farji-Brener and Sierra;

2016). While foraging material appears abundant in tropical forests, high-quality resource distribution is patchy (Wirth et al.; 2003a), and a reduction in territory could conceivably be costly. This raises the question of how such seemingly adverse spatial effects have influenced the evolutionary trajectory of the behaviour. In Chapter 4 – Evolution of trail clearing behaviour, I quantified the influence of these effects, and in an evolutionary game theory setting investigated how they influenced the evolution of trail-clearing.

I reviewed the results of my thesis and the direction for subsequent research in Chapter 5 – Conclusions.

Chapter 2

Energetics of trail clearing

Note that the results presented in this chapter have been published in a journal article (Bochynek et al.; 2017). The content of this chapter is partially identical with that of the publication. While I wrote most of this article, individual sentences were revised by my Ph.D. supervisors and co-authors Prof. Bernd Meyer and A/Prof. Martin Burd, and might be present in this chapter. Work on this chapter profited from the feedback of two anonymous reviewers, for which I am grateful.

2.1 Introduction

In Chapter 1 – Introduction I reviewed the occurrence of cleared trails amongst ants and the extraordinary quality of those in *Atta*. Here, I review published work on the costs and benefits in this genus.

2.1.1 Costs of foraging in *Atta*

Networks encompassing several kilometres of cleared trails conceivably require a substantial energetic investment. Shepherd (1982) hypothesised that the benefits of improved foraging through the ease and speed of movement (Rockwood and Hubbell; 1987; Fewell; 1988) on cleared trails would outweigh the costs of time and effort devoted to construction and maintenance of trails. In a case study with *A. colombica*, Howard (2001) explored this suggestion by making estimates of the annual energetic and time costs of trail construction. His estimates were based on observation of ants cutting and removing leaf litter from trails and measurements of leaf litter fall rates. Leaf removal required around 11,000 ant-days of

effort, an annual energetic cost approximately equivalent to the intake of 8000 leaf fragments. Considering that colonies often field more than $10,000$ foragers (Lugo et al.; 1973; Howard; 2001; Bruce and Burd; 2012) at a given time, Howard concluded that the payback for trail clearing by an entire colony "can be accomplished in a matter of days" (Howard; 2001).

These measurements, useful though they are, do not compare the energetics of trail construction against its alternative: what would happen if leaf-cutter ants did not clear their trails? Any adaptive advantage of *Atta* trails can be understood only in reference to the alternative of walking over forest floor litter to retrieve the same quantity of leaf fragments while avoiding the investment in trail clearance and maintenance. In the field, *Atta* colonies do indeed sometimes forage on uncleared trails. While this can be a temporary necessity (e.g. during the establishment of a trail to a new resource site, or during collection of ephemeral, scattered resources like fallen fruit), other uncleared trails are used for weeks of foraging (personal observation). The use of such uncleared trails suggests that trail clearing may not be profitable under all conditions.

2.1.2 Standby clearing workforce

The model incorporated an aspect of trail clearance that has been previously overlooked. Long-term use of a trail requires continuous clearing of newly occurring obstructions. Howard (2001) considered only the immediate energetics of cutting and removing such obstructions from a trail, but not the cost to the colony of fielding the extra workforce to carry out these maintenance tasks. I postulated that maintenance requires more workers to be present on a trail than would otherwise be needed for foraging, defence, and other tasks.

There are indications that these maintenance workers are partially distinct from other ants on foraging trails. Trail clearers tend to be larger than leaf-carrying foragers (about 15% greater head width, on average), but are rarely drawn from the largest workers, soldiers with head widths above 3.0 mm (Howard; 2001). Laden workers do not readily drop their fragments to remove obstacles they encounter (personal observation), leaving trail clearing to be performed by unladen workers, as previously suggested by Lugo et al. (1973) and Lewis et al. (1974). Ants marked during trail clearance displayed a higher probability than foragers

of being recruited to experimental trail debris **24** hours later (Howard; 2001).

The observations of trail clearing in laboratory colonies of *Atta* (see Chapter 3 for details) suggest that about a quarter of the obstructions removed from the path are displaced by ants meandering on the trail outskirts rather than actively moving with the traffic stream. Although trail clearers are statistically distinguishable from other workers, they are possibly recruited to obstacles by known mechanisms of response thresholds (Theraulaz et al.; 1998) from the pool of workers available on a trail, and may return to other tasks after trail clearing. The time they spend clearing, however, must reduce the overall rate at which other tasks are performed throughout the trail. In order to redress the shortfall, a colony would need to field additional workers so that all tasks are performed at the desired rate, given the need for redundancy that probabilistic response thresholds entail (Herbers; 1981). Moreover, a multi-tasking workforce sufficient to perform all other tasks at the needed rates would not be sufficient to maintain trails if trail clearance is the most demanding task. Colonies in the field seem capable, however, of rapid response to trail obstructions. Howard (2001) noted that clearing ants were recruited to experimental obstacles in a mean time of **123** s after placement of the obstacle on the trail, implying that a reserve of workers is available throughout the trail to respond to unpredictable need for clearance.

It is difficult to estimate empirically how much the traffic stream of a colony is expanded to satisfy this need, but since laden ants do not generally remove obstacles, it is likely that some fraction of the unladen ants account for the additional capacity. In my model I represented this fraction as a "clearing workforce" specified by an adjustable parameter that reflects its size. I then explored variation in this parameter within the observed range of unladen ants in natural traffic flows. This new perspective highlights the need for research on the enigmatic presence of the large fraction of ants on a trail seemingly not performing any task.

2.1.3   Chapter Scope

I calculated both the costs and benefits of trail clearing in a common energetic currency by drawing on a variety of published sources and my own measurements of movement on cleared and uncleared trails of field

colonies of *Atta*. I used the model to examine how the energetic profitability of trail clearing changes with the length and usage of trails. I particularly investigated the effect of altering the proportion of unladen ants in the traffic stream and their contribution to a stand-by clearer workforce.

I find that trail clearing costs could be recovered within a few days under many realistic foraging parameters, but that there were also realistic conditions under which amortisation takes weeks or months, or was never achieved. The composition of the workforce was one of the deciding factors in the cost/benefit balance, and I made predictions about behaviour that are testable in the field.

I outline the conceptual structure of the model here, while giving a full account of all parameters and equations used in 2.1.8 – Model details.

My strategy for calculating the energetic value of trail clearance was to estimate the metabolic expenditure due to all ant activity on a cleared trail and compare this to the total expenditure needed for an equal leaf harvest over an uncleared trail. Locomotion and load carriage will be less energetically demanding on a cleared trail, but the colony incurs the cost of clearing and maintaining the trail. In contrast, workers will expend more energy traversing an uncleared trail, but the colony avoids construction and maintenance expenses. The balance of these effects yields the net benefit of trail clearance to the colony:

$$B(t) = E_{UC}(t) - E_C(t) \text{ [J]}, \tag{2.1}$$

where B denotes the benefit in Joules; E the energetic cost of all worker activity on the trail including any clearance, outbound and inbound travel, and load carriage; subscripts UC and C represent uncleared and cleared trails, respectively; and t the duration of foraging (see Equation 2.30). The development of new trails carries an initial clearance cost, so the net benefit, B, starts out negative at $t = 0$ in my model. But if cleared trails deliver a net advantage, the energetic savings will accumulate as foraging progresses, so that the initial trail investment is recuperated and B eventually rises to zero. Further foraging on the trail then delivers a positive net benefit (see Figure 2.1).
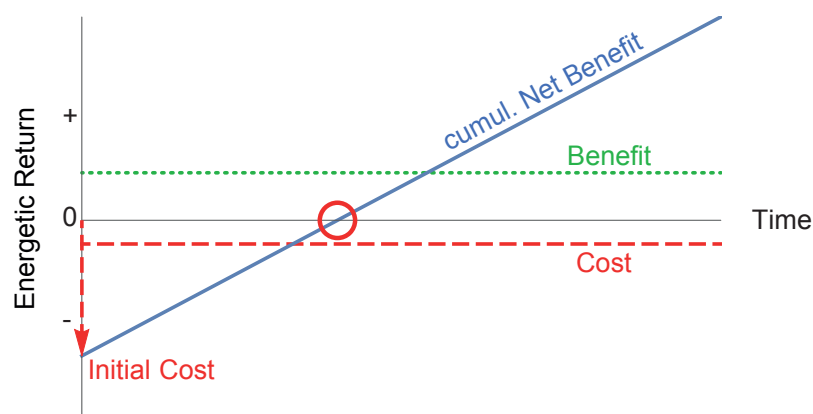
Figure 2.1: Cost/benefit model concept. One-time initial clearing cost and continuous daily costs (indicated by dashed line) are amortised by the continued energetic savings of foraging on a cleared trail. Continuous costs are a combination of maintenance costs and the cost of patrolling standby clearers. As long as the energetic savings (indicated by dotted line) on a cleared trail outweigh the cost of keeping it cleared, a break- even point (indicated by red circle) is reached

I used the time at which $B = 0$ (the "break-even time") as the measure of model performance in order to compare the effect of changing parameter values.

2.1.4   Components of trail clearing and maintenance

How much material must be removed to create a cleared trail? Howard (2001) measured standing leaf litter on the ground of the BCI rainforest and estimated that colonies of *A. colombica* removed approximately **1.22** kg of leaf litter per square meter of trail area during the initial construction of a trail. A similar amount of fresh litter fall, **1.44** kg, needed to be continuously removed from an existing trail over the course of a year (Leigh et al.; 1982). Howard (2001) then measured the time cost (ant-hours) needed to cut and remove natural obstacles of known mass from active trails. He extrapolated these experimental measurements to the time investment needed to remove **1.22** or **1.44** kg of litter per square meter of trail surface and translated the time costs to energetic costs based on metabolic expenditures during leaf cutting and load carriage reported by Roces and Lighton (1995) and Lighton et al. (1987). I used these estimates of energetic cost directly in my model.

Howard's estimates referred to the direct metabolic costs of removing obstacles, but not to the costs of making workers available to respond to unpredictable needs for trail maintenance at all times. I incorporated this cost in my model with a term representing a stand-by clearing workforce. As noted earlier, this workforce needs not exclusively perform trail maintenance and so needs not be a completely distinct portion of the trail traffic. Nonetheless, the need for trail clearance would add to the workforce needed on the trail, and I accounted for the extra demand by adding a term in the model to represent the trail clearers as a fraction of the unladen ants occurring on the trail.

The proportion of unladen workers observed on trails varies greatly, from 13% to 80% of a traffic stream (Lutz; 1929; Cherrett; 1968, 1972; Hodgson; 1955; Lewis et al.; 1974)(see Table 2.3). Not all of these unladen workers are necessarily stand-by clearers, but little comprehensive research exists on what function they perform. Bollazzi and Roces (2011) argued that during establishment of foraging traffic, workers returned to the nest without carrying leaves to maximise recruitment speed, which would give some unladen workers a role in information transfer. The smallest unladen workers (referred to as "minims") are known to hitchhike on and clean leaf fragments during transport (Griffiths and Hughes; 2010) and to play a role in pheromone trail maintenance (Evison et al.; 2008). Littledyke and Cherrett (1976) noted that unladen workers aid in leaf-sap transport and Da-Silva et al. (2012) observed water transport by unladen workers, although these observations came from a laboratory setting.

Thus, it is challenging to make informed estimates of the number of extra workers present on a trail due to the need for trail maintenance. I explored the range of possible variation up to the extreme point at which all unladen workers are taken to be a stand-by workforce for trail clearing. I represented this workforce with a model parameter u_S designating a fraction of the unladen ants on the trail and analysed how the energetics of trail clearing change with this proportion.

2.1.5 Effects of cleared and uncleared trails on movement

The net benefit of clearing, if one exists despite the investment costs, would come from easier locomotion along a cleared trail. That is, workers using an uncleared trail move more slowly (which might provide energetic savings), but thus expend metabolic energy for a longer time on a given journey than they would if the trail were cleared. To measure this effect, I recorded the traffic of an *A. colombica* and an *A. cephalotes* colony over cleared and uncleared portions of their foraging trails in April 2014. Each colony had a number of foraging trails that persisted during several weeks. I recorded two-minute segments of traffic flow during peak foraging hours on three and four cleared segments and two uncleared segments of the trails of each colony, respectively. The cleared trails had been cleared down to the soil and featured no obvious obstructions. For uncleared trails, I only sampled sections of trails that showed no visible indication of leaf litter removal. From the recordings, I measured the time needed for randomly selected ants (529 in total) to cover 30 cm of trail length, and calculated their speed. Because of the nature of the recordings, data extraction was non-blind. I found an approximately two-fold greater speed on cleared trails than on uncleared trails (see Table 2.2 for averaged results, and electronic supplementary material for the full dataset). This is smaller than the suggested four- to ten-fold increase reported by Rockwood and Hubbell (1987). The effect is likely to vary with the abundance and nature of the trail obstructions. A greater locomotion advantage of cleared trails would yield a greater energetic advantage in my model.

2.1.6 Translating movement to metabolic cost

In my model, a colony's energetic costs were calculated from the time used to perform various tasks, the cost of an individual ant undertaking it, and the number of ants performing them. As described above, I estimated metabolic expenditure given by Howard (2001) per unit area of trail surface to account for activities directly involved in clearing vegetative litter from a trail. The other costs in the model came from locomotion between the nest and the foraging site by foragers, stand-by clearers, and other ants. Journey time was a function of speed, and individual workers could be unladen or laden with leaf fragments. The model calculations reported here assumed that locomotion speed on a cleared trail was a function of

ant size and load mass as reported for *A. colombica* by Burd (1996), and that speed on an uncleared trail was approximately half that on a cleared trail (see Table 2.2). I further assumed that laden foragers carried leaf fragments related to their size according to the average loading pattern observed by Burd (1996) for *A. colombica* harvesting leaves of *Cordia alliodora*. These assumptions were well within the range of typical behaviours for *A. colombica* and *A. cephalotes* in tropical forest, but the model could be parameterised to represent other circumstances, such as the activity of grass-cutting *Atta* species that carry elongated fragments of grass leaves (Moll et al.; 2012).

From walking speed I then estimated metabolic expenditure. Lighton et al. (1987) measured oxygen consumption of *A. colombica* ants at rest and during locomotion and found that the rate of consumption per unit body mass rose linearly with speed. The net rate – consumption while walking less resting consumption – divided by walking speed yielded the net cost of transport (NCOT), i.e. the volume of oxygen consumed in moving a unit mass a unit distance. Taking account of their measured scaling of NCOT with body mass and the well established equivalence of body mass and external load mass on the cost of transport yielded an expression for NCOT as a function of ant mass, load mass, and speed (Lighton et al.; 1987, Equation 16). Oxygen consumption was then converted to Joules of metabolic energy (Lighton et al.; 1987, Equations 3 and 11). See 2.1.8 – Model details for details on the calculation of metabolic costs.

2.1.7 Complete trail activity

The model represented the simple case of a single trail of length *l*, which may be completely cleared or completely uncleared. *Atta* colonies opportunistically exploit fallen branches, rocks, or other naturally exposed features as part of their trails. Howard (2001) estimated of clearing cost accounted for such features as 9.3% of the total length of a trail, but Farji-Brener et al. (2007) found that naturally exposed branches made up 30% of the length of *A. cephalotes* trails in Costa Rica. For simplicity, I used estimates from Howard (2001) with their implied proportion of 9.3%, but other values could be modelled by simple adjustment of the clearing cost per unit of trail length. Ants on the model trail followed a body size distribution based on the measurements of Howard (2001), in which trail-clearing workers were about one-third heavier than foragers

(see Equation 2.5). The number of laden ants using the trail was esti-
mated from the scaling of traffic flow with trail length reported for 18
Atta colonies in Costa Rica and Panama (Bruce and Burd; 2012). The
number of laden ants returning to the nest per minute scaled as a slightly
superlinear function of trail length (scaling exponent = 1.28). I calcu-
lated the number of round trips per day needed to maintain these laden
traffic flows, assuming 10 hours of foraging per day and accounting for
the typical daily rhythm of foraging activity (see 2.1.8 – Model details).
For a 100 m trail, my model implied 6933 leaf fragments harvested per
day.

Laden ants in the traffic flow were accompanied by unladen nest
mates, which make up a fraction u of the total traffic. I explored a
range of values for u from the lowest to highest values reported in the
literature (see Table 2.3). To account for unladen workers that might
perform tasks not related to trail clearing, I introduced the parameter
u_S to represent the fraction of unladen ants working as trail clearers.
Because the potential energetic cost of trail clearers had not previously
been recognised, the fraction u_S has never been investigated empirically.
I considered the full range of possible values from 0 to 1 for calculating
model results. In particular, I searched for the parameters conditions
under which trail clearing was or was not an energetically beneficial be-
haviour, and investigated the dynamics at the transition between these
phases.

In sum, a given trail length in the model implied a certain number
of laden and unladen round trips per day by ants of a certain size dis-
tribution. Ants moved at speeds determined by their size and, if laden,
the typical pattern of loading, and by the state of the trail, cleared or
uncleared. The metabolic cost of round trips at these speeds were de-
rived from the physiological measurements of Lighton et al. (1987), and
the model thus calculated the total metabolic expenditure involved in all
trail activity. A comparison of the energetic costs on cleared and un-
cleared trails yielded the net benefit of clearing, B, as outlined above.
Below, I give the full details of the model.

2.1.8 Model details

This section lists all parameters and functions of the used model in full detail. In the original publication (Bochynek et al.; 2017) it was included as Appendices A and B.

Model parameters

Here, I give the structure of the model and its parameters, and list the sources for parameters values. Many of the relationships described below are functions of ant size, but because they are drawn from diverse literature, use different measures of size, either body mass, head width (longest dimension of the head capsule), or metathoracic femur length. I used body mass in grams as the fundamental measure of size in my model calculations. In order to translate among the three measures, I used scaling relationships determined by reduced major axis regressions for size measurements of 352 workers of an *Atta colombica* colony on Barro Colorado Island (BCI), Panama (Burd, unpublished data). The relationships among ant mass, a, in g; head width, h, in mm; and femur length, fl, in mm, are given by:

$$a_h(h) = 1.047 \cdot h^{2.954} \text{ [g]}, \tag{2.2}$$

$$fl_a(a) = 1.1173 + 1.2408 \cdot \ln(a \cdot 1000) \text{ [mm]}. \tag{2.3}$$

$$a_{fl}(fl) = e^{(fl-1.1173)/1.2408}/1000 \text{ [g]}, \tag{2.4}$$

While the near-cubic relationship in Equation 2.2 is somewhat intuitive, the relationship between femur length and body mass is more complicated: Feener et al. (1988) fit a third-order polynomial to their empirical data. Instead, data analysed by Burd(unpublished) fits well to the equation given in Equation 2.3; Equation 2.4 is obtained by solving Equation 2.3 for a.

Worker size distribution

Howard (2001) measured the head width of foragers transporting leaf fragments and of workers clearing obstacles from trails at three colonies of *A. colombica* on BCI. I combined his data from the three colonies and determined the mean and standard deviation of size for laden foragers and for trail clearers (Table 2.1), translating head width to body mass

using the allometric equations described above. I assumed in the model calculations that both groups of workers had normally distributed body mass with probability density function $\xi_F(a)$ for foragers and $\xi_S(a)$ for stand-by clearers, with the parameters specified in Table 2.1. For numerical calculation, I truncated the tails of both distributions at six standard deviations below and above the mean.

Table 2.1: Worker size metrics, measured in head with (mm) by Howard (2001) and translated to body mass (g)

Parameter	Worker type	Mean	S.D
Head width h [mm]	Forager	1.992	0.271
	Standby clearer	2.245	0.302
Ant mass a [g]	Forager	0.00802	0.00109
	Standby clearer	0.1141	0.00154

Clearing costs

Howard (2001) estimated the energetic costs of clearing a new trail by measuring the amount of standing forest floor litter that needed to be removed and the time taken by workers to perform the removal tasks. He found that colonies removed an average of **14.4 kilog** of leaf litter from **267 m** of trail (**16.5 m^2** of trail area). He then translated the time cost of removal tasks into energetic expenditure using the respirometry measurements of leaf cutting and load transport by *A. colombica* provided by Lighton et al. (1987) and Roces and Lighton (1995). Howard (2001, Table 3) estimated that an average of **66.3 kJ** was expended to clear **267 m** of trail, which gives the initial cost x_0 for clearing a single meter of trail:

$$x_0 = 248.3 \; [\text{J}\,\text{m}^{-1}] \tag{2.5}$$

Using estimates of annual leaf litter fall per unit area in the BCI forest rather than the standing crop of litter, Howard (2001) further estimated the energetic costs of maintaining a cleared trail. The average energetic cost of removing new leaf fall from the same trail system of **267 m** amounted to **109.5 kJ** per year, or

$$x_m = 1.1236 \ \text{J}\,\text{m}^{-1}\,\text{d}^{-1} \tag{2.6}$$

Leaf fragment size

Like locomotion speed in relation to ant size, the pattern of load carriage in relation to ant size has been measured repeatedly. I used the loading pattern determined by Burd (1996, Table 1) for an *A. colombica* colony harvesting leaves of *Cordia alliodora* to give load mass m in g, as function of femur length fl in mm:

$$m(fl) = 0.000229 \cdot fl^{3.26} \ \text{g}. \tag{2.7}$$

Cordia leaves had a density close to the average to that of harvested plants as measured by Wetterer (1994). There are contrasting indications about whether the loading pattern depends on trail length (see Roces (1990) and Wetterer (1991)); for simplicity, I assumed that load size does not change with trail length.

Movement speed

The speed of leaf-cutting ants moving on cleared trails has been measured by many researchers. I used the relation found by Burd (1996, Table 2) for *A. colombica* ants on BCI, which gave velocity as a function of ant size in femur length, fl, and leaf fragment mass, m:

$$v_{C}^{L}(fl, m) = (-7.83 - 920 \ m(fl) + 16.41fl) \cdot 0.0036 \ [\text{km}\,\text{h}^{-1}]. \tag{2.8}$$

The parameter values accommodate fragment mass measured in grams and the factor 0.0036 converts speed from $\text{mm}\,\text{s}^{-1}$ (the units used by Burd (1996)), to speed in $\text{km}\,\text{h}^{-1}$, the units used by Lighton et al. (1987). The load mass for unladen workers equals zero, and so the velocity expression for these ants can be written:

$$v_{C}^{UL} = v_{C}^{L}(fl, 0) \ [\text{km}\,\text{h}^{-1}]. \tag{2.9}$$

In these expressions, I used subscript C to indicate cleared trails and superscripts L and UL to indicate laden and unladen workers, respectively.

Table 2.2: Movement speed on cleared and uncleared trails. Relative speed, w, is the ratio of mean speed on uncleared trails to the mean on cleared trails

	Cleared trail		Uncleared trail		
	Mean speed $(\mathrm{cm\,s^{-1}})$	S.D. speed $(\mathrm{cm\,s^{-1}})$	Mean speed $(\mathrm{cm\,s^{-1}})$	S.D. speed $(\mathrm{cm\,s^{-1}})$	Relative speed (w)
Laden workers	3.14 (n=157)	0.83	1.37 (n=110)	0.64	0.44
Unladen workers	4 ($n = 150$)	1.1	2 (n=112)	0.53	0.5

To account for the effect of trail obstructions on locomotion speed, I measured the velocity of **529** laden and unladen *A. colombica* and *A. cephalotes* ants as they travelled over cleared and uncleared portions of foraging trails. Ants on uncleared trails moved, on average, at a fraction w of their speed on cleared trail (Table 2.2). Thus, in calculating the energetic costs as benefits of trail clearance, I assumed that travel speed on an uncleared trail is $w_L \cdot v_C^L$ for laden ants and $w_{UL} \cdot v_C^{UL}$ for unladen ants.

Oxygen consumption

I estimated metabolic costs in my model from the respirometry measurements of metabolism in *A. colombica* made by Lighton et al. (1987). They found that the standard (i.e. resting) respiratory rate r_S (volume of oxygen consumption per unit body mass per unit time) scaled as a function of ant body mass, a:

$$r_s(a) = 0.074 \cdot a^{-0.38} \; [\mathrm{ml_{O_2}\,g^{-1}\,h^{-1}}] \tag{2.10}$$

(derived from Lighton et al. (1987, Equation 9), with the scaling exponent on ant body mass in the equation above equal to the exponent in Equation 9 minus unity to account for standardisation of the respiratory rate per unit body mass). The respiratory rate of a moving unladen ant on a cleared trail, r, measured in $\mathrm{ml_{O_2}\,g^{-1}\,h^{-1}}$, varied as a function of the ant's speed v in $\mathrm{km\,h^{-1}}$ (Lighton et al.; 1987, Figure 6):

$$r(v) = 0.72 + 10.05v \; [\mathrm{ml_{O_2}\,g^{-1}\,h^{-1}}]. \tag{2.11}$$

The net cost of transport ($NCOT$) gives the oxygen consumption required to move a unit mass a unit distance. I used Equation 16 of Lighton et al. (1987) to calculate $NCOT$ based on the net oxygen consumption (i.e. the total oxygen consumption $r(v)$ minus the standard rate of oxygen consumption, r_s). Dividing this net rate by movement speed provided transport costs per unit distance. This term was adjusted to account for scaling of $NCOT$ with body mass (see explanation of Equation 16 in Lighton et al. (1987)). To calculate $NCOT$ for laden ants, I relied on the equivalence of load mass and body mass in their effect on the cost of transport (Lighton et al.; 1987). I introduced an additional term, the loading ratio $(1 + \frac{m}{a})$, which evaluated to 1 for unladen ants (cf. Lighton et al.; 1987, Equation 19). Combining these elements, I calculated $NCOT$:

$$NCOT(v, a, m) = \frac{r(v_C^{UL}(fl_a(a))) - r_s(a)}{v} \cdot (\frac{a}{0.0314})^{-0.60} \cdot$$
$$(1 + \frac{m}{a}) \ [\text{ml}_{O_2} \ \text{g}^{-1} \ \text{km}^{-1}]. \tag{2.12}$$

The relationship between respiration rate and speed was measured by Lighton et al. (1987) for unladen ants on cleared trails, and so I used the expected velocity for an unladen ant on a cleared trail, $v_C^{UL}(fl)$, in the expression above. To account for the effect of a carried load and the state of trail clearance on speed, the velocity v in the denominator of the expression above could take on the value appropriate to the loading and trail state (e.g. v_C^L). Thus, the expression accounted for the longer time over which a slowly moving ant incurred a metabolic cost in order to traverse a unit distance.

Metabolic energy

Metabolism measured by oxygen consumption can be converted to Joules of energy expenditure using a mass-dependent conversion factor, $K(a)$, based on Lighton et al. (1987, Equations 3 and 11)

$$K(a) = 15.97 + 7.71 \cdot a^{0.18} \ [\text{J} \, \text{ml}_{O_2}{}^{-1}]. \tag{2.13}$$

To calculate the net energetic expenditure in Joules per g of body mass a and load mass m at velocity v and per metre:

$$NCOT^J(v, a, m) = NCOT(v, a, m) \cdot K(a)/1000 \ [\text{J} \, \text{g}^{-1} \text{m}^{-1}]. \tag{2.14}$$

Number of round trips in a day

I calculated the number of round trips performed by a colony in a day based on data by Bruce and Burd (2012), who measured the returning flow of laden workers per minute at the entrances of 18 colonies of *A. colombia* and *A. cephalotes* during peak foraging activity. The flow of laden ants per minute scaled with trail length l in metres:

$$q(l) = 0.008 \cdot l^{1.281} \ [\text{s}^{-1}]. \tag{2.15}$$

Peak flow, however, is not maintained throughout the day. I therefore introduced a modifier that took into account the typical daily rhythm of foraging activity. The rate of leaf fragment delivery to the nest at sampling times over the course of a foraging day has been repeatedly measured (Hodgson; 1955; Cherrett; 1968; Lewis et al.; 1974; Wirth et al.; 2003a). From their plots, I calculated the daily number of fragments taken into the nest relative to the number that would have been retrieved had peak inflow been sustained for the entire day. The mean fraction was 0.66 ± 0.08, which I used to modify the rate $q(l)$ to calculate the total number of foraging round trips a colony makes during a day.

For an assumed 10 hour (or 36,000 second) foraging day, a colony made n_F foraging trips:

$$n_F(l) = 0.66 \cdot 36000 \cdot q(l) \ [\text{day}^{-1}] \tag{2.16}$$

In contrast to laden workers, unladen workers move along the trail without obvious function. They can make up a fraction u of all foragers as small as 13% and as large as 80% of the trail traffic flow (Lutz; 1929; Hodgson; 1955; Cherrett; 1968, 1972; Lugo et al.; 1973; Lewis et al.; 1974)(see Table 2.3). Thus the total number of trips n_T that occurred was

$$n_T(l) = n_F(l)/(1-u) \ [\text{day}^{-1}] \tag{2.17}$$

with $0.13 < u < 0.8$, and the number of unladen trips was

$$n_U(l) = n_T(l) - n_F(l) \ [\text{day}^{-1}]. \tag{2.18}$$

I assumed that stand-by trail clearing workers performed a fraction u_S of all unladen trips n_U. Thus the total number of trips by the stand-by

clearing workforce n_S was

$$n_S(l, u, u_S) = u_S \cdot (n_T(l) - n_F(l)) = u/(1 - u) \cdot n_F(l) \cdot u_S \ [\text{day}^{-1}]. \quad (2.19)$$

Range of observed unladen worker proportions

Table 2.3 gives all mentions of unladen worker proportions from the literature that I am aware of, along with the measurement method. It is perceivable that unladen workers are not uniformly distributed along the trail and that their distribution depends on the distance to the nest. The literature records however do not specify at what location along the trails measurements were undertaken. This might be the case because their potential function as trail clearers has not been recognised. In absence of contrary evidence, the parsimonious interpretation of the literature record is to assume uniform distribution.

Summary of all used parameters

Table 2.4 summarises all used parameters.

Model construction

This section details the construction of the cost/benefit model from the components introduced above.

I calculated the energetic cost of building and maintaining a trail as a function $C(l, t, u, u_S)$ of trail length l and elapsed foraging time t. I calculated the differential energetic benefit $B(l, t, u, u_S)$ of foraging on a cleared trail versus an uncleared trail as a function of the same parameters. To determine the break-even time for establishing and using a cleared trail, I solved equation

$$B(l, t, u, u_S) = C(l, t, u, u_S) \quad (2.20)$$

Energetic cost of clearing

The energetic cost of clearing C as described by Howard (2001) comprised two components of metabolic expenditure: an initial cost $C_0(l)$ for clearing the trail of length l and a continuous time-dependent expenditure $C_C(l, t)$ for maintaining this trail, for example for removing leaf litter falling on

Table 2.3: Literature records of the proportion of unladen workers in foraging traffic and method used during measurement

Unladen workers on trail (%)	Species	Measurement method	Source
13.1	*A. cephalotes*	Average of 150 one-minute counts over five days (Table 2).	Lewis et al. (1974)
55.8	*A. cephalotes*	One count of laden and unladen ants per hour for 24 hours, for one colony (Figure 2). I compared area under curves for laden and unladen workers.	Cherrett (1968)
35.8	*A. cephalotes*	$n = 22,101$ ants observed during three-minute counts in morning and evening in one colony, for 52 days.	Cherrett (1972)
75	*A. colombica*	Hourly measurements on one trail during one day. Unladen worker ratio calculated from observed average unladen-to-laden worker ratio of 2.81 (Table 4).	Lugo et al. (1973)
28.4, 36, 26.5, 31.9	*A. cephalotes*	Count of all laden and unladen workers on two trails, during two days. From these, I calculated daily averages per trail.	Hodgson (1955)
30.8, 80, 42.7, 43.1	*A. cephalotes*	57 five-minute counts of all laden and unladen workers over four days, Tables 1-4.	Lutz (1929)

Table 2.4: List of parameters from empirical results and literature

Description	Symbol	Value	Unit	Given in
Initial clearing cost	x_0	248.3	$\mathrm{J\,m^{-1}}$	Equation 2.5
Continuous trail maintenance cost	x_m	1.1236	$\mathrm{J\,m^{-1}\,day^{-1}}$	Equation 2.6
Mean mass of foragers	$\bar{a}_F$	0.00802	g	Table 2.1
Mean mass of stand-by clearers	$\bar{a}_S$	0.00109	g	Table 2.1
S.D. mass of foragers	s_F	0.01141	g	Table 2.1
S.D. mass of stand-by clearers	s_C	0.00154	g	Table 2.1
Uncleared trail speed factor for laden ants	w_L	0.44	-	Table 2.2
Uncleared trail speed factor for unladen ants	w_{UL}	0.5	-	Table 2.2

an existing trail. I added to this the cost of providing a stand-by clearer workforce, $C_S(l, t, u, u_S)$:

$$C(l, t, u, u_S) = C_0(l) + C_C(l, t) + C_S(l, t, u, u_S) \ [\mathrm{J}]. \qquad (2.21)$$

Initial cost of trail construction

To simplify the model, I assumed $C_0(l)$ to be a fixed amount of energy that is expended instantly at $t = 0$, i.e. at the beginning of foraging:

$$C_0(l) = x_0 \cdot l \ [\mathrm{J}]. \qquad (2.22)$$

Maintenance cost

The maintenance cost has two components: Firstly, the actual cost for continuous cutting and transporting obstructing leaf litter as it occurs, C_C, as estimated by Howard (2001). For a trail of length l and a duration of t days, this is

$$C_C(l, t) = x_m \cdot l \cdot t \ [\text{J day}^{-1}]. \tag{2.23}$$

Secondly, the cost C_S of provisioning a stand-by workforce to patrol the trail and thus be available to clear new obstructions as they occur. I assumed that, like foragers, stand-by clearers undertook complete round-trip journeys between the nest and the foraging site. For n_S stand-by clearers patrolling a trail of length l in both directions for duration t, this cost was

$$C_S(l, t, u, u_S) = t \cdot n_S(l, u, u_S) \cdot 2l \cdot i_S \ [\text{J}], \tag{2.24}$$

where i_S was the expected cost of a single stand-by clearer to walk a unit of distance.

In general, the cost of transport COT for an ant of mass a carrying load mass m to travel at velocity v could be derived from the net cost of transport in Joules Equation 2.24 as

$$COT(v, a, m) = a \cdot NCOT^J(v, a, m) \ [\text{J m}^{-1}]. \tag{2.25}$$

Note that $NCOT^J$ quantified the energy expenditure in $\text{J g}^{-1}\text{m}^{-1}$, whereas COT quantified the expenditure in J m^{-1} for a single ant of mass a.

Because the load size m could be expressed as a function of body mass a converted to femur length fl, I simplified the cost of transporting a load to

$$COT^L(v, a) = COT(v, a, m(fl_a(a))) \ [\text{J m}^{-1}]. \tag{2.26}$$

The cost of travelling without carrying a load likewise could be expressed as

$$COT^{UL}(v, a) = COT(v, a, 0) \ [\text{J m}^{-1}]. \tag{2.27}$$

To calculate the costs of the unladen stand-by clearers patrolling a cleared trail, I substituted the expected velocity of unladen workers on cleared trails, which could be expressed as a function of body mass a converted to femur length fl:

$$COT_S^{UL}(a) = COT(v_C^{UL}(fl_a(a)), a, 0) \ [\text{J m}^{-1}]. \tag{2.28}$$

The expected energetic cost i_S of a single stand-by clearer depended on the size of the ant and its velocity. Assuming that the stand-by clearer have a size distribution with the probability density function $\xi_S(a)$, the average expected cost per clearer was

$$i_S = \int COT_S^{UL}(a) \cdot \xi_S(a) \ \text{d}a \ [\text{J m}^{-1}], \tag{2.29}$$

integrated over all possible body sizes.

Energetic benefit

The differential energetic benefit B was given by the difference between the energy expenditure for foraging E, on cleared versus uncleared trails:

$$B(l, t, u, u_S) = E_{UC}(l, t) - E_C(l, t, u, u_S) \ [\text{J}]. \tag{2.30}$$

Influence of uncleared trails on worker locomotion

The benefit of trail clearing arose from two factors:

- ants could walk faster on cleared trails,
- they walked less distance on cleared trails, because they did not need to follow surface modulations.

In other words, the effective trail length was shorter on cleared trails.

From field observations, I estimated the relationship w between the velocity on cleared and on uncleared trails (Table 2.2), for both laden (w_L) and unladen workers (w_{UL}). Since this speed was measured on the projected 2-dimensional surface, it accounted for both factors mentioned above. I adapted movement speeds for uncleared paths, for laden workers

$$v_{UC}^L(fl, m) = w_L \cdot v_C^L(fl, m) \ [\text{km h}^{-1}], \tag{2.31}$$

and for unladen workers,

$$v_{UC}^{UL}(fl) = w_{UL} \cdot v_C^{UL}(fl) \; [\mathrm{km\,h^{-1}}]. \tag{2.32}$$

Cost of Walking and Transport

On an uncleared trail, the total energetic cost of foraging E_{UC} was a combination of the cost of all outgoing workers E_{UC}^o and all incoming laden workers E_{UC}^{iL}, both functions of trail length l and elapsed foraging duration t. I dropped arguments l, t for clarity:

$$E_{UC} = E_{UC}^o + E_{UC}^{iL} \; [\mathrm{J}]. \tag{2.33}$$

In contrast, cleared trails required the presence of a stand-by clearing workforce. The total foraging costs on cleared trails E_C hence included costs for outgoing (E_C^o) and incoming laden (E_C^{iL}) workers, but additionally contained the cost of the stand-by clearing workforce, $C_S(l, t, u, uS)$, as calculated in Equation 2.30. Again I dropped function arguments for clarity:

$$E_C = E_C^o + E_C^{iL} + C_S \; [\mathrm{J}]. \tag{2.34}$$

Because no information exists on their function, I did not account for unladen workers that did not belong to the stand-by clearing workforce (e.g., fraction $1 - u_S$). However, I assumed their function to be identical on cleared and uncleared trails; it hence did not impact my calculations.

As for the stand-by clearers before, I first calculated the expected cost j for a single foraging ant of mass a to walk a unit distance carrying the expected load mass $m(fl)$ and moving at the expected speed v. The population of foraging workers had the size distribution of $\xi_F(a)$. On cleared trails, expected costs for a laden worker moving a unit of distance were

$$j_C^L = \int COT^L(v_C^L(fl_a(a), m(fl_a(a))), a) \cdot \xi_F(a) \; \mathrm{d}a \; [\mathrm{J\,m^{-1}}], \tag{2.35}$$

and for an unladen worker were

$$j_C^{UL} = \int COT^{UL}(v_C^{UL}, a) \cdot \xi_F(a) \; \mathrm{d}a \; [\mathrm{J\,m^{-1}}]. \tag{2.36}$$

On uncleared paths, the expected costs for laden workers were

$$j_{UC}^L = \int COT^L(v_{UC}^L(fl_a(a), m(fl_a(a))), a) \cdot \xi_F(a) \, da \, [\text{J m}^{-1}], \qquad (2.37)$$

and for unladen workers

$$j_{UC}^{UL} = \int COT^{UL}(v_{UC}^{UL}(fl_a(a), 0), a, 0) \cdot \xi_S(a) \, da \, [\text{J m}^{-1}]. \qquad (2.38)$$

I obtained the total foraging cost for each group by multiplying the corresponding expected cost for a trip j with the number of foragers n_F, the trail length l and the duration of foraging t:

$$E_{UC}^o(l, t) = t \cdot n_F(l) \cdot l \cdot j_{UC}^{UL} \, [\text{J}] \qquad (2.39)$$

and

$$E_{UC}^{iL}(l, t) = t \cdot n_F(l) \cdot l \cdot j_{UC}^L \, [\text{J}]. \qquad (2.40)$$

The same costs on cleared trail were, respectively,

$$E_C^o(l, t) = t \cdot n_F(l) \cdot l \cdot j_C^{UL} \, [\text{J}] \qquad (2.41)$$

and

$$E_C^{iL}(l, t) = t \cdot n_F(l) \cdot l \cdot j_C^L \, [\text{J}]. \qquad (2.42)$$

The additional cost C_S incurred on cleared trails through the movement of the stand-by clearing workforce is calculated above in Equation 2.24.

I substituted my calculations for costs and benefits into Equation 2.20 and solved for the break-even time t.

2.2 Results

Despite the seemingly large costs of initial clearing, continuous maintenance and patrolling, trails could quickly achieve energetic profitability under a wide variety of parameter values. An example representative of field conditions is shown in Figure 2.2, for a 50 m trail with unladen workers comprising 42% of traffic ($u = 0.42$, the average value from the studies cited Table 2.3, of which half are assumed to be stand-by clearers

($u_S = 0.5$). At the typical foraging rate reported by Bruce and Burd (2012), clearing a 50 m trail would become profitable from 0.5 days of use onwards. That is, in less than a single day the energetic savings from foraging on a cleared trail would have amortised the cumulative costs of clearing. Note that the cost incurred by the stand-by workforce, although modest, was many times larger than the cost for the actual removal of obstructions Figure 2.2.
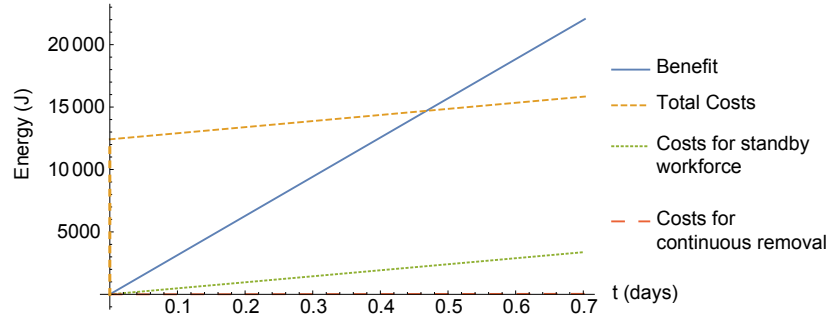
Figure 2.2: Cumulative energetic foraging expenditure over time. Trail length = 50 m, percentage of unladen workers $u = 0.42$, stand-by workers as fraction of all unladen workers $u_S = 0.5$. After the break-even point at 0.5 days of use, the cumulative benefit of foraging on a cleared trail was larger than the cumulative costs of trail clearing. The offset of total costs at $t = 0$ represents the initial clearing cost

2.2.1 Effect of trail length on trail profitability

With otherwise fixed conditions ($u = 0.42$, $u_S = 0.5$), trail length affected a colony's energetic benefit in a counter-intuitive way: the benefit of trail clearing B were proportionally higher on longer trails (see Figure 2.3). As a result, longer trails achieved profitability earlier than short ones (also see Figure 2.5). This relationship follows from the superlinear scaling of forager numbers with trail length (Bruce and Burd; 2012), which I incorporated in the model (see Equation 2.14). Given the scaling relationship, the pattern in Figure 2.3 reflects the intuitive idea that the benefit of a cleared trail depended on how much it was used.
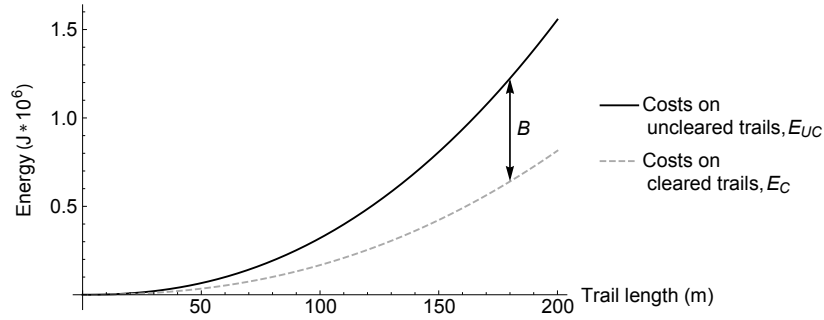
Figure 2.3: Scaling of daily energetic expenditure with trail length, on cleared and on uncleared trails, assuming $u = 0.42$ and $u_S = 0.5$. The energetic benefit of trail clearing B (i.e. the difference between foraging costs on cleared and uncleared trails, E_C and E_{UC} increases with trail length

2.2.2 Effect of stand-by workforce proportion on trail profitability

Because the proportion of stand-by trail clearers among the unladen workers in the traffic stream is unknown, I determined the ranges for u and u_S under which trail clearing can be profitable (i.e. a break-even point is reached in a finite time). I considered a lower boundary for u at 13.1% and an upper boundary at 80%, the range of unladen workers observed in field colonies (see Table 2.3). Except at the highest values of u and u_S, trail clearing could be profitable (Figure 2.4). At the maximum observed fraction of unladen workers, $u = 0.8$, a cleared 50 m trail could remain profitable with a stand-by workforce of up to $u_S \approx 0.3$. Even if all unladen workers were stand-by clearers (i.e. $u_S = 1$), such a trail remained profitable if a fraction up to $u = 0.54$ of workers in the traffic stream were unladen. Only for values of u between 0.54 and 0.8 did the fraction u_S need to be lower than unity for trails to remain profitable (Figure 2.4).

The duration of trail usage needed for clearing to be an energetically profitable option depended non-linearly on the size of the stand-by workforce, especially near the boundaries of profitability. Profitability landscapes for $u_S = 1$ and 0.3 are shown in Figure 2.5.

For most trail lengths and proportions of unladen workers, cleared trails reached energetic profitability in less than a day of use. At the
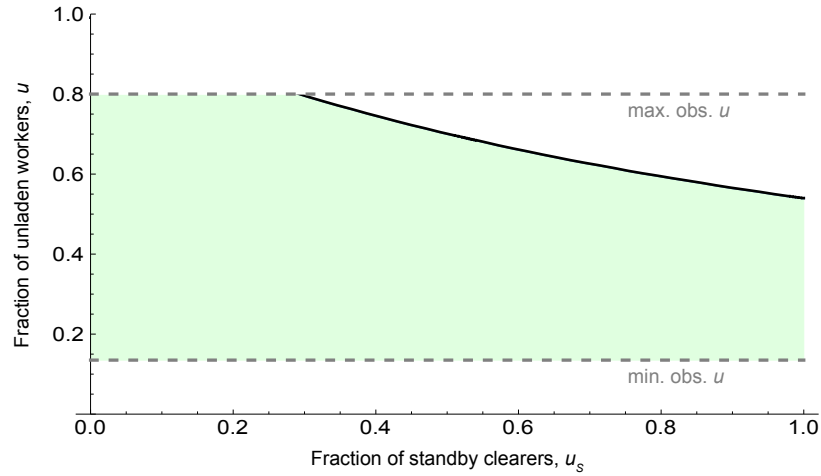
Figure 2.4: Maximum sustainable proportion of unladen workers u for any stand-by workforce size u_S (given as fraction of u), on a 50 m trail. Dashed lines indicate range of observed proportions of u in the field. The intersection of this range and the area under the curve gives the expected conditions under which I expect to find profitable trails. Under most observed values for u, trails remained profitable even if the entire unladen workforce acted as stand-by clearers (i.e. $u_S = 1$) and incurred costs accordingly

boundary of unprofitability, however, the break-even times rapidly increased to weeks, months, and even years. The point at which this occurs depended only on the proportion of unladen workers u and stand-by workers u_S, but was independent of trail length. Under plausible but relatively narrow ranges of parameter values, then, it was possible for trail clearing to require long pay-back times.

2.2.3 Effect of speed gain on cleared trails

My measurements showed that on uncleared trails ants moved about half as fast as on cleared trails (i.e. speed coefficient for laden ants on uncleared trails $w_L = 0.44$ and for unladen ants $w_{UL} = 0.5$, see Table 2.2). If I assumed a lesser disadvantage on uncleared trails (i.e. a higher value of w), the conditions under which trail clearing was profitable were narrower. In particular, the maximum sustainable proportion of unladen workers (as shown in Figure 2.4) decreased (see Figure 2.6). In line with this, break-even times increased (e.g. from 0.5 to 4.1 days for $w = w_L = w_{UL} = 0.7$, in the previous example trail of $l = 50$ m, $u = 0.42$, $u_S = 0.5$). Thus, the
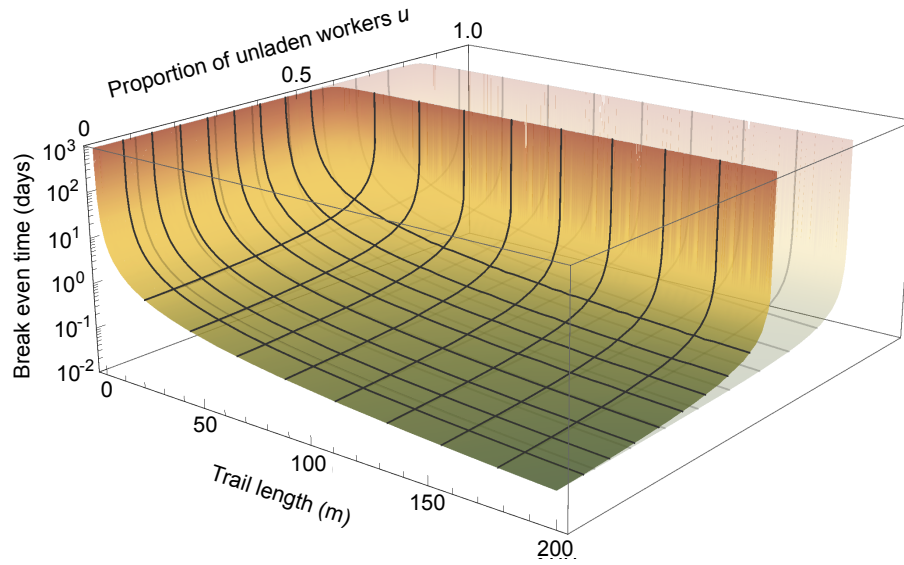
Figure 2.5: Energetic break-even times as a function of both trail length and percentage of unladen workers in the traffic flow, u. The solid surface shows results for the assumption that all unladen ants are stand-by clearing ants ($u_S = 1$), while the transparent surface corresponds to $u_S = 0.3$, implying that most unladen ants perform tasks other than clearing. The graph shows that trail clearing achieved rapid break-even times for most combinations of trail length and unladen worker fraction. However, the time to reach a break-even point rose sharply, to months and years, when the proportion of unladen workers was high or trails were short.

obstructing effect of an uncleared trail and the potential speed gain on a cleared surface were a deciding factor on whether trail clearing can be profitable, and on how long it took to reach profitability.

2.3 Discussion

2.3.1 Conditions of profitability

Profitability depended on a number of factors, including the number of stand-by clearers a colony needed to deploy on its trails for maintenance. If the proportion of unladen ants in the traffic flow was high, trail clearing could be profitable only if the stand-by workers made up a small fraction of the unladen total (Figure 2.4). If the stand-by clearers comprised too large a fraction, trail clearing could be energetically unprofitable. Furthermore, as the speed advantage provided by a cleared trail surface diminishes, the tolerable fraction of stand-by clearing ants declined Figure 2.6). Thus, the speed gain on cleared trails had a strong influence on trail profitability. If it was not high enough, trail clearing could never pay off, no matter how much the cleared trail is used. This suggests that it would be advantageous for *Atta* colonies to possess a regulatory mechanism for clearing behaviour that takes speed gain into account. Such a mechanism might involve direct assessment of trail conditions on speed, or use indirect cues of the expected average speed in relation to trail conditions.

2.3.2 Model limitations

My calculations of energetic benefits of trail clearing are conservative and represent a lower bound: in addition to my modelled assumptions, travelling over leaf litter obstruction might be proportionally costlier for laden workers; reduced net energetic expenditures of foraging on cleared trails might lead to larger fragments being cut; and reduced round trip times on cleared trails might lead to a higher overall number of foraging trips. While my approach focused on the benefit derived by foraging workers, unladen workers not involved in trail clearing might also benefit from using cleared trails. The model compared cleared and uncleared trails assuming equivalent amounts of leaf harvesting. However, faster movement on cleared trails increases the potential number of round-trips per forager, and trail clearing might therefore increase the potential resource
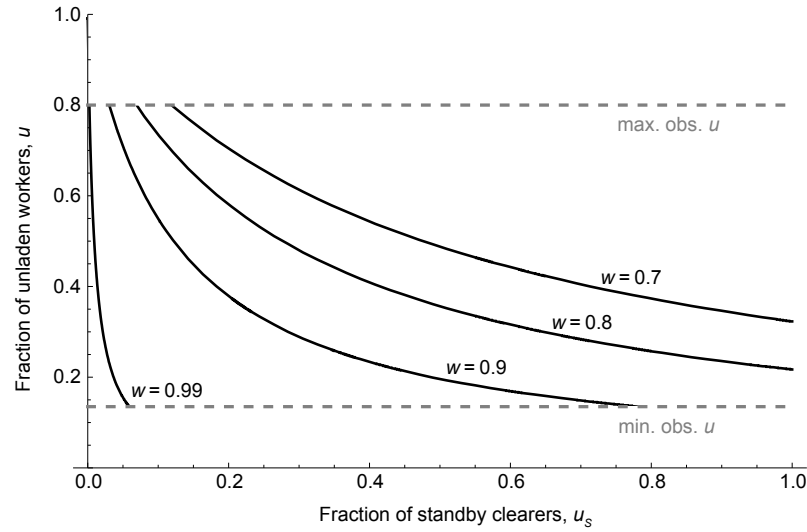
Figure 2.6: Influence of different values for expected speed gain w on clearing profitability (cf. Figure 2.4). As the expected speed gain decreases, (e.g., as $w = w_L = w_{UL}$ approaches 1), the range of conditions for profitable trail clearing (as given by the intersection of area under the curve and the observed range of unladen workers u) decreases superlinearly. Dashed lines indicate minimum and maximum observed values of u

intake during a foraging day. *Atta* colonies opportunistically exploit fallen branches, rocks, or other naturally exposed features as part of their trail networks. While these trail segments might introduce slight detours from the direct path to the harvest site, they also require no clearing or maintenance and offer a movement speed benefit (Freeman and Chaves-Campos; 2016). Howard's calculation of clearing costs (Howard; 2001) accounted for such features as 9.3% of the total trail length. Farji-Brener et al. (2007) found that naturally exposed branches made up 30% of the length of *A. cephalotes* trails in Costa Rica. For simplicity, I used estimates by Howard (2001) with their implied proportion of 9.3%, but other values could be modelled by simple adjustment of the clearing cost per unit of trail length.

2.3.3 Model applicability to other collective infrastructure construction

The genus *Atta* occurs in diverse environments including both rainforests and grassland. Qualitative predictions derived from the model should

be largely transferable to other rainforest-living *Atta* species: parameters used in my model (i.e. rate of leaf litter fall, effect of leaf litter on movement speed, achievable speed gain) would be similar among tropical lowland forests. Quantitative differences (e.g. in the rate of leaf-fall, or in the impact of morphological differences on the movement speed), could, however, be effortlessly integrated into the model. For grassland *Atta* species, litter fall on trail surfaces is likely to be reduced, so that trails need less maintenance. If, as a result, a smaller reserve of potential trail clearers is needed on trails, then the cost of trail clearance is lowered. The advantage of cleared trails would remain nearly unchanged: laden workers of the grassland species *A. laevigata* moved **2.4** times faster on cleared than on uncleared trails (**4.1** vs. **1.7** cm/s, respectively, (Bouchebti; 2015)), similar to the advantage I found for forest species of *Atta* (**1.37** vs. **3.14** cm/s, respectively – see Table 2.3). With lowered maintenance cost and similar locomotion benefits, I expect that foraging trails would be cleared more readily and be more prevalent among grassland than among forest species.

Other than *Atta*, the ant genera *Formica, Lasius, Pogonomyrmex* (Hölldobler and Wilson; 1990), *Messor* (Acosta et al.; 1993; Plowes et al.; 2013), *Camponotus* (Marlin; 1971), *Iridomyrmex* (Greaves and Hughes; 1974), and *Acromyrmex* (Gamboa; 1975; Wetterer; 1995) are known to likewise construct cleared trails. My approach is applicable for those genera as well but will require a detailed investigation of the costs of trail construction and the speed-based benefit they each derive. Extrapolated, our model can serve as a template for other infrastructure constructing social insects, e.g. tunneling ants or termites. However, assessing the benefit they receive from constructing tunnels will likely be more complex than the speed-based benefit in *Atta* and involve predator avoidance and protection from environmental conditions.

Literature on *Atta* trails overwhelmingly focussed on cleared trails. However, uncleared trails do exist in the field: I have observed such trails persisting for weeks. This may reflect a delayed onset of clearing, or indicate that the conditions on this trail do not allow for trail clearing to be profitable. It would be interesting for future research to investigate whether there is any correlation between actual trail clearance in the field and the predicted profitability of clearing it.

2.3.4 Model predictions

Based on the model results, I make qualitative predictions about behaviour I expect to find in the field and suggest experimental approaches for their investigation: The proportion of unladen workers on trails should coincide with the intensity of leaf litter fall. In environments where the rate of leaf fall is low (e.g., in grassland), I expect to find fewer unladen workers than in forest habitats. *Atta* colonies have been shown to be sensitive to litter fall in their trail design, Farji-Brener et al. (2015) found that the branching angles of newly constructed paths from trunk trails to harvesting sites differed between forest and grassland environments. The resulting geometry minimised maintenance costs in the forest where long-term maintenance costs were high, but minimised travel times in grassland areas with high sun exposure and increased desiccation risk but little litter fall. Likewise, I expect that within the same colony, trails receiving higher leaf fall should show more unladen workers. A detailed investigation of unladen worker numbers under these conditions, as well as potential differences in their behaviour, could offer indications as to the validity of my assumptions.

I further predict that trails should remain uncleared if the duration of resource exploitation will be shorter than the break-even time. Such short exploitation times are conceivable for scattered or ephemeral resources, e.g. fallen flowers or ripe fallen fruit, resources that would decompose rapidly in a tropical rainforest. Previous research on *Atta* foraging has focused almost exclusively on their use of cleared trails, but I have observed foragers collecting fallen *Guapira standleyana* fruit on uncleared trails (manuscript in review). Offering resources that in nature would be ephemeral near established trails – and observing trail clearing to these – would be a possible method to investigate this prediction.

Likewise, I expect the quality of the foraged resource to correlate with the trail clearing intensity: a low-quality resource will provide comparably less energy to the colony, while the cost of trail clearing will be identical. Controlled offering of different resources – and the observation of time of onset and intensity of trail clearance – would provide insight into the influence of resource quality. Shepherd (1982) also inferred that trail construction would be most beneficial for colonies exploiting high quality, long-lasting resources. Likewise, I agree with his assumption that

larger colonies should more frequently clear trails. However, my reasoning on this point is based on the observed superlinear scaling of forager numbers with trail length (Bruce and Burd; 2012), and the corresponding higher relative resource intake on longer trails. An investigation into trail clearing occurrence in colonies of different sizes could investigate this hypothesis.

The extended use of uncleared trails by leaf-cutting ants has not been the focus of research, and is poorly described. Research on uncleared trails, the duration of their use, the conditions under which they occur, and the functional role of unladen workers might shine further light on trail clearing profitability and deserve further study.

2.3.5   Summary

I present a comprehensive model of the energetic balance of trail clearing in leaf-cutting ants. The model allows to calculate the conditions under which trail clearing can be energetically profitable, and how long it takes before trails reach profitability. Previous work by Howard (2001) had suggested that the volume of foraging traffic carried by a cleared trail would easily make trail clearing energetically favourable. My model largely confirmed this idea: in many scenarios, the time to energetic break-even can be very short. Nonetheless, a full exploration of the parameter space shows that under an array of realistic circumstances, the maintenance of a cleared trail can be very expensive. In such scenarios, cleared trails may only amortise after months or years – or may even never be profitable at all.

Chapter 3

Mechanism of trail clearing

3.1 Introduction

In Chapter 1 – Introduction, I examined the basic self-organisation processes that underlie complex group level behaviour in social insects. While little research exists on the construction of cleared trails, the adaptive excavation of nests and tunnels has been extensively investigated. In the case of the latter, functional aspects (e.g. movement of foragers and transport of resources) are comparable to those of cleared trails. Further, excavation in both nests and tunnels is done via gradual removal of soil, much like trail construction requires removal of trail obstructions (e.g. fallen leaves and other organic debris). Here, I review mechanisms that regulate the construction of these structures.

3.1.1 Adaptive mechanisms in nest construction

As highlighted before, colony-level nest construction behaviour is not hard-coded. Nests and tunnels are adaptive structures that need to reflect local environmental conditions and to change with the changing needs of the inhabitant (Hansell; 1993). Indeed, relationships between nest volume and colony size have been found to be relatively constant in numerous examples (e.g. Franks et al.; 1992; Tschinkel; 1999a,b; Mikheyev and Tschinkel; 2004; Tschinkel; 2014; Murdock and Tschinkel; 2015). To assess the need for nest expansion, workers need to estimate the current nest size and relate it to the number of occupants. This does not require communication between individuals: Deneubourg and Franks (1995) suggested a mechanism in which workers estimate global properties by determining

local worker density (e.g. via proxy of collisions with other workers) or CO_2 concentration, and expand nest size until density matches an internal reference value. The same mechanism has been suggested for the construction of tunnels, in termites (Su and Lee; 2009) and ants (Gordon et al.; 1993). In the above cases, adaptive infrastructure sizes are achieved without need of communication between workers.

Signals like soil-borne vibrations (Markl; 1965; Roces et al.; 1993) or digging pheromones (Wilson; 1958; Imamumra; 1982; Chen and Zhang; 2013) have been shown to localise excavation behaviour. Here, evaporation of pheromones functioned as negative feedback that prevented over-expansion (Pielstrom and Roces; 2013). At the same time, physical cues of existing structures are used to guide the direction of nest and tunnel expansions (Sudd; 1970); in termite tunnels, workers unable to reach the digging face of a tunnel due to overcrowding did instead expand the width of tunnels, leading to a traffic-adaptive tunnel sizing (Bardunias and Su; 2010).

3.1.2   Adaptive properties of cleared trails

Just as nests and tunnels, cleared trails need to be considered as adaptive structures. In *Atta*, network architecture has been shown to reflect environmental conditions like leaf fall rate and risk of desiccation (see Chapter 2 – Energetics of trail clearing, page 38).

The cost of infrastructure construction and maintenance imposes further restrictions on trail clearing. In the cost/benefit model, I showed that trail clearing is only energetically profitable under certain conditions, including potential speed gain (itself dependent on the environment) and workforce composition. Indeed, this prediction corresponds with observations of persisting uncleared trails in nature; in Barro Colorado Island, Panama, I saw foraging occur on uncleared trails for several weeks. This suggests that clearing might only occur if the resulting trail is energetically profitable.

Likewise, trail widths should be adaptive to the amount of traffic they receive. Trail widths measured in nature varied between 4 and 20 cm (Farji-Brener et al.; 2012; Bruce and Burd; 2012), and varied as a function of distance to the nest (Bruce and Burd; 2012). Intriguingly, Farji-Brener et al. (2012) found that the sum of widths of sub-branches

is typically greater than the width of the parent branch. However, it is unknown what dynamics shape the individual branch sizes.

3.1.3 Chapter scope

Little is known about the individual behaviours, the dynamics, and the control mechanisms underlying the construction of cleared trails. It is reasonable to assume that outgoing workers should perform trail clearing, while incoming workers should concern themselves only with resource transport. However, on average 41% of workers on observed trails returned from foraging without carrying resources (see Table 2.3 in Chapter 2 – Energetics of trail clearing), and this unladen subset plays an important role in trail clearing.

In this chapter, I investigated the dynamics of trail clearing via a set of experiments and the subsequent construction of an individual-based model. I asked:

- what individual behaviours contribute to trail clearing, and what are the rates of their occurrence?
- what role do encounter rates (and their dynamics) play?

I aim to produce a model that correctly replicates the colony-level behaviour.

3.1.4 Modelling approach

I conducted behavioural experiments, in which I obstructed a cleared trail in a laboratory setting and recorded the clearing dynamics. From recordings, I manually and automatically extracted behavioural probabilities of individuals and interaction dynamics of workers and obstructions.

In a second step, I simulated the clearing behaviour. Assuming workers encounter obstructions with a constant rate and have a fixed probability to interact with them), the observation of removal rates k and change of number of obstructions on trail O is sufficient to construct a simple deterministic model, e.g. via a decay process,

$$\frac{\mathrm{d}O}{\mathrm{d}t} = -kO. \tag{3.1}$$

However, such a model would add little knowledge about the behaviour of the individuals. Rather than using a deterministic model, I opted for a

stochastic individual-based model that described the interplay of workers and obstructions as a physical process: individual moving workers randomly encountered stationary obstructions; global encounter propensities depended on the worker flow rate and obstruction numbers. I assigned to individual workers a probability of removing an obstruction at the time of encounter, p_{clear}.

3.2 Behavioural Experiments

3.2.1 Experiment methods

Experiment setup

I obstructed an artificial cleared trail in two *Atta vollenweideri* colony fragments to investigate the dynamics of trail clearing. One colony contained a queen the other was a fragment of the mother colony. The queen, alongside with workers and fungus, was collected from a colony in Argentina of approximately 4 years of age in November 2013. The separation aimed to create two identical-sized colony fragments of around $50,000$ individuals; both fragments contained several litres of fungus. All experiments were conducted in a temperature control room at 25°C and $40 - 60\%$ LFR, in a 12/12 hour light/dark cycle.

Colonies were connected to their respective food containers via 8 meters of clear plastic tube. Halfway, I inserted arenas measuring $100 * 50$ cm. In nature, cleared trails are framed by the surrounding leaf litter. To simulate this trail delineation in the laboratory arena, I framed the length of the trail with a perimeter of expanded clay pellets. The path's length spanned the arena. The width of the path was 8 cm, approximately twice as wide as the colony's unconstrained traffic flow during peak foraging activity. Figure 3.1 gives an overview of the arena.

Trail obstructions were made from thin blue cardboard of weight 2.56 $\mathrm{mg\,mm}^{-2}$. From this, I cut strips of $25 * 5$ mm length. In preliminary experiments, I tested how foraging workers react to the material under different circumstances. I noted the following interactions:

- when unfolded paper strips were placed flat on the foraging trail, they were ignored.

Figure 3.1: Overview of foraging arena with central cleared trail and 10x8 cm test area marked in red, and blue paper strips as obstructions. Inset on bottom left shows magnification of image centre The arena was inserted between the nest and the feeding site.

- when treated with sugar-water, they were collected and integrated into the fungus. (The colony on which this experiment was performed was not subsequently used for removal experiments.)
- when folded in half at approximately 90° and placed upright on the trail as a 3-dimensional obstruction (see inset of Figure 3.1), they were picked up, carried to the side of the outlined trail, and dropped among the expanded clay pebbles.

I found that the behavioural response at encounter depended on the encounter context. The removal of folded paper strips was identical to trail clearing as observed in nature. I hence concluded that the material itself was neutral, and that the presentation as folded paper strips serves the function of a trail obstruction akin to their natural equivalent.

Experiments were only performed during steady foraging, when traffic flow had reached a balance of inbound and outbound workers. Colonies were fed *Rubus sp.* leaves every morning, and were given at least one hour to recruit to the feeding site and to establish foraging activity. On days on which foraging activity was low or inconsistent, experiments were forgone. Each experiment was run for three hours. Where possible, two replicates were recorded during one day. After each experiment the colony was rested for one hour.

Table 3.1: Overview of experimental treatments

	Exp. Set A	Exp. Set B
Number of obstructions	20	20
Obstruction area size	$5 * 8$ cm	$10 * 8$ cm
Relative obstruction density	1	0.5
Number of replicates	19	16

Experiments were recorded with a GoPro 4 camera suspended centrally above the arena and capturing its entire expanse. Resolution of the recordings was $1920 * 1080$ px, at 24 frames per second. All experiments were conducted in temperature-controlled rooms at the Behavioural Ecology and Neurobiology workgroup at Konstanz University in Germany.

Experiment procedure

In order to provoke trail clearing behaviour, I placed 20 equally spaced paper obstructions in an obstruction area in the centre of the cleared trail. In two experiment sets, I tested the influence of obstruction density on the trail clearing behaviour. I varied obstruction density by changing the size of the obstruction area: In Set A, the area measured $5 * 8$ cm; in Set B, it measured $10 * 8$ cm. Density of obstructions hence was half as high in Set B. I performed 19 and 16 replicates in Sets A and B, respectively (see summary of treatments in Table 3.1). Figure 3.1 shows paper obstructions within a $5 * 8$ cm obstruction area, early in the experiment.

Recording clearing dynamics

Workers often antennated obstructions for several seconds before removal; I recorded the time at which a worker first grabbed an obstruction in its mandibles as the time of removal. An obstruction was considered as removed if it was grasped with the mandibles and moved for at least 5 mm. Rarely, carried obstructions were subsequently dropped back on the trail and abandoned. I ignored those obstructions for the remainder of the experiment, and did not record any further removal. All removals took place within the first hour of each experiment, after which point I stopped data extraction. During the experiments, the majority of foraging activity occurred on half of the available width of the predefined trail. Hence, I

only recorded removals of the ten obstructions placed on the used half of the trail.

To investigate which workers engage in trail clearing, I manually tracked any clearing ant backwards in time as far as possible – under optimal conditions, until it entered the arena on either side. Based on the side of entry, I identified clearing workers as in- or outbound when they encounter the obstruction. In some cases, clearers moving along the trail and performed frequent U-turns. I termed this behaviour "meandering". However, I did not define a criteria for the latter; a potential discrimination could have been that ants need to perform a fixed number of U-turns in a period of time.

Automated flow rate extraction

The rate of object removal was likely dependent on the worker flow rate, and the consequent worker/obstruction encounter rate. In order to quantify flow in each experiment, I tested manual extraction by volunteers watching slow motion videos. However, counts of the same video segment were highly variable, and data extraction was time consuming. Instead, I used an automated method to track worker movement in a trail segment approximately 10 cm adjacent to the obstruction zone. In each frame, individual ants were identified using an image-based classifier, provided by Professor Bernd Meyer. It extracted ant locations and associated them in consecutive frames via a nearest-neighbour algorithm. I tested the generated movement trajectories for the crossing of a virtual "finish line" in either direction (see Figure 3.3). I viewed eight of these 30 second video fragments frame-by-frame and manually counted the number of line crossings, and then compared the manual and machine counts in order to test the accuracy of the tracking algorithm. Figure 3.2 shows the comparison of human and machine counts. On average, the automated count deviated by 8% from the manual count. I applied this method to 30 second video segments at 2, 20, and 40 minutes into each experiment, and averaged the three measurements to obtain mean flow rates. Worker flow rate in Set A ($W = 3.95$ s^{-1}) was about a quarter higher than in Set B ($W = 2.82$ s^{-1}).

The tracking algorithm used is given in Chapter 6 – Supplementary material.
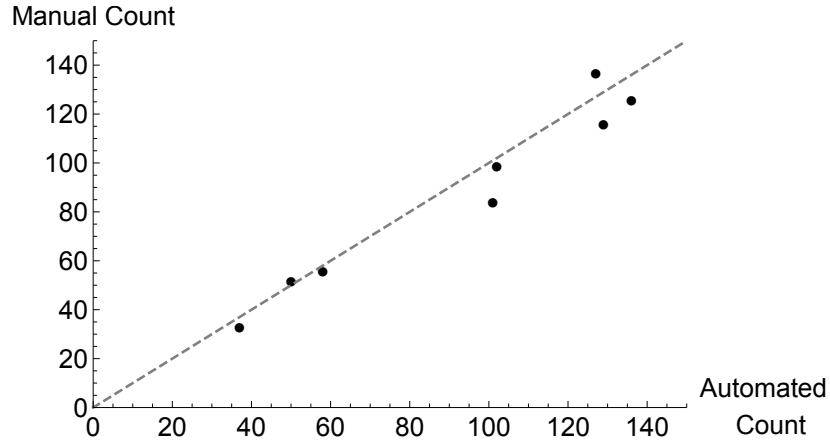
Figure 3.2: Automated versus manual counts of worker crossing a virtual "finish line" during a 30 second window of observation. Dashed line indicates unity.

Encounter rate extraction

I hypothesised that the number of encounters between workers and a given obstruction influences the speed of this obstruction's removal. To investigate these dynamics, I quantified encounters in consecutive video segments of 30 seconds length. In each video fragment, I measured the amount of traffic each area of the trail received by comparing pixel values in consecutive, grey-scale converted frames. I used a value difference of *threshold* = 30 to differentiate between unoccupied and occupied pixels in consecutive frames. Algorithm 1 gives the pseudo-code of the algorithm used; a Mathematica notebook with the model can be found in Chapter 6 – Supplementary material.

Figure 3.3: Magnified output sample of ant-tracking algorithm. Red trajectory gives the position of respective ants in the last three frames. Black line in image centre indicates virtual "finish line" at which passings ants (indicated by green trajectory segment) are counted. White scale bar indicates length of 1 cm

$threshold = 30$;

$summary\_image$ = empty image of same dimensions as frame;

while *extraction running* **do**

    for *each frame* **do**

        for *each pixel_value^{x,y} in frame* **do**

            if *pixel_value$_{t-1}^{x,y}$ > pixel_value$_{t}^{x,y}$ + threshold*

            then

                summary_image^{x,y} $\overset{+}{=}$ 1

            end

        end

    end

    load next frame

end

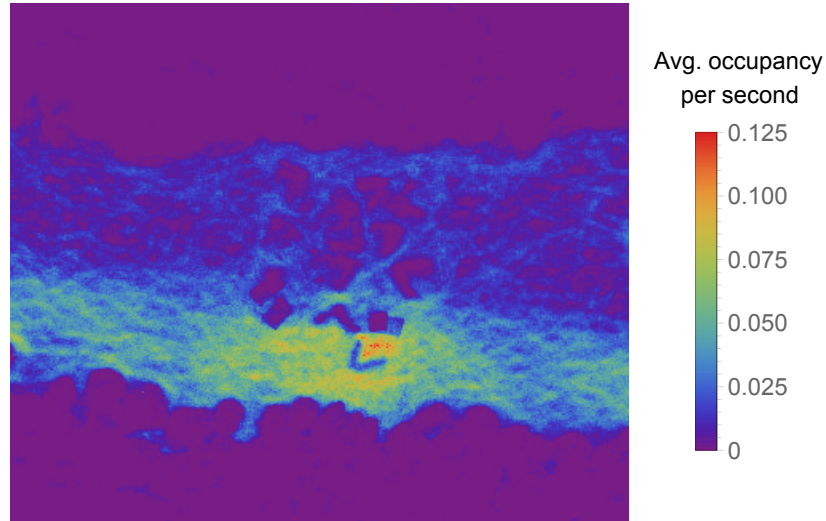**Algorithm 1:** Pseudo-code of encounter quantification algorithm

Figure 3.4: Visualisation of ant movement over a 30 second period in (and adjacent to) the 5 ∗ 8 cm obstruction zone, 8 minutes into an experiment. For visualisation, pixel values in the original grey-scale image have been scaled to a maximum of 1, and the image was mapped to a colour image using the Mathematica "Rainbow" colour scheme. Purple colours indicate absence of any movement, and show the folded paper obstructions and the expanded clay pellets delineating the trail. Brighter colours indicate ant movement. Legend gives average flow per second

After 30 seconds, *summary_image* gave the aggregated movement of ants in that video segment.

This method does not take into account differences in body sizes or angle of trajectory when passing through obstruction masks. In absence of a feasible method for tracking ants as entities in high-density areas (i.e. near obstructions), I rely on the large number of sampled ants to provide a meaningful average as proxy for ant/obstruction encounters.

This array can be represented as a grey-scale or colour-mapped image; an example is given in Figure 3.4.

At the beginning of each 30 second video fragment, I manually recorded obstruction locations. Around each obstruction, I placed a circular mask with a diameter of 2 cm, large enough to capture any ant that came within an antenna's length of the obstruction and can therefore be considered to have encountered it. I used the pixel values in the mask v_i as proxy for the number of ant encounters with each obstruction i. The
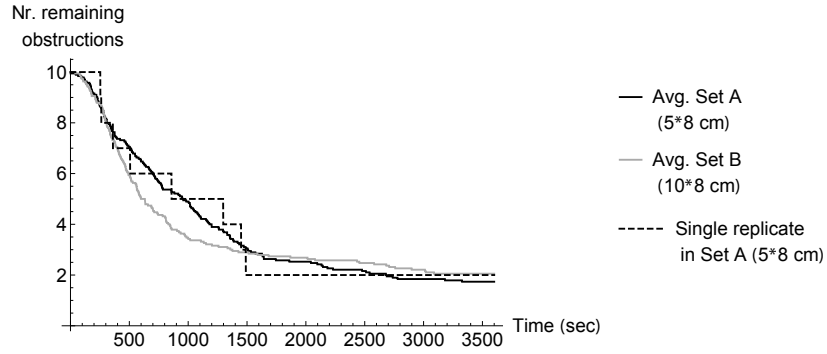
Figure 3.5: Plot of colony-level removal dynamics, averaged over all experiments in both sets (solid lines), and in a representative replicate of Set A (dashed line)

average number of encounters enc_t each obstruction receives at any time t is:

$$enc_t = (\sum_{i=1}^{O} v_i)/O,$$ (3.2)

where O is the number of remaining obstructions.

The encounter extraction algorithm is provided in Chapter 6 – Supplementary material.

3.2.2 Experiment results

Colony-level clearing dynamics

Plotting remaining obstructions against the time of the experiment provides a summary of the colony-level clearing dynamics. Figure 3.5 shows the average number of remaining obstructions over all experiments in Set A and B, and the trajectory of a representative experiment from Set A.

Overall, removal occurred most frequently at the beginning of the experiments, and progressed faster in Set B than in Set A. Often, obstructions were removed in bursts. As mentioned above, no further removals occurred after an hour of observation; at the end of the experiment, on average 1.7 obstructions remained in Set A, and 2 in Set B.

Table 3.2: Movement direction prior to removal. N gives number of totally observed ants

Movement direction	Exp. Set A (N = 114)	Exp. Set B (N = 34)	Sum (N=148)	Percentage of total removals
Outbound	48	12	60	40.54%
Inbound	39	12	51	34.46%
Meandering	27	10	37	25.00%

Individual clearing behaviours

In addition to the colony-level behaviour, I extracted data on individual behaviours during the experiments.

To shine a light on how movement direction influences trail clearing behaviour, I tracked a total of **148** clearing workers across the two experimental sets (see Table 3.2). I classified workers by tracking them from entering to leaving the recorded arena, and assigned the label "inbound" and "outbound" based on the direction in which they entered the arena. Both inbound and outbound workers contributed approximately equally to the trail clearing (binomial test, $p = 0.5$). A quarter of all removals were undertaken by workers that did not actively follow the trail. Observing individual behaviour after removal, I identified two different trail clearing methods: predominantly, workers performed "one-off" trail clearing, in which unladen workers encountering obstructions lifted them up in their mandibles, carried them off the trail, deposited them, and continued their progress along the trail. However, a portion of these clearers, after deposition, returned directly to the obstruction zone and continued clearing. Such "repeaters" removed several objects in sequence before rejoining the trail traffic. I differentiated between them by following each removing worker both backwards and forwards in time on the video recordings, until it left the experimental arena captured on video. Workers were classified as repeaters if they removed more than one obstruction during passage of the arena. I cannot however rule out that workers I classified as one-off clearers, after re-entering the arena at a later point, continued to remove obstructions.

I found that even though the number of repeaters was small, because of their repeated obstruction removal they accounted for a significant

Table 3.3: Frequency of different clearer behaviours. N gives total number of removals

Removal type	Exp. Set A (N=167)	Exp. Set B (N=189)	Sum (N=356)	Percentage of total removals
One-off	118	122	240	67%
Repeater	49	67	116	33%

Table 3.4: Repeater statistics for both experimental Sets

	Exp. Set A	Exp. Set B
Number repeaters	18	24
Number removals by repeaters	49	67
Average number removals per repeater	2.72	2.79
Mean repeater bout duration (sec)	113	223
S.D. repeater bout duration (sec)	69	184
Mean interval length between repeater removals (sec)	66	124
S.D. interval length between repeater removals (sec)	31	118

portion of the trail clearing: Of a total of **356** removals recorded in experimental sets A and B repeaters were responsible for **116** (i.e. **33%**). These **116** removals were performed by **42** individuals only, accounting on average for **2.73** removals each. Variation in the number of repeat removers therefore had the potential to significantly influence the trajectory of the experiment. I therefore quantified the occurrence and number of repeat removals, as well as duration of repeater bouts and intra-bout intervals in both experimental sets (see Table 3.4).
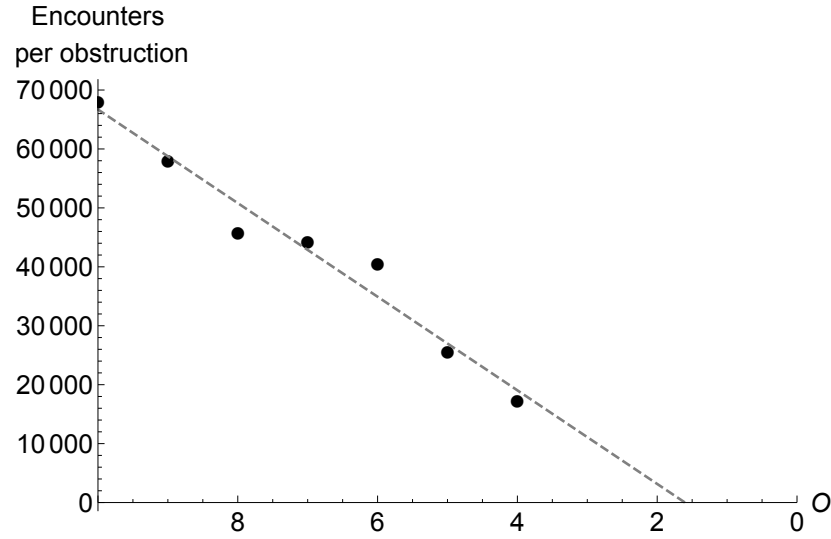
Figure 3.6: Number of encounters per obstruction declined linearly with the number of remaining obstructions O. Data from a representative experiment of Set A. Dashed line gives linear approximation $y = -12725.8 + 7941.84x$ ($R^2 = 0.96$, ANOVA: F=133.057, df=1, $p < 0.0001$)

Encounter rate dynamics

I calculated how the number of encounters per obstruction changed as the experiment progressed. Figure 3.6 shows a plot of the dynamics for a representative experiment. The number of encounters per obstruction decreased approximately linearly as obstructions were removed (with fitted function $y = -12725.8 + 7941.84x$ ($R^2 = 0.96$, ANOVA: F=133.057, df=1, $p < 0.0001$).

For the construction of the removal model, I therefore assumed that there was a linear relationship between the number of removed obstructions and the number of encounters.

3.3   Removal simulation

3.3.1   Model methods

I assumed that workers have a fixed probability to remove an obstruction when they encounter it, and that the trajectory of the overall removal process was subject to the probabilistic processes of these encounters. I

Figure 3.7: State diagram of removal behaviours (starting state marked with asterisk). Filled arrows represent the encounter and subsequent removal of an obstruction. The open arrow indicates spontaneous (i.e. not requiring obstruction encounter or removal) reversal of repeaters to trail following behaviour
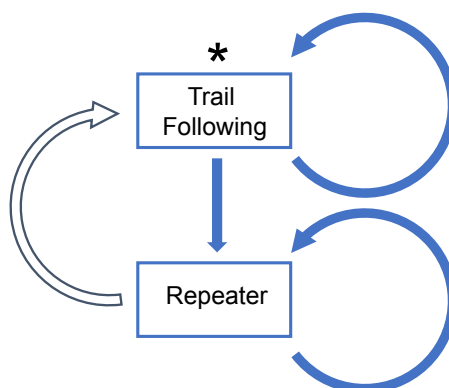
further assumed that the system is well mixed, and that all obstructions have the same probability of being encountered by workers.

To account for repeaters, I assumed that clearing workers – upon removal of an obstruction – had a probability to switch to repeater state in which they continue removing encountered obstructions. They could leave this state spontaneously without need to encounter or remove further obstructions.

The different modes of trail clearing behaviour are represented with the below finite state diagram Figure 3.7.

I have shown that relative obstruction encounters decreased as more obstructions were removed (Figure 3.6) and included these dynamics via an encounter modifier, which reduced the probability of removing an encountered object. Encounter data and Mathematica script for calculation of encounters per obstruction are given in Chapter 6 – Supplementary material.

To simulate the transitions between behaviours depicted in Figure 3.7 I used the Gillespie algorithm (Gillespie; 1977), which allows the simulation of parallel Poisson processes. It was originally conceived to give stochastic trajectories for molecular concentrations during chemical reactions, as opposed to the traditional deterministic description via differential equations. I largely used method and notation for the Gillespie algorithm given by Twomey (2007, Equations 3.1 - 3.5).

Table 3.5: Possible removal behaviours, notation, and dependencies. Workers, Repeaters, and Obstructions are given as W, R, O, respectively; k is the corresponding reaction rate in s^{-1}

Removal Reaction	Symbol	Reaction equation
One-off obstruction removal	R_1	$W + O \xrightarrow{k_{WO}} W$
One-off removal and switch to repeater behaviour	R_2	$W + O \xrightarrow{k_{WR}} R$
Repeater removal	R_3	$R + O \xrightarrow{k_{RR}} R$
Repeater stopping	R_4	$R \xrightarrow{k_{R0}} W$

I formulated the removal behaviour as reactions in which workers and obstructions are the reagents, and removal events and workers (or repeaters) are the product. In Table 3.5, I described each individual action in the behavioural sequences shown in Figure 3.7 with a reaction-based notation; with workers W, repeating clearers R, and obstructions O; reaction rates (in s^{-1}) are denoted k.

Given an initial amount of reagents and rates for each possible reaction, the algorithm after every removal calculated the propensities $\alpha_i, \{i = 1, \ldots, 4\}$ for each possible removal reaction $R_i, \{i = 1, \ldots, 4\}$. It then generated the next reaction time τ and reaction type $R_\mu, \mu = \{1 \ldots, 4\}$ via two random numbers $r_{\{1,2\}}$, see Algorithm 2.

$t = 0$;

Initialise reagent counts;

while *simulation running* **do**

$\quad \alpha_0 = \sum_{i=1}^{4} \alpha_i$;

$\quad r_{1,2} \sim U[(0, 1)]$;

$\quad \tau = \frac{1}{\alpha_0} \ln\left[\frac{1}{r_1}\right]$;

$\quad \mu = \min\{m \in M = \{1 \ldots 4\} : \frac{1}{\alpha_0} \sum_{i=1}^{m} \alpha_i > r_2\}$;

$\quad t = t + \tau$;

$\quad$ execute reaction R_μ;

end

Algorithm 2: Gillespie algorithm

The reaction rates were calculated as below:

The rate of workers removing obstructions was

$$k_{WO} = N_O / t_{Max}, \tag{3.3}$$

where N_O is the number of one-off clearers, and t_{Max} the duration of the experiment. The rate of workers becoming repeaters was

$$k_{WR} = k_{WO} \cdot p_{WR}; \tag{3.4}$$

where p_{WR} is the probability of workers becoming repeaters:

$$p_{WR} = N_R / N_O, \tag{3.5}$$

with N_R being he numbers of repeaters, and N_O the number of one-off clearers.

The rate of repeaters removing further obstructions was

$$k_{RR} = 1 / t_i, \tag{3.6}$$

where t_i is average repeater inter-removal duration. The rate of repeaters stopping clearing behaviour,

$$k_{R0} = 1 / t_d, \tag{3.7}$$

where t_d is average repeater bout duration.

Table 3.6: Reaction rates for repeater events: method of calculation and result

	Symbol	Value Set A	Value Set B
Probability of clearers becoming repeaters	p_{WR}	0.153	0.197
Rate or repeaters removing obstructions	k_{RR}	0.0184	0.0129
Rate of repeaters stopping clearing	k_{RO}	0.0127	0.0091

In Table 3.6, I listed the calculated reaction rates and probability for $R_{2\dots4}$. I determined k_{WO} by fitting the model to the empirical data.

To calculate the propensities α_i (in s^{-1}) for each reaction, I identified all involved reactants and determined their relationship.

α_1 is the propensity for workers to encounter and remove an obstruction. It was dependent on worker flow rate W and on the number of remaining obstructions O (which give the probability of an encounter) and the probability at which the encounter results in a removal. I assume worker flow rate to be constant throughout an experiment.

Due to the linear decrease of encounters as removal progressed, I adjusted the encounter probability with encounter modifier γ. This function returned 1 when no obstructions were removed, and lower values as clearing progressed:

$$\gamma(O) = 1 + m \cdot (O_{max} - O), \tag{3.8}$$

where O_{max} is the number of obstructions at the beginning of the experiment (i.e. 10), O is the number of remaining obstructions, and m is negative.

For α_1, this gave

$$\alpha_1 = k_{WO} \cdot W \cdot O \cdot \gamma(O). \tag{3.9}$$

where W is worker flow rate.

After a removal, a fraction of removers became repeaters. To calculate the propensity of this occurrence, α_2, I multiplied α_1 by the observed probability of switching:

$$\alpha_2 = \alpha_1 \cdot p_{WR}. \tag{3.10}$$

Workers made the decision to become a repeater as they removed an obstruction – I observed repeaters directly returning to the obstruction zone after each removal. Hence the propensity for repeater clearing events to occur was independent of the number of remaining obstructions (or their encounter rates); it depended solely on the current number of repeaters. The propensity for removals by repeaters, α_3, is

$$\alpha_3 = N_R \cdot k_{RR}, \tag{3.11}$$

where N_R is the number of repeaters.

Repeaters stopped removing obstructions at propensity α_4. As above, this was independent of the number of remaining obstructions.

$$\alpha_4 = N_R \cdot k_{R0}. \tag{3.12}$$

Exploring the parameter space for the rate of one-off removals k_{WO} and slope m of encounter modifier $\gamma(O)$, I compared the average of $20,000$ simulations to the averaged empirical data. Via squared-error minimisation, I selected parameter values that provided the best fit.

A Mathematica notebook containing the full simulation (including results) can be found in Chapter 6 – Supplementary material.

3.3.2   Model results

The simulation (average trajectory of $20,000$ replicates) provided the best fit to the averaged empirical data for k_{WO} of 0.00018 and 0.00037, and slope m of the encounter modifier $\gamma(O)$ of -0.076 and -0.125 in experiment Sets A and B, respectively. In both cases, the model provided a convincing fit to the empirical data (see Figure 3.8). Figure 3.9 shows the plot for the encounter modifier functions with the fitted slope parameters, in both sets.

Contrary to initial expectations, the removal rate k_{WO} was smaller in Set A than in Set B. I assume that the removal process was too noisy to allow for a distinction of obstruction densities as I used them, or that they had no influence (see 3.4.2 – Model validity and limitations for a discussion).
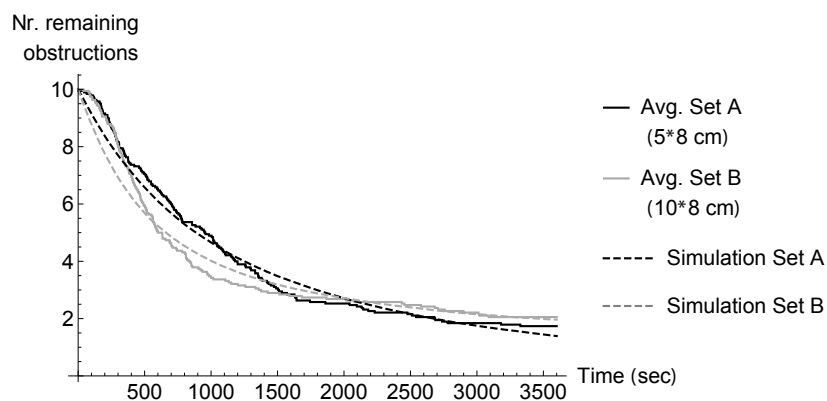
Figure 3.8: Fit of removal simulations (dashed line; mean of 20,000 replicates) to averaged empirical data from both experimental set (solid lines)
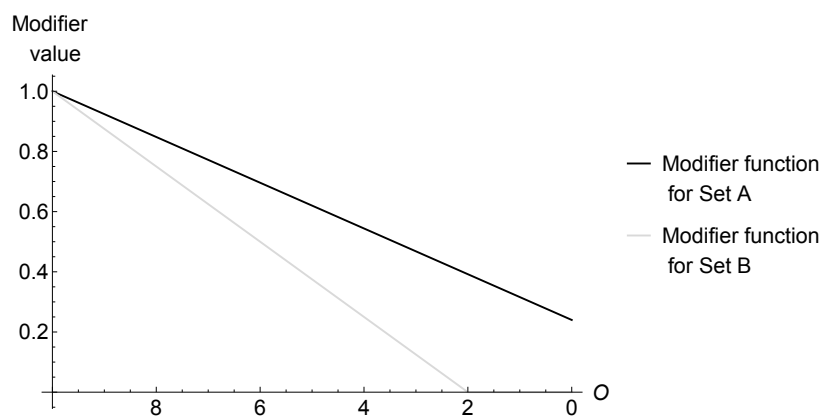

Figure 3.9: Plot for encounter modifier function $\gamma(O)$ with fitted slopes, for both experimental sets. O is number of remaining obstructions

All clearing was undertaken by the subset of workers I previously named the "standby workforce". However, my tracking method captured all workers. The flow of clearers within that total flow, c, was not determinable by observation (see Chapter 2 – Energetics of trail clearing), but can be expressed with a set of place-holder variables,

$$c = W \cdot u \cdot u_S \qquad (3.13)$$

where u is the fraction of unladen workers in the traffic, to which clearers contribute fraction u_S (see Chapter 2 for review of these variables). From this, individual probability of removing an object on encounter, p_{clear}, could conceptionally be calculated as

$$p_{clear} = k_{WO}/c. \qquad (3.14)$$

Because of the uncertainty surrounding c, I could not determine the value for p_{clear}. By entering the measured values for flow W (3.95 and 2.82 for Set A and B, respectively) in Equation 3.13, and for k_{WO} (0.00018 and 0.00037) into Equation 3.14, I showed that $p_{clear} < 0.000045$ and < 0.00013 for both sets.

3.4   Discussion

3.4.1   Individual clearer behaviour

The assumption that all trail clearing might be done by outbound workers, and that inbound workers exclusively forage, does not hold. Instead, I found that inbound and outbound workers contributed equally to trail clearing.

Of all observed removals, 25% were undertaken by workers which meandered along the trail, suggesting that standby clearers might not always follow the main traffic flow.

In Chapter 2, I showed that the fielding of this workforce was the largest cost associated with the trail clearing behaviour. Considering this, the probability of individuals to remove an object upon encounter was surprisingly low ($p_{clear} < 0.000045$ and < 0.00013 in Sets A and B, but see Equation 3.13 and Equation 3.14). In this light, a smaller workforce of individuals with a higher probability of removing obstructions (which

would have the same clearing potential) might have been a more economic solution. The underlying cause for the large workforce might be the unpredictable nature of leaf-fall. In nature, obstructed trails quickly congested with laden workers, which delayed resource flow and worker movement to and from the nest. Laden workers did not readily drop their carried fragments (personal observation) and hence did not contribute to the clearing effort. Hence to minimise delays, clearers need to be able to quickly respond anywhere on the trail. A larger workforce would lead to a faster encounter of obstructions, and would allow for a more rapid response time. At the same time, a higher probability to remove objects might over-allocate clearers to obstructions, and deplete potential clearers from a stretch of trail. Additionally, Herbers (1981) noted that an abundance of "inept" workers might, by virtue of redundancy, form a reliable and well working system.

The workforce size and clearing probability observed in *Atta* probably represented a balance between those factors.

The behaviour of repeat-clearing seems not to have been noticed or investigated previously. Unlike one-off clearers, repeaters removed several obstructions: while they only made up **12**% of the clearing individuals, they removed an average of **2.7** obstructions each, and thereby contributed **32**% of the overall trail clearing effort. This raised the question of the adaptive value of a two-pronged clearing behaviour.

In absence of repeaters, clearing only occurred when potential clearers encounter an obstruction. Clearing was hence dependent on a probabilistic process. Repeaters, however, appeared to seek out obstructions and not to require random encounters for continued clearing. They thereby focussed clearing efforts spatially as well as temporally, allowing for a faster removal of an obstruction. As such, repeat behaviour might be adaptive to the patchy nature of trail obstructions: fallen leaves or branches require continued removal effort, during which they are progressively cut up and dragged of the trail (Howard; 2001).

A clearing workforce composed solely of repeaters would appear to provide the same clearing potential as a approximately **3** times larger workforce made up from one-off clearers only, at a fraction of the metabolic costs associated with additional workers. Why, then, was the repeater behaviour not ubiquitous?

Although repeaters seemed to explicitly search out obstructions, their first removal depended on a random obstruction encounter. As in the considerations on workforce size above, reduction in the size of the workforce in favour of more repeaters could reduce the propensity for encounters to occur, and thereby increase the response time.

I acknowledge that the obstruction area covered a relatively short trail segment (5 and 10 centimetres wide), and that workers observed to be one-off clearers might have, had they encountered obstructions further along the trail, continued clearing. However, I argued above that repeaters made the decision to continue clearing at the time of the previous removal and then directly returned to the obstruction site, unlike one-off clearers. To substantiate the exact nature of repeated clearing behaviour will need to be the focus of further research.

3.4.2 Model validity and limitations

Comparison of data for Set A and B showed that although average flow rate was lower, clearing initially progressed faster in Set B than in Set A, despite the reduced obstruction density in Set B (see Figure 3.5). This was reflected in the slightly higher fitted value for k_{WO}. In the same set, first-time clearers had a higher probability to become repeaters (see Table 3.3). However, these variations between Sets A and B need to be considered the result of a noisy process with a small number of removals and repeaters per experiment. Overall, my model provided a convincing fit to the empirical data, which suggests that I correctly captured key individual behaviours and removal dynamics underlying the colony-level clearing behaviour.

I designed the model with as few assumptions as possible. It only captures the interplay between worker numbers, obstruction numbers, and the different individual modes of trail clearing; I did not allow for direct or indirect (e.g. via stygmergy) interaction between workers, or any resulting positive or negative feedback. The model fit suggests that there exists no active regulation of trail clearing. The occurrence of removals is dependent only on the stochastic process of obstruction encounter and fixed individual probabilities of removal. At the end of the experiments, on average 1.7 (Set A) and 2 (Set B) obstructions remained on the trail. These were predominantly situated at the outskirts of the cleared trail,

where they were encountered less frequently than earlier removed obstructions (see Figure 3.6). Their reduced encounter rate lead to a much lower overall probability that they would still be removed. In effect, trail width emerged as a function of the local traffic volume.

3.4.3 Other potential adaptive properties of trail clearing

Field observations showed that trails can remain uncleared for extended periods of times, which might indicate that clearing these trails would be unprofitable (see Chapter 2 – Energetics of trail clearing).

To avoid wasting energy by trail clearing under these conditions, individuals would need to be able to assess the benefit of trail clearing. This would require an appreciation of the obstruction levels along the trail, for example by assessing their impact on movement speed. In absence of any global knowledge about this, workers could use their current movement speed as proxy. If individuals used this information to decide on whether or not to remove encountered obstructions (e.g. via a response threshold), this would implement an obstruction-dependent trail clearing behaviour. I have shown that potential speed gain played an important role in the energetics of trail clearing (Figure 2.6), and that clearing became more expensive as the potential gain became lower. This mechanism would, via the response threshold, be evolutionarily addressable and could thereby have lead to the proposed "energy-aware" trail clearing behaviour.

I found indications for the existence of this mechanism in the preliminary experiments I conducted: I showed that flattened obstructions in the same density as in experiment Set A did not elicit any trail clearing behaviour. Arguably, they were encountered at the approximately same rate as normal obstructions; workers readily walked over them. The differences in obstruction quality clearly played a role in the decision for or against trail clearing, and the flattened obstructions may not have impacted the movement speed of workers enough to warrant potentially uneconomical removal.

In previous work, Bruce et al. (2017) described an additional behaviour influencing clearing dynamics. They suspended a metal wire above a foraging trail in the field, creating an obstruction too high for unladen workers to encounter, but low enough to interfere with leaf-carrying workers. However, they observed unladen workers climbing the suspended wire and

trying to cut through it. After experimental removal of all laden work-
ers, attempts at wire cutting occurred at a greatly diminished rate. This
observation suggests that there might exist an exchange of information
between foragers and clearers in the case of clearing above-trail obstruc-
tions, even though I did not find any evidence for this in my experiments.

3.4.4   Summary

In behavioural experiments, I recorded the colony-level dynamics of clear-
ing and identified underlying individual behaviours. I further showed how
the progression of clearing influences the rate of subsequent removals.

From empirical parameters, I constructed a simple stochastic model
that determined time and type of the next removal reaction. I found that
the model of these individual clearing behaviours correctly replicated the
colony-level dynamics I observed.

At the same time, I described the self-regulation of trail clearing that
lead to the observed trail dimensions of cleared trails, and suggested a
mechanism that would allow assessment of energetic profitability of any
potential cleared trail.

Chapter 4

Evolution of trail clearing behaviour

4.1 Introduction

As explored in the previous chapters, trail clearing is linked to substantial costs but can provide an energetic net benefit under some circumstances. While this provides the explanation for why the behaviour could evolve, it does not explain the trajectory of evolution: trail clearing occurs in an environment filled with competing conspecific colonies, and the evolutionary trajectory of the behaviour needs to take interaction between neighbours into account.

A number of studies have remarked on spatial effects that cleared trails imposed on colonies. They localised workforce to the trails themselves, and scouting predominantly occurred from the end of existing trails (Shepherd; 1982; Elizalde and Farji-Brener; 2012; Farji-Brener and Sierra; 2016). While rainforests (which form the habitat of many *Atta* species) may appear to offer over-abundant resources, resource quality is variable (Howard; 1990; Nichols-Orians and Schultz; 1990; Nichols-Orians; 1991), and distribution of high quality resources is patchy (Wirth et al.; 2003a) (which explains why colonies are known to exploit trees distant from the nest although the same host species would be available in closer proximity (Rockwood and Hubbell; 1987)). A smaller territory and a reduced resource discovery rate result in a potentially lower number of exploitable high quality resources (Stradling; 1978; Shepherd; 1982; Reed and Cherrett; 1990). These effects directly benefit neighbouring colonies,

which gain access to additional territory and reduced competition in the exploitation of high quality resources. At the same time, trails serve as territory markers, and reduce aggressive interaction between neighbours (Wirth et al.; 2003a).

What influence did these interaction dynamics between colonies have on the evolutionary trajectory of trail clearing? A toolkit to investigate such interactions can be found in game theory.

4.1.1 Game theory

The concept of game theory considers strategic interactions between actors (referred to as "players"), and was initially conceived for the study of social games (von Neumann; 1928) and economic processes (von Neumann and Morgenstern; 1944). The term "game" is used for any exchange between at least two players, in which the pay-offs received by a player depend not just on its strategy choice, but also on that of the opponent. Strategies are selected from a finite set to which all players have equal access. The purpose in a game is for players to maximise their respective pay-offs. Pay-offs are case-dependent utilities: in the case of financial interactions, they could be monetary profit; in leaf-cutter ants, they could be resource accumulation or evolutionary fitness.

A way to visualise such games between two players A, B is via the "normal form" matrix, Table 4.1. For two strategies C, D, pay-offs for all possible strategy pairings are given in the matrix cells; each cell contains pay-offs for players A and B respectively. Where, as in this example, pay-offs are symmetrical (i.e. pay-offs depend on the strategy only, independent of which players selects it), cells conventionally only give the pay-off value for the row player A.

Players are assumed to have access to perfect information about available strategies and potential pay-offs. Further, it is assumed that players are rational; that is, that they select a strategy which will provide them the greatest benefit given the opponent's possible choices. Such a strategy set, in which no player can gain a higher pay-off by changing its strategy, is referred to as Nash equilibrium (Nash; 1950).

4.1.2 Evolutionary game theory

Evolutionary game theory (EGT) considers strategies in evolving populations (Maynard Smith; 1982). Instead of rational actors analysing

Table 4.1: Normal form game matrix. Cells give pay-offs for Players A and B respectively

Player B

		Strategy C	Strategy D
	Strategy C	(e, e)	(f, g)
Player A	Strategy D	(g, f)	(e, e)

the game before selecting a strategy, players in EGT games are biological entities with a genetically fixed strategy and do not require perfect knowledge about game conditions or the ability to assess potential game outcomes. Pay-offs gained from interactions are measured in terms of fitness, and define a player's potential to replicate. Strategies propagate via reproduction of its carriers: offspring inherit from their parents the genetic material defining the strategy. Increased reproductive success of a well-performing player therefore leads to a spread of its strategy through a population (Maynard Smith; 1982). A strategy that eventually dominates an initially heterogeneous population, and cannot be invaded by a newly occurring mutant strategy, is an evolutionary stable strategy (ESS) (Maynard Smith; 1972).

Here, I considered trail clearing as a strategy, and in an EGT setting explored how the behaviour evolved, and what role the coupling with territory interactions played.

4.1.3 Definition of pay-off terms

I differentiated between effects that trail clearing has on the clearing colony itself, and that on neighbouring colonies.

For the clearing colony, trail use allowed more effective resource exploitation (i.e. more energy returned per unit resource), which I captured in the benefit-factor α ($\alpha > 1$). At the same time, the reduction of territory incurred cost factor β ($\beta < 1$) in reduced resource discovery rate and availability. I collected these internal effects in term $\gamma = \alpha \cdot \beta$. Neighbouring colonies gained from the increased availability of resources and territory, and receive benefit δ ($\delta > 1$).

Pay-off values are dependent on overall availability of resources. I defined each pay-off term as function of resource availability k, ranging from total absence ($k = 0$) to abundance ($k = 1$). I calculated game pay-offs multiplicatively; pay-offs of 1 indicated no change in fitness.

Where resources are absent, trails do not provide any benefit ($\alpha(0) = 1$). For $k > 0$, every unit of resource provides proportionally higher energetic pay-offs; I hence postulated a linear relationship with slope $m_\alpha = 1$,

$$\alpha(k) = 1 + m_\alpha \cdot k. \tag{4.1}$$

The cost of using trails (in terms of reduction in exploitable colony territory) is high where resources are scarce, and so the cost parameter β is small. β increases as resources become more abundant, and at maximum abundance $k = 1$, β reaches unity and thereby has no effect in the pay-off calculation. As above, I assumed a linear relationship. Previously, I showed that foraging-related benefits can outweigh clearing costs. I assumed that this was also true for the relationship of $\alpha(k)$ and $\beta(k)$, and reflected this by assigning a lower slope $m_\beta = 0.7$:

$$\beta(k) = (1 - m_\beta) + m_\beta \cdot k. \tag{4.2}$$

Internal effect $\gamma(k)$ are hence

$$\gamma(k) = \alpha(k) \cdot \beta(k). \tag{4.3}$$

Pay-off δ describes the benefit neighbouring colonies accrue from access to additional territory relinquished by a focal trail-clearing colony. This benefit is likely to provide diminishing marginal returns, a concept firmly established in ecological thinking (e.g. parental investment in offspring fitness (Smith and Fretwell; 1974) and resource intake from time spent foraging in a resource patch (Charnov; 1976)). When resources are scarce, colonies are unlikely to be near a point of resource saturation, and the marginal benefit derived from access to additional resources will be large. As the resource environment becomes richer, the marginal benefit of the additional resources will grow smaller. The diminishing marginal benefit as a function of resource abundance $\delta(k)$ can be represented by the relationship

$$\delta(k) = e^{-m_\delta k} + 1, \tag{4.4}$$

where $m_\delta = 3$ was chosen for a gradual decline over range $0 < k < 1$.

Plots for all pay-off are shown in Figure 4.1; a pay-off matrix for the trail clearing game is given in Table 4.2.
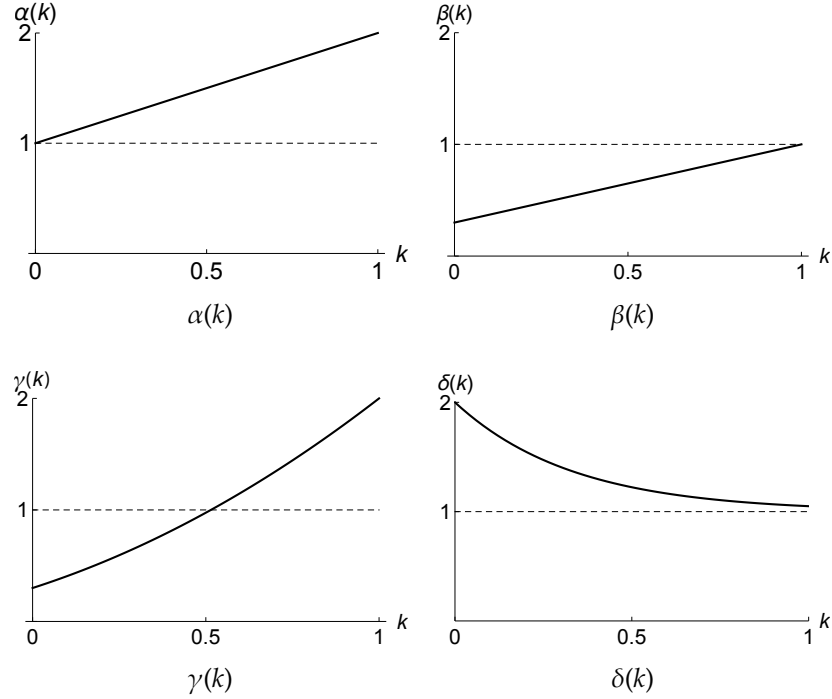

Figure 4.1: Plots of pay-off functions $\alpha(k), \beta(k), \gamma(k)$ and $\delta(k)$. See text for details

I explored the influence of k on the relative values of the pay-off terms. There existed three distinct phases in the rankings between the pay-off values. For $k < k_\downarrow$, clearing incurred a loss of fitness, and should therefore never evolve. For $k > k^\uparrow$, $\gamma \cdot \delta$ was the highest pay-off term; here, clearing was the optimal strategy.

In range $k_\downarrow < k < k^\uparrow$, pay-offs were ranked $\delta > \gamma \cdot \delta > 1 > \beta$, which is the defining criterion of a "Prisoner's Dilemma" (Maynard Smith; 1982; Axelrod and Hamilton; 1981), a type of game I review below.

In my parameterisation, this range was $0.31 < k < 0.52$. I arbitrarily chose $k = 0.5$ for the parameterisation of the game; the resulting pay-off matrix is given in Table 4.3.

A link to a Mathematica notebook with calculations of pay-off functions can is provided in Chapter 6 – Supplementary material.

Table 4.2: Pay-off terms for trail clearing game. Cells give pay-offs for Players A as function of resource abundance k

Player B

		Clear	Don't clear
	Clear	$\gamma(k) \cdot \delta(k)$	$\gamma(k)$
Player A	Don't clear	$\delta(k)$	1

Table 4.3: Pay-off matrix for trail clearing, for $k = 0.5$

Player B

		Clear	Don't clear
	Clear	1.19255	0.975
Player A	Don't clear	1.22313	1

4.1.4 The Prisoner's Dilemma

The Prisoner's Dilemma (PD) is a game characterised by a conflict of interest. It is usually applied within the context of cooperative behaviour (Axelrod and Hamilton; 1981), and I introduce it in this nomenclature.

Players A and B choose whether to cooperate with each other (strategy C) or to defect (strategy D). Mutually cooperating players receive a significant "Reward" R; if both defect, they receive a "Penalty" P. However, a player that unilaterally defects against a cooperating opponent will reap the largest possible reward T ("Temptation"), while the opponent receives the "Sucker" pay-off S. The pay-off matrix for such games is given in Table 4.4. The relative values of pay-offs in a PD game are $T > R > P > S$ (which match the ranking I obtained from corresponding pay-off values for $0.31 < k < 0.52$ above).

Table 4.4: Pay-off matrix in PD games. Cells give pay-offs for Player A. R, S, T, P stand for "Reward", "Sucker", "Temptation", and "Penalty". PD's are defined by the ranking of terms $T > R > P > S$

Player B

		Cooperate	Defect
Player A	Cooperate	R	S
	Defect	T	P

In a classical example, two prisoners (A and B) have been caught committing a crime together and are being interrogated in separate cells without means of communication with each other. Because of the lack of evidence, prosecution is offering each a deal: if they testify against their partner (i.e. defect), they are set free (receive pay-off T), while their partner goes to jail for three years. If both prisoners testify against each other, they are both jailed for two years. However, if both remain silent (i.e. cooperate), lack of evidence means each is only jailed for one year.

Players could obtain the highest pay-off (i.e. the collective optimum) if they would both cooperate. However, a cooperating player is always in danger of encountering a defecting opponent and incurring the sucker's pay-off S. The Nash equilibrium, which independent of opponent action provides the highest benefit to a player, is therefore mutual defection.

Independent of the original context of cooperation, PD games can be used to explore arbitrary strategy-based interactions in which the dominant strategy offers a lower pay-off than mutual selection of the dominated strategy.

While players can encounter each other repeatedly, such iterated games are less relevant in the consideration of evolutionary dynamics in biological populations; here, each player only plays a single game before being succeeded by its offspring. In such one-shot games (and assuming large, well mixed populations), the collective optimum can never be

reached (Axelrod and Hamilton; 1981; Nowak and Sigmund; 2004). However, trail clearing coincides with spatial effects, which can aid in the propagation of collectively optimal strategies.

4.1.5   Spatiality in games

Spatiality can be introduced into games by placing players on lattices and limiting interaction ranges (e.g. Nowak and May; 1992; Killingback and Doebeli; 1996; Killingback et al.; 1999; Doebeli and Hauert; 2005; Langer et al.; 2008; Lion and Baalen; 2008; Szabó and Szolnoki; 2009), or by representing them as nodes on graphs, in which connected nodes play against each other (e.g. Lieberman et al.; 2005; Santos et al.; 2006b; Ohtsuki et al.; 2006; Szabó and Fáth; 2007; Wu et al.; 2010). In both cases, such constraints on spatial interaction can lead to the propagation of collectively optimal strategies. In static neighbourhoods, the condition for this to occur is that the number of neighbours a player has on a lattice, or the average number of nodes on a network, is smaller than a threshold value (Ohtsuki et al.; 2006).

Neighbourhoods in natural systems are rarely static, and their dynamics are important: relocation of influential players (Droz et al.; 2009) and dynamically adjusting interaction neighbourhoods based on previous encounters was shown to promote the propagation of strategies (Santos et al.; 2006a; Pacheco et al.; 2006b,a). Such rules can themselves become the subject of evolutionary dynamics (Gross and Blasius; 2007; Perc and Szolnoki; 2010).

4.1.6   Chapter scope

I considered the evolution of trail clearing behaviour as a strategy in a spatial evolutionary Prisoner's Dilemma game, and modelled the interaction in games via the pay-off functions described above.

I further assumed that localisation of workforce and scouting behaviour lead to a reduction of colony territory and of neighbourhood interactions. In the simulation, I implemented this by assuming that players are mobile, and that a reduction in player mobility corresponds to a reduction in neighbourhood dynamics.

Rather than restricting players to a binary strategy choice (e.g. "clear" or "don't clear"), I allowed continuous-valued investment, reflecting the

gradual evolution of the behaviour. In the below experiments, I investigated how a coupling of investment and relocation probability influenced the evolution of trail clearing behaviour.

4.2 Methods

Continuous-valued PD

The classical binary strategy choice provides "extreme" strategies: either full investment into a behaviour, or none whatsoever. However, not all behaviours are linked to a binary choice. In the case of trail clearing, I assumed that the behaviour evolved gradually via partially cleared trails, and that even partial clearing provided energetic benefits.

I reflected such partial investment via a continuous pay-off function, in which players can invest an amount I in range $0 < I < 1$. I further assumed that the benefit of cleared trails increases linearly with investment. I use a linear extrapolation between rows and columns of the binary pay-off matrix, as given by Verhoeff (1998, Equation 7): when two players A and B competed, I used terms a and b to denote their investment I into trail clearing. They obtained pay-offs p_A and p_B, respectively:

$$p_A(a, b) = ab(\gamma \cdot \delta) \cdot a\bar{b}(\gamma) \cdot \bar{a}b(\delta) \cdot \bar{a}\bar{b}(1)$$
$$p_B(a, b) = ba(\gamma \cdot \delta) \cdot b\bar{a}(\gamma) \cdot \bar{b}a(\delta) \cdot \bar{b}\bar{a}(1)$$

$$(4.5)$$

where $\bar{x} = 1 - x$. Note that I dropped pay-off function argument k; in the remainder of the chapter, each was taken to be parameterised with $k = 0.5$.

For extreme values of investment (i.e. $a, b = 0, 1$) the function returns the terms of binary pay-off matrix Table 4.2:

$$p_A(1, 1) = \gamma \cdot \delta, \quad p_A(1, 0) = \gamma,$$
$$p_A(0, 1) = \delta, \quad p_A(0, 0) = 1.$$

A pay-off landscape is given in Figure 4.2; values on plot corners correspond to those in Table 4.3.

4.2.1 Model rationale

I simulated the spatial interplay between clearing and non-clearing players in a 2-dimensional lattice. Players were created with an initial investment
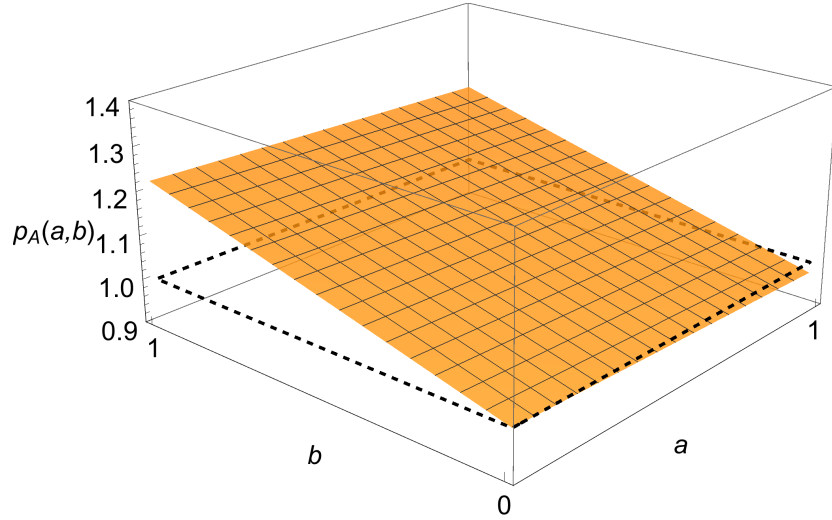
Figure 4.2: Pay-off landscape for Player A, for own investment a and opponent investment b (see Equation 4.5)

value $I_0 \sim U(0, 0.0001)$. In each generation, a player competed against all opponents in its Moore neighbourhood (i.e. the $N = 8$ players in all adjacent cells, cf. (Nowak and May; 1992; Killingback and Doebeli; 1996; Killingback et al.; 1999)). Based on the investment of the players, each received a pay-off as given by Equation 4.5.

Normalised relative fitness of each player was calculated as

$$\omega = \frac{\sum\limits_{i=1}^{N} p_A(a, I_i)}{N},\qquad(4.6)$$

where a is player investment, N is the number of neighbours, and I_i is investment of neighbour i.

To represent the displacement of players by more successful competitors, in each generation every cell was occupied by the offspring of the player in its neighbourhood that had the highest relative fitness in the previous generation.

To allow for evolution of the population, players mutated with a probability of $p_{mutate} = 0.01$ at the end of each round. A mutating player drew a new investment value $I_n \sim N(I, 0.1 \cdot I)$.

Trail clearing leads to a reduction of neighbourhood interactions. I introduced neighbourhood dynamics by relocating players across the lattice: players were assigned a relocation probability p_{move} in range $[0, 1]$

with which they switch their location with a randomly chosen other player. This allowed for the modification of interaction dynamics via a continuous variable. By randomly relocating players, I interrupted the forming of clearing clusters, from which strategies can propagate (Houston; 1993; Vainstein et al.; 2007, cf.).

Algorithm 3 shows pseudo-code for the simulation.

---

initialise 2d player lattice;
assign random strategy to each player;
while *experiment running* **do**
    for *each player in the game* **do**
        play against every player in neighbourhood;
        update own fitness to normalised sum of payoffs;
    end
    for *each player in the game* **do**
        update own strategy to winning strategy in
        neighbourhood;
    end
    for *each player in the game* **do**
        assign random value $r_1 \sim U[0, 1]$;
        if $r_1 < p_{mutate}$ **then**
            apply mutation;
        end
    end
    for *each player in the game* **do**
        assign random value $r_2 \sim U[0, 1]$;
        if $r_2 < p_{move}$ **then**
            switch player location with random other player;
        end
    end
end

Algorithm 3: Pseudo-code of spatial trail clearing simulation

Chapter 6 – Supplementary material provides a link to the Python scripts containing the model.

Table 4.5: Summary of parameters used in the model

Parameter	Symbol	Definition / Range
Investment	I	$I \in [0,1]$
Initial investment	I_0	$I_0 \sim U[0, 0.0001]$
Probability of mutation	p_{mutate}	$p_{mutate} = 0.01$
Probability of relocation	p_{move}	$p_{move} \in [0,1]$
Normalised relative player fitness	ω	$\omega = \dfrac{\sum\limits_{i=1}^{N} p_A(a, I_i)}{N}$

Experimental program

On a $70 * 70$ lattice with a continuous boundary, I conducted two sets of experiments. In the first, I investigated how different values of relocation probability p_{move} influenced average investment levels.

In the second experiment, I simulated the progressive reduction of neighbourhood dynamics caused by the reduction of colony territory by coupling increasing investment into trail clearing with the corresponding decrease of relocation probability p_{move}.

All relevant parameters are summarised in Table 4.5.

4.3 Results

4.3.1 Influence of relocation probability p_{move}

Players were assigned a fixed probability p_{move} of switching positions with a randomly selected player. With higher values of p_{move}, the asymptotic levels I_{max} decreased (see plot for $\delta = 1.22$ in Figure 4.3). For $0 < p_{move} < 0.052$, I_{max} declined monotonically; at $p_{move} \sim 0.052$, the system had a turning point: for higher values, trail clearing did not evolve.

To investigate whether the model parameters influence the location of the turning point, I increased the value for δ (initially 1.22313, see Table 4.3), thereby increasing the pay-off gained by non-clearing players when playing against clearing players.
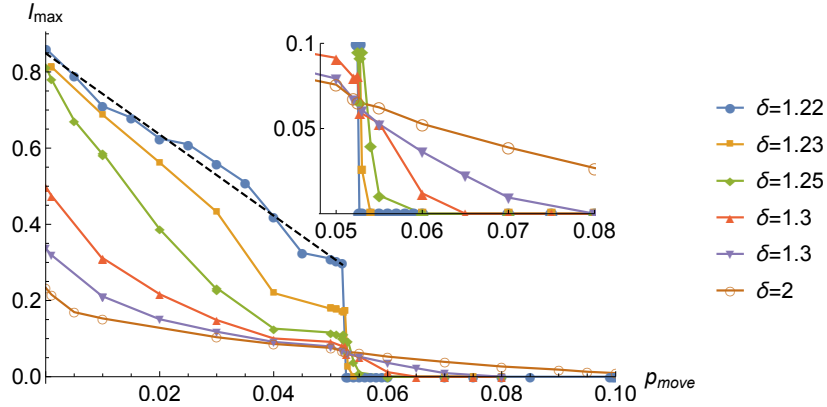
Figure 4.3: Effect of different values for δ on the turning point in the original parameterisation of $\delta = 1.22$ at $p_{move} \sim 0.052$. Dashed line is linear approximation $I(p_{move}) = 0.85 - 10.66 \, p_{move}$ of plot $\delta = 1.22$, fitted to the data for $0 < p_{move} < 0.052$ ($R^2 = 0.98$, ANOVA: F=776.6, df=1, $p < 0.0001$). Inset shows magnification of area surrounding turning point

Figure 4.3 shows plots for different values of δ. Higher values of δ lead to a higher investment in static populations (e.g. for $p_{move} = 0$). In the range $0 < p_{move} < 0.052$, I_{max} decreased non-linearly; for $\delta > 1.23$, plots resembled decay functions in which higher values of δ correlate with higher decay rates. The turning point observed for the initial parameters existed in all tested variations of δ, at approximately $p_{move} \sim 0.052$. For $p_{move} > 0.052$, graphs continued their monotonic decline with an inverse relationship between speed of decline and δ (see inset in Figure 4.3).

4.3.2 Coupling of p_{move} and individual investment I

Via regulatory function $p_{move}(I)$, I coupled investment and relocation rate. To be consistent with the initial parameterisation, I estimated their relationship with the linear approximation given in Figure 4.3. From it, I calculated the inverse relationship $p_{move}(I_{max}) = 0.079 - 0.092 \, I_{max}$.

Relocation probability was adjusted by the piecewise function $p_{move}(I)$:

$$p_{move}(I) = \begin{cases} 0.052 & I \leq 0.3 \\ 0.079 - 0.092 \, I & I > 0.3, \end{cases} \tag{4.7}$$

visualised in Figure 4.4. After $50,000$ generations, an asymptotic value of $I_{max} \sim 0.62$ was reached (see Figure 4.5); indication that p_{move} had indeed decreased from initial values of 0.052 to $p_{move} \sim 0.02$ (cf. Figure 4.3).
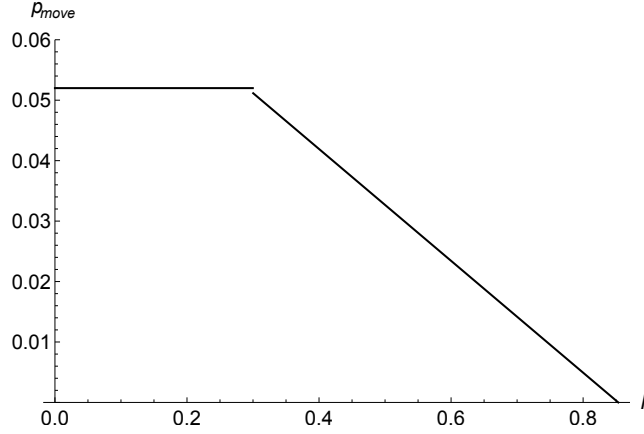

Figure 4.4: Plot of the piecewise function in Equation 4.7, which decreased individual probability of relocation p_{move} as function of current player investment I. For all values $I < 0.3$, it assigned $p_{move} = 0.052$, the largest value for which trail clearing evolved in static experiments
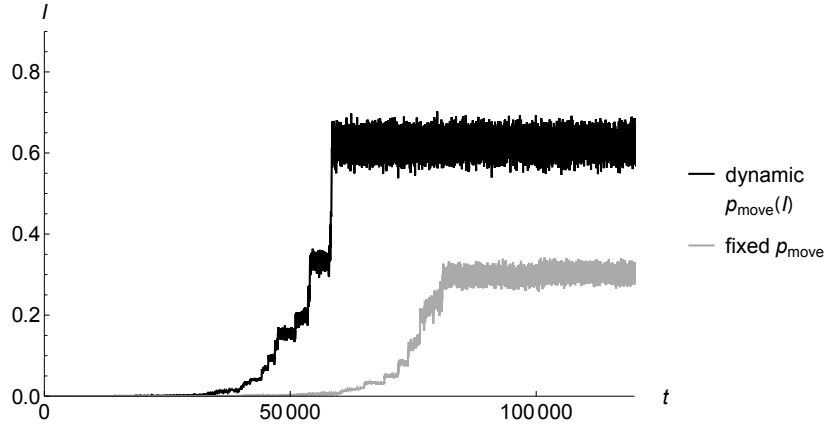
Figure 4.5: Comparison of evolutionary trajectories for players with fixed $p_{move} = 0.052$, and for players with coupled I and p_{move} as given by Equation 4.7. t is time in number of generations. Investment in the population with coupled properties evolved beyond the limits imposed by the initial parameterisation.

4.4 Discussion

4.4.1 Influence of relocation probability p_{move}

As p_{move} increased, I found a progressive reduction of average investment in populations. Mobility lead to mixing of the population, a factor that is well documented to inhibit the evolution of collectively optimal strategies (Axelrod and Hamilton; 1981; Nowak and Sigmund; 2004). I explored at which level of mixing the evolution of clearing could prevail; for my parameterisation, this was the case for $p_{move} < 0.052$ (but see below notes on influence of value δ).

Killingback et al. (1999) found that the condition under which higher investing mutants could propagate depended on their fraction n in a given neighbourhood, and player mobility likely interfered with a build-up of n to critical levels.

4.4.2 Coupling of interaction dynamics with strategy choice

The coupling of interactions dynamics and player investment lead to an auto-catalytic reduction of neighbourhood perturbation, which drove the investment of trail clearing along the trajectory of the monotonically increasing plot in Figure 4.3.

This method is fundamentally different to other proposed coupling mechanisms influencing neighbourhood dynamics, e.g. merit-based neighbourhood readjustment on graphs (Santos et al.; 2006a; Pacheco et al.; 2006b,a). In these, players prune nodes to opponents against which they receive low pay-offs, and select new ones from the neighbourhood of opponents against which they perform well. These methods require rational players with a number of complex traits: the cognitive capacity to memorise previous games and to assess performance of opponents, and a strategy for (and capacity of) adjusting their neighbourhood ties accordingly (Perc and Szolnoki; 2010)

Such requirements of player complexity however conceivably limit the range of organisms for which these mechanisms are applicable. Instead, I showed that self-organised spatial processes can introduce structure in unstructured populations of biological players, with no requirement of player complexity.

4.4.3 Model limitation

I did not investigate different pay-off functions and only explored a single, arbitrary parameter set (i.e. for $k = 0.5$). Variation of pay-off value δ influenced maximum achievable investment levels, and identified a turning point $p_{move} \sim 0.052$. Future work will hence be needed to investigate parameters under which spatial self-organisation can propagate strategy evolution, and under which conditions this effect disappears. In general, conditions for the evolution of collectively optimal strategies include a term to asses cost/benefit relations (e.g. Nowak; 2006; Ohtsuki et al.; 2006). I assume that this will apply here as well and that such rules can be formulated for the dynamical introduction of structure.

4.4.4 Summary

Collectively optimal strategies (like cooperative behaviour) do not readily evolve in unstructured populations, and omnipresence of such behaviours in nature has baffled scientists since times of Darwin. A plethora of theories exist to explore the apparent conundrum of their existence (see e.g. Doebeli and Hauert; 2005; Nowak; 2006, for reviews). Spatial structure has been recognised to propagate their evolution. Structure can be introduced by different processes, e.g. group cohesion (Wilson; 2007) or dynamic social network structures (Santos et al.; 2006a; Pacheco et al.;

2006b,a). As an extension to these findings I showed – by the example of trail clearing – that even in populations of simple players, self-organised spatial processes can drive the evolution of a behaviour. To the best of my knowledge, this interplay of spatial self-organisation and the evolution of collectively optimal strategies has not been previously recognised.

Chapter 5

Conclusions

The diversity of infrastructure construction in social insects has been described in numerous publications, but most of this research has focused on the construction of nests and tunnels (see 3.1.1 – Adaptive mechanisms in nest construction). In this thesis, I investigated the construction of cleared trails, a behaviour known from a small number of ant species (Lanan; 2014).

Some of the functional aspects of pheromone-based trail networks have been investigated (e.g. Jackson et al.; 2004; Latty et al.; 2011), but little was known of the construction and functional consequence of ant trail networks that involved investment in clearing and maintenance. I used a multi-pronged approach to investigate this topic, addressing the energetic costs and benefits of trail use in *Atta*, the mechanism of self-organisation that regulates investment in trail clearing, and the game theoretic considerations that may govern the evolutionary trajectory of investment in trail clearing. My results advance the understanding of the use of physical trails and the investment in transport infrastructure by ants, and create a new framework for addressing some outstanding questions about the ecology of *Atta* ants and of infrastructure investment by social organisms in general.

The construction of trail networks by *Atta* colonies reduce their foraging costs but require an energetically expensive investment. That cleared trails provide benefits by reducing the energetic expenditure of foraging has long been suspected (Shepherd; 1985), and trails could provide other benefits like reduction of pathogen intake into the nest that might threaten ants or the symbiotic fungus. That cutting and clearing leaf

litter and other vegetative material from pathways requires an energetic investment is immediately apparent, and the energetic costs of this activity had been partly quantified by Howard (2001) in a case study. Howard (2001) reached the conclusion that trail clearing was energetically inexpensive relative to the use a trail receives, that is, that clearing trails was cheap for an *Atta* colony.

Despite the groundbreaking nature of the measurements Howard (2001) made, they were incomplete and his comparison of costs and benefits (in terms of ant-hours invested in clearing vs. ant-hours of foraging traffic) did not allow a full assessment of costs, and did not calculate benefits in terms in incremental gains from a cleared trail relative to use of an uncleared trail. In my work, I explicitly modelled the entire cost associated with the trail clearing, including the previously unrecognised expense of maintaining a portion of the workforce on cleared trails to be available for removal of obstacles that arise during active trail traffic. I also calculated energetic benefits of trail use relative to equivalent foraging in the absence of a cleared trail. With this model, I was able to show that for a wide range of realistic foraging conditions, trail clearing indeed provides a net energetic benefit. My model showed that costs of maintaining a cleared trail could be large enough to outweigh the energetic benefit. In particular, the metabolic costs of fielding a proportion of the foraging workforce to patrols trails and respond to obstructions that arise haphazardly could outweigh the immediate costs of cutting and pulling obstructing material off the trail (see Chapter 2 – Energetics of trail clearing). This model confirms that the evolution of trail construction by *Atta* ants can be explained by a cost-benefit calculation, but it provides a new perspective on the nature of the costs.

Field biologists studying leaf-cutting ant foraging have long noted the large number of seemingly unsuccessful foragers returning to the nest without leaf fragments (see Table 2.3). Their function was unknown. My work suggests that a fraction of these unladen workers function as trail clearers, and that their numbers immediately influence the speed of trail clearing (see Chapter 3 – Mechanism of trail clearing). I made predictions about how I expect this to be reflected in field observations: I would expect this variation in unladen traffic to be correlated with rates of leaf litter fall and the consequent need for trail maintenance, which itself could vary geographically depending on ecological factors. The geography

of variation in the economics of leaf-cutting has never been considered, but could be an informative test of the ideas in Chapter 2 – Energetics of trail clearing.

A further avenue of investigation would be to focus on individual behavioural elements of trail clearing. Howard (2001) noted a difference in the size distributions of workers that engage in maintenance clearing or foraging, and measured the fidelity of workers to leaf transportation or trail clearing over 24 hours. These elements of attine foraging efforts need more thorough characterisation. No one, for example, has ever documented the complete repertoire of behaviours of workers throughout the duration of outbound and return journeys along trails. It is unknown if trail maintenance workers engage exclusively in patrolling and clearing during their activity outside the nest, how they move along trails, or whether their activity patterns differ from foragers. A promising venture for future research would be to investigate what dynamics underlie behavioural differences, what their function is (e.g. in optimising response times to unpredictable trail obstructions), and whether or not (and at what time scale) these patterns of behaviour are adaptive to environmental conditions. Long-term manipulative experiments reducing or increasing obstruction occurrence might be particularly informative, and might provide further elements for the mechanistic model of trail clearing (see Chapter 3 – Mechanism of trail clearing.

I showed that the self-organised spatial restriction incurred by trail clearing (in terms of workforce localisation) could have been a driving force behind the evolution of the trail clearing behaviour (see Chapter 4 – Evolution of trail clearing behaviour). Here, spatiality was not the result of a complex individual behaviour (which is a commonly assumed requirement for the evolution of collective optimal strategies (cf. Santos et al.; 2006a; Pacheco et al.; 2006b,a)) or imposed externally by experimenters, but was a "side-effect" of the progressive increase in investment into trail clearing.

To the best of my knowledge, such a mechanism has previously not been recognised. It suggests a novel pathway for the evolution of behaviours, and encourages the search of comparable spatial dynamics in other biological systems, particularly in the domain of "cognitively simple" organisms.

Research into the evolution of behaviours focusses on optimisation of resource intake. In the consideration of the evolutionary success of foraging behaviours, research frequently focusses on potential optimisation of resource intake (e.g. Stephens and Krebs; 1986). In the case of trail clearing, I investigated these aspects in Chapter 2 – Energetics of trail clearing. In Chapter 4 – Evolution of trail clearing behaviour, I additionally suggested that trail clearing influences neighbourhood interactions. This provides a novel perspective on "secondary" benefits of foraging behaviour derived from dynamics independent of resource intake optimisation, and suggests that ecological effects of investigated behaviours should need to be taken into account in the consideration of evolutionary dynamics.

Chapter 6

Supplementary material

Here, I list and links all relevant supplementary material. Where needed, I included a readme.txt file with instructions on use.

Below Python scripts use Python 2.7.11, NumPy 1.10.4, and OpenCV 3.1.0. Mathematica version used was Mathematica 11.0.0.0.

Supplementary material for Chapter 2 – Energetics of trail clearing

Mathematica Notebook of cost/benefit model: `https://figshare.com/articles/Chapter_2_-_Energetics_of_trail_clearing_-_Cost_Benefit_Model_nb/4796581`

Supplementary material for Chapter 3 – Mechanism of trail clearing

Archive containing removal simulation, tracking algorithm, encounter extraction script, and analysis of tracking data. `https://figshare.com/articles/Chapter_3_-_Mechanism_of_trail_clearing/4800874`

Supplementary material for Chapter 4 – Evolution of trail clearing behaviour

Mathematica Notebook calculating pay-off functions; Python scripts containing simulation, readme file with instructions on simulation use: `https://figshare.com/articles/Chapter_4_-_Evolution_of_trail_clearing_zip/4796635`

Vita

Relevant publications arising from thesis

The following publication contains results of Chapter 2 – Energetics of trail clearing:

> Bochynek, T., Meyer, B. and Burd, M. (2017). Energetics of trail clearing in the leaf-cutter ant Atta, *Behavioral Ecology and Sociobiology* **71**(1): 14.

Further publications are planned, which will contain results of Chapter 3 – Mechanism of trail clearing and Chapter 4 – Evolution of trail clearing behaviour. An additional publication is in revision after favourable review, and will present results on foraging activity regulation in *Atta* which are not included in this thesis.

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This thesis was typeset with LaTeX 2_ε[1] by the author.

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[1] LaTeX 2_ε is an extension of LaTeX. LaTeX is a collection of macros for TeX. TeX is a trademark of the American Mathematical Society. The macros used in formatting this thesis were written by Glenn Maughan and modified by Dean Thompson and David Squire of Monash University.

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