Dynamics of the aphid-ant mutualism

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Abstract

An appreciation of the role of mutualism is essential when studying ecology and evolution in most ecosystems. Information covering aspects of mutualistic interactions can serve as a complement to the somewhat one-sided perspective from the 1950's and 60's that is used when teaching biology. In this thesis I applied an in-depth approach in which variation in the interspecific interaction between *Aphis fabae* aphids and *Lasius niger* ants was studied both in the field and in the laboratory. An emphasis was put on studies spanning several consecutive aphid generations. This approach revealed important differences between ant tended aphids and those without ants. In the lab, I found an initial decrease in aphid adult size and reproductive investment in the first generations after the start of ant tending, which was followed by a recovery to the pre-tending situation after about four generations. Another laboratory experiment showed an increase in alate (winged aphid) production from exposure to aphid alarm pheromones, and an even stronger decrease in alate production from ant attendance, suggesting that ants have gained the upper hand in an evolutionary conflict over aphid dispersal. Results from a field experiment further emphasized the possibility of negative effects of ants on aphids, showing that ant-tended aphid colonies experienced a higher rate of parasitoid attacks, produced fewer alates and embryos in adult aphids. The thesis highlights the scope for variation in the net effect of the interaction for aphids, and argues that, depending on the environmental circumstances, the interaction may sometimes and perhaps even often not really be a case of mutualism.

Keywords: mutualism, transgenerational, aphids, aphid-ant, conflict of interest, (*E*)-*β*-farnesene, phenotypic plasticity, predation risk, wing induction, maternal effect, reproductive investment, embryo size

List of papers

This thesis is based on the following papers, which will be referred to by their roman numbers (I – IV).

I. **Tegelaar, K., Hagman, M., Glinwood, R., Pettersson, J., Leimar, O.** 2012. Ant-aphid mutualism: the influence of ants on the aphid summer cycle. **Oikos** 121:61-66

II. **Tegelaar, K., Glinwood, R., Petterson, J., Leimar, O.** 2013. Transgenerational effects and the cost of ant tending in aphids. **Oecologia** 173: 779-790.

III. **Tegelaar, K., Leimar, O.** 2014. Alate production in an aphid in relation to ant tending and alarm pheromone. **Ecological Entomology** 39: 664-666.

IV. **Tegelaar, K., Leimar. O**. The influence of ants and parasitoids on aphid reproduction in the field. Manuscript.

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Ethically, there is nothing in the phenomena of symbiosis to justify the sentimentalism they have excited in certain writers. Practically, in some instances, symbiosis seems to result in mutual advantage. In all cases it results advantageously to one of the parties, and we can never be sure that the other would not have been nearly as well off, if left to itself (Roscoe Pound 1893).

Cited from a speech held 1892 and published in The American Naturalist 1893

Introduction

Mutualism

Our understanding of how evolution works is always changing. Mutualism was, and is still, defined as an interaction where both parties experience benefits from engaging with each other. In recent decades, traditional explanations of mutualistic interactions have been challenged, perhaps not by a new theory but by a wider understanding of all the circumstances that influence the outcome. Mutualism is a widely used term in behavioural biology and a ubiquitous phenomenon in nature and has a historical tendency to be viewed as a fixed property of an interaction, as pointed out by Bronstein (1994), but since that time a wider perspective has become established. This includes a larger number of contexts as well as a larger number of species involved in the interaction. Aphid-ant mutualism has served as one of the models in general descriptions of mutualism (Stadler & Dixon 2008). In this thesis the interspecific relation between a myrmecophilous aphid species and one of its tending ant species is studied in relation to aphid dispersal and reproductive investment, and to the risk of parasitoid attack on aphids. The studies were carried out over several consecutive aphid generations, which turned out to provide new information about the interaction.

The term mutualism in itself is broad and includes rather different species interactions, ranging from pollination to symbiosis. Mutualism can be divided into several categories where costs and benefits are used for classification, for instance pseudo-reciprocity, reciprocity and altruism. The term mutualism as it is used today might be translated into "interspecific engagement where organisms can gain benefits from interacting with other species, and where costs can vary with fluctuating conditions". The persistence of cooperation is sometimes presented as an evolutionary paradox, because of the potential advantages of exploitation. Hence, it is not surprising that recent studies of interactions between aphids and ants find that it is a complex relation where the ants sometimes exploit the aphids. The interaction between different species of aphids and ants ranges from ant predation to obligate mutualism and there is a complex variation during the length of the interaction.

In the late 1950s and early 1960s the relation between aphids and ants was described as an uncomplicated example of mutualism, ants protected aphids and in return received nutritious honeydew. Ants also caused enhanced rates of feeding and excretion (Banks 1958) and more rapid development and colony growth (El Ziady & Kennedy 1956, El Ziady 1960). Exclusion experiments showed that aphids were protected against predation (Völkl 1992, Takizawa & Yasuda 2006) and gained in colony growth and reproductive rate (El Ziady & Kennedy 1956, El Ziady 1960, Flatt & Weisser 2000). Ants are also known to clean honeydew residue from host plants, reducing pathogen incidence and promoting a healthy aphid colony and host plant (Way 1963, Nielsen *et al.* 2010). In the last decades experiments have showed that the relation is not that simple (Völkl 1992, Billick *et al.* 2007, Völkl & Stechmann 1998, Jung *et al.* 2011, Endo & Itino 2012, Yao 2014, this thesis). Mooney (2011) found genetic variation linked to attractiveness to ants and recent studies revealed that ants are able to distinguish between aphids previously tended by their colony and untended aphids (Glinwood *et al.* 2003). Aphids in low density colonies are tended by a higher ratio of ants and therefore get marked by ant cuticular carbohydrates (CHCs) and are

not predated on to the same extent (Endo & Itino 2012). Endo & Itino (2013) were also able to show that the aphids can produce these CHCs themselves and this discovery gives us a new perspective of the interaction. The long-term stability of any mutualism is threatened by cheaters and by shifts towards parasitism and the outcome can vary within the same aphid species depending on habitat and conditions (Stadler & Dixon 2005). Palmer *et al.* (2010) argued that ant symbionts only could be understood by considering the timing, duration and sequence of mutualistic engagements. The expected lifetime of individuals of the species involved may be important, such that long-lived species are more often mutualists whereas exploiter species tend to be short lived.
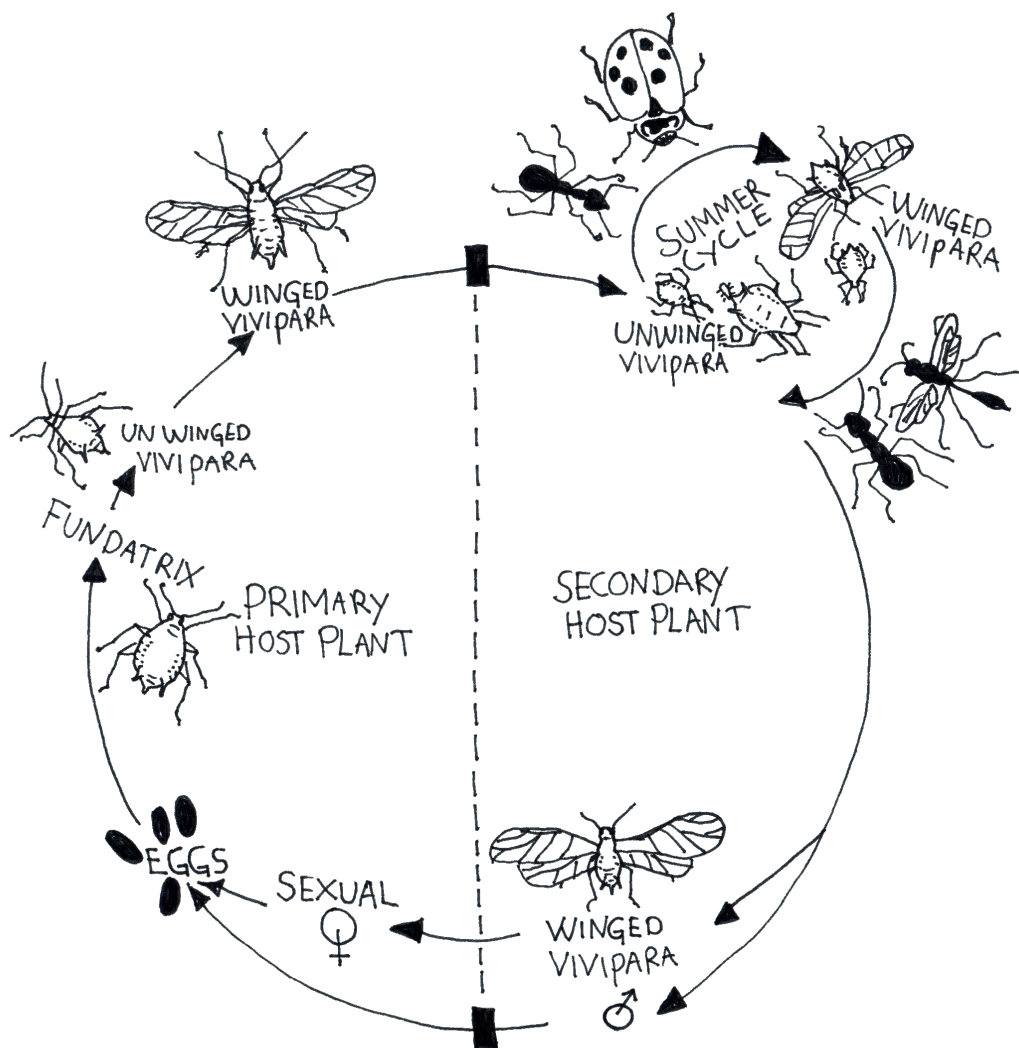

Figure 1. The life cycle of *Aphis fabae* illustrating host change and parthenogenetic summer cycle and the associated morphs.

Aphid life cycles

Aphids evolved a series of life cycle characteristics that are unusual compared to most other herbivorous insects. In most aphids, both sexual and asexual reproduction is present. This cyclic parthenogenesis combines the advantages of each of the two strategies, a sexual phase for variation and an asexual phase for high reproductive capacity (Heie 1980). There are several differences between sexual and

parthenogenetic reproduction that have an impact on their occurrence in nature. Asexual reproduction involves no fusion of gametes and therefore no recombination, leading to accumulation of deleterious mutations (Dixon 1998, Blackman 1981). In a longer perspective, evolving asexuality might thus cause extinction of a lineage but in the short term the twofold reproductive rate is an important advantage. Partial loss of sexual reproduction is uncommon in nature (Maynard Smith 1975, Blackman 1981, Hales *et al*. 1997, von Dohlen & Moran 2000), but all extant aphids reproduce parthenogenetically during some stage of their life cycle (Moran 1992). Aphid phenotypic variation within and between generations is a characteristic that holds the key to their life cycles (Fig. 1). During parthenogenetic summer cycles, clone growth is prioritised, whereas dispersal between host plants reduces aphid numbers radically (Ward et al. 1998). The high reproductive rate in aphids makes density control vital (Blackman 1981). Different morphs are used for aphid colony growth, dispersal and reproduction, in response to fluctuating conditions such as perceived predation risk, host plant quality and photoperiod.
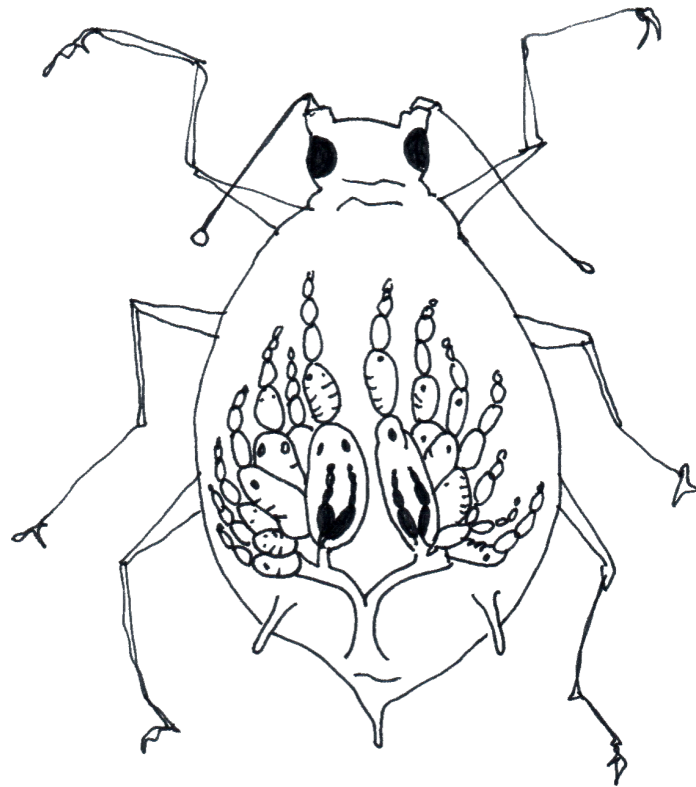

Figure 2. Parthenogenetic female with several ovariole strings visible. A female can have up to 12 ovariole strings. In the largest embryos the telescoping third generation can be seen.

Two characteristic features of aphids are cyclic parthenogenesis and viviparity (giving birth to live young), and these traits in turn result in an increased possibility of several generations per season. Cyclic parthenogenesis dominates over totally parthenogenetic species (Simon & Sunnucks 2002). The combination of cyclic parthenogenesis, a high degree of polyphenism and seasonal host plant alternation make aphid life cycles both complex and unique. Cyclic parthenogenesis presents aphids with means of reproduction that allows a high reproductive rate. Parthenogenesis allows a life style without restrictions from fertilization and gives the organism an ability to start up a colony from a single individual and the egg to start developing without fertilization directly after ovulation. A parthenogenetic aphid

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invests in both somatic and gonadal cells in early development and starts oogonial division in their grandmother, thereby shortening the generation time (Blackman 1981, Kindlmann & Dixon 1989). In such 'telescopic' reproduction (Fig. 2), several generations coexist in one aphid female (Dixon 1985, Dixon 1998) and within the first day of adulthood the parthenogenetic female is able to give birth to the first nymphs that become adult through four successive nymphal stages (Blackman 1981, Hales *et al*. 1997). Parthenogenetic reproduction also contributes to aphid colony growth because it results in an intrinsic rate of increase twice that of a sexual lineage (Cuellar 1977). In aphids parthenogenesis together with a shortening of the generation time from telescoping can result in a three-fold increase in reproductive rate compared to sexually reproducing animals (Dixon 1998). Host plants, predators, parasites and herbivore competitors all have the evolutionary advantage of variability through sexual reproduction, but the rarity of parthenogenesis in those groups also means that predators and parasitoids in most cases are reproducing sexually and cannot keep the same rate of increase as the aphids they utilize.

Dispersal

Aphids are one of the best examples of trade-offs between investment in reproduction and dispersal. Various types of seasonal host alternation between woody and herbaceous plants occur among aphids and enable them to gain high productivity from each season (Fig. 1). The alate morph has wings and a reproductive physiology that is adapted to function during dispersal, whereas the apterous morph has no wings but maximal fecundity. The production of alate individuals prolongs development and the alate aphid has a gonad that is 20% smaller (Dixon et al. 1993). The percentage of alate aphids that survive the dispersal to a new host has been estimated at 1% or less (Taylor 1977, Ward *et al*. 1998), so a high productivity of the aphid colony on a host plant is a crucial ingredient in the summer cycle. The number of alate aphids a clone is able to produce before the host switch is a useful measure of the reproductive success of the summer cycle. When aphids have the means of reproduction they should produce alate aphids to a higher extent, since their investment in nymphs is otherwise consumed by the ants in a higher percentage (Endo & Itino 2012).

Winged development can be induced by multiple cues (Kunert *et al*. 2005, Mehrparvar *et al*. 2013), and these can act inside the mother (pre-natal) or in the nymph (post-natal), and in some aphid species both of these possibilities are present (Müller *et al*. 2001). Winged aphids are often induced by interactions with other aphids due to crowding, or with other insects, such as predators. Aphids interacting with ants produce fewer alates, their alates has a higher wing load and do not disperse as much as untended alates (Yao 2011, Yao 2012) or aptera (Oliver *et al*. 2007). Ant tended aphid species are more likely to be wingless (Stadler *et al*. 2003), and this can also been seen from their genetic diversity, where tended aphid species have fewer haplotypes (Yao & Kanbe 2012). An elevated risk of predation can induce a phenotypic change that lasts several generations (Mondor *et al*. 2005). Production of winged aphids as a response to predation risk has not been found for *A. fabae* (Kunert *et al*. 2008, Dixon & Agarwala 1999), but for other aphid species that are not attended by ants. The relation to ants has previous been used as an explanation for why predation risk does not induce wing formation in myrmecophilous species (Müller *et al*. 2001). Previous studies that did not find an increased alate production as a

response to predation risk examined the generation born within a few days after a predation cue (Dixon & Agarwala 1999) or aphids born within three days of the predator treatment (Kunert *et al.* 2008). This time might not be sufficient to find a transgenerational wing induction effect. The predation pressure by developing ladybird larvae on an aphid colony increases even after three aphid generations and a facultative switch from wingless to winged individuals should be beneficial if a ladybird visits the colony (Dixon & Agarwala 1999).

Kindlmann *et al.* (2007) found that ant-attended aphid species disperse significantly later in the summer to their secondary host plant. Whether this is an aphid adaptation or results from manipulation by the ants is an interesting question. Ants are known to influence aphids in the direction of reduced mobility and alate production, which might lead to the production of larger aphid colonies and a higher production of honeydew in the area near an ant colony. This is an example of how the interests of aphids and ants differ, as aphids depend on dispersal to complete their yearly life cycle.

Phloem feeding and honeydew secretion

Aphids are phloem sap feeders, inserting their stylets into the host plant phloem tissue. Feeding on the inner phloem, by having a longer stylet, increases the likelihood for the aphid species to be myrmecophilous (Shingleton *et al.* 2005, Oliver *et al.* 2008). The honeydew produced is utilized by a large number of organisms (Völkl *et al.* 1999) and honeydew quality depends on host plant species and quality (Fisher & Shingleton 2001). Different species of aphids produce different levels of sugar and amino acid content and aphids are able to alter their honeydew excretion composition and rate (Yao *et al.* 2000, Yao & Akimoto 2001, Yao & Akimoto 2002, Woodring *et al.* 2004, Fisher *et al.* 2005) and there are large differences in the honeydew produced in the presence of ants for facultative species (Vantaux *et al.* 2011). Aphids have a chance of signalling to ants that they are not producing high quality honeydew by CHCs (Land & Menzel 2011) and it has been shown that aphid clones that are not attracting ants with altered honeydew rate or composition still exist in the gene pool (Vantaux *et al.* 2011).

Purpose of the experimental set-up for each paper

The questions used as a guideline in each of the papers are described shortly below:

Paper I: How does ant-tending in the field affect a growing colony over a longer period of time.

Paper II: How does ant tending affect an aphid colony in a controlled environment. Reproductive investment is measured as colony growth, aphid weight and embryo size distribution
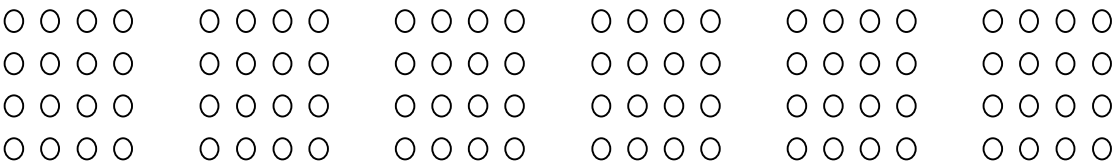
Paper III: How does the presence of alarm pheromone (EBF) affect dispersal investments in aphid colonies tended or untended by ants.

Paper IV: How do reproductive investment and parasitoid attacks in the field change with ant exclusion.

PAPER I

Method

In this study we examined colony growth in *Aphis fabae* in the field in relation to presence or absence of ants (*Lasius niger*). The experiment was performed in an experimental field at the Swedish Agricultural University in Uppsala. Potted scentless mayweed was placed in patches of 16 pots, either with Tanglefoot on the sides and a mesh covering the holes in the bottom or with free access for ants. In each patch, pots were placed 50 cm from each other and each patch was placed 150 cm apart, illustrated below. Data on numbers of aptera, alates, ants and parasitoids were collected over five weeks. The aim was to investigate changes over the summer cycle in a faculatively myrmecophile aphid, both in the presence and in the absence of ants.

O O O O   O O O O   O O O O   O O O O   O O O O   O O O O
O O O O   O O O O   O O O O   O O O O   O O O O   O O O O
O O O O   O O O O   O O O O   O O O O   O O O O   O O O O
O O O O   O O O O   O O O O   O O O O   O O O O   O O O O

Results

The results of the study showed that aphids tended by ants initially grew faster than untended colonies, but there were fewer colonies, aphids and alates present on plants with ant access in the later part of the experiment (Fig. 3a), indicating decreased production and dispersal possibilities on plants with ant attendance. Aphid colonies tended by ants had a higher observed number of parasitoids (Fig. 3b) and a statistically significant higher proportion of parasitized aphid mummies.
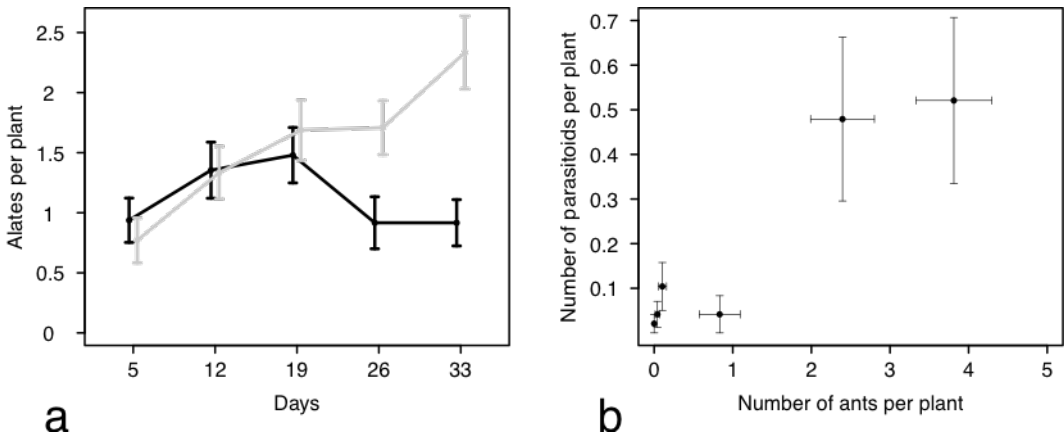
Figure 3. (a) Total number of alates (winged) aphids on tended and untended plants during the five-week experiment (mean value ± SE). (b) The correlation between number of ants present on plant and parasitoid numbers.

Conclusion

The results in this study show that there is a large cost associated with ant tending for a colony of aphids, supporting the conclusion that ant attendance increased the risk of parasitoid attack. The possibility of conditionality in the outcome of a seemingly mutualistic interaction is concluded and the presence of benefits for the parasitoid in the case of an interaction between aphids and ants.

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PAPER II

Method

In PAPER II we investigate long-term changes in growing aphid colonies. We developed a novel measurement of aphid reproductive investment by comparing the distribution of embryos of different sizes in the ovarioles of apterous (wingless) females. This novel dissection and measuring of embryos is described in Fig. 4.
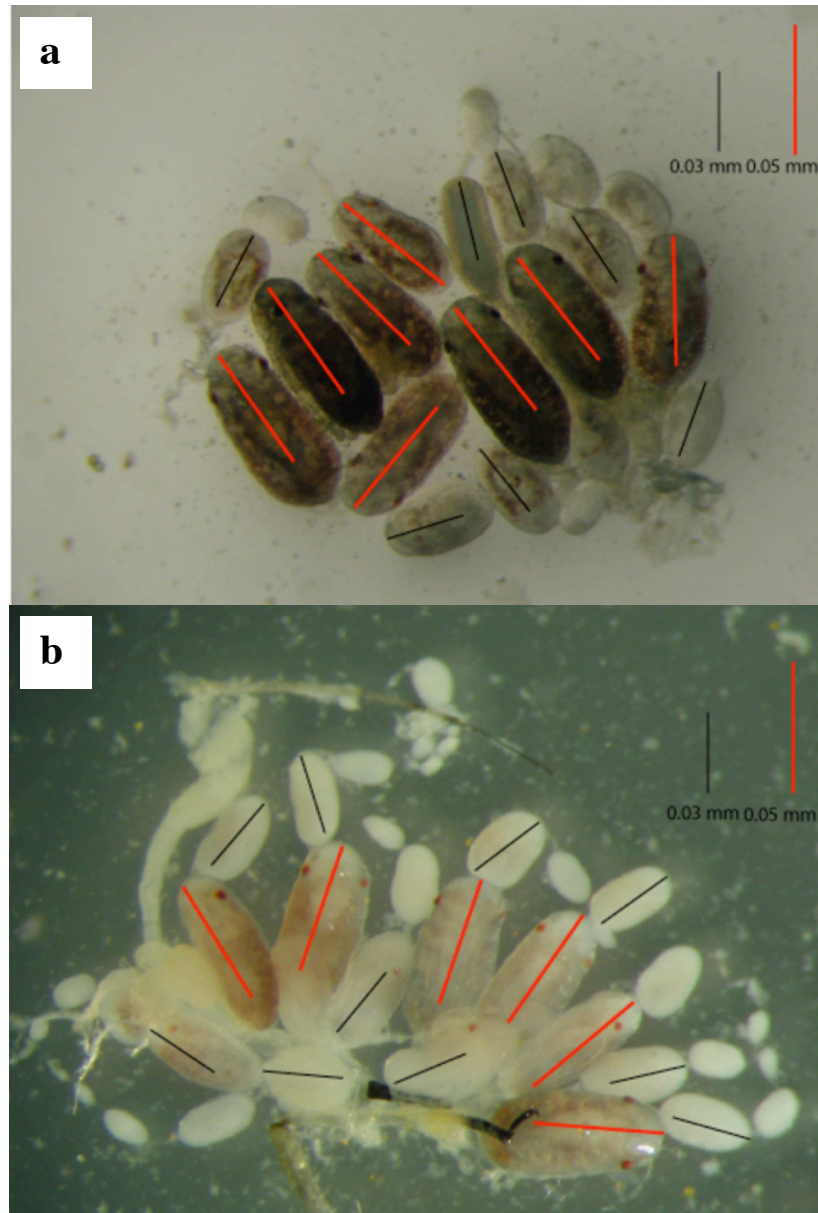

Figure 4. Embryos from one adult aphid dissected directly after euthanization (b) and embryos from one adult aphid, preserved in ethanol for 3 days (a). Notice the darker colour in (a) compared to (b). The number of large embryos are indicated by the red measuring lines (0.05mm in length). Number of medium embryos are indicated by the black measuring lines (0.03mm in length). The distribution for large/medium embryos differs between the two aphids with 6/10 (a) and 8/5 (b).
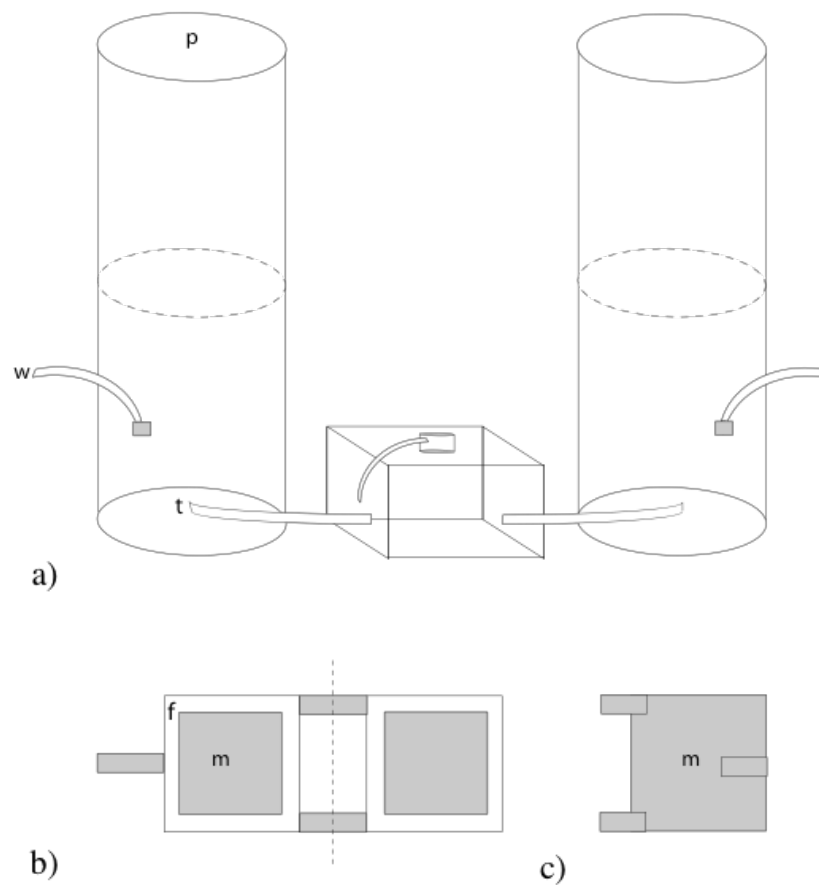
Figure 5. Experimental set up. (a) A pair consists of two plexiglas cylinders 40 cm high, openable at 20 cm, sealed in the bottom and connected to an ant colony with a open/close tap. The cylinders each have a watering tube placed in the bean plant pot to minimize daily disturbance. A perforated sheet of plastic, allowing light intake and air circulation, covers each cylinder. Ant colonies have access to a feeding arena at the top of the nest by tubes. (b) Small clip cages (3x3cm) made of fine metallic mesh (m) and foam rubber (f) were used to enclose groups of aphids on plants at the start of each experimental generation; this was done to increase the likelihood that aphids stayed together in a group. Aphids were kept in closed (c) cages for 24 hours when introduced.

Laboratory experiments in this paper were conducted in 12 pairs of plexiglas cylinders connected to a nest with ants with closable taps to permit changes in ant tending (Fig. 5a). This system enabled ant access to only one of the cages in a pair, with the other cage acting as a control. Over 13 successive weeks the production in aphid colonies was recorded over alternating ant tending. During the first two generations all cages were untended; in the four following generations one of each pair was ant tended; in the two following generations all cages were untended and during the last four generations a reversal of the first ant treatment was conducted. We measured aphid growth in wet weight and made a correlation of wet and dry weight to assert that wet weight was applicable as a measure of aphid weight as dry weight mainly has been used previously. Our correlation from comparing wet weight to dry weight shows that both dry and wet weight can work as a measure of growth.
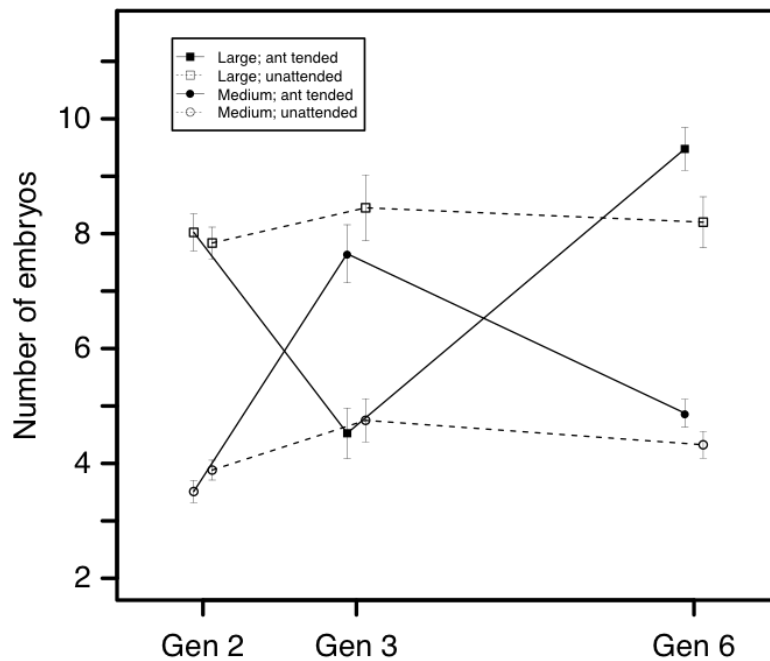
Results

Figure 6. Number of large (greater than 0.05mm) medium sized (0.03mm - 0.05mm) and small (0.005 mm - 0.03mm) embryos in dissected aphids. In generation 2 cages were untended; generation 3 to 6 one cage of each pair was ant tended (first ant treatment).

There was a strong effect of treatments on the embryo size distribution (Fig. 6) and the difference in total aphid colony weight between the paired cages over the duration of the experiment. Weight difference did not just depend on the ant tending in the current generation but was also influenced by the previous history of tending and the effect decreased and could no longer be detected after four generations of ant tending (Fig. 7). For the embryo distribution there was a shift in size categories (Fig. 6) and ant tended aphids had a higher proportion medium sized embryos compared to untended aphids. We also found a significant correlation between risk of fungus infection on bean plants infested with *A. fabae* and ant tending. Ant tended aphid colonies had a significantly reduced risk of growing fungus (5,1%) compared to untended aphid colonies (70,6%).
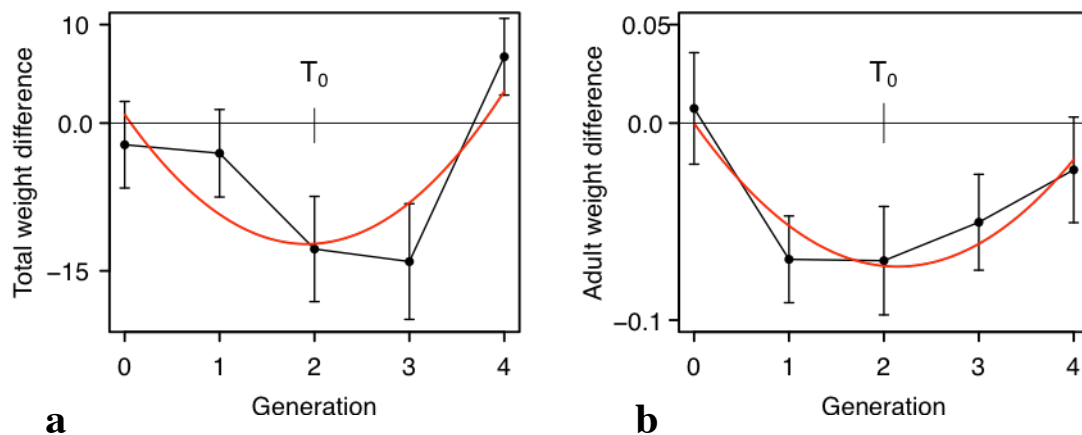

Figure 7. Observed and model fitted (parabola) total aphid colony weight difference (a), average adult weight difference (b) between tended and untended cages in pairs. The two ant treatment periods are analysed together and time is measured such that generation 1 is the start of ant tending in each period. Data are given as mean ± SE (mg) for the pairs present at that time. T_0 is the generation used for the intercept in the model fitting. There were 12 pairs at the start of the first ant treatment and 9 at the start of the reversed ant treatment.

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Conclusion
We where able to show how the growth of the aphid colony changed with access to tending ants. The results imply that there can be a large reproductive cost associated with ant tending, but the decrease in growth disappeared after four generations, perhaps indicating that telescoping of generations allows aphids to decrease investments and costs over time. The interaction is classified as a case of pseudo-reciprocity where the aphid colony at least to some extent makes costly investments to obtain by-product benefits the form of the ant tending behavior or reduced predation by ants. Further, our results are the first to investigate how aphid colony growth respond to ant tending changes over a succession of generations. A novel aspect of our work is the role of transgenerational effects in this kind of flexibility.

PAPER III

Methods
We investigate how aphids are influenced by mutualistic relations to ants and how reproductive investment alters when faced with induced predation threat by alarm pheromone (*E*)-β-farnesene (EBF). We used the laboratory set-up in PAPER II. Both cages in a pair were either with or without ant tending, and in each pair one plant was treated with alarm pheromone (EBF), and the other with hexane as a control. We used 4 exposures of EBF each day, delivered within an hour and approximately 15 minutes apart. This was meant to simulate EBF production during repeated predator attacks. The experiment was continued for 12 – 16 days, until the colony of aphids exceeded plant capacity and it no longer was suitable as a host plant.

Results
Ant tending and alarm pheromone both influenced the production of alates. Ant tending resulted in fewer produced alates and induced risk of predation in a higher production (Fig. 8). The effect of ant tending was stronger than that of EBF. Ant attendance decreased alate production by a factor of 4.3 and the EBF treatment increased it by a factor of 2.2.
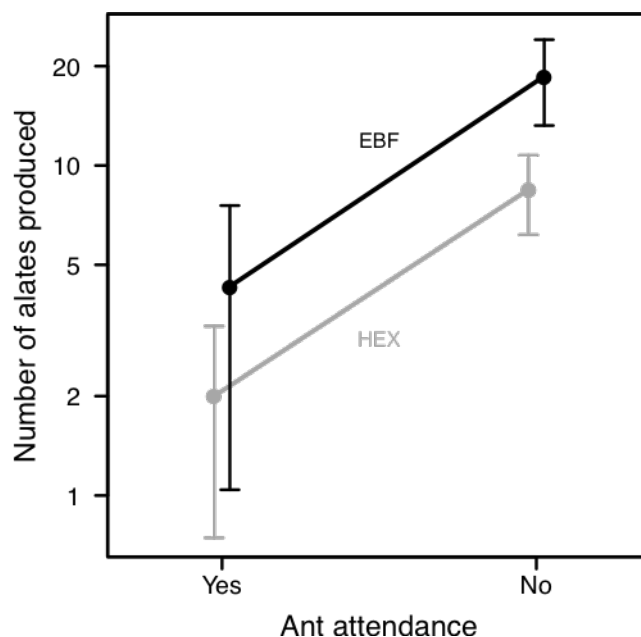

Figure 8. Number of alates produced at the time of collection of the aphid cages, shown as a function of ant attendance and EBF treatment vs. hexane control. Note the log scale of the y-axis, making the error bars appear asymmetric around the mean. Data are presented as mean ± SE.

Conclusion
This paper investigates the effect of ant tending and risk of predation in the ant-aphid mutualism and we found, in contrast to previous studies, that a cue of predation risk (EBF) increased alate production. Our main conclusion is that ant tending reduces aphid production of alates and therefore their means of dispersal. We argue that our observations illustrate a flexibility and condition sensitivity that might be present in many mutualistic interactions. A tentative conclusion is that ants to some extent have gained the upper hand in an evolutionary conflict about aphid dispersal.

PAPER IV

Methods
In this study aphids collected during the filed experiments for PAPER I were dissected to investigate how the presence of ants affected reproductive investment in aphids in the field. Number of embryos in different size categories were recorded and the presence of parasitoid larvae and signs of a large immune responses with hemocytes was also recorded.
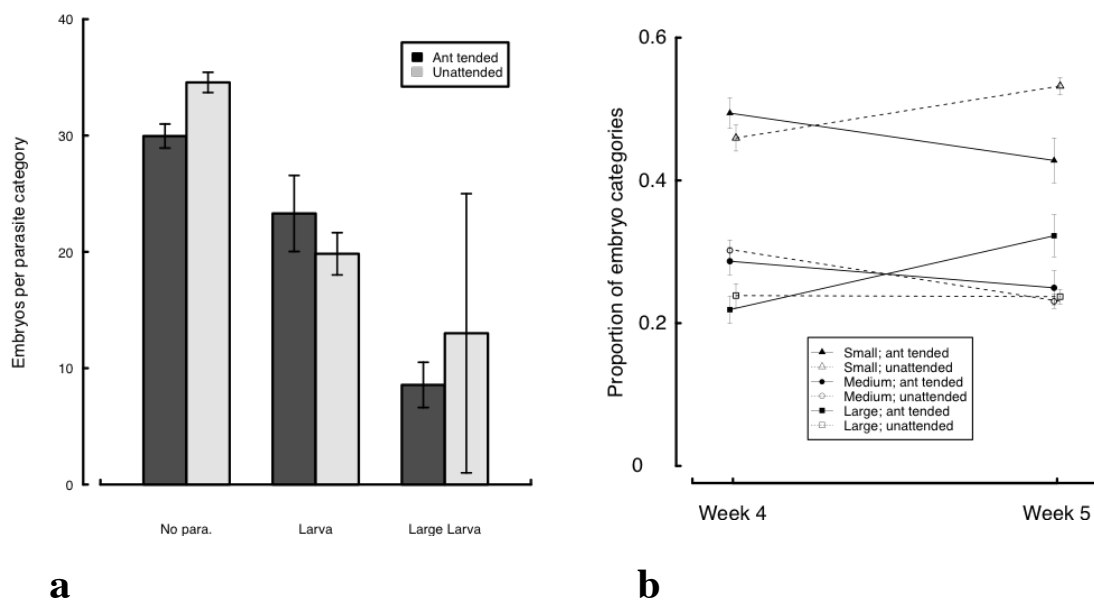
Results

Figure 9. (a) Number embryos in adult aphids (mean ± SE) for different ant treatments and stages of parasitism. Data are pooled over the two periods of sampling for both (a) and (b). (b) Proportion of embryos (mean ± SE) (Small, Medium, Large) in aphids that did not contain a parasitoid larva, for ant tended and unattended. Dashed lines and open symbols indicate unattended aphids and solid lines and filled symbols indicate ant-tended aphids.

Total embryo production increased on the patches where ants had been prevented access. During the two last weeks of the experiment the presence of parasitoid larvae was significantly higher in ant tended aphid colonies. The proportion of large embryos increased in ant tended aphid colonies, as the proportion of small embryos decreased, the same shift was not seen in untended colonies (Fig. 9b). We recorded presence of a probable immune response with large hemocytes in a high proportion of adult aphids. In analyses for publishing data these are counted as unparasitized aphids as no sign of larvae or egg could be seen. These possible hermocytes were present in a significantly higher proportion of ant-tended aphids and it further reduced the number of embryos in ovarioles (Fig. 10b).
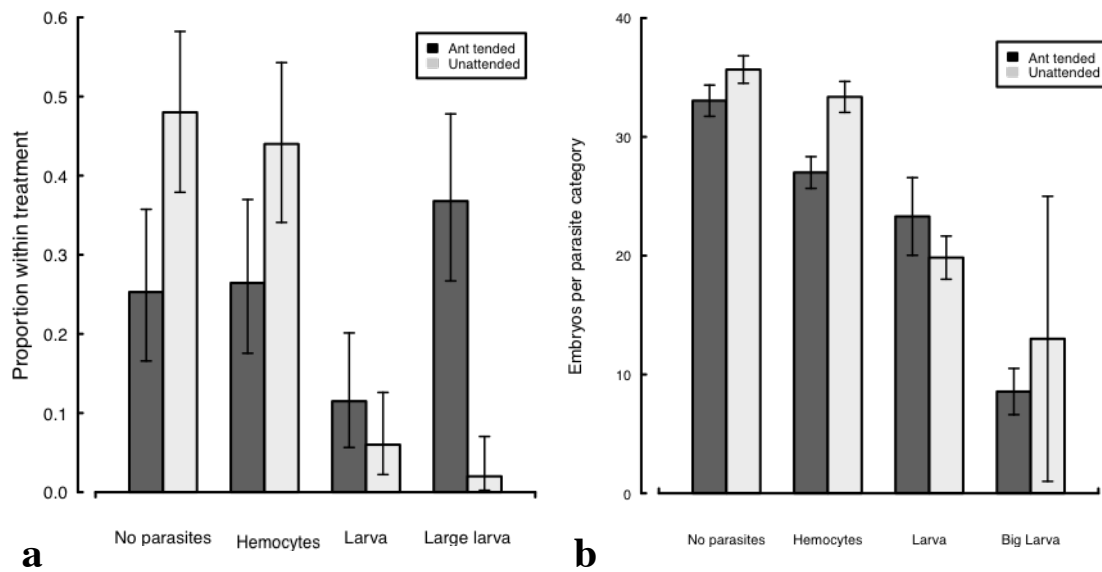
Figure 10. Proportion of aphids in different stages of parasitism (No parasite, Hemocytes, Larva of parasitoid, Large larva of parasitoid). (b) Number embryos in adult aphids (mean ± SE) for different ant treatments and stages of parasitism. Error bars show 95% binomial confidence intervals. Data are pooled over the two periods of sampling for both (a) and (b).

The immuno defence of *A. fabae* reaction to parasitoid eggs or foreign objects is not known, after dissecting field reared adult aphids it seems they have a response similar to the pea aphid with large spherulocytes or similar hemocytes found at the base of the cornicles (Schmid et al. 2012). Eggs are partially covered and not encapsulated fully and the egg can then be incorporated in the aphid's own tissue and are physiologically restrained from developing (Henter & Via 1995). Large granulate round cells, hemocytes, (Fig. 10a, 11b) and a high number of smaller round cells could be seen in a high proportion of the females after storing in 70% alcohol, these were not seen on females reared in a parasitoid-free laboratory environment.

Figure 11. Hymenopteran parasitoid life cycle in aphids resulting in parasitoid mummies and a new generation of parasitoids (a). Adult *Aphis fabae* female preserved in ethanol, here with possible hemocytes showing as ligher clusters in the abdomen in microscope backlight (b).

Conclusion

Our main conclusion is that ant tending reduces aphid reproductive investment, as seen in PAPER II, here mainly due to an increased level of parasitoid infestations, not a parameter in PAPER II, and this further strengthens the reduction of alates, embryonal and colony growth. The internal response to parasitoid eggs and larvae needs to be further studied to understand the formations seen in dissected aphids.

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Discussion

Phenotypic plasticity plays an important role in current evolutionary thinking. This is in contrast to the situation 50 years ago, when genetic variation was thought to be the most interesting factor behind phenotypic variation. Phenotypic plasticity might be especially important for species that do not use sexual reproduction in certain parts of their life cycles (Whitman & Ananthakrishnan 2009). When a plant is infested with aphids and the summer cycle starts, the clone on any plant should adjust its phenotypes to the conditions on that spot, in order to produce a high number of winged aphids for dispersal. Aphids need to adapt to the conditions imposed by ant tending and possible ant manipulation. In this thesis I have investigated reproductive investment and dispersal in a facultative myrmecophilous aphid that is commonly used as an example of mutualism. By providing evidence for negative effects of ants on aphids in a supposedly mutualistic interaction, the thesis reveals the complex system in need of further description.

Costs and benefits associated with an aphid-ant interaction are hard to measure. In particular, it is difficult to know if changes in aphid phenotypes and reproductive investments resulting from changes in ant tending are instances of beneficial changes expressed by aphids when they are tended by ants or instances of ant manipulation of aphids. In early studies of ant-aphid interactions only benefits where reported, but nowadays we assume that there is always a cost-benefit scale. There are many parameters to account for, amongst others internal symbionts acting within the aphids (Polin *et al*. 2014), intraguild predation between various predators (Meyhöfer & Klug 2002) and selective removal of parasitized aphids by tending ants (Frazer & van den Bosch 1973, Vinson & Scarborough 1991). These levels make for a complicated web of interactions. As I pointed out in the introduction, mutualistic interactions are dynamic. Current research in mutualism has reached an understanding of the nature of cooperation between species, but the variability in the interactions, e.g., depending on environmental conditions, is less well understood. For a mutualistic interaction to first evolve and then be further maintained, benefits must outweigh costs (Sachs & Simms 2006). If mutualisms were only beneficial in some circumstances it would seem reasonable that interactions do not persist unchanged after initiation but rather adjust to varying conditions. As long as one of the participants benefit, the other participant may have to adjust, finding itself in a costly interaction. Because of the context dependence of the balance between costs and benefits, it may be difficult to estimate if the interaction is truly mutualistic (Bronstein 1994, Bronstein 2001a, Bronstein 2001b). When a shift occurs such that costs outweigh benefits, a mutualism is predicted to break down or shift to a parasitic association. Little & Currie (2009) showed that parasitism can be a stabilizing agent in mutualism, making cooperative strategies stable, whereas they might not be when parasitism is not taken into account. Their conclusion is that the interests of the co-operators are aligned due to the common enemy. Together with reviews such as Yao (2014), the interpretation of

these systems will probably be characterized by more caution and wider interpretation of results in the future.

There are few multi-generational studies on the effects on aphids of ant tending. This thesis is aimed at investigating how the presence of ants influences growth and dispersal of facultative aphids over several generations. PAPER II shows some of the dynamics over an extended period of time. The increased aphid size might either be a benefit for the aphids or for the tending ants. Since we know the numbers of alates ultimately are reduced (PAPER I, PAPER III), it seems that the changes mainly benefit the ants. PAPER I shows that after the initial benefit the aphids experience from the ant attendance, there is a loss in clone growth and dispersal toward the end of a summer cycle.

By dissecting the adult aphids (PAPER II, PAPER IV) I was able to further strengthen the results presented in the thesis. As the number of ovarioles is fixed during the summer cycle (Dixon & Dharma 1980a), altering embryo size distribution is the adult aphid's only means of changing reproductive strategy as conditions change. Embryo size categories in these studies are based on embryo length, and change in embryo size distribution observed in connection to ant tending corresponds to a substantial difference in reproductive investment as volume of the developing embryo is increased several-fold between the different categories. Large aphids tend to produce larger and more offspring and they also reproduce earlier than smaller aphids (Dixon & Dharma 1980b, Ward *et al*. 1983). This indicates that aphids try to compensate for loss induced by the tending ants or are able to increase their investment due to the protection provided.

The results in this thesis will not likely apply to all instances of aphid-ant interactions, but the possibility of conditionality in mutualism should always be taken into account, and the results found are not unique among present studies. Thus Völkl (1992), Woodring *et al*. (2004), Billick *et al*. (2007), Jung *et al*. (2011), Yao (2014), and Endo & Itino (2012) suggest that these patterns, where ants have a negative influence on aphid reproduction, are widespread. Since ants attack aphids that are not myrmecophilous (Lang & Menzell 2011), myrmecophilous aphids might experience an improvement in the form of reduced ant attacks. In fact, there is still a risk of being predated on as a mutualistic aphid depending on other resources available (Offenberg 2001, Völkl 1992, Billick *et al*. 2007). Investing resources in an interaction is costly and is only expected to occur if it increases the benefit received by the investing organism, but if the investment is an adaptation to mutualism or a bribe (Zachariades *et al*. 2009) to reduce predation is not clear. The same difficulty concerning conclusions about cost- benefit ratios between ant tended and untended aphids are present for stylet length (Shingleton *et al*. 2005, Oliver *et al*. 2008). The question is whether their relation with ants makes the adaptation possible or whether they are more easily utilized by the ants due to increased immobility connected with feeding
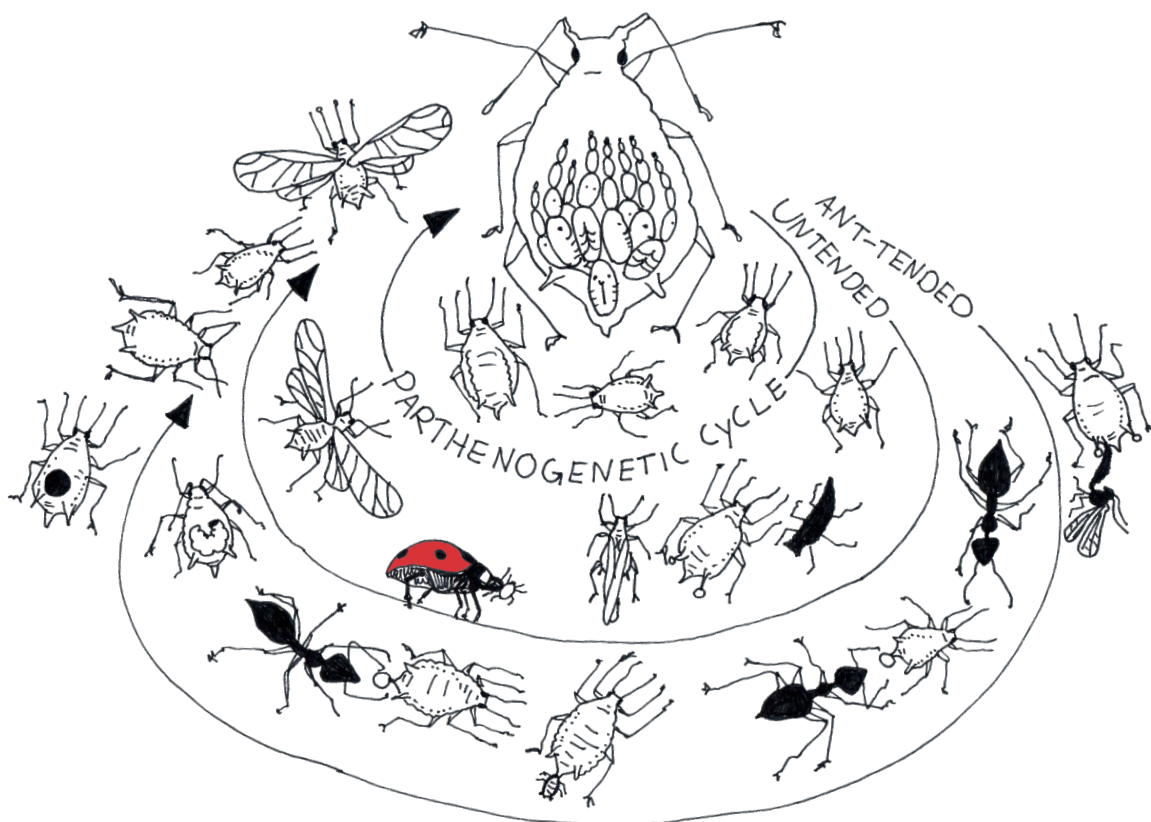
on inner floem. Reduced cornicle length in ant-tended species (Mondor *et al*. 2002) is more likely related to reduced predator avoidance.

The secretion of honeydew could both be strategy by the aphids to prevent the ants from predation or a result of ant manipulation, to sustain a sugar resource. If this mutualism is considered as a protection against ant predation, the practice of mimicking the ant CHCs fit well into this theory (Endo & Itino 2013). Vantaux and associates (2012) find that aphids that do not produce the ants' favorite sugar are not punished, and ask why they can persist in such a high percentage in nature. The answer might be that these are the aphids that get away with the ability to mimic CHCs that indicates a high honeydew production rate. A speculation is that aphid species unable to produce and mimic ant CHCs do not interact with ants but rather let go of the plant if ants approach. Aphid-ant mutualism is likely to be an example where the aphids are first adapted to escape ant predation, and the possible benefits of being tended by ants is related to varying conditions, possibly mainly to escape ant-predation and gain some protection from other predators. The long-term benefits might therefore not always be clear, as it is the ants themselves that pose the threat of predation if aphids are not investing in the interaction. Aphids cannot easily terminate a less profitable interaction with ants, and with fluctuating conditions the benefits might never occur, a low reproductive success is better than no reproductive success as would be if the ant instead of tended the aphid, predated on them (Sakata 1994, Billick *et al*. 2007, Offenberg 2001).

Conclusion

After several years of both laboratory and field experiments, we still cannot draw any definite conclusions about how aphid-ant mutualisms are regulated or how the costs, benefits and constraints of the interaction should be assessed. We can however conclude that there are substantial costs for the aphids and that they need to adjust to fluctuating conditions. An experiment that only manipulates one aspect of an interaction will of course yield a partial picture, but several studies combined can give us a broader view. This thesis raises the issue that many mutualistic interactions might fluctuate between being beneficial to all parties and being costly to some parties, depending on the circumstances. Species interactions have previously been classified as neutral, beneficial or negative, but a new way to interpret these interactions is to consider these possibilities to apply to essentially every interaction, depending on conditions, thus combining these possibilities. Mutualism simultaneously has positive and negative effects upon the individuals involved. Thinking in these terms one might rather use the terminology cost-constraint instead of cost-benefit, which was also introduced by Yao (2014) in his recent review. Each example of mutualism should perhaps be examined as to how conditions affect the interaction. Parasitoids, predators and fluctuating environments shift the interaction, within and between species, ranging from beneficial to costly.

The aphid honeydew delivered by myrmecophilous aphids has been termed danegeld by Zachariades *et al.* (2009), comparing it to the gold tax paid to Vikings to save the land from being plundered, and perhaps this is an apt comparison. The aphids may have evolved from being prey to becoming a mutualist and a food source for ant colonies. This might have happened through changes in the rate of delivery of honeydew and an increase in the level of nutrition in it. There are undoubtedly both pros and cons resulting from this evolutionary change, and as most often in science, these studies seems to raise more questions than they answer.

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