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UNIVERSITY OF ALBERTA

CONDITIONAL MUTUALISM: DENSITY- AND QUALITY-DEPENDENT RESPONSES OF APHIDS TO TENDING BY ANTS

by

LORRAINE M. BRETON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled CONDITIONAL MUTUALISM: DENSITY- AND QUALITY-DEPENDENT RESPONSES OF APHIDS TO TENDING BY ANTS submitted by LORRAINE M. BRETON in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE.

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Date: October 3, 1990

Abstract

The influence of conditionality on the reproductive success of the organisms involved in mutualistic systems, and on the stability and evolution of mutualism was reviewed. The review develops a context for the experimental work.

Experimental objectives were to determine if the effect of ant tending on aphid population growth varies with aphid density and host plant quality, and to consider possible mechanisms explaining the conditionality of the association. Four experiments involving aphids (*Aphis varians*) and ants (*Formica cinerea*) on fireweed (*Epilobium angustifolium*) were conducted in Colorado in 1988 and 1989.

Ant tending was examined in three density experiments using naturally occurring low and high ranges of aphid density, and a manipulated range of aphid density, respectively. Ant tending significantly improved the growth of small aphid populations. However, the benefit from ant tending decreased as aphid density increased, as there was no difference in growth between tended and untended populations at high aphid densities.

Possible mechanisms of this density-dependence were considered by looking at how changes in aphid density affected number of ants tending, alate and predator abundance, and host plant quality. The following results were observed: (1) the relative number of ants tending declined as aphid density increased, (2) some evidence suggested that ant tending inhibited alate production at high aphid densities, (3) the effect of ant tending on predator abundance did not vary with aphid density, and (4) ant tending reduced predator abundance in only one experiment. Although the ant-aphid association is density-dependent, mechanisms of the pattern are not clear.

To assess the effect of plant quality, aphids were artificially introduced to a wide range of fireweed (*Epilobium angustifolium*) shoots. Aphid population growth was significantly greater on higher quality (i.e. taller) plants. The effect of ant tending on population growth did not vary with plant quality. Therefore, the ant-aphid association is not dependent on plant quality.

Density-dependence of the ant-aphid association is important in generating stability in the system. A lack of benefit from ant tending at high aphid densities may slow population increase and prevent aphids from over-exploiting their host plant.

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Structure and objectives of the thesis

This thesis is composed of two chapters. Chapter 1 is a literature review of conditionality in mutualistic systems. The objectives were: (1) to establish the extent to which variation exists in the problems faced by mutualists, in the ability of a partner to solve those problems, and in the ability of a mutualist to respond to its partner, (2) to determine the importance of this variation for the reproductive success of mutualists, and for the evolution and stability of mutualism in general, and (3) to introduce the broader context for the experimental work.

Chapter 2 describes experiments examining conditionality in an ant-aphid mutualism. Objectives were to determine if the effect of ant tending on aphid population growth varies with aphid density and host plant quality, and to consider possible mechanisms explaining the conditionality of the association. Manipulations of aphid density and plant quality were performed to directly establish the existence of conditionality in this system.

CHAPTER 1

A REVIEW OF CONDITIONAL MUTUALISM

INTRODUCTION

Mutualism is a process that results in reciprocal net benefits, at either an individual or population level, for two or more species (Boucher et al. 1982). Although the widespread existence of mutualism has been well documented (see Way 1963; Boucher et al. 1982), the structure, dynamics, stability, and regulation of these systems are still poorly understood. The paucity of theoretical and empirical studies of these aspects of mutualistic systems has lead to the formulation of inappropriate conclusions (for discussion see Addicott 1981; Addicott and Freedman 1984). For instance, it is often stated that mutualism is an unstable process (Levins 1974; May 1976; Case and Casten 1979) because increased population density of one partner is believed to lead to unhindered increases in the population density of the second partner. However, for a mutualistic interaction to result in the uncontrolled increase of one partner the intensity of the mutualism must remain constant as the density of the mutualist partners increases. Variation in the intensity and outcome of the mutualism with changes in mutualist density could, therefore, have important effects on the stability and evolution of mutualistic systems (Addicott 1981; Udovic 1981; Addicott and Freedman 1984). Conclusions inferred from point-estimates of the magnitude of an interaction will often be misleading because the strength of an interaction will not remain constant as factors internal and external to the interaction vary. Therefore, conclusions regarding the properties of mutualistic systems must be based on either longer-term studies in which a variety of factors are considered in relation to their effect on the interaction, or on studies designed specifically to address the conditional nature of mutualistic systems.

Mutualism can be conditional in two fundamentally different ways (Cushman and Whitham 1989). First, the extent to which a species benefits from a mutualistic association may depend on which species it associates with, because the partners may differ in the quality of services (or resources) they provide. Second, the benefits provided by a mutualistic partner may vary depending on the biological and physical environment. Such variation affects the problems faced by an organism, the ability of a mutualistic partner to solve these problems, and the ability of a mutualist to respond to its partner. These three types of variation can arise from changes both internal and external to an association. The following review deals with these types of variation and illustrates how they arise in protection, pollination, seed dispersal, and feeding mutualisms. The review also discusses the consequences of conditionality for the reproductive success of an organism and for the evolution and stability of mutualistic systems. Finally, the review shows that, although the existence of conditionality is recognized, most examples are strictly descriptive. More experimental evidence is required in order to demonstrate the widespread existence of conditionality and the importance of this conditionality for the stability and evolution of mutualism.

This review begins with a discussion of the four main types of mutualism: protection, pollination, seed dispersal and feeding. Next, variation in the problems faced by a mutualist are discussed in relation to changes in the density, species and life-stage of the mutualist, and in relation to changes in enemy abundance and environmental nutrient levels. Third, variation in a partner's ability to solve the problems faced by its mutualist is discussed in relation to changes in partner species and density, and in relation to changes in the mutualist's density. Fourth, variation in a mutualist's ability to respond to its partner is discussed in relation to changes in the species involved, and in relation to changes in environmental nutrient levels. Finally, the consequences of these patterns of variation are discussed.

TYPES OF MUTUALISMS

Most of the mutualistic associations mentioned in this review are non-specific, in that a given mutualist can interact with a number of different partner species. Because they are non-specific, these mutualisms are relatively loose associations. The occurrence of conditionality has been examined more in non-specific mutualisms, and it is this class of mutualisms that is dealt with in the review.

In protection mutualisms, a partner protects its mutualist from predators, disease or environmental stress, and receives food, housing or reciprocal protection. For example, ants often protect homopterans and plants from natural enemies, and receive food such as honeydew or extra-floral nectar respectively. In pollination mutualisms, a pollinator transfers pollen from an anther of one plant to the stigma of the same or another plant. The pollinator, in turn, usually feeds on pollen and/or nectar. For example, many bee species pollinate flowers while collecting nectar and pollen to rear their broods. In seed-dispersal mutualisms, animals transport a plant's seeds to areas suitable for germination and survival, and, in turn, receives nutrients from the fruit or the seed elaiosomes. For example, ants often carry seeds to their nest where the seed elaiosomes are fed to the developing brood. The seeds remain and subsequently germinate in the ant nest. Finally, feeding mutualisms are associations in which a partner provides nutrients for or enhances the nutritional uptake of its mutualist. For example, ant tending stimulates aphid feeding rate resulting in an increase in the nutritional uptake of the aphids.

TERMINOLOGY

A problem of terminology arises when discussing mutualistic systems. Due to the reciprocal nature of the interaction, distinctive names, such as "predator" and "prey" in predator-prey systems, are not easily assigned to the species involved in the mutualism. Here, for the sake of clarity, the target or focal species is referred to as the "mutualist", and emphasis is placed on the benefit obtained by the mutualist. The species providing the benefit is referred to as the "partner" or "mutualistic partner".

VARIATION IN THE PROBLEM

Mutualist density

The existence or importance of the ecological problems faced by a mutualist may change with its density. Therefore, a partner potentially capable of providing a solution would be needed only when the mutualist was at a density at which it was faced with a significant problem. For instance, how vulnerable a mutualist is to predation, hence how much protection it requires, is often dependent on its population size. For example, small aphid populations are at a high risk of extinction from predation, but this risk decreases as aphid population size increases (Banks 1962; Addicott 1979). Hence, ant protection is far more critical for small aphid populations than for large ones. Although mutualists may receive little or no benefit from their partners when the mutualist's density is high, their likelihood of persisting may be extremely low if not associated with a partner during their early stages of establishment (Bentley 1977; Addicott 1978, 1979; Cushman and Addicott 1989).

In seed dispersal mutualisms the benefit is lower when the density of the mutualist is low. One of the advantages of seed dispersal is decreased intraspecific competition (Pudlo et al. 1980; Heithaus 1986). If plant density is continually low, due to high mortality at another stage, then at least one advantage of widespread dispersal is lost (Pudlo et al. 1980).

Mutualist species

All species involved in an interaction will not necessarily benefit from it. Similar species may face different problems; therefore, their interactions with the same partner can have different outcomes. For instance, in the mutualism between aphids and ants, *Aphis salicariae* does not benefit from ant tending, although two other species in the same genus do. *A. salicariae*'s feeding position on the underside of leaves may make it less vulnerable to predation. This species, therefore, might not require ant protection (Addicott 1979). Consequently, detection of a mutualistic interaction should not lead to the assumption that similar species involved in similar interactions also obtain benefits.

Life-history stage

Although a mutualist may benefit from a mutualistic interaction during some stages in its life, it will not necessarily benefit during all stages if the problems the mutualist faces depend on its life-stage. For instance, mutualists are not usually equally vulnerable to predation or parasitism at all life-stages; therefore, their need for protection will vary accordingly. For example, herbivory can often cause mortality in young plants; so, protection may be critical only at the seedling stage (Koptur 1984). In an ant-membracid mutualism, membracids do not benefit from ant tending during mid-season. At this time the membracid populations consist largely of fourth and fifth instar nymphs which are not parasitized and do not require protection (Buckley 1983). In another system, ant tending benefits membracid nymphs but not adults (Cushman and Whitham 1989). Since adults are heavily sclerotized and more mobile than nymphs, they are seldom preyed upon by spiders, and so, do not need ant protection.

Natural enemy abundance and distribution

In associations where the benefit arises mainly through protection, the mutualism will be dependent on predator and parasite abundance. Therefore, in areas or at times where few enemies are present, mutualists may experience low predation rates and receive little or no protection benefit from their partners (Bentley 1977; Inouye and Taylor 1979; Laine and Niemelä 1980; Beckmann and Stucky 1981; Messina 1981; Bristow 1984; Barton 1986; Putz and Holbrook 1988; Cushman and Whitham 1989). For instance, ant-protection increases plant fitness (Barton 1986) and survival (Putz and Holbrook 1988), but only in areas where herbivore abundance is high. Similarly, Cushman and Whitham (1989) observed a positive influence of ants on membracids in three out of four years of study. They suggested that the absence of a tending effect the fourth year was due to the low abundance of a predatory spider. Consequently, an external factor leading to the conditionality of protection mutualisms is variation in the abundance and distribution of natural enemies.

Nutrient levels

Biotic and abiotic factors apparently external to an association can have profound effects on the magnitude or direction of its outcome. Some interactions are mutualistic under stressful conditions but antagonistic under nutrient-rich conditions (Janos 1980; Allen et al. 1987; Thompson 1987). For instance, plant growth is affected by the fertility of the soil and by the presence of mycorrhizal fungi on the roots (Janzen 1974; Janos 1980; Allen et al. 1987). The nutrient content of the soil largely determines the extent to which the presence of mycorrhizal fungi benefits the plant because the soil nutrient content governs the degree to which the plant requires assistance to grow and survive (Janos 1980; Allen et al. 1987). In nutrient-poor soils, mycorrhizae improve host plant water uptake, growth, and seedling survival. However, in fertile soils, the advantage of an expanded absorbing surface is lost and continued association with the mycorrhizae can be detrimental to plant growth (Janos 1980).

The fertility of the soil can also govern whether a mutualistic association will be obligate or facultative. Obligate associations can become facultative in nutrient rich environments. Some plants require the additional nutrients provided by their mutualistic partner in order to survive or reach sexual maturity when growing in infertile soils. For example, epiphytic myrmecophytes have hollow tubers into which ants place plant and animal debris (Janzen 1974). This debris is a source of nutrients for the plant. Epiphytes growing in nutrient poor white sand require ant "feeding" in order to survive. However, in more fertile soils, although epiphyte growth may still be improved through the association with ants, the plant is able to grow and survive in their absence.

VARIATION IN THE ABILITY TO PROVIDE A SOLUTION

Partner species

In non-specific mutualisms, all partners that an organism can potentially associate with will not necessarily provide equivalent benefits. Therefore, variation in the distribution of partner species will result in changes in which partners are available to a mutualist. Consequently, the reproductive success of the mutualist will also vary. The best examples of this are mutualisms involving ants, such as protection and seed-dispersal mutualisms. Ant distributions are usually heterogeneous and highly patchy (Sanders 1970; Inouye and Taylor 1979; Mesler and Lu 1983). Due to this patchiness, mutualists in different areas could be exposed to different ant partners; therefore, they could experience different reproductive success. In contrast, variation in pollinator assemblages often results in individual plants being exposed to a number of different pollinators because pollinators usually cover large distances while foraging (Brown and Kodric-Brown 1979; Willson and Bertin 1979; Augspurger 1981; Thompson 1982; Herrera 1988).

For variation in the distribution of mutualistic partners to result in variation in the reproductive success of a mutualist, the partners must differ significantly in their ability to solve the problems faced by the mutualist. For example, in many protection mutualisms involving ants that defend either plants or homopterans against insect predators and parasitoids, ant species differ in size, aggressiveness and foraging behaviour (Bradley and Hinks 1968; Bentley 1977; Addicott 1978; Laine and Niemelä 1980; Beckmann and Stucky 1981; Messina 1981; Thompson 1982; Bristow 1984; Horvitz and Schemske 1984; Koptur 1984; Barton 1986; Smiley 1986; Cushman and Addicott 1989). These differences alter the ability of the ants to provide protection in the following ways.

First, smaller ants are often unable to remove predator larvae once the larvae have reached a certain size (Tilman 1978; Beckmann and Stucky 1981; Messina 1981). Thus, the ants' ability to provide protection declines as the season progresses. Second, ants differ in how quickly they respond to the presence of enemies (Schemske 1980; Bristow 1984), and predators can, at times, continue feeding for an extended period before being chased away. Third, some ants simply abandon their mutualist due to, for example, changing nutrient requirements (Messina 1981; Bristow 1984; Sudd and Sudd 1985; Smiley 1986), and leave their partner without protection. Finally, some ants simply drop to the ground if the plant they are on is disturbed (Bradley and Hinks 1968; Addicott 1978, 1979). Although the presence of ants may deter enemies from landing on the plant (Bentley 1977), ants may do nothing to expel the enemy once the enemy is present. In contrast, some ant species are highly aggressive or display a swarming behaviour in response to enemies, and so, provide effective defense (Bradley and Hinks 1968; Bentley 1977; Beckmann and Stucky 1981).

In seed-dispersal mutualisms, transport of seeds beyond the canopy of the parent plant decreases intraspecific competition (Pudlo <u>et</u> <u>al</u>. 1980; Heithaus 1986), decreases predation risk (Bond and Slingsby 1984; Heithaus 1986; Horvitz and Schemske 1986), increases the probability that some seeds will end up in favourable locations (Herrera 1985), and increases outcrossing (Horvitz and Schemske 1986). Therefore, the advantage for the plant of seed dispersal depends on the distance that dispersers carry the seeds away from the parent, and where the seeds are placed after dispersal (Pudlo <u>et</u> <u>al</u>. 1980; Mesler and Lu 1983; Bond and Slingsby 1984; Herrera 1985; Horvitz and Schemske 1986). However, there is wide variation in the behaviour exhibited by disperser species. First, some species of ants do not [illegible] seeds beyond the canopy of the parent plant; [illegible]re, they do not increase a seed's likelihood of escaping intraspecific competition, of experiencing higher outcrossing, or of finding a favourable location for germination (Pudlo <u>et</u> <u>al</u>. 1980; Horvitz and Schemske 1986). Second, some species of ants do not begin dispersing seeds immediately after the seeds have dropped to the ground or the fruit containing them have ripened. This delay increases the number of seeds lost to predation before dispersal occurs (Pudlo *et al.* 1980; Bond and Slingsby 1984). Third, not all ant species place seeds in areas equally suitable for germination. For example, some ant species place seeds in their nests which are suitable areas for germination. In contrast, other ant species place seeds in shallow hollows which are not suitable areas for germination (Bond and Slingsby 1984).

In pollination systems, the animals arriving at a flower range from flower visitors, which do not effect pollination, to highly effective pollinators, whose activity results in the production of a full complement of seeds (Lindsey 1984). Therefore, a plant can be exposed to animals that fall anywhere along this pollination continuum. For instance, pollinators differ with respect to the frequency of pollen transfer (Lindsey 1984; Montalvo and Ackerman 1986; Herrera 1988), the number (Lindsey 1984; Herrera 1988) and purity (Lindsey 1984; Montalvo and Ackerman 1986) of pollen grains they carry, and the distance they fly between flowers (Willson and Bertin 1979; Lindsey 1984; Montalvo and Ackerman 1986; Herrera 1988). Insufficient transfer of pollen often increases fruit abortion rates (Augspurger 1981; Udovic 1981; Herrera 1988), and short flight distances lower the rate of outcrossing (Willson and Bertin 1979; Montalvo and Ackerman 1986; Herrera 1988). These two factors can decrease plant reproductive success (Willson and Bertin 1979; Augspurger

1981; Udovic 1981; Montalvo and Ackerman 1986; Herrera 1988).

In summary, the distribution of mutualistic partners varies spatially and temporally; therefore, a mutualist will be exposed to partners which differ in their ability to solve the problems faced by the mutualist. Consequently, the reproductive success of the mutualist will vary over time and space.

Density

The ability of a partner to solve the problems its mutualist encounters usually increases as the density of the partner increases. The number of partners available to a mutualist will vary with changes in the absolute density of the partner and with changes in the density of the mutualist itself. These two factors will be dealt with separately.

Partner density:

Spatial and temporal variation in the abundance of dispersers (Mesler and Lu 1983; Herrera 1988), ant protectors (Bentley 1976; Inouye and Taylor 1979), and pollinators (Brown and Kodric-Brown 1979; Willson and Bertin 1979; Kephart 1983) is common. Consequently, the number of partners available to a mutualist will change continually. For example, in some habitats, the absolute number of ant dispersers is so low that seeds are rarely found and dispersed (Mesler and Lu 1983). In ant-protection systems, ants are not only more numerous when tending close to their nests (Bentley 1977; Tilman 1978; Laine and Niemelä 1980; Beckmann and Stucky 1981; Messina 1981; Horvitz and Schemske 1984), but also more aggressive (Bentley 1977). Therefore, the location of a mutualist with respect to its partner can influence the number of partners available to it.

Mutualist density:

If many mutualists require the services of a limited number of partners, then some individuals or populations will be unable to attract a sufficient number of partners. Competition for mutualistic partners is related to the regional density of a mutualist. Regional density refers to the number of mutualists in an area requiring the services of a partner. For competition to occur, neighbouring mutualists must reduce the number of partners that each attracts, and the fitness of a mutualist must increase as the number of partners increases (Cushman and Addicott 1989).

Competition for mutualists occurs in protection (Addicott 1978; Cushman and Addicott 1989; Cushman and Whitham in press), seed-dispersal (Manasse and Howe 1983; Herrera 1986), and pollination (Beattie et al. 1973; Waser 1978; Brown and Kodric-Brown 1979; Kephart 1983; Campbell 1985) mutualisms. For example, the presence of large aphid populations reduces the number of ants tending neighbouring aphid populations, and consequently reduces the fitness of these neighbours (Cushman and Addicott 1989). In dispersal systems, a high density of fruiting plants results in a low relative disperser abundance and decreases the likelihood of an individual plant having many of its seeds dispersed (Manasse and Howe 1983; Herrera 1986).

In pollination systems, geographic, yearly or seasonal changes in the length and time of flowering will influence the degree to which plant species overlap in flowering (Waser 1978, 1979; Brown and Kodric-Brown 1979; Kephart 1983; Campbell 1985). The degree of overlap will affect the abundance of flowers available to pollinators. For example, early flowers of *Stellaria pubera* overlap more highly with flowers of *Claytonia virginica* than late ones. Early flowers experience increased pollinator limitation resulting in decreased seed set (Campbell 1985). Competition for pollinators can result in a significant decrease in the number of visits to a plant (Beattie et al. 1973; Brown and Kodric-Brown 1979; Kephart 1983; Herrera 1988), or in a decrease in the amount of intraspecific pollen transferred (Waser 1978; Kephart 1983; Campbell 1985; Campbell and Motten 1985). The latter results in the loss of effective pollinator visits and of stigmatic surfaces. Both a decrease in pollinator visits and a decrease in intraspecific pollen transferred lead to lowered seed set and lower reproductive success (Waser 1978; Augspurger 1981; Kephart 1983; Campbell 1985; Campbell and Motten 1985). However, not all plants are equally vulnerable to competition. Some plants offer higher nectar rewards which increases their attractiveness to pollinators (Galen and Plowright 1985). In addition, specialist pollinators will often search out their host plant even if the host is in low abundance or surrounded by other plant species (Lindsey 1984). Both differential attractiveness and specialist pollinators make certain plant species less susceptible to competition for pollinators.

Competition for pollinators is a problem that arises due to high flower density. However, low flower density can also cause problems for the plant because flowers at low density are often unable to attract and sustain pollinators (Augspurger 1981; Udovic 1981; Aker 1982; Montalvo and Ackerman 1986). *Hybanthus prunifolius* plants flowering in spatial or temporal isolation are unable to attract an adequate number of pollinators and experience lowered reproductive success. However, the detrimental effect of asynchronous flowering is less pronounced for large individuals (Augspurger 1981). Large flowering individuals often receive relatively more pollinator visits than small individuals (Willson and Bertin 1979; Augspurger 1981; Udovic 1981; Aker 1982). The abundant, sometimes excessive, production of flowers has been proposed to be a 'bet-hedging' tactic. Large numbers of flowers would ensure pollination in years when pollinator abundance was low (Willson and Bertin 1979; Udovic 1981; Aker 1982).

In summary, a decline in the relative density of partners results in competition for the services provided by the partner and in a subsequent decline in the fitness of the mutualist. However, both mutualist and partner abundance are highly variable and thus, so are competition and reproductive success.

The number of partners a mutualist is able to attract is influenced not only by the regional density of the mutualist but also by its local density. For example, the relative number of ants tending an homopteran population is related to the number of neighbouring homopteran populations (i.e. regional density), and to the size of the homopteran population itself (i.e. local density). The relative number of ants tending an homopteran population has been found to both increase (Wood 1982; Cushman and Whitham 1989) and decrease (Banks 1962; Addicott 1979) as local homopteran density increases. Cushman and Whitham (1989) observed that large membracid aggregations attracted more ants and benefitted more from ant tending than small aggregations. In contrast, Addicott (1979) observed that large aphid populations had relatively fewer ants tending and did not benefit from ant tending.

VARIATION IN THE ABILITY TO OBTAIN BENEFITS

Mutualist species

Similar species may differ in ways which are relatively subtle, yet important enough to prevent one of the species from benefitting from an association that is mutualistic for the other species. For example, in an ant-homopteran association, ant tending increases the size and development rate of membracids but has no effect on these two parameters in aphids (Bristow 1984). In another system, fungal endophytes decrease the reproductive success of most host plants by inhibiting flowering or causing fruit abortion. However, since grasses are capable of vegetative reproduction, fungal endophyte infection does not lower their reproductive success. On the contrary, endophyte infection leads to decreased herbivory and increased growth in grasses (Clay 1988). Consequently, it cannot be assumed that all species involved in similar interactions, or even in interactions with the same partner, are able to obtain the same level of benefit.

Nutrient levels

The ability of a mutualist to respond to a situation or to its partner can be controlled by environmental conditions. For example, herbivory, which is usually thought to be detrimental to plants, can become beneficial when plants are growing in nutrient-rich environments (McNaughton 1986; Maschinski and Whitham 1989). High nutrient levels not only enable plants to replace tissue lost to herbivory but also enables them to produce additional tissue. *Ipomopsis arizonica*, growing under conditions of low competition and high nutrients, overcompensated in response to early-season mammalian herbivory. Grazed plants put out more inflorescences, more flowers, and had a higher seed set than ungrazed plants (Maschinski and Whitham 1989). A similar response to herbivory was observed by Paige and Whitham (1987). Consequently, some antagonistic interactions become beneficial under good or fertile conditions because high nutrient levels enable organisms to respond positively to an otherwise negative interaction (McNaughton 1986; Carroll 1988; Maschinski and Whitham 1989).

CONSEQUENCES OF CONDITIONALITY

Selection

Mutualistic partners differ in the quality of services and resources they provide for their mutualists. Therefore, certain species interactions should be favoured over others (Bristow 1984; Horvitz and Schemske 1986) and should become more closely linked over time (Keeler 1981; Louda 1982; Herrera 1986). However, a prerequisite to the evolution of such close interdependence is a long period of interaction once the relationship is established (Keeler 1981; Herrera 1986).

Species assemblages (Aker 1982; Thompson 1982; Howe 1984; Montalvo and Ackerman 1986; Herrera 1988) and environmental conditions (Bentley 1976; Schemske 1980;

Herrera 1985, 1986) change spatially and temporally. Therefore, a mutualist has to deal with continually changing conditions and has to interact with a number of different partner species. Local variation in a mutualist's neighbourhood tends to decrease the force of selection of any single partner (Thompson 1982; Howe 1984). A plant species continually exposed to different pollinators will be faced with shifting selection pressures. This inconsistency will decrease the possibility of a plant adapting to any particular pollinator (Aker 1982; Montalvo and Ackerman 1986; Herrera 1988). In addition, variation in the magnitude of benefits obtained from a mutualistic partner will limit the extent to which characteristics used to attract the partner are selected for (Inouye and Taylor 1979; Horvitz and Schemske 1984; Barton 1986). Finally, if the intensity or outcome of an interaction varies across environments, then selection could favour different species combinations in different environments (Thompson 1987).

In addition, mutualist species, although at times providing each other benefit, may have conflicting requirements. For instance, spiders foraging on the inflorescences of *Haplopappus venetus* prey on insects arriving at the plant. The plant is visited by both herbivores and pollinators. Therefore, the spiders have a positive impact on the plant by decreasing the number of herbivores the plant is exposed to. However, they also have a negative impact by decreasing pollinator visits (Louda 1982). The net outcome of the interaction for the plant is dependent on the relative timing of flowering and spider activity. Similarly, *Acacia decurrens* has extra-floral nectaries (EFN) that attract tending ants. However, the presence of membracids draws the ants away from the EFNs; hence, plant protection declines (Buckley 1983). In this example, the net outcome of the interaction is dependent on the relative timing of EFN production and membracid activity. Because the timing of plant flowering, EFN production, and insect activity vary spatially and temporally, the intensity and outcome of the interactions will shift continually. The lack of a continuous positive relationship decreases the force of selection potentially drawing the organisms together.

It is evident that the magnitude of mutualistic interactions is strongly influenced by the biotic and abiotic environment, which in non-specific mutualisms can be highly variable. Species rarely experience long periods of unvarying association; thus, the likelihood of a species combination becoming closely linked is greatly reduced.

Stability

Mutualism is said to be an unstable process by many authors (Levins 1974; May 1976; Case and Casten 1979). Increase in the population density of one mutualist is believed to result in unchecked increase in the density of a second mutualist. However, these conclusions regarding the stability of mutualism are based upon unrealistic assumptions (Addicott 1981; Udovic 1981; Addicott and Freedman 1984). For instance, the magnitude of mutualistic interactions does not usually remain constant as the density of one or both partners increases. For example, in a *Yucca*-yucca moth system, if moth density rises above the point of fruit set satiation, then seed survival declines due to predation by the moth larvae (Udovic 1981). In addition, in an ant-aphid association, ant tending benefits aphids at low density, but does not benefit aphids at high density (Addicott 1979). Models of facultative mutualism have further demonstrated that density-dependence leads to stability (Addicott 1981; Addicott and Freedman 1984; Wolin and Lawlor 1984).

Although the importance of conditionality is being recognized, much of the evidence remains anecdotal. Direct experimental testing is required to determine the extent of the existence of conditionality in mutualism. The chapter to follow is an experimental examination of conditionality in the mutualism between aphids and ants. Manipulations were conducted to directly observe how the benefits aphids obtain from ant tending vary with local aphid density and host plant quality. Mutualistic interactions are perhaps far more dynamic than previously thought and this complexity has important implications for the stability, evolution and dynamics of these systems.

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CHAPTER 2

DENSITY- AND QUALITY-DEPENDENT RESPONSES OF APHIDS TO TENDING BY ANTS

INTRODUCTION

The biotic and abiotic conditions faced by organisms are spatially and temporally variable. For instance, habitats change with respect to species abundance and distribution (Barton 1986; Edson 1985), nutrient availability (Whitham 1978, 1981; Wisdom 1985; Maschinski and Whitham 1989), population density (Platt et al. 1974; Augspurger 1981), level of precipitation (Dunham 1980; Gibbs and Grant 1987), and degree of physical stress (Bertness 1989). Therefore, the intensity and outcome of species interactions should vary in space and time. Consequently, point estimates of the existence and magnitude of species interactions are unreliable and do not allow for the progression past simple typological studies (Wiens 1977; Dunham 1980). In other words, classification of systems into absolute categories such as competitive, mutualistic or parasitic, based on data collected at one point in time does little to further our understanding of the underlying mechanisms and complexity of these systems.

A number of studies have illustrated that the intensity of species associations may be highly conditional. For example, coexistence of two aphid species on goldenrod is possible only where plant dispersion is patchy and density-regulating predators are present. Because the proximity of neighbouring plants allows aphids to move to plants freed for colonization by predators, interspecific competition is reduced (Edson 1985). Second, the compensatory response of *Ipomopsis arizonica* to herbivory can be positive or negative depending on nutrient availability, the number of other plants present and the timing of herbivory (Maschinski and Whitham 1989). Third, the survival of acorn barnacles, at low tidal heights, is drastically reduced at high densities due to crowding. However, at high tidal heights increased density acts as a buffer against physical stress leading to improved survival (Bertness 1989).

Although much of the work on conditionality has dealt with competitive interactions, some studies have documented its occurrence in mutualistic associations. For instance, ants on the extrafloral nectaries of *Cassia fasciculata* increase plant reproductive success by decreasing seed predation, but only when the abundance of both ants and herbivores is high (Barton 1986). *Heliconius* butterflies interacting as Müllerian mimics benefit each other at low density because their combined numbers allow predators to learn more quickly to avoid them, but this advantage is lost at higher densities (Gilbert 1983). And in an ant-membracid mutualism, the degree to which membracids benefit from ant tending depends on membracid aggregation size and age, and on predator abundance (Cushman and Whitham 1989). Nevertheless, much of the work on conditionality is observational. There is a need for direct study of how variation internal and external to mutualistic systems alters the magnitude and outcome of these interactions to prevent the formulation of inappropriate conclusions.

The present study considers how the existence and magnitude of the mutualistic association between aphids and ants varies with aphid density and host plant quality. It also considers the mechanisms leading to the conditionality of the association.

The association between most aphids and ants is facultative and non-specific (Addicott 1978; Buckley 1987). Honeydew excreted by aphids provides a food source for tending ants (Dixon 1973), and there are three main benefits which aphids are thought to receive from ant tending. First, ants protect aphids against predators and parasitoids (Banks 1962; Tilles and Wood 1982; Buckley 1987). Second, ants stimulate aphid feeding rate, leading to increased aphid growth rate, size and fecundity (Way 1963). Third, ants delay dispersal by alatae (i.e. individuals with wings), resulting in increased aphid survival because dispersal tends to be hazardous (Tilles and Wood 1982).

The existence and magnitude of each of these benefits could vary with aphid density or host plant quality. First, ants may be unable to respond numerically to the rapid increase in aphid population size; so, tending levels may become inadequate at high aphid density (Addicott 1979). A decrease in the relative number of ants tending at high aphid densities may make ants unable to provide aphids with sufficient protection from natural enemies. In addition, if predators cue into large aphid aggregations (Whitham 1978; Wellings and Dixon 1987), then aphids at high densities may experience elevated predation pressure even with ants present. Second, if host plant quality deteriorates substantially as aphid density rises (Way and Cammell 1970; Edson 1985), then increased feeding rate may have a relatively small effect on population growth at high aphid density. Likewise, increased feeding rate, due to ant tending, may have a small effect on the growth of small aphid populations if the aphids are on poor quality host plants (van Emden 1963). Finally, an ant-induced decrease in alate production at high aphid density could lead to increased aggregation which, in turn, could lead to higher predation pressure, to overcrowding, or to a more rapid decline of host plant quality. Therefore, decreased alate production at high aphid density could result in a decrease in population growth (Addicott 1979).

Direct examinations of conditionality, such as the one described in this chapter, are needed because conditionality can have important consequences for species coexistence, selection, stability, and community structure (Dunham 1980;

Gibbs and Grant 1987). First, if competition between two species depends on the level of fluctuating resources, then the degree to which the species compete at any one time will vary. Dunham (1980) suggested that periods of relaxed competition can increase the potential for species coexistence by allowing a competitively inferior species to re-establish itself. Welden and Slauson (1986) addressed this issue by making the distinction between the "intensity" of competition and its "importance" in the community relative to other processes. They stated that two species that compete intensely can still coexist if competition is unimportant relative to other factors, such as predation or climatic stress. Correspondingly, competition would also be unimportant if its occurrence or intensity was highly variable due to changing resource availability.

Second, environmental variation may alter which individuals or traits are selected at a given time or place, making selection pressure insufficiently consistent to influence the direction of evolutionary change (Barton 1986). For example, spatio-temporal variation in the abundance and composition of pollinators, differing in effectiveness, will impede a plant's morphological convergence towards a form which promotes pollinator specialization. That is, coevolution between plant and pollinator will be less likely to occur (Herrera 1988). This process has been proposed to explain why some populations of plants in more variable environments lack given floral characteristics that individuals of the species usually possess (Barton 1986).

Third, the conditionality of some associations can generate stability. For instance, many studies have suggested that mutualism is an inherently unstable interaction (see May 1976). However, mutualistic systems are perhaps more dynamic than previously thought and must be considered accordingly (Addicott 1981; Addicott and Freedman 1984).

Addicott (1981) showed, by means of computer simulations, that the stability of mutualistic associations can arise from the density-dependence of the interaction. For example, in an ant-aphid system, ant tending increases aphid population growth when aphid density is low allowing a population to quickly grow above the size where the threat of extinction is high. Conversely, ant tending has no effect at high aphid density, when a population is no longer likely to go extinct, decreasing the likelihood of it over-exploiting its host plant. Both the positive effect of ant tending at low aphid density and the absence of an effect at high aphid density stabilize the interaction (Addicott 1979). Wolin and Lawlor (1984), in modelling facultative mutualisms, also found that the interaction will have a stabilizing effect if the greatest benefits are obtained at low density, thus reducing the time taken for the system to return to equilibrium.

Fourth, variation in the outcome of species interactions at one trophic level will indirectly affect species at higher trophic levels. Consequently, variation between single pairs of species will influence community structure (Dunham 1980; Clancy and Price 1986; Cushman and Whitham 1989). For example, variation in host plant quality influences herbivore success. This variation, in turn, affects predators and parasitoids that prey on the herbivore (Price et al. 1980). Therefore, to begin to understand community level patterns, we must be aware of the variation in the species associations making up the community.

Although the existence of mutualism between aphids and ants is well recognized (see Way 1963), little is known about how aphid density and plant quality influence the outcome of the mutualism, and how these factors operate. The present study will address the following questions. First, does the magnitude of the benefits aphids receive from ants vary with aphid density and/or host plant quality? Second, can this variation in the ant-aphid mutualism be explained by changes in the number of ants tending, in alate production, in enemy abundance, or in plant quality?

Study system

Experiments were conducted on *Aphis varians* Patch tended by *Formica cinerea* Wheeler, feeding on *Epilobium angustifolium* L. at two sites along Spring Creek, Gunnison County, Colorado (38° 47'N, 106° 45'W) during the summers of 1988 and 1989. The same sites were used by Addicott (1978; 1979) and are designated as SC38 and SC22. The main predators in the system are coccinellid larvae and adults, chamaemyiid and syrphid fly larvae, and a parasitoid wasp.

The aphid-ant system has advantages for the study of conditionality of mutualism. First, *Aphis varians* Patch is parthenogenic and viviparous during most of its life-cycle; therefore, it has a short generation time. Second, the majority of individuals produced throughout the summer are apterous (i.e. without wings) and remain on the plant on which they were born. Populations, therefore, can be defined as the individuals on a single fireweed shoot and be easily censused and monitored (Addicott _et_ _al_. 1987; Antolin and Addicott in press). Also, aphid population size can be increased or decreased to address questions of density-dependence. Third, ants can be easily excluded from tending aphids so that the effect of removing a mutualist can be ascertained.

METHODS

Natural density experiments

Low density experiment (LDE):

To determine if the effect of ant tending on aphid population growth varies over a range of aphid densities occurring naturally at a given time, an experiment was conducted which consisted of a series of tended and untended aphid populations ranging in size from 10 to 100 individuals. This experiment was the first of two dealing with a natural range of aphid densities. The difference in growth between tended and untended populations, as well as the change in the magnitude of this difference as aphid density increased (Table 1), was determined over a two-week period.

Before the start of the experiment, 50 enemy-free aphid populations were established at SC38. On 18 June, 1988, tended aphid populations on fireweed were individually covered with netting to exclude predators and parasitoids. Because an aphid that has been parasitized (parasitized aphids are referred to as mummies) becomes noticeable within approximately 10 days, populations were observed for the next 12 days so that all parasitized aphids could be removed.

At the start of the experiment on 30 June, the net bags were removed, the initial number of individuals in each population was counted, and plant height and stem diameter

TABLE 1. Summary of the experiments conducted at Spring Creek during the summers of 1988 and 1989. LDE and HDE: low and high natural density experiments. MDE: manipulated density experiment. PQE: plant quality experiment. The primary dependent variable is the per capita growth rate.

Experiment	Date	Density range	Plant height range (cm)	Primary analysis	Covariates
LDE	June 30 - July 14 1988	10-100	43-104	1-way ANCOVA	density & height
HDE	July 19 - Aug 2 1988	250-1300	57-121	1-way ANCOVA	density & height
MDE	July 16 - July 28 1989	10-1000	39-120	3-way ANCOVA	height
PQE	Aug 3 - Aug 15 1989	20-200	37-145	2-way ANCOVA	density

were measured. The latter two variables were used as covariates in the analyses to decrease within treatment variation because they have been shown to be highly correlated with aphid growth and fecundity (Addicott pers. comm.).

To observe directly the interaction between ant tending, aphid density and aphid population growth, an ant-exclusion treatment was applied. To prevent ants from reaching the aphids, Tanglefoot^{TM} gel was applied to half the fireweed shoots which had been chosen randomly. Tanglefoot was applied to a ring of paper below the inflorescence to avoid any possible effects on the plant. The paper rings were placed on all fireweed shoots for consistency.

Every second day from 30 June to 14 July, populations were censused, in random order, for number of ants tending and number of predators, parasitoids and mummies. On 14 July, all populations were re-bagged after the length and the percentage of the shoot covered with aphids (an indirect measure of population size) were measured. For the next two weeks, natural enemy larvae, eggs and mummies were counted and removed, as they became noticeable, from the bagged aphid populations in the field. These counts provided an estimate of the number of enemies an aphid population had been exposed to during the experimental period.

High density experiment (HDE):

A second experiment was conducted at the same site between 6 July and 2 August to determine if the effect of ant tending on aphid population growth varies over a naturally occurring range of higher aphid densities (250 - 1300) (Table 1). This experiment was the second dealing with a natural range of aphid densities. Approximately one third of the aphid populations used in the HDE had been used in the LDE. Treatments in the HDE were assigned randomly among all populations. The LDE and the HDE differed in the following two ways. First, the mean starting population density in the HDE was larger than in the LDE (see Table 1). Second, at the end of the HDE each population was harvested and stored individually in AFA instead of being re-bagged. In the laboratory, aphids were removed from the fireweed stalks using a fine paintbrush, placed into a gridded petri-dish and counted under a dissecting microscope.

From these counts and from measurements of the length and percent of each fireweed shoot covered with aphids taken at the end of the HDE, a linear regression relating aphid number to the length and the percent of the shoot covered with aphids was calculated ($r=0.798$). The resulting equation [number of aphids = $- 1754.0 + (118.6 \times \text{length}) + (33.9 \times \text{percent})$] was used to estimate the final population size in the LDE and starting population size in the HDE, from length and percent coverage measurements, because the aphids were not counted directly in either case.

Manipulated density experiment (MDE)

During the LDE and the HDE, the effect of ant tending was considered over a total range of aphid densities of 10-1300 individuals (see Table 1). However, within each experiment the density range was much narrower. In addition, the two experiments were conducted 2.5 weeks apart. To observe directly how the effect of ant tending on aphid population growth varies over a large range (10 - 1000) of aphid population densities, aphid population sizes were artificially manipulated to extend the range of densities naturally seen at a given time. These densities were well within the natural range, because a single aphid population can easily exceed 10,000 individuals. The experiment was a two block, randomized block design. The blocks, SC22 and SC38, were approximately 1 km apart. The experiment consisted of tended and untended aphid populations within each of three density classes (low: ≤ 100, mid: 101 - 249, high: ≥ 250 individuals) at each of two sites. The density level at which ant tending no longer benefitted aphids in terms of increased population growth was determined (Table 1). Fewer aphid populations were established at SC22 (N=118) than at SC38 (N=251) due to a lower abundance of fireweed at this site (ca. 250 shoots at

SC22 vs. ca. 500 shoots at SC38).

On 12 June 1989, net bags were placed over aphid populations on *Ribes* sp., the winter host of *Aphis varians*, to catch dispersing alate females. Alates were transferred daily from the bags to fireweed shoots at both SC38 and SC22 using a fine paintbrush. Some fireweed shoots were colonized naturally by dispersing alate females. A number of populations were started earlier than others (max. 10 days) in order to create the density range needed. In addition, the size of some populations was increased by transferring aphids to them in the following manner. An inflorescence having aphids was clipped and loosely tied to the inflorescence of a receiving shoot. After 36 hours most aphids had moved from the dying cutting to the live plant. A maximum of two such transfers was performed for any one population. Transfer of aphids continued until one week before the start of the experiment.

Three weeks prior to the start of the experiment, all aphid populations were covered with net bags to exclude predators and parasitoids. During these three weeks any mummies and predaceous larvae were removed.

On 12 and 13 July, aphid population density was estimated on each shoot. Ant-exclusion treatments were applied randomly within the three density classes, populations were censused from 16 July to 28 July, and aphid populations were harvested on 29 and 30 July, using the same methods applied to the HDE. In the laboratory, aphids were evenly spread into a petri-dish that was divided into eight equal-area sections. Due to limited available time, five sections were randomly chosen and counted for each population, and the total population size was estimated from this count. Small aphid populations (< 200) were counted in full.

Plant quality experiment (PQE)

To determine how the effect of ant tending on aphid population growth varies over a large range of host plant quality, an experiment which included tended and untended aphid populations within each of three plant quality classes (poor: ≤ 70 cm, mid: 71 - 90 cm, high: ≥ 91 cm in height) was conducted during the summer of 1989. The growth of tended and untended populations was compared within and across quality classes (Table 1).

At a site approximately 4 km upstream from SC22, aphids were artificially introduced to 120 fireweed shoots varying in height from 37 cm to 145 cm. Plant height is an index of plant quality, as increased height is associated with increased rate of development, fecundity and survivorship in aphids (Addicott pers. comm.). On 20 July, aphids were transferred to fireweed shoots from other fireweed inflorescences by clipping as described for the MDE. A maximum of approximately 100 individuals was transferred to each plant to reduce variation in population growth within treatments resulting from unequal starting population densities. All aphid populations were covered with net bags to exclude predators and parasitoids. The populations remained covered throughout the experimental period to isolate the aphid-ant-plant interaction, and reduce within treatment variation. Plant quality will influence the amount of nutrients aphids ingest. If ant tending does stimulate aphid feeding rate, resulting in an increase in aphid population growth, then host plant quality may affect the magnitude of benefit aphids receive. Although the net bags exclude flying insects, thereby excluding most natural enemies, they do not prevent ants, who climb up from the bottom of the plant, from tending the aphids. Therefore, excluding natural enemies simplified the system while still allowing the aphid-ant-plant interaction to be studied.

On 1 August, the size of each aphid population was estimated, and the height and stem diameter of each fireweed shoot was measured.

To observe directly the interactions between ant tending, plant quality and aphid population growth, an ant-exclusion treatment was applied. Tanglefoot^{TM} gel was applied to half of the fireweed shoots randomly chosen within each of the three quality classes. Tanglefoot was applied to a ring of paper below the inflorescence. Paper rings were placed on all shoots for consistency.

Every second day from 3 August to 15 August, populations were censused for the number of ants tending and the number of alates. On 15 August, each aphid population was harvested and stored individually in 70% ethanol. Aphids were later counted using the same methods as in the MDE.

Data analysis:

Analyses were performed on SPSS-PC+, version 3.0. The principal dependent variable in the analyses was the final aphid population size divided by the initial population size (i.e. the size at the start of the experiment). This measure is the per capita growth rate and allowed the magnitude of population growth to be compared among treatment groups. It is also simply referred to as population growth. The difference in growth between tended and untended aphid populations was compared across aphid density and plant quality levels.

Population growth data were log transformed in all but the LDE to obtain homogeneous variances. In all analyses of covariance where no interaction was observed between the treatment effect and the covariate, the analyses were repeated using the assumption of parallel slopes. In all of the experiments, except at SC38 in the MDE, a few aphid populations were lost due to aphid colony failure or destruction of the host plant. However, at SC38 in the MDE, 93 aphid populations were lost as a result of herbivory and trampling by deer.

RESULTS

Existence of density-dependence

In the LDE and the HDE, the per capita growth rates of tended and untended aphid populations were compared over a range of aphid densities using a one-way analysis of covariance.

Aphid populations tended by ants grew significantly more than untended populations in the LDE; however, this positive effect declined as aphid density increased (Fig. 1a; Table 2). In the HDE, where mean starting densities were considerably higher than in the LDE (Fig. 2a), the effect of ant tending did not change with aphid density ($F_{22,1} = 2.39$, $p > 0.05$), nor did ant tending improve aphid population growth overall (Fig. 1b; Table 3). The results from the LDE suggest that ant tending benefits aphids only when aphid population density is low, and that the benefit from ant tending had already declined substantially at initial densities well below 100 individuals. These findings are strengthened by comparing the results of the LDE and the HDE which showed a positive effect of ant tending at low aphid densities (LDE) and no effect of ant tending at high aphid densities (HDE). However, the HDE was conducted 2.5 weeks later than the LDE; therefore, conclusions based on the comparison of the results are not completely valid due to a possible confounding time effect.

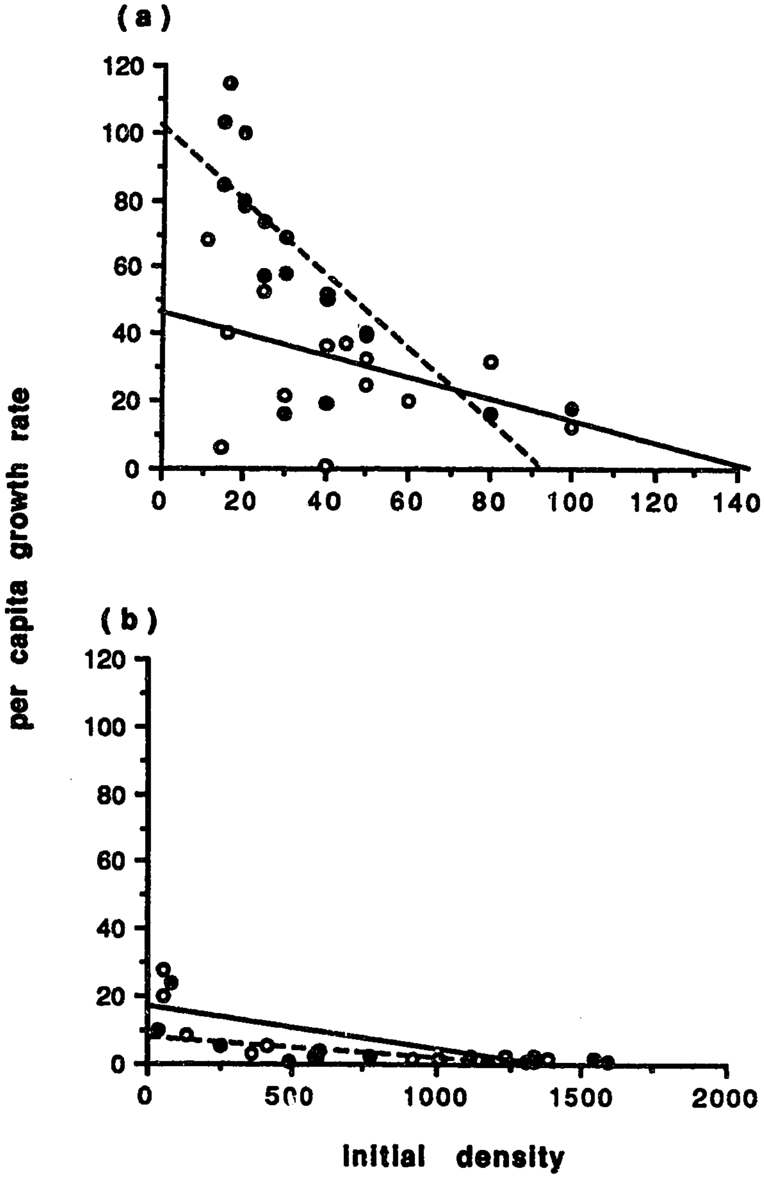

FIGURE 1: Per capita growth rates of untended (open circles, solid line) and tended (closed circles, dashed line) aphids in the LDE (a) and the HDE (b). Significance levels are given in Tables 2 and 3.

TABLE 2. Analysis of covariance of the effect of ant tending and initial aphid population density on aphid population growth in the LDE, 1988. Covariates: aphid density and plant height. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Source of variation	SS	DF	MS	F	
ant treatment	6362.01	1	6362.01	13.40	**
initial density	8912.62	1	8912.62	18.78	***
ant X density	2685.78	1	2685.78	5.66	*
within+residual	13290.51	28	474.66		

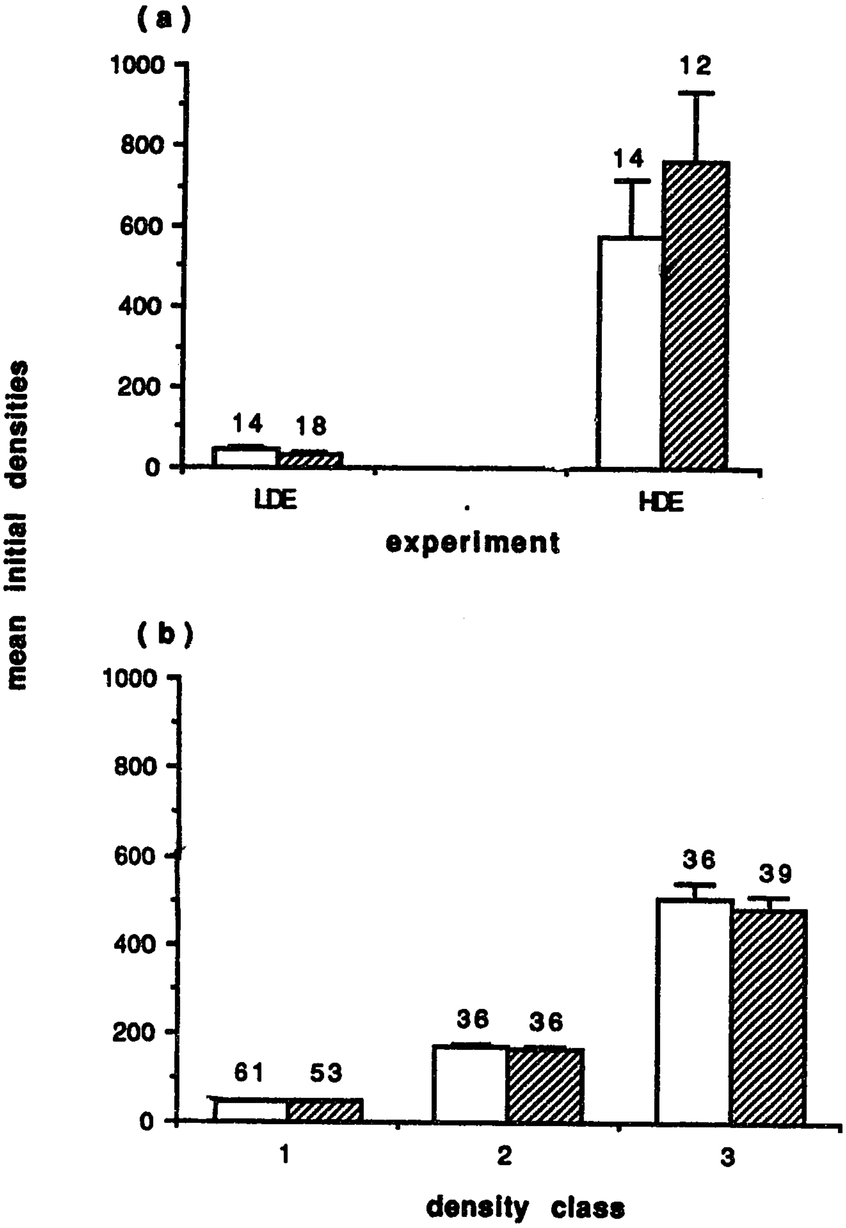

FIGURE 2: Mean initial densities (+ SE) and sample sizes for untended (open) and tended (hatched) aphid populations in the LDE and the HDE (a), and in the MDE (b).

TABLE 3. Analysis of covariance assuming parallel slopes of the effect of ant tending on aphid population growth in the HDE, 1988. Covariates: aphid density and plant height. NS not significant.

Source of variation	SS	DF	MS	F	
ant treatment	0.23	1	0.23	1.53	NS
within cells	3.28	22	0.15		

The confounding effect of time was eliminated in the MDE conducted in 1989. A three-way analysis of variance was performed to compare the effect of ant tending on population growth across density classes and across sites. Orthogonal contrasts were used to determine at which density level the ant tending by aphid density interaction occurred. The mean initial population sizes for each density class are shown by ant treatment in Figure 2b. Although there was a significant density by site interaction, the change in the effect of ant tending with changing aphid density did not differ between the two sites (ant X density X site) (Table 4). As in the LDE, the positive effect of ants on aphid population growth declined with increasing aphid density (Fig. 3). Direct comparison of the effect of ant tending on aphid population growth at low, mid and high initial aphid densities revealed that the benefit from ant tending varied significantly across density classes (Table 4).

The benefit from ant tending began to decline at aphid densities between 100 and 250 individuals. Density class 2 seemed to be the transitional zone between being small enough to benefit from ant tending (density class 1) and being too large to benefit (density class 3). Specifically, there was a significant ant by density interaction between the low density class and the higher two classes ($F_{1,247} = 4.24$, $p < 0.05$), and between the high density class and the lower two classes ($F_{1,247} = 4.60$, $p < 0.05$).

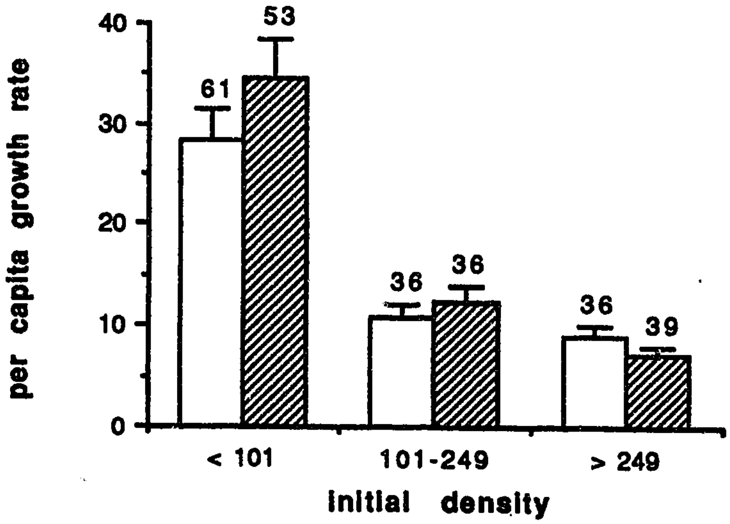

FIGURE 3: Mean per capita growth rates (+ SE) and sample sizes for untended (open) and tended (hatched) aphids in the MDE. Significance levels are given in Table 4.

TABLE 4. Analysis of covariance of the effects of ant tending, initial aphid population density and site on aphid population growth in the MDE, 1989. Covariate: plant height. * $p < 0.05$, NS not significant.

Source of variation	SS	DF	MS	F	
ant treatment	0.1467	1	0.1467	1.54	NS
initial density	0.2972	2	0.1486	1.56	NS
site	0.0012	1	0.0012	0.01	NS
ant X density	0.5919	2	0.2959	3.11	*
ant X site	0.0652	1	0.0652	0.68	NS
density X site	0.7676	2	0.3838	4.03	*
ant X density X site	0.1268	2	0.0634	0.67	NS
within cells	23.5098	247	0.0952		

Mechanisms of density-dependence

I. Number of ants tending

The decrease in benefit from ant tending as aphid density increases may be the result of tending levels becoming inadequate at high aphid density. In other words, the number of ants per aphid may be substantially lower when aphid density is high. The mean number of ants tending per aphid in the LDE ($\overline{x}$ = 0.148, SE = 0.047, n = 14) was more than four times greater than the mean number during the HDE ($\overline{x}$ = 0.035, SE = 0.02, n = 12). Therefore, it is possible that there were not enough ants tending to benefit aphid populations at high densities.

The results from the MDE suggest that the change in the relative number of ants tending was due to increasing aphid density rather than to seasonal changes in ant foraging activity. Although tending levels were significantly higher at SC22 than at SC38 ($F_{1,122} = 31.91$, $p < 0.001$; Fig. 4), the same interaction between tending level and aphid density was seen at both sites as in the LDE and the HDE.

At both sites, the number of ants tending per aphid was significantly lower when aphid populations were large (Fig. 4; Table 5). The number of ants recruited to large aphid populations was not proportional to the aphid population size. As a result, the relative number of ants tending large aphid populations was approximately three times lower than the number tending small aphid populations.

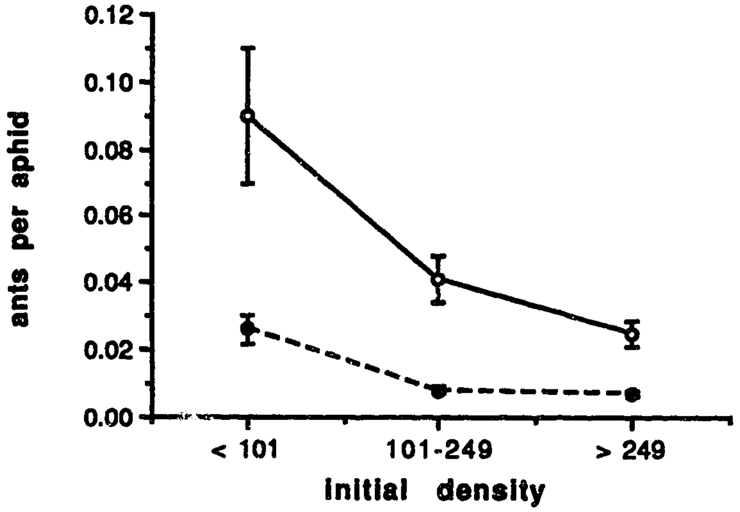

FIGURE 4: Mean number of ants tending per aphid (+ SE) at SC22 (solid line) and SC38 (dashed line) in the MDE. Significance levels are given in Table 5.

TABLE 5. Analysis of variance of the effect of initial aphid population density on the number of ants tending per aphid at SC22 and SC38 in the MDE, 1989.
** $p < 0.01$, *** $p < 0.001$.

Site	Source of variation	SS	DF	MS	F	
SC22	initial density	0.0460	2	0.0230	7.72	**
	within cells	0.1430	48	0.0030		
SC38	initial density	0.0038	2	0.0019	8.50	***
	within cells	0.0160	73	0.0002		

However, it is interesting to note that large aphid populations at SC22 had the same relative number of ants to aphids as small aphid populations at SC38 (Fig. 4). In other words, small aphid populations at SC38, that did benefit from ant tending, had the same relative number of ants tending as large populations at SC22 that did not benefit. This observation implies that although the relative number of ants to aphids decreased as aphid density increased, ant tending levels seen at high densities still should have been adequate to benefit aphids.

Consequently, it does not appear that the decline in benefit from ant tending at high aphid densities is primarily due to a decrease in the relative number of ants to aphids.

II. Alate abundance

The production of alatae occurs mainly at high densities. The alates disperse and prevent further population increase on the plant on which they were born. If the inhibition of alate production by ant tending leads to the continued production of apterous individuals, population density will continue to rise. At elevated aphid densities plant quality will deteriorate (Way and Cammell 1970), and aphid growth and fecundity will decline (Wellings and Dixon 1987) resulting in a decrease in population growth.

If a similar process was occurring, at high densities untended populations would have a greater proportion of alates than tended populations. As a result, the fecundity and population growth of tended aphids would decline. In contrast, untended aphids would continue producing alates.

Since aphid population size was only measured at the beginning and end of the experiments, the above mechanism can only be considered by looking at the final proportion of alates (i.e. final number of alates / final population size). Untended populations should have a greater proportion of alates than tended populations. This difference should increase in magnitude as aphid density increases because the production of alates increases with density.

Tended and untended populations had approximately the same proportion of alates at the end of the LDE (Fig. 5; Table 6), and there was no change with aphid density in the effect of ant tending on the final proportion of alates ($F_{1,28} = 0.004$, $p > 0.05$). However, untended populations had a greater final proportion of alates than tended populations in the HDE (Fig. 5; Table 6), although this effect was only borderline significant. There was no interaction between ant tending and aphid density ($F_{1,19} = 0.41$, $p > 0.05$).

The results of the MDE lend some support to the above findings in that the pattern seen in the HDE was also observed in the MDE at SC22, but not at SC38. The effect of

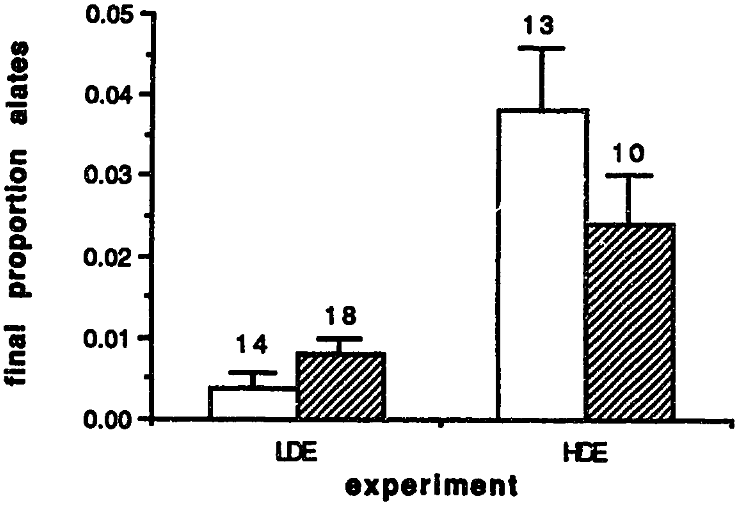

FIGURE 5. Mean final proportion of alates (+ SE) and sample sizes for untended (open) and tended (hatched) aphids in the LDE and the HDE. Significance levels are given in Table 6.

TABLE 6. Analysis of covariance of the effect of ant tending on the final proportion of alates in the LDE and the HDE, 1988. Covariates: aphid density and plant height. NS not significant.

Experiment	Source of variation	SS	DF	MS	F	
LDE	ant treatment	0.000091	1	0.000091	1.54	NS
	within cells	0.001647	28	0.000059		
HDE	ant treatment	0.001371	1	0.001371	3.51	NS[a]
	within cells	0.007428	19	0.000391		

[a] p = 0.077.

ant tending on the final proportion of alates was significantly different between the two sites in the MDE ($F_{1,247} = 4.38$, $p < 0.05$).

At SC22, mid- and large size untended aphid populations had a significantly greater proportion of alates than tended populations of the same size, whereas the proportion of alates was similar for small tended and untended populations (Fig. 6a; Table 7). Specifically, there was a significant ant by density interaction between the low density class and the higher two classes ($F_{1,97} = 5.02$, $p < 0.05$). This finding strengthens the result obtained in the HDE which showed the same pattern yet was only borderline significant. Conversely, at SC38, the effect of ant tending did not vary with aphid density, nor did it influence the final proportion of alates overall (Fig. 6b; Table 7).

In summary, in the HDE and at SC22 in the MDE, large untended populations had a greater final proportion of alates than tended populations which implies that ant tending inhibited alate production. The inhibition of alate production may have resulted in overcrowding and in a subsequent decline in growth of large tended populations. However, at SC38 in the MDE, the growth of large tended populations decreased without a corresponding decline in alate production. Therefore, the data do not confirm the importance of ant-inhibition of alate production to the density-dependence of the ant-aphid association.

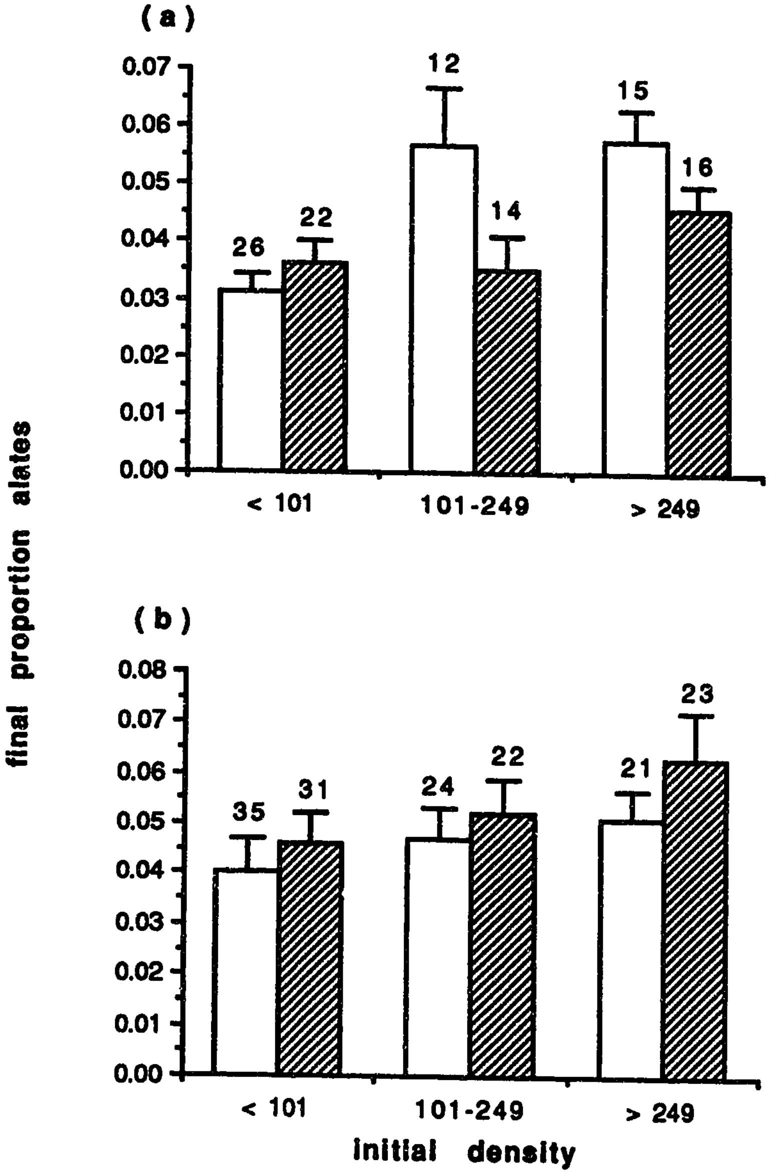

FIGURE 6. Mean final proportion of alates (+ SE) and sample sizes for untended (open) and tended (hatched) aphids at SC22 (a) and SC38 (b) in the MDE. Significance levels are given in Table 7.

TABLE 7. Analysis of covariance of the effects of ant tending and initial aphid population density on the final proportion of alates at SC22 and SC38 in the MDE, 1989. Covariate: plant height. NS not significant.

Site	Source of variation	SS	DF	MS	F	
SC22	ant treatment	0.002209	1	0.002209	3.56	NS[a]
	initial density	0.002676	2	0.001338	2.16	NS
	ant X density	0.003375	2	0.001688	2.72	NS[b]
	within cells	0.060206	97	0.000621		
SC38	ant treatment	0.002390	1	0.002390	1.86	NS
	initial density	0.000563	2	0.000282	0.22	NS
	ant X density	0.000293	2	0.000146	0.11	NS
	within cells	0.190661	148	0.001288		

a: p = 0.062; b: p = 0.071.

III. *Predator abundance*

One of the main benefits provided by ants may be protection from predators and parasitoids. Given that the relative number of ants to aphids decreases at high aphid density, protection by ants may become inadequate when aphid population size is large. This result may account for the observed decrease in benefit. Ants could influence the abundance of enemies preying on an aphid population and/or the distribution of enemy attack (i.e. the probability of a single population being found by enemies). Analyses were performed to address these two possible mechanisms.

First, in the LDE and the HDE, a one-way analysis of covariance was performed to determine the effect of ant tending and its interaction with aphid density on the final number of predators per aphid. Tended aphid populations had significantly fewer predators per aphid than untended populations at the end of the LDE (Fig. 7; Table 8), but this protection effect decreased as aphid density increased (Table 8). Conversely, in the HDE, tended populations had significantly more predators per aphid than untended populations (Fig. 7; Table 9). The effect of ant tending did not vary significantly with aphid density ($F_{1,21} = 0.19$; $p > 0.05$).

Although ant tending was associated with a lower

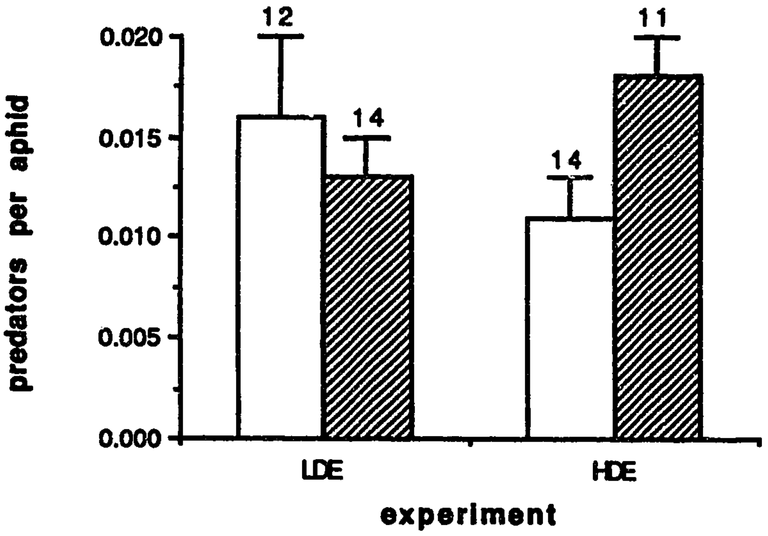

FIGURE 7: Mean final number of predators per aphid (+ SE) and sample sizes for untended (open) and tended (hatched) aphids in the LDE and the HDE. Significance levels given in Tables 8 and 9.

TABLE 8. Analysis of covariance of the effect of ant tending and initial aphid density on the final number of predators per aphid in the LDE, 1988. Covariate: aphid density. * $p < 0.05$, NS not significant.

Source of variation	SS	DF	MS	F	
ant treatment	0.000524	1	0.000524	4.95	*
initial density	0.000019	1	0.000019	0.18	NS
ant X density	0.000433	1	0.000433	4.09	NS[a]
within+residual	0.002328	22	0.000106		

a: $p = 0.055$.

TABLE 9. Analysis of covariance assuming parallel slopes of the effect of ant tending on the final number of predators per aphid in the HDE, 1988. Covariate: aphid density. * $p < 0.05$.

Source of variation	SS	DF	MS	F
ant treatment	0.000225	1	0.000225	4.48 *
within cells	0.001105	22	0.000050	

relative number of predators at low aphid densities, the reverse was true at high aphid densities. This result could explain the reduced growth of large tended populations. However, the pattern could also be related to seasonal changes in predator abundance rather than to changes in aphid density.

The possibility of a seasonal effect was eliminated in the MDE. A two-way analysis of variance was performed to determine the effect of ant tending on the final number of predators per aphid, and its interaction with aphid density. The final number of predators per aphid was significantly higher at SC22 than at SC38 ($F_{1,249} = 30.69$, $p < 0.001$; Fig. 8); therefore, predator abundance data were analyzed by site.

The loss of benefit from ant tending at high aphid density was not due to a reduction in protection by ants. There was no significant decrease in the effect of ant tending on the final number of predators per aphid with increasing aphid density at either site (Fig. 8; Table 10: ant X density). In addition, ant tending did not reduce the number of predators per aphid at any density level (Fig. 8; Table 10: ant treatment).

The second possible protection mechanism, the effect of ant tending on the probability of an aphid population being found by predators, was addressed using a Hierarchical Log-linear Model. This analysis tests relationships among

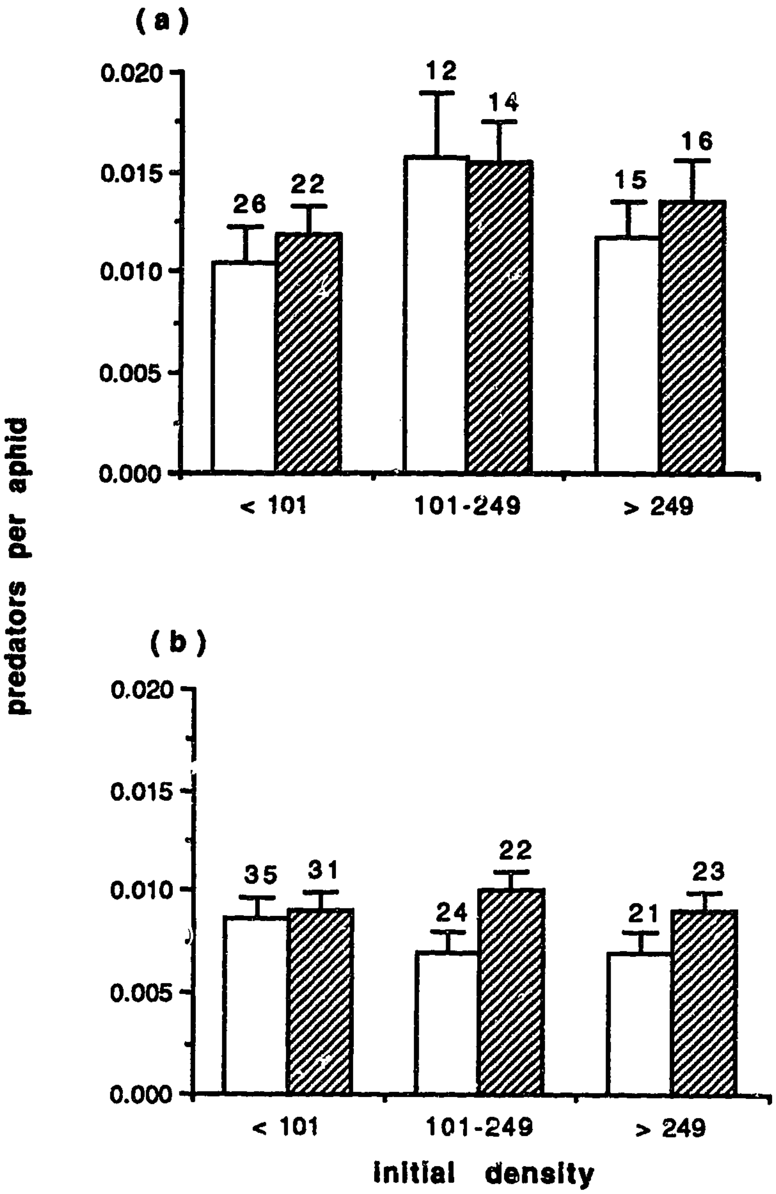

FIGURE 8. Mean final number of predators per aphid (+SE) and sample sizes for untended (open) and tended (hatched) aphids at SC22 (a) and SC38 (b) in the MDE. Significance levels are given in Table 10.

TABLE 10. Analysis of variance of the effects of ant tending and initial aphid population density on the final number of predators per aphid at SC22 and SC38 in the MDE, 1989. NS not significant.

Site	Source of variation	SS	DF	MS	F	
SC22	ant treatment	0.000022	1	0.000022	0.32	NS
	initial density	0.000335	2	0.000167	2.42	NS
	ant X density	0.000020	2	0.000010	0.14	NS
	within cells	0.006855	99	0.000069		
SC38	ant treatment	0.000091	1	0.000091	3.04	NS
	initial density	0.000040	2	0.000020	0.67	NS
	ant X density	0.000035	2	0.000017	0.58	NS
	within cells	0.004501	150	0.000030		

variables using the natural log of their cell frequencies. Aphid populations were classified into categories and the frequency of populations in each category was compared in the analysis. Specifically, each aphid population was classified with the following variables: number of predators per aphid (PRED) and ants tending (ANT). In the MDE, a third variable was used: aphid population size per plant (DEN). PRED was divided into two levels: above and below the median number of predators per aphid. ANT was divided into two levels: ants tending and no ants tending. DEN was divided into three levels based on initial population densities: ≤ 100, 101 - 249 and ≥ 250 individuals.

In neither the LDE nor the HDE was there any association between ant tending and the probability of having more predators per aphid (LDE: $x^2 = 0.23$, $p > 0.05$; HDE: $x^2 = 0.008$, $p > 0.05$). Similarly, there was no association between ant tending and predator distribution seen at SC22 in the MDE ($x^2 = 0.023$, $p > 0.05$). However, at SC38, tended populations were more likely to have more predators per aphid ($x^2 = 4.55$, $p < 0.05$) than untended populations.

In summary, ant tending seemed to reduce the number of predators in the LDE but not in the HDE, and in neither experiment did tending influence the distribution of predator attack. In the MDE, ant tending had no influence on predator abundance or distribution, except at SC38 where ant tending was associated with a distribution of predator attack in a direction opposite to that most often stated in the literature (see Way 1963). Therefore, although protection may be a benefit aphids occasionally receive from ants, it does not seem to be a benefit regularly received. Change in the level of protection is not likely a primary factor explaining the decline in benefit from ant tending at high aphid density.

Plant quality

Effect of plant quality within density experiments

To determine the effect of plant quality on aphid population growth, and to determine if plant quality influences the degree to which ant tending benefits aphids, analyses of covariance were performed in each of the three density experiments.

Host plant quality did not significantly influence aphid population growth in either the LDE or the HDE (Fig. 9; Table 11). In contrast, it had a significant positive influence in the MDE (Fig. 10; Table 12). Also, the effect of ant tending on aphid population growth did not vary significantly with plant quality within either the LDE, the HDE or the MDE (Tables 11 and 12).

The absence of a plant quality effect in the LDE and the HDE may have been due to the fact that the range of plant heights (43 - 108 cm) in those experiments was

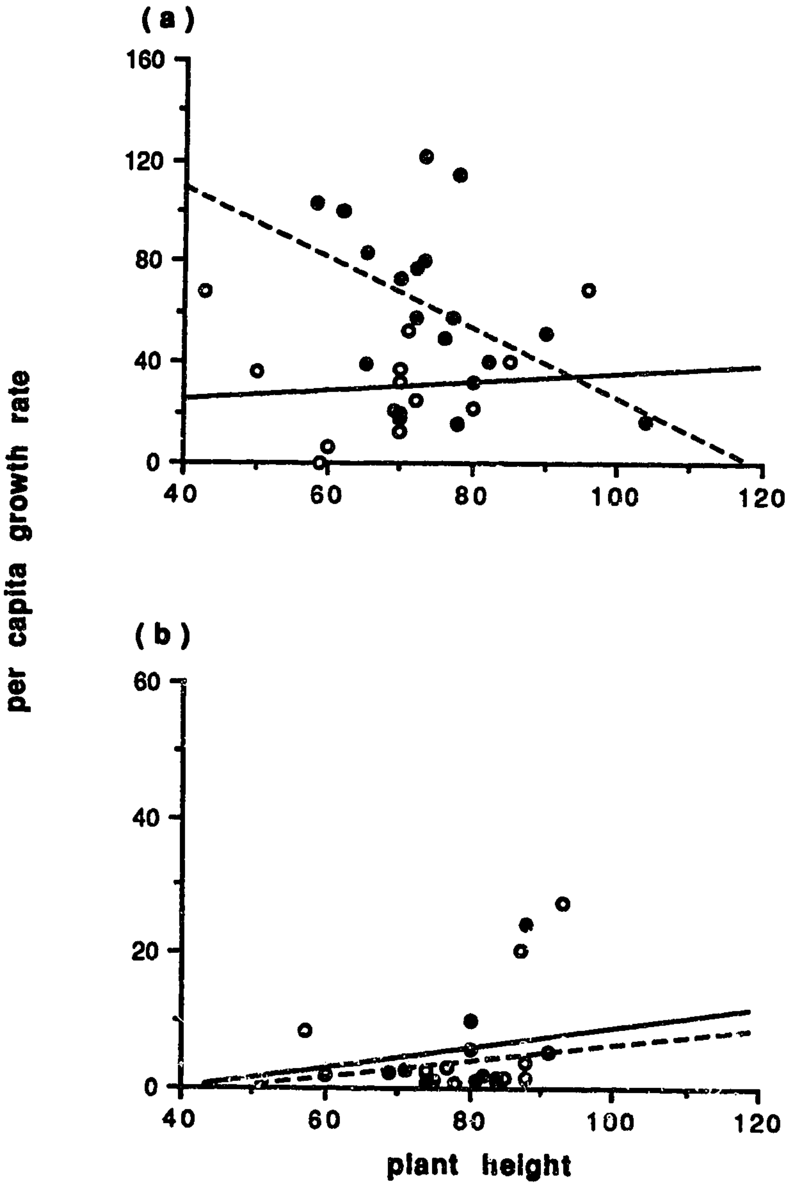

FIGURE 9. Per capita growth rates of untended (open circles, solid line) and tended (closed circles, dashed line) aphids as a function of plant height in the LDE (a) and the HDE (b).

TABLE 11. Analysis of covariance of the effect of plant quality, expressed in terms of height, and its interaction with ant tending on aphid population growth in the LDE and the HDE, 1988. In the HDE, growth data were log transformed. Covariates: aphid density and plant height. NS not significant.

Experiment	Source of variation	SS	DF	MS	F	
LDE	plant height	1463.15	1	1463.15	1.88	NS
	height X ant	2747.37	1	2747.37	3.53	NS
	within+residual	21767.72	28	777.42		
HDE	plant height	0.08	1	0.08	0.37	NS
	height X ant	0.04	1	0.04	0.18	NS
	within+residual	4.72	22	0.18		

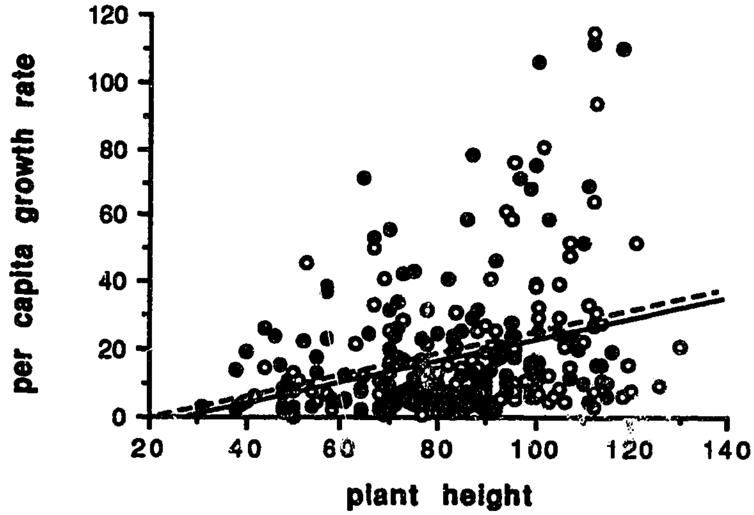

FIGURE 10. Per capita growth rates of untended (open circles; solid line) and tended (closed circles; dashed line) aphids as a function of plant height in the MDE.

TABLE 12. Analysis of covariance of the effect of host plant quality, expressed in terms of height, and its interaction with ant tending and initial aphid population density on aphid population growth in the MDE, 1989. Covariate: plant height. *** p < 0.001, NS not significant.

Source of variation	SS	DF	MS	F	
plant height	5.750	1	5.750	48.79	***
height X ant	0.003	1	0.003	0.03	NS
height X density	0.093	2	0.046	0.39	NS
height X ant X density	0.534	2	0.267	2.27	NS
within+residual	29.584	251	0.118		

narrower than the size range of plants aphids colonize. In the two experiments, plants of similar size were specifically chosen to reduce variation in population growth related to plant quality as this was not the focus of the experiments. Therefore the size range may not have been broad enough to detect a plant quality effect.

Quality effect within PQE

Plant quality had a significant positive effect on the growth of both tended and untended populations in the PQE (Fig. 11; Table 13). Specifically, host plant quality explained 42% of the variation in growth of tended aphid populations ($F_{1,38} = 26.48$, $p < 0.001$) and 40% of the variation in growth of untended aphid populations ($F_{1,37} = 16.91$, $p < 0.001$).

Low host plant quality is believed to trigger an increase in the production of alates (El-Ziady and Kennedy 1956). The alates could then disperse with the potential of finding a better host. The proportion of alates produced would, therefore, increase as plant quality decreased. However, the quality of the plant did not influence the proportion of alates produced (Table 14). Aphid populations on poor quality plants produced the same relative abundance of alates, over the two-week experimental period, as aphid populations on higher quality plants.

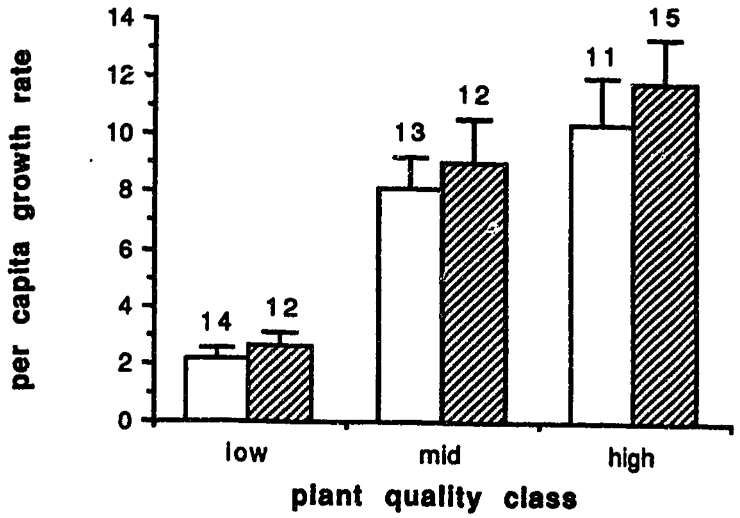

FIGURE 11. Mean per capita growth rates (+SE) and sample sizes for untended (open) and tended (hatched) aphids in the PQE. Significance levels are given in Table 13.

TABLE 13. Analysis of covariance of the effects of ant tending and host plant quality on aphid population growth in the PQE, 1989. Covariate: aphid density. *** $p < 0.001$, NS not significant.

Source of variation	SS	DF	MS	F	
ant treatment	0.02	1	0.02	0.20	NS
plant height	1.77	2	0.88	10.55	***
ant X height	0.03	2	0.02	0.20	NS
within cells	5.78	69	0.08		

T covariance of the effects of ant tending and host
P portion of alates produced in the PQE, 1989.
C NS not significant.

So ation	SS	DF	MS	F	
ant treatment	0.0029	1	0.0029	1.25	NS
plant height	0.0106	2	0.0053	2.28	NS
ant X height	0.0024	2	0.0012	0.51	NS
within cells	0.1398	60	0.0023		

Ant tending by quality interaction within PQE

A direct comparison of the effect of ant tending on population growth of aphids on poor, average and high quality plants showed that the effect of ant tending did not vary significantly with host plant quality (Table 13). Unexpectedly, the effect of ant tending was not significant at any plant quality level (Fig. 11; Table 13). However, all aphid populations were covered with net bags that excluded natural enemies throughout the experimental period. If the benefit from ant tending arises, at least in part, from protection from predators and parasitoids, then the fact that enemies were excluded from all populations may explain the absence of a tending effect.

DISCUSSION

Ant tending significantly increased the growth of low density aphid populations, but the positive effect of ant tending decreased as aphid density increased. This corroborates the observational results of Addicott (1979) which showed that ant tending lowered the probability of a population declining at low aphid densities, but had no effect or even increased the probability of a population declining at high aphid densities. The decrease in benefit from ant tending became apparent within the range of densities (10 - 100) in the LDE, and within the mid-range of densities (101 - 249) in the MDE. Consequently, the benefit aphids obtain from ants, in terms of increased population growth, begins to decline when aphid population density is still fairly low.

Contrary to this result, Wood (1982) and Cushman and Whitham (1989) found that the benefit membracids obtained from ants increased as membracid density increased. They suggested that large membracid aggregations produce more honeydew which attracts a larger number of tending ants resulting in increased benefit.

Addicott (1979) proposed just the opposite to explain the negative density-dependence of an ant-aphid association. He stated that as aphid density increases, the relative number of ants tending decreases due to the ants' inability to respond to the rapid increase in aphid density, or to their limited requirement for honeydew. Many studies of mutualism involving ants have shown that the ants' mutualist partner receives greater benefit or benefits only when tending levels are high (Addicott 1978; Messina 1981; Barton 1986; Cushman and Addicott 1989).

In the present study, the relative number of ants tending within sites was significantly lower at high aphid density. Correspondingly, the benefit aphids received from ants, in terms of increased population growth, was lower at high aphid density. However, small aphid populations at SC38, that benefitted from ant tending, were tended by the same relative number of ants as large aphid populations at SC22, that did not benefit. Consequently, the tending levels present at high density should have been adequate to benefit large aphid populations given that equivalent tending levels were sufficient to benefit small populations. The failure of ant tending to enhance aphid population growth at high aphid density cannot be due solely to inadequate tending levels.

Furthermore, a decrease or loss of benefit from ant tending when tending levels are low is often associated with a decrease in protection from predators (Barton 1986; Laine and Niemelä 1980; Messina 1981; Smiley 1986; Cushman and Whitham 1989). The results of the present study suggest a protection effect from ant tending only in the LDE. At no other time did ant tending reduce the number of predators present regardless of the number of ants tending or of whether or not tended populations had improved growth. Therefore, there does not seem to be any relationship between the number of ants tending and the level of protection received by aphids or aphid population growth.

A second possible explanation for the negative density-dependence of the association is that ant tending can lead to increased aggregation of aphids at high densities by decreasing the production of alatae. Increased aggregation can lead to higher predation pressure, to overcrowding, or to a more rapid decline of host plant quality, resulting in a decrease in population growth (Addicott 1979).

Decreased aphid fitness at high density as a result of increased competition and predation pressure has been demonstrated (Whitham 1978, 1981; Wellings and Dixon 1987), and high aphid density can lead to the deterioration of the host plant (Way and Cammell 1970; Edson 1985). However, whether the decreased growth of high-density, tended aphid populations in the present study was related to a decrease in alate production or simply occurred as a result of high initial aphid density is not known. Except at SC38 in the MDE, large tended populations had a lower final proportion of alates and their magnitude of population growth was slightly lower than that of large untended populations. This result suggests that ant inhibition of alate production may have been involved in the density-dependence of the ant-aphid association. Although decreased alate production may be involved, it is not a necessary factor because negative density-dependence was seen at SC38 in the MDE without a corresponding decrease in the production of alates.

Even if aphid population growth declines at high density regardless of ant inhibition of alate production, ant tending may still be disadvantageous at high aphid density. An aphid population will reach a size where it is more advantageous to expend energy producing alates to exploit new plants, than it is to continue growing in a limited environment. The inhibition of alate production by ant tending would greatly reduce the possibility of a population exploiting new hosts.

A third possible explanation for the density-dependence stems from the fact that tended aphids may experience greater population growth because of an ant-induced increase in the rate of aphid feeding. However, if host plant quality deteriorates substantially as aphid density rises, then at high aphid density feeding more quickly may not result in improved population growth. This could explain the decline in benefit from ant tending as aphid density increases. For a change in plant quality to be responsible for the negative density-dependence of the interaction, the following three requirements must be satisfied. First, plant quality must influence aphid population growth. Second, plant quality must decline as aphid density increases. Third, the effect of ant tending on aphid population growth must vary with plant quality.

Results of the MDE and of the PQE illustrated that aphid population growth was significantly elevated on higher quality plants. Also, although a decline in plant quality with increasing aphid density cannot be ascertained from the present study, some studies have clearly shown plant quality deterioration as a result of high insect infestation (Way and Cammell 1970; Edson 1985). The third requirement, however, does not seem to be fulfilled. In none of the experiments was an interaction between ant tending and plant quality observed.

Messina (1981) and Strauss (1987) both found that the benefit membracids obtained from ant tending was greater on higher quality plants. However, the ant by quality interaction occurred because membracids on higher quality plants formed larger aggregations and attracted more ants. As a result, membracids received greater benefits due to the elevated tending levels.

In a previous discussion it was argued that this phenomenon did not occur in the present system since larger aggregations were tended by fewer ants, and received less, not more, benefit from ant tending. Consequently, it is not possible to suggest the role that changes in plant quality might play in explaining the density related change in benefit from ant tending.

CONCLUSIONS

Although the density-dependence of the ant-aphid association was clearly established, no definite mechanism was found to explain the decline in benefit from ant tending as aphid density increased. First, tending levels dropped as a result of increasing aphid density, but not below the level seen in cases where benefits were obtained. Second, ant tending generally did not affect predator levels whether aphid density was high or low. Third, growth of large tended aphid populations declined substantially whether or not the production of alates also declined. Finally, high aphid density may have resulted in a decrease in plant quality, but nothing was found to indicate that the effect of ant tending on aphid population growth varied with plant quality.

It is likely that more than one of the above mechanisms was acting concurrently; therefore, the effect of no one single factor was outwardly apparent. Further experiments could be conducted to try to isolate a given mechanism by eliminating other possible factors. For example, natural enemies could be excluded to eliminate variation due to the differential effects of enemies on aphid population growth. Ant tending levels could be manipulated to prevent the decline in the relative number of ants tending seen at high aphid densities. Finally, the PQE could be repeated without excluding natural enemies to determine if the effect of ant tending on aphid population growth is, in fact, dependent on host plant quality.

Another possible explanation for the density-dependence of the association is related to the fact that the growth of both tended and untended aphid populations declined substantially at high densities. Density-related decreases in growth and fecundity have been documented in many systems (e.g. Smith 1963; Shaw 1970; Wilbur 1976; Antonovics and Levin 1980; Murdoch and McCauley 1985). The negative effect of density on aphid population growth may simply have overridden the positive effect of ant tending, leading to a convergence of the growth of tended and untended populations at high densities. In conjunction with this possibility, one or any combination of the above mechanisms may explain why the growth of tended populations dropped off more rapidly, and to a slightly lower level, than the growth of untended populations (Figs. 1 and 3).

In summary, the mutualistic association between aphids and ants is density-dependent, but it is not evident how changes in the number of ants tending, alate and predator abundance, and plant quality affect the density-dependence.

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