Effects of host-plant population size and plant sex on a specialist leaf-miner

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Abstract

Animal population density has been related to resource patch size through various hypotheses such as those derived from island biogeography and resource concentration theory. This theoretical framework can be also applied to plant-herbivore interactions, and it can be modified by the sex of the host plant, and density-dependent relationships. Leaf-miners are specialised herbivores that leave distinct traces on infested leaves in the form of egg scars, mines, signs of predation and emergence holes. This allows the life cycle of the insect to be reconstructed and the success at the different stages to be estimated. The main stages of the leaf-miner *Phytomyza ilicis* were recorded in eleven populations of the evergreen host *Ilex aquifolium* in Denmark. Survival rates were calculated and related to population size, sex of the host plant, and egg and mine densities. Host population size was negatively related to leaf-miner prevalence, with larger egg and mine densities in small populations. Percentage of eggs hatching and developing into mines, and percentage of adult flies emerging from mines also differed among host populations, but were not related to population size or host cover. Feeding punctures left by adults were marginally more frequent on male plants, whereas egg scars and mines were more common on females. Overall survival rate from egg stage to adult emergence was higher on female plants. Egg density was negatively correlated with hatching, while mine density was positively correlated with emergence of the larvae. The inverse effects of host population size were not in line with predictions based on island biogeography and resource concentration theory. We discuss how a thorough knowledge of the immigration behaviour of this fly might help to understand the patterns found.

Keywords: animal density, gender-biased leaf mining, *Ilex aquifolium*, patch area, *Phytomyza ilicis*, survival rates

1. Introduction

Animal population density has been related to resource patch size through diverse hypotheses leading to different predictions. Island biogeography (MacArthur and Wilson, 1967), density compensation phenomenon (MacArthur *et al.*, 1972), resource concentration theory (Root, 1973), and mixed hypotheses derived from them, have been interpreted and applied broadly to a variety of spatial scales and taxa. This includes plant-herbivore interactions, suggesting a diversity of factors and conditions acting at the same time, and influencing animal population densities (see reviews by Connor *et al.*, 2000; Gaston and Matter, 2002). (1) According to island biogeography, herbivore abundance is expected to increase linearly with the population size of the host plant, such that density should be constant with area (MacArthur and Wilson, 1967). (2) Following from this, the density compensation hypothesis accounts for observations that the accumulated density of herbivore species on small islands or habitat patches equals that of a larger island or habitat patch. Since total number of species is expected to be lower in smaller islands, the average population density of each species should be greater on smaller islands or patches if herbivore density would be independent of island size (MacArthur *et al.*, 1972). (3) Related to these theories, but only focusing on plant–animal interactions, the ‘resource concentration hypothesis’ (Root 1973) describes an insect–plant feedback mechanism whereby “herbivores are more likely to find and remain on hosts that are growing in dense or nearly pure stands”, suggesting that small host populations are difficult for a specialist herbivore to detect, which, eventually, might lead to disproportionally lower densities of the specialist in these small populations. (4) The ‘trophic-level hypothesis of island biogeography’ takes this idea further, setting it into a food web framework. It suggests that organisms in a relatively higher trophic position in the food web (specialist herbivores in this case) may suffer more from reduction in habitat area and isolation of populations than organisms in a lower trophic position (their hosts in this case) (Holt, 1996; Carlsson-Granér and Thrall, 2002). This could be caused by inverse density effects leading to a loss of genetic variability and demographic stochasticity.

These hypotheses (with the exception of the density compensation hypothesis when considering individual species) predict a neutral or positive relationship between host plant population size and herbivore density, whereas empirical results on effects of plant population size are equivocal: Small plant population size has been related to low densities of pollinators or fruit dispersers, which reduces plant reproductive success (Olesen and Jain, 1994; Kearns *et al.*, 1998). Small plant populations have also been associated with lower incidence of seed predation and diseases, which would increase plant fitness (Kéry *et al.*, 2001; Colling and Matthies, 2004). However, there are also several studies that failed to find any effect of plant population size (Benitez-Malvido, 1998) or reported negative correlations (Dooley Jr and Bowers, 1998; reviewed by Bowers and Matter, 1997; Bender *et al.*, 1998; Debinski and Holt, 2000).

Immigration behaviour has been proposed as an additional factor influencing the effects of plant population size on plant–animal interactions, in order to explain apparently contradictory results (Bowman *et al.*, 2002). This hypothesis considers the probability of patch interception as proportional to a linear patch dimension rather than to patch area. Since patch area increases quadratically with its linear dimension, the number of immigrants per unit area would decrease with increasing patch size, leading to a negative relationship between host patch size and the population density of a specific herbivore. An exception could be organisms with active searching behaviour which enables them to detect a preferred habitat or resource from some distance (Bowman *et al.*, 2002). There are other factors besides immigration that can affect density (i.e. birth, death, emigration), and these may also differ with patch size, resulting in a different final pattern. The hypothesis therefore applies particularly to situations in which immigration is the dominant process, and should be treated with caution in other cases. Nevertheless, it is frequently suggested that immigration has important effects on population density in habitat fragments, even for established populations (Fahrig and Paloheimo, 1988; Bowman *et al.*, 2002 and references therein).

In dioecious plant species effects of population size might be modified by the sex ratio of the host. This is due to sexual differences in plant palatability, with the larger reproductive allocation in females forcing them to grow slower, with higher leaf concentration of carbon-based secondary metabolites (Reekie and Bazzaz, 1987a) and lower nitrogen concentration (Reekie and Bazzaz, 1987b; reviewed by Ågren *et al.*, 1999; Cornelissen and Stiling, 2005). Empirical studies have shown that male plants are often more damaged by herbivores, although the mechanism is not yet completely clear. Moreover, if there is a sexual difference in food quality, then herbivores should perform better on the preferred sex. However, the only two studies that have compared herbivore performance in dioecious plants do not support this hypothesis, although the herbivores showed a clear preference for male leaves (Krischik and Denno, 1990; Strauss, 1990). However, these studies only considered one aspect of the life cycle of the herbivore, i.e. adult fecundity in the first study, and larval growth and survival in the second. To add some more complexity, any gender-biased activity of the herbivore and any population size effect may be modulated by the density-dependence of the insect population.

The relative effects and potential interactions of host population size, plant sex and density dependence of a monophagous herbivore have not been studied before, and are the aim of our study. We will focus on the monophagous holly leaf-miner (*Phytomyza ilicis* Curtis, Diptera: Agromyzidae) and its host plant, the European holly (*Ilex aquifolium* L., Aquifoliaceae; Cameron, 1939). Macroecological variation (Brewer and Gaston, 2002), population dynamics (Valladares and Lawton, 1991; Brewer and Gaston, 2003), and abiotic factors controlling this interaction are well understood (Klok *et al.*, 2003). There is also some evidence that egg and mine density can limit the final success of the leaf-miner (Valladares and Lawton, 1991; Eber, 2004). However, nothing is known about potential effects of population size and sex of the host plant on final density. In this paper we test the following hypotheses: (1) leaf-miner density is lower in small populations of the host plant; (2) the leaf-miner prefers male plants; (3) leaf mining is less successful in females; and (4) low egg and mine densities lead to increased hatching and higher larval emergence rates.

2. Material and methods

2.1. LEAF-MINER

Leaf-miners are immature stages of moths, beetles, flies or sawflies that feed on tissue between the upper and lower surface of leaves (Needham *et al.*, 1928; Connor and Taverner, 1997). They usually cause little damage, but some species are pests on ornamental shrubs and trees (Weintraub and Horowitz, 1995; Eber *et al.*, 2001; Salvo and Valladares, 2007). Leaf-miners leave characteristic signatures on infested leaves in the form of egg scars, mines, signs of predation and emergence holes. This allows reconstruction of the life cycle of the insect and estimation of their success at the different stages, especially on evergreen hosts. Monophagous leaf-miners can also be used for studying plant–herbivore interactions over large areas (Brewer and Gaston, 2002; Brewer and Gaston, 2003; Gaston *et al.*, 2004).

The holly leaf-miner (*Phytomyza ilicis* Curtis, Diptera: Agromyzidae) is a small fly, which is a specialist herbivore on the dioecious evergreen European holly (*Ilex aquifolium* L., Aquifoliaceae; Cameron, 1939). The insect is monophagous, and thus its geographical range and local distribution are limited by the presence of the host species. The leaf-miner has the following main developmental stages: In summer, adult females damage leaves with their ovipositors, causing small punctures from which sap exudes. Both males and females feed on leaf sap, and after healing the puncture looks like a pock mark; large numbers of punctures lead to stunted or twisted leaves. At the same time, females lay eggs in the midrib of young leaves, resulting in another type of scar. In late summer, the maggots eat and form mines from the midrib to other central parts of the leaf. The larvae pupate in March, and in late May until early June new adults emerge, leaving round holes of around 1 mm diameter.

Some maggots become parasitized, and their mines remain small and undeveloped; the most common parasitoid is *Chrysocharis gemma* (Hymenoptera: Eulophidae; Eber *et al.*, 2001). Some larvae are eaten by birds, resulting in open mines with a V-form mark. Maggot death due to parasitoids can happen at any stage of larval development, while bird predation occurs most often in late spring. The dispersing adult flies cover distances of at least several hundred meters (Eber, 2004).

2.2 HOST PLANT

Ilex aquifolium is a shrub or small tree (<25 m height, <250 years) that occurs sparsely but usually gregariously in deciduous forests of western and southern Europe (Peterken, 1966). Its north-eastern border in Denmark has been associated with winter frost (Iversen, 1944; Peterken and Tubbs, 1965; Walther *et al.*, 2005). Naturally, the species is most common in old-grown beech forests on moist, nutrient-rich soils (Lawesson, 2000; Arrieta and Suárez, 2004), but it is also found in other forest types suggesting a relatively broad habitat niche (Bañuelos *et al.*, 2004). Seeds are dispersed mainly by thrushes and other frugivorous passerines, with most seedlings establishing directly underneath female trees (García *et al.*, 2005). In addition, the species exhibits clonal spread by suckering from roots and layering from branches (Peterken and Lloyd, 1967).

In Denmark, several populations of *I. aquifolium* are found in eastern Jutland, some on the island of Funen and a few on Lolland and Zealand (Ødum, 1968). In reassessing this distribution there was no evidence of extinction of the historical populations, while numerous new populations were observed in the eastern parts of Denmark, especially in areas with high proportions of forest cover and urbanization (Bañuelos *et al.*, 2004).

2.3 HOST PLANT POPULATIONS

Eleven *I. aquifolium* populations of differing size and cover were chosen within the natural range of the species in eastern Jutland (see electronic supplementary material). Suburban or disturbed populations were excluded, and most sites were situated in semi-natural deciduous forests.

Each population was characterized in terms of number of *I. aquifolium* plants, mean plant cover and sex ratio. A population was delimited when we could not find any other *I. aquifolium* plant within a radius of ca. 500 m. Since it was difficult to accurately estimate population size due to clonal spread of the species, we used the following categories: 1, <50 plants; 2, 50–150 plants; and 3, >150 plants. Mean cover abundance of *I. aquifolium* was estimated in randomly placed 20 m x 20 m plots (4–9 plots, depending on population area).

2.4 LEAF SAMPLING

In June 2003, 15–20 leaves were randomly collected from six male and six female shrubs (1–3 m tall) per population (a total of 2559 leaves). Since all leaves were 1 year old, the observations corresponded to events from the previous year. Abundance of *P. ilicis* is relatively stable over years (Valladares and Lawton, 1991), and thus the observed patterns can be considered as representative for average densities over several generations.

Leaves of *I. aquifolium* have a lifespan of 2–5 years, thus allowing us to reconstruct the complete history of the plant–herbivore interaction throughout one season (2002–2003). The leaves were analysed for the distinctive marks left by *P. ilicis* at the different stages of larval development. For each leaf we counted the number of (1) feeding punctures (feeding marks left by adults in summer 2002), (2) egg scars (midrib punctures due to oviposition in summer 2002), (3) mines (winter 2002–2003 till spring 2003), and (4) adult emergence holes (round holes due to adult emergence, spring 2003). We also recorded mine density in the field by counting the number of mines in 100 random leaves per shrub in seven populations, a measure used in related studies (e.g. Brewer and Gaston, 2002). Bird predation on developed mines was also identified (spring 2003), using the characteristic marks left.

To be sure that all viable adults had emerged at the sampling date, we picked some leaves with mines that were still closed in June 2003, and preserved them at room temperature for 2 months. Since none of the mines opened, we assumed that all adults had emerged. One possible reason for emergence failure is infestation by parasites, the rate of which is considered to be quite stable over years (Valladares and Lawton, 1991).

2.5 LEAF-MINERS AND LEAF SHEDDING

If egg laying and mine development result in premature leaf shedding, as observed by Owen, (1978) and Stiling and Simberloff (1989), there is a possibility underestimating survival rates. Thus, in October 2003, ten leaves per shrub with and without egg scars were tagged in 4–5 shrubs within seven populations; they were checked for mine development and leaf shedding in June 2004. The tagged leaves were attached to the branch with a thread to avoid losing them.

Egg laying and mine development significantly affected leaf shedding in our study. While normal leaf fall usually occurs in June–July, most leaves shed in January–May were mined. Also, while only 2% of 1-yr old tagged leaves without eggs fell, this value was 10% for 1-yr old tagged leaves with egg punctures; of the latter, 98% showed fully developed mines. A three-factor nominal logistic model was significant (egg presence, population and female nested within population as nominal factors, $\chi^2_{31} = 105.3$, $P < 0.0001$), and the Wald test indicated that this was mainly due to the presence of egg punctures in the shed leaves. Thus, mined leaves both fall earlier in the season and persist for fewer years. As a consequence, survival rates at the different larval stages might be slightly underestimated due to the infected leaves which have already been shed in the sampling moment, this bias probably being higher in most attacked populations. Thus, special care should be taken when comparing survival rates among populations.

2.6 STATISTICAL ANALYSES

The relationship between host population size and leaf-miner density was analysed using linear mixed models with ‘population’ (random factor) nested within ‘population size class’, ‘sex’ as a fixed factor, and ‘*I. aquifolium* cover’ as covariate. The minimal adequate model for the density of feeding punctures, egg scars and mines was determined by stepwise selection from the full model based on model comparison, using Akaike Information Criterior (AIC). This method weighs the significance of a model by its complexity (Crawley, 2007). Specifically, models were fitted with *lmer* (R Development Core Team, 2009), and P-values were computed based on a Monte Carlo Markov Chain sample.

Densities of feeding punctures, egg scars and developed mines were arc-sin transformed to achieve normal distributions of the residuals. This analysis also allowed us to test for differences in leaf-miner density and feeding activity between sexes. Differences between populations and sexes in hatching and emergence rates were analyzed with G-tests (GLMs with Poisson errors and log-link function) by comparing the residual deviance with the χ^2 statistic. The relationship between incidence of eggs or mine development and feeding activity was tested with logistic regressions including sex as a fixed factor. Density dependence of hatching and emergence rates was tested by means of parametric ANCOVAs, including sex as a fixed factor. All statistical analyses were done in the R statistical environment (R Development Core Team, 2009).

Results

3.1. OVERALL PATTERNS IN LEAF MINING

We found feeding punctures left by adult *P. ilicis* leafminers in all *I. aquifolium* plants studied ($N = 132$ plants), and in 70% of the leaves ($N = 8200$ leaves). On average, there were 60 ± 2.6 (mean ± SE) egg scars per 100 leaves ($N = 2559$), and mean number of egg scars per oviposited leaf was 1.59 ± 0.04 ($N = 964$; range 1–13).

Most mined leaves had only one mine (95.4% of cases), although in a few cases two (3.7%), three (0.7%) or four (0.2%) mines per leaf were found. However, even in these less common cases only one adult emerged per leaf, with a single exception where two adults emerged from two mines in the same leaf.

Overall mine density was 15.7 per 100 leaves ($N = 8200$ leaves, field estimate). This value was slightly higher when only the leaves collected for the detailed analysis were considered, i.e. 18.0 mines per 100 leaves ($N = 2559$ leaves).

2.7 HOST POPULATION SIZE AND LEAF-MINER PREVALENCE

Host population size was significantly related to leaf-miner prevalence, with small populations having higher egg and mine densities per 100 leaves (Fig. 1a; $F_{2,128} = 4.57$, $P = 0.012$; Fig. 1b; $F_{2,67} = 3.48$, $P = 0.036$ for egg and mine densities, respectively). Populations with sparse *I. aquifolium* cover also showed higher mine densities ($F_{1,67} = 6.50$, $P = 0.013$). In contrast, adult feeding activity, estimated from the number of feeding punctures per 100 leaves, was not significantly related to any of the population characteristics measured (Fig. 1c; $P > 0.2$ for all factors considered).

Hatching success (as indicated by the number of mines per 100 eggs), and emerging success (i.e. adult flies per 100 mines) differed significantly among host populations ($G = 69.6$, $df = 10$, $P < 0.001$ for egg hatching rate; $G = 57.9$, $P < 0.001$ for emergence rate). However, neither host population size nor cover were related to survival rates at any of the stages ($F_{2,8} < 1.5$, $P > 0.2$ for population size; $F_{1,9} < 1.45$, $P > 0.2$ for population cover); mean values per population were used to calculate both survival rates from egg hatching to developed mines and from mines to adult emergence.

2.8 LEAF-MINER ABUNDANCE AND SUCCESS ON MALE AND FEMALE HOST PLANTS

Feeding records (i.e. feeding punctures per 100 leaves) were more frequent in male host plants, although this difference was only marginally significant ($F_{1,129} = 3.08$, $P = 0.08$). Mean values of egg and mine densities were also higher in males, but this difference was not statistically significant, and the fixed factor ‘sex’ did not enter the full minimal models.

Survival rates at the various stages were different for male and female *I. aquifolium* (all populations pooled; Fig. 2). Egg hatching rate was significantly higher in females than males ($G = 7.94$, $df = 1$, $P < 0.01$). Adult emergence rate was also higher in female than in male plants ($G = 3.99$, $P < 0.05$). Combining both results, the overall survival rate of the leaf-miner from egg laying to adult emergence was higher in female than in male plants ($G = 11.7$, $P < 0.001$).

Mortality values were also higher in male plants when considering mortality due to bird predation (29.3% and 21.2% for mines on male and female plants, respectively), as well as the additional mortality of remaining mines due to parasites, diseases, etc. (53.7% and 55.2% for mines on male and female hosts, respectively). However, these differences were not significant ($G = 1.99$, $P > 0.1$ for predation rate; $G = 2.47$, $P > 0.1$ for additional mortality), although this result might be partly due to low sample size.

2.9 DENSITY-DEPENDENCE OF FEEDING, EGG LAYING, HATCHING AND EMERGENCE

Leaf infestation rate, i.e. incidence of eggs or mines, was higher in leaves with more intense feeding by adult leaf-miners (logistic regression; $Z = 12.86$, $P < 0.001$, $df = 2558$ for egg scars; $Z = 8.15$, $P < 0.001$, $df = 2558$ for mines), and the pattern was similar for both sexes (in both logistic regressions, $Z < 0.5$, $P > 0.1$ for ‘sex’ as factor).

Egg density was negatively correlated with egg hatching, and both male and female host plants showed similar patterns ($F_{1,19} = 9.61$, $P = 0.005$; Fig. 3a). However, mine density was positively correlated with emergence rate of the larvae ($F_{1,19} = 8.81$, $P = 0.007$; Fig. 3b).

3 Discussion

4.1 EFFECTS OF HOST POPULATION SIZE AND COVER

The density of *P. ilicis* (i.e. number of eggs and mines per 100 leaves) was higher in small host populations. This result contradicts the hypothesis based on island biogeography (MacArthur and Wilson, 1967) or the resource concentration hypothesis (Root, 1973). However, it does fit with the predictions of the density compensation hypothesis (MacArthur *et al.*, 1972). It has been suggested that certain autoecological factors related to population dynamics and migration behaviour (Bowman *et al.*, 2002; Hambäck and Englund, 2005), should be taken into account to understand these patterns. In a review of available data on migration rates from patches of different sizes, Hambäck and Englund (2005) found a large variability among broad taxonomic groups of insects, with mean slope in density–area relationships being non different from zero in the broad group of Diptera. Moreover, they found significant heterogeneity within groups, especifically within Diptera the flies, suggesting a finer taxonomic or functional grouping. As a consequence, they suggest that a thorough understanding of the searching mode of each species is required to understand the relationship between animal density and patch size.

According to Bowman *et al.* (2002), the pattern we found would be expected if *P. ilicis* were an organism with aerial movement and random searching from a distance. This small fly certainly shows aerial movement, but little is known about its searching behaviour for new host patches, apart from its ability to disperse at least several hundred meters (Eber, 2004). We also know that a related leaf-miner, *Phytomyza ilicicola*, feeding on *Ilex opaca* (American holly) (Marino and Cornell, 1992), has no preference for concentrated resources (i.e. larger area patches) in comparison to single *I. opaca* plants, suggesting a random searching behaviour (i.e. no orientation toward larger patches), which might eventually lead to only slightly larger immigration rates to larger patches, and to negative slopes in density-area relationships (Bowman *et al.*, 2002). According to a review by Prokopy and Owens (1983) on plant detection by different insect groups, including some herbivorous flies, *P.ilicis* might disperse in the 'aerial plankton' with random searching from a distance, and a final approach to patches using some combination of visual and olfactory cues. If this were confirmed, *P.ilicis* might be another example supporting Bowman's theory.

We also found that sparser *I. aquifolium* populations had higher *P. ilicis* densities. This finding contrasts with predictions of low densities of leaf-miners in regions where *I. aquifolium* is sparse, due to 'metapopulation' processes (Brewer and Gaston, 2003). However, our result is not surprising if immigration behaviour is taken into account, since a single sparse *I. aquifolium* population might be equivalent to many small resource patches for *P. ilicis*, which according to the immigration behaviour hypothesis should result in higher densities of leaf-miners.

4.2 DIFFERENTIAL SURVIVAL IN MALE AND FEMALE HOSTS

We found similar feeding records of adult leaf-miners on male and female *I. aquifolium* plants, albeit with a trend towards more feeding records on males. Other studies with dioecious species reported that male plants are more intensely used than females (Ågren, *et al.* 1999; Cornelissen and Stiling, 2005). This has been associated with higher male palatability as a result of trade-offs in resource allocation (Reekie and Bazzaz, 1987b). In our study variability among shrubs in feeding records (100–7320 feeding punctures per 100 leaves) was much larger than variability between sexes. Although no gender-related plant trait was measured, this suggests that leaf quality, including allelochemicals, might be more variable among shrubs than between sexes. Moreover, adult *P. ilicis* feed on leaf exudates and leaf sap, especially on phloem sap, the latter being a sugar-rich resource, with nitrogen predominantly in the form of free amino acids, and generally free of toxins and feeding deterrents (Douglas, 2006). Thus, it is possible that leaf thickness, sugar levels or amino acid composition are more relevant traits than secondary compounds for feeding behaviour of adult leaf-miners when selecting specific populations, shrubs or leaves (Douglas, 2006).

Furthermore, shrub phenology might be involved in the patterns observed in our study. In a closely related species, *Phytomyza ilicicola,* it has been observed that adult leaf-miners showed feeding preferences for terminal and expanding leaves, although oviposition rates were independent of leaf age and position (Marino and Cornell, 1993). It might be that slight phenological differences between plants contribute to the differences observed in such a way that shrubs with expanding leaves during the peak of adult emergence get more feeding records.

Larval development, on the other hand, has to cope with secondary compounds and palatability of leaf mesophyll cells. Our results indicate that adult feeding records per shrub are positively correlated with larval infestation rates. However, differences in mine densities do not simply reflect differences in the number of eggs laid. Thus, differential larval or pupal mortality between sexes due to parasitism or to other causes might also play a role. Contrary to the expectations set out previously, we found that survival of *P. ilicis* was higher on female *I. aquifolium*. This result might be due to differences in secondary compounds which act as deterrents for herbivores. Classical ecological hypotheses consider that trade-offs occur between two traits, when all resources are consumed (Coley *et al.*, 1985; Reekie and Bazzaz, 1987c). As a consequence of the diversion of nutrients from vegetative to reproductive structures, these models assume that female plants are better defended than males, due to a higher concentration of carbon-based secondary metabolites in female leaves. More recent models, however, consider that trade-offs can involve unforeseen ’third party traits’, and thus results become less clear when resources are limited (Mole, 1994). In species producing costly fruits, such as *I. aquifolium*, and growing at the edge of the distribution, as is the case for this species in Denmark, females might have favoured reproduction at the expense of growth and defence (Mole, 1994), as has been found in other fleshy-fruited species (Massei *et al.*, 2006).

Previous results relating palatability and both leaf-miner abundance and success are not conclusive. Nitrogen is considered as a critical nutrient for phytophagous insects, and in fact other agromyzids have been found to lay more eggs and to perform better on high-nitrogen hosts (Minkenberg and Fredrix, 1989). Negative relationships have also been found (Faeth *et al.*, 1981; Valladares and Lawton, 1991) together with large variation among shrubs (Valladares and Lawton, 1991). The mechanism for the increased success of *P. ilicis* developing on female hosts remains unresolved, but as far as we are aware, this is the first time that differences in survival rates of a leaf-miner, or any parasite, have been related to the sex of the host plant.

4.3 DENSITY-DEPENDENT SURVIVAL OF *PHYTOMYZA ILICIS*

The negative density-dependent survival in the early development of the larvae after egg hatching is consistent with the results of other studies (Valladares and Lawton, 1991; Eber, 2004). It has been suggested that this negative density-dependence occurs at leaf level, probably due to competition among first-instar larvae as they advance through the midrib of the leaf, possibly a limiting resource. In this paper we show that this relationship is also observed at the population level, and, furthermore, that both male and female host plants show a similar pattern.

At the mining stage, as a contrast, we did not expect such a negative density-dependent survival, since interference and competence among mines is not probable, yet in most cases only one mine develops per leaf. Besides, we did not expect a high mortality due to parasitoids, because of their lower incidence at the limit of the distribution range of the host species in Denmark (cf. Fig. 2c in Brewer and Gaston, 2003). However, all these facts suggest density-independent survival curves, while what we found in both sexes was a positive density-dependent survival, so that adult emergence rate was higher in populations with higher mine density (Valladares and Lawton, 1991; Eber, 2004). Further work is needed to identify the mechanism involved.

4.4 LEAF-MINER INFESTATION AT THE EDGE OF THE DISTRIBUTION RANGE

We found the leaf-miner *P. ilicis* in all host populations, and mortality rate from egg stage to emergence was relatively high, compared to previously reported values, and also to predicted values according to spatially explicit models previously developed by Brewer and Gaston (2003).

Hatching failure or early larval mortality was 14% higher than the previously reported mean European value (52%; Brewer and Gaston, 2003), and 41% higher than the predicted rate for the geographic area of this study (25%; Brewer and Gaston 2003). Emergence failure from the initiated mines was 35% higher than predicted values (ca. 40%; Brewer and Gaston, 2003). Nevertheless, this comparison should be treated with caution, since interpolated maps come from relatively few data points and might not be representative of the specific study area represented herein. In addition, bird predation was higher than described in the literature: 24% of all mines were predated, (population range 0–57%) *vs.* ca. 10–15% estimated by Brewer and Gaston (2003).

In general, the high variability in leaf-miner performance found in the study populations is interesting, as it was sometimes larger than the variability predicted for the whole range (Brewer and Gaston, 2003). This might be at least partly explained by regional differences in plant palatability as observed in other studies (e.g. Keller *et al.*, 1999).

5 Conclusions

Plant–leaf-miner interactions are more complex than previously assumed, and thus we advocate that all key factors should be combined in future studies. Experimental approaches are needed if we are to reach a clear understanding of the significance and potential interactions of these factors. External and internal factors influence this specific plant–leaf-miner interaction at three levels: (1) intrinsic density-dependence patterns of the leaf-miner population at different stages, (2) individual host plant characteristics (e.g. for example plant sex, as a proxy of nutrient content, hardness or secondary compounds), and (3) host population traits in a spatial context (such as host population size or host cover). For the latter, the specific immigration behaviour of the animal appears to be essential to understand the spatio-temporal interactions involved.

Considering the whole organism cycle in plant–leaf-miner interactions provides a much more realistic perspective, since different forces may drive the interaction at different stages. The study system of *P. ilicis* and *I. aquifolium* allows for all these spatial and temporal considerations. Existing variability and previous knowledge of this interaction open the door for manipulative experiments. This will lead to a deeper understanding of the specific system and may allow better prediction of the relevant forces that drive other plant–herbivore interactions.

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FIGURE CAPTIONS

Figure 1. Intensity of host use by the leaf-miner *Phytomyza ilicis* (egg scars, mines and feeding punctures per 100 leaves) in populations of *Ilex aquifolium* of different sizes (small, <50 plants; medium, 50–150 plants; large, >150 plants). Mean values ± SE are shown (one value per plant, all plants from populations of a similar size class pooled). Different superscripts indicate significant differences after Tukey post-hoc tests. Statistical results included in the text.

Figure 2. Differences in survival of the leaf-miner *Phytomyza ilicis* on male and female *Ilex aquifolium* plants (sample size specified in the text).

Figure 3. Relationship between egg density and egg hatching (above), and between mine density and adult emergence rate (below) of *Phytomyza ilicis* on male and female host plants in eleven Danish populations of *Ilex aquifolium*.

SUPPLEMENTARY ELECTRONIC MATERIAL

S Table 1. Characteristics of eleven study populations of the host plant *Ilex aquifolium* in eastern Jutland, Denmark. Population size is described in three classes (1, <50 plants; 2, 50–150 plants; 3, >150 plants); population order follows sex ratio

Study site	UTM32 X/Y coordinates	Forest vegetation	Population size	Cover (%)	Males (%)
Trelde Næs	553666/6164593	Mixed deciduous	2	1.7	76.7
Andkær Vig	540944/6171937	Beech	3	7.5	69.0
Sjelle Overskov	555946/6228397	Mixed conifer-deciduous	3	2.7	60.8
Københoved Skov	503700/6141100	Mixed conifer-deciduous	2	1.0	63.0
Rødding Skov	506216/6137191	Beech	2	3.1	56.5
Hovens Odde	536653/6152767	Beech	1	4.5	59.1
Grøn Skov	543795/6209070	Beech	1	2.7	56.0
Gauerslund Skov	543809/6168432	Mixed conifer-deciduous	1	<1	53.3
Hemstok Skov	553668/6216902	Mixed conifer-deciduous	1	1.1	50.0
Gjern Bakker	543454/6231111	Mixed deciduous	3	8.7	49.6
Skodborg Præsteskov	510930/6138593	Beech	2	19.0	43.8

S Table 2. Leaf mining of *Phytomyza ilicis* in eleven populations of *Ilex aquifolium* (N, number of leaves sampled per population). Population order follows sex ratio (see S Table 1)

Population	Pop size Class	N	Leaf-miner traces (per 100 leaves)					Leaf-miner success (%)	
			Feeding punctures	Egg scars	Mines	Emergence holes	Predation	Eggs to mines	Mines to emergence
Trelde Næs	2	231	2702	103.0	27.3	4.76	6.93	26.5	17.5
Andkær Vig	3	230	1915	52.2	16.5	6.52	5.22	31.7	39.5
Sjelle Overskov	3	233	1599	48.5	11.2	0.00	5.58	23.0	0.0
Københoved Skov	2	239	2004	40.2	19.3	5.86	7.11	47.9	30.4
Rødding Skov	2	238	2019	33.6	14.7	4.20	2.94	43.8	28.6
Hovens Odde	1	229	2680	101.8	32.3	14.0	3.06	31.8	43.2
Grøn Skov	1	234	1594	31.2	12.0	1.71	0.85	38.4	14.3
Gauerslund Skov	1	216	1746	144.4	24.1	7.41	0.00	16.8	30.8
Hemstok Skov	1	240	1583	52.1	14.2	1.25	1.25	27.2	8.8
Gjern Bakker	3	233	1448	34.8	15.9	0.86	3.00	45.7	5.4
Skodborg Præsteskov	2	236	1270	29.2	11.9	0.85	6.78	40.6	7.1

S Table 3. Leaf-miner traces of *Phytomyza ilicis* recorded in leaves from male and female plants in eleven Danish populations of *Ilex aquifolium* (2003–2004) (means ± SE; sample size in brackets)

Leaf-miner traces	Male leaves (1274)	Female leaves (1285)
Feeding punctures		
Feeding punctures per infested leaf	29.0 ± 1.1 (940)	25.0 ± 1.0 (874)
Leaves with feeding punctures (%)	72.3	68.0
Egg scars		
Egg scars per infested leaf	1.62 ± 0.05 (504)	1.56 ± 0.05 (460)
Leaves with egg scars (%)	38.8	35.8
Mines		
Leaves with mines* (%)	17.0	16.2
Leaves with egg scars that developed mines (%)	43.7 (504)	46.0 (460)
Mines per mined leaf	1.06 ± 0.02 (221)	1.05 ± 0.01 (214)
Leaves with emergence holes (%)	3.5	4.7
Survival		
Hatching (% eggs that developed mines)	31.5 (821)	37.2 (719)
Mines predated by birds (%)	29.3 (236)	21.2 (225)
Additional mine mortality (%)	53.7 (236)	55.2 (225)
Emergence (% completed mines)	17.0 (236)	23.6 (225)
Eggs producing adult flies (%)	5.5 (821)	8.7 (719)

*Percent of the total number of leaves in the population with developed mines.

Figure 1

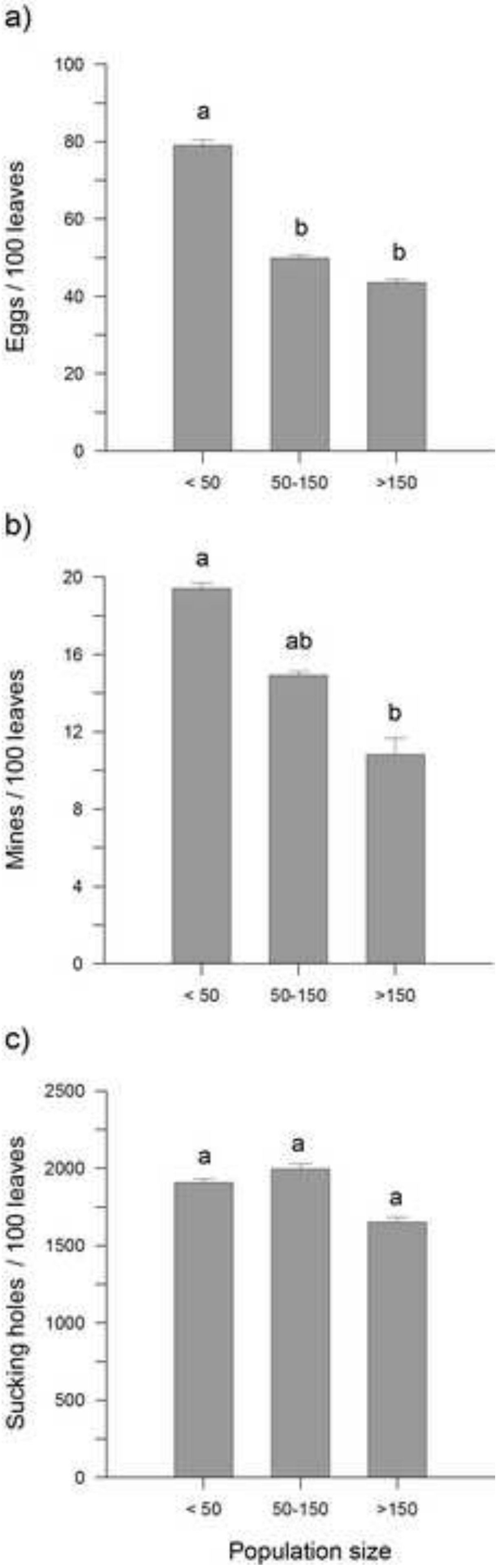

a)
Eggs / 100 leaves
0
20
40
60
80
100
a
b
b
< 50
50-150
>150
b)
Mines / 100 leaves
0
4
8
12
16
20
a
ab
b
< 50
50-150
>150
c)
Sucking holes / 100 leaves
0
500
1000
1500
2000
2500
a
a
a
< 50
50-150
>150
Population size

Figure 2

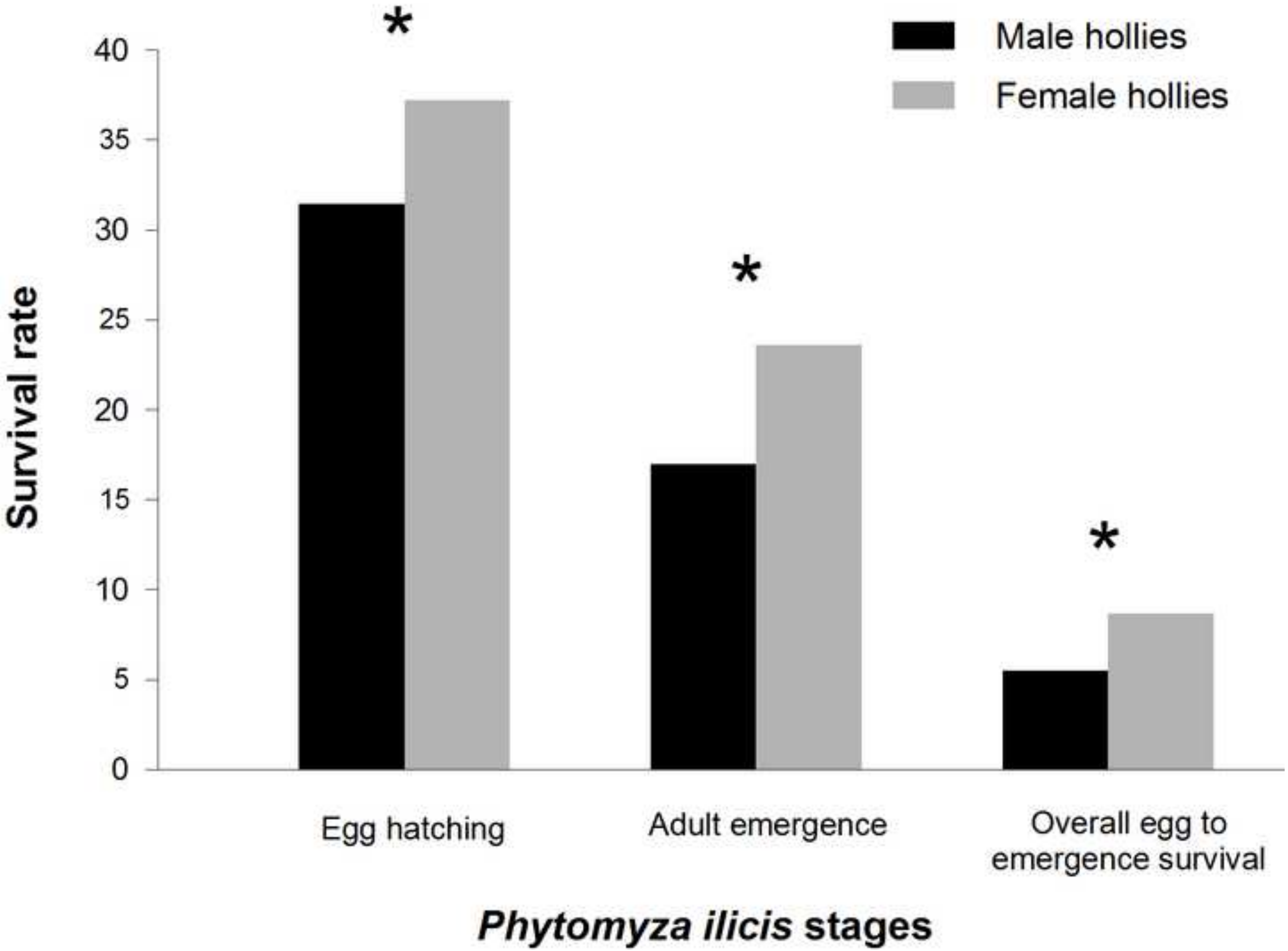
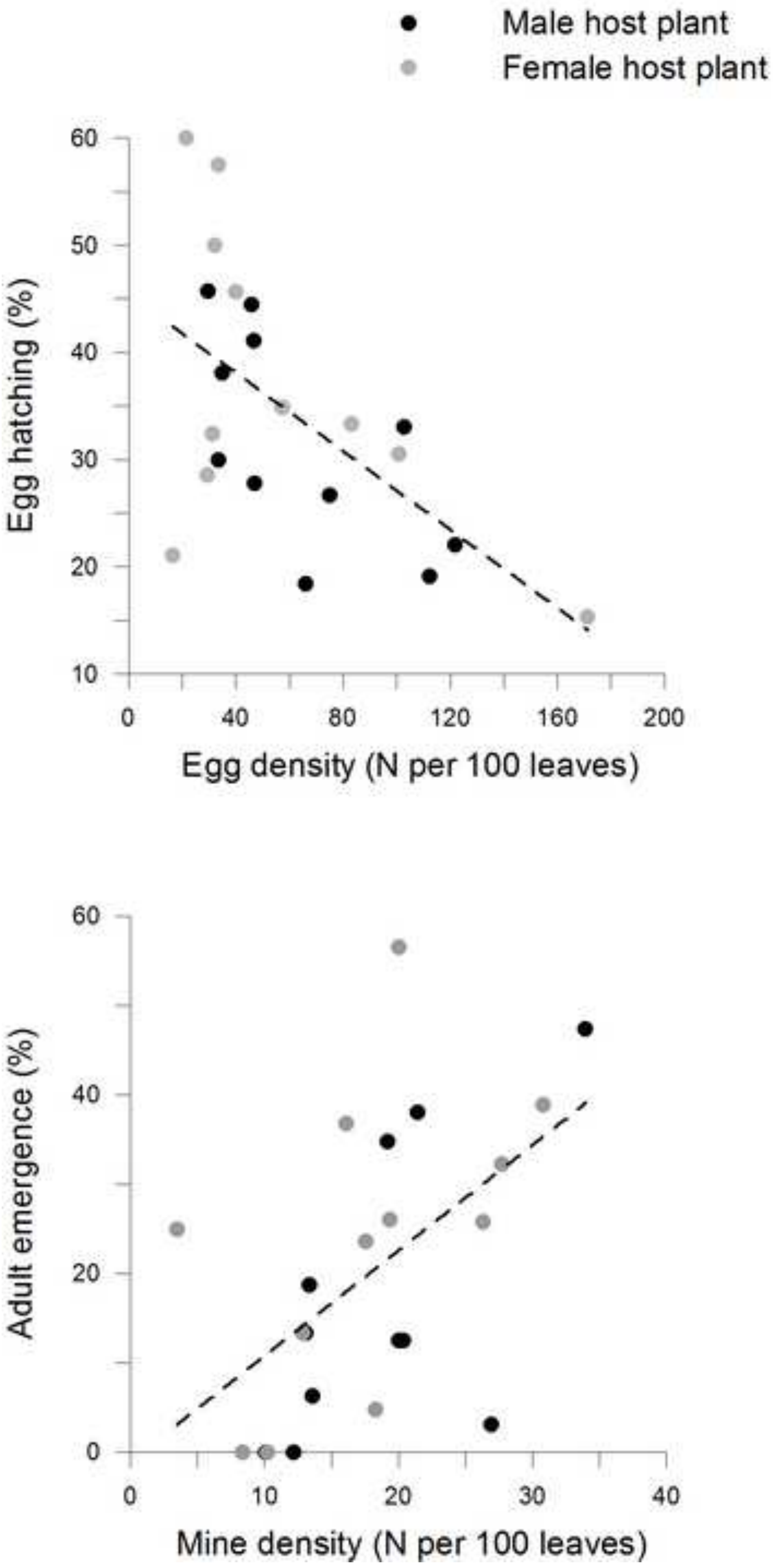

*
*
*
40
35
30
25
20
15
10
5
0
Survival rate
Male hollies
Female hollies
Egg hatching
Adult emergence
Overall egg to emergence survival
Phytomyza ilicis stages

Figure 3


Male host plant
Female host plant
Egg hatching (%)
60
50
40
30
20
10
0
40
80
120
160
200
Egg density (N per 100 leaves)
Adult emergence (%)
60
40
20
0
0
10
20
30
40
Mine density (N per 100 leaves)