

Clumped distribution of oak leaf miners between and within plants

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Abstract

Leaf miners typically show non-random distributions both between and within plants. We tested the hypothesis that leaf miners on two oak species were clumped on individual host trees and individual branches and addressed whether clumping was influenced by aspects of plant quality and how clumping and/or interactions with other oak herbivores affected leaf-miner survivorship. Null models were used to test whether oak herbivores and different herbivore guilds co-occur at the plant scale. Twenty individual *Quercus geminata* plants and 20 *Quercus laevis* plants were followed over the season for the appearance of leaf miners and other herbivores, and foliar nitrogen, tannin concentration, leaf toughness and leaf water content were evaluated monthly for each individual tree. The survivorship of the most common leaf miners was evaluated by following the fate of marked mines in several combinations that involved intra- and inter-specific associations. We observed that all leaf miners studied were clumped at the plant and branch scale, and the abundance of most leaf-miner species was influenced by plant quality traits. Mines that occurred singly on leaves exhibited significantly higher survivorship than double and triple mines and leaves that contained a mine or a leaf gall and a mine and damage by chewers exhibited lowest survivorship. Although leaf miners were clumped at individual host trees, null model analyses indicated that oak herbivores do not co-occur significantly less than expected by chance and there was no evidence for biological mechanisms such as inter-specific competition determining community structure at the plant scale. Thus, despite co-occurrence resulting in reduced survivorship at the leaf scale, such competition was not strong enough to structure separation of these oak herbivore communities.

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Zusammenfassung

Blattminierer zeigen typischerweise nichtzufällige Verteilungen sowohl zwischen den Pflanzen als auch auf den Pflanzen. Wir testeten die Hypothese, dass die Blattminierer auf zwei Eichenarten auf den einzelnen Wirtsbäumen und einzelnen Ästen geklumpt verteilt sein würden und beschäftigten uns damit, ob die Gruppierung von Aspekten der Pflanzenqualität beeinflusst war und wie die Gruppierung und/oder die Interaktionen mit anderen Eichenherbivoren das Überleben der Blattminierer beeinflusste. Es wurden Nullmodelle genutzt, um zu testen, ob die Eichenherbivoren und verschiedene Herbivorengilden auf der Pflanzenebene gemeinsam vorkommen. Über die Saison wurde bei 20 *Quercus geminata* und 20 *Quercus laevis* Bäumen das Auftreten von Blattminierern und anderen Herbivoren verfolgt, sowie für jeden einzelnen Baum monatlich der Blattstickstoff, die Tanninkonzentration, die Blattfestigkeit und der Blattwassergehalt erfasst. Das Überleben der häufigsten Blattminierer wurde erfasst, indem das Schicksal markierter Minen bei verschiedenen Kombinationen verfolgt wurde, die intra- und interspezifische Assoziationen beinhalteten.

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Wir beobachteten, dass alle untersuchten Blattminierer auf der Pflanzen- und Astebene geklumpt vorkamen und dass die Abundanz der meisten Blattminierer von Merkmalen der Pflanzenqualität beeinflusst wurde. Minen, die einzeln in den Blättern vorkamen, zeigten eine signifikant höhere Überlebensrate als doppelte oder dreifache Minen. Blätter, die eine Mine oder ein Blattgalle und eine Mine und Fraßschäden aufwiesen, zeigten die geringste Überlebensrate. Obwohl die Blattminierer auf einzelnen Wirtsbäumen geklumpt waren, zeigte die Analyse der Nullmodelle, dass die Eichenherbivoren nicht signifikant seltener gemeinsam auftreten als aufgrund des Zufalls erwartet wurde. Es gab keine Hinweise auf biologische Mechanismen wie interspezifische Konkurrenz, welche die Struktur der Gemeinschaften auf der Pflanzebene bestimmen. Also war die Konkurrenz nicht stark genug, um die Separation von Gemeinschaften dieser Eichenherbivoren zu strukturieren, obwohl gemeinsames Auftreten auf der Blattebene in einer reduzierten Überlebensrate resultierte.

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Keywords: Insect competition; Plant quality; Leaf-miner survivorship; Null models; Inter-specific associations; Intra-specific associations

Introduction

Patterns of distribution of insects on plants are strongly determined by host plant variation. A question of great interest in herbivory-related studies is how plant traits affect attack rates by phytophagous insects and how variation in plant quality affects the distribution and performance of herbivores both within and among host plants. Recently, it has become clear that differences between individuals can have profound effects upon the kind of dynamics exhibited by herbivorous populations, their stability and their mean levels of abundance (Crawley & Akhteruzzaman, 1988) and the study of individual variation and its consequences for population dynamics has become a priority. For folivorous insects, it is not unusual for populations to be aggregated on their host plants (Stanton, 1983; Faeth, 1990). For leaf-mining insects, oviposition site selection by females may be highly influenced by variation in leaf structure (Reavey & Gaston, 1991), leaf age and size (Faeth, 1991; Faeth, Mopper, & Simberloff, 1981), leaf chemistry (Stiling, Brodbeck, & Strong, 1982) and effects of the third trophic level (reviewed by Connor & Taverner, 1997) and leaf miners usually show non-random distributions among plants and among leaves within an individual tree (Shibata et al., 2001; Stiling, Simberloff, & Anderson, 1987). One recurring explanation for clumped patterns of leaf miners in particular is that insect distribution reflects variation in nutritional quality and/or secondary chemistry among and within the host plants (Faeth, 1990). Because leaf quality is a major determinant of host choice by many herbivores (Strong, Lawton, & Southwood, 1984), variations in leaf quality are expected to influence leaf-miner distribution, abundance and survivorship.

Besides variations in leaf quality, interactions with other herbivores, including external and other internal feeders may affect the distribution and abundance of leaf-mining insects. The distribution of endophagous

insects, such as gall formers and leaf miners, is typically aggregated and when selection for tissues of better nutritional quality does occur, competition between insects that utilize the same type of resource can arise. The role of intra- and inter-specific competition among insects in ecological theory has changed throughout the years, from the argument that competition was weak and infrequent on phytophagous insect communities in the early 80s, to the resurrection of the importance of competition between phytophagous insects in the 90s (Denno, McClure, & Ott, 1995; Reitz & Trumble, 2002). For leaf miners, competition may arise from the presence of conspecifics and/or from the presence of other feeding guilds, such as gall formers and free-feeding herbivores. The quality of resources available to leaf miners, and hence their performance, may be modified by the feeding of other folivores (Faeth, 1992). Leaf chewers, in particular, can affect the foliage on which leaf miners are feeding by changing plant quality and by altering or redirecting plant resources (Shibata et al., 2001). Damage caused by leaf chewers can, for example, lead to an increase in secondary compounds, such as phenolics, and a decrease in nitrogen concentration (Hartley & Lawton, 1992). Gall formers also have the potential to modify plant quality by physically and/or chemically modifying plant vasculature, architecture and nutritional quality (Johnson, Mayhew, Douglas, & Hartley, 2002), and some studies have demonstrated that galled leaves exhibit higher nutritional quality than neighboring non-galled leaves (e.g., Abrahamson & Weis, 1986, but see Hartley & Lawton, 1992). For sessile insects and free-feeding herbivores in general, most of the studies that indirectly addressed inter-specific competition by analyzing species co-occurrence have used presence-absence distributional data as a surrogate for competition (e.g., Kagata & Ohgushi, 2001; Stiling, Rossi, Catell, & Bowdish, 1999, and examples in Denno et al., 1995), although more recent studies advocate for the use of more refined statistical tests based on random distributions such as

null models (e.g., Gotelli & Graves, 1996; Ribas & Schoereder, 2002; Zwölfer & Stadler, 2004).

The purpose of this study was to investigate how differences in plant phenology and nutritional quality influence the distribution and abundance of leaf miners on *Quercus geminata* (Fagaceae) and *Quercus laevis*, both between and within plants. We also aimed to examine the effects of other herbivores co-occurring on the same host plants on both the abundance and survivorship of the most common leaf miners on both oak species. The specific aims of this study were: (1) to examine variation in the spatial distribution of leaf miners among plants, within plants and according to canopy position, (2) to determine how leaf-miner abundance was affected by plant nutritional quality in terms of foliar nitrogen, water content, tannin concentration and leaf toughness, (3) to observe how the presence of other herbivore guilds such as chewers and gall formers affect the abundance and survivorship of leaf miners on oaks, and (4) to determine whether herbivore guilds and herbivore species co-occur significantly less or more than expected on oak host plants using null models.

Study systems

The sand live oak, *Q. geminata* (Fagaceae), is a semi-evergreen oak associated with sand hill and scrub communities in Florida and, typically, old leaves abscise and new leaves appear in late April or early May, reaching full size in approximately 2 weeks. *Stilbosis quadripustulatus* (Lepidoptera: Cosmopterygidae) is a moth whose larvae induce mines on the adaxial surfaces of *Q. geminata*. *S. quadripustulatus* is a univoltine species, whose adults emerge in early summer (from May to June) from pupae that overwinter in soil and litter. Oviposition occurs approximately in early June, when females oviposit at the junction of the midvein and a major lateral vein. Larvae take from 60 to 90 days to complete their 5 instars and mines may reach 3.0 cm in length (Simberloff & Stiling, 1987). Many other herbivores compose the insect community associated with *Q. geminata* (Supplementary Plate S1). Leaves are frequently found damaged by chewing insects such as the eastern buck moth *Hemileuca maia* (Lepidoptera: Saturniidae), and at least four cynipid species (Hymenoptera: Cynipidae) of galling insects are commonly observed on sand live oak leaves and stems, but *Andricus quercusfoliatus* and *Disholcaspis quercussuccinipes* are the most common stem galls. *Andricus quercusfoliatus* induces white flower-like galls on sand live oak stems, whereas *D. quercussuccinipes* wasps induce clusters of 5–20 yellowish brown galls usually crowded around a terminal oak twig. Unidentified eyespot galls (Diptera: Cecidomyiidae) are the most common leaf galls and are recognized as circular spots, usually 8–10 mm in diameter. The adults emerge from the soil in the spring and lay eggs in the upper leaf surface. As the larva grows,

the leaf tissue surrounding it swells slightly and red rings are seen around the galls. Larvae complete their development in 8–12 days and pupate in the soil. This is the most common gall found on sand live oak leaves, often reaching densities of 5 galls per leaf.

The turkey oak *Q. laevis* is one of the characteristic trees associated with the sand hill community over much of Florida. *Q. laevis* is a moderately fast to fast-growing tree and presents deciduous simple leaves, alternately arranged with usually five lobes, although this number may vary from 3 to 7. *Acrocercops albinatella* (Lepidoptera: Gracillariidae) is a microlepidopteran species whose larval stages feed on young leaves, creating distinct linear-blotch mines on the lower surface of *Q. laevis* leaves (Supplementary Plate S2). Larvae typically feed on the palisade parenchyma cells and deposit frass throughout the mine, completing their development in approximately 10 days. Larvae emerge from the blotch mine and usually pupate on the same leaf from which they emerge (T. Cornelissen, pers. obs.). *Brachys tessellatus* (Coleoptera: Buprestidae) is a univoltine species that also forms distinct blotch mines in *Q. laevis* leaves. The adults emerge in Mid-March to Mid-April, coinciding with budburst of turkey oak. Adults initially feed on the early leaves and flowers until mating and oviposition. Eggs are deposited singly on the upper surface of the leaves and after hatching the larvae mine into the mesophyll creating distinct, characteristic damage. *Brachys* in our study sites go through two generations and the first mines appear in early April and remain active until late June, when larvae complete their development and exit mines to pupate on the soil. New adults emerge in early July and oviposit to form new *Brachys* mines that remain active until September–October. Pupation and overwintering of this second generation occurs within the leaves after they have senesced and abscised from the tree. New adults emerge from the leaf litter in the following spring (Waddell, Fox, White, & Mousseau, 2001). Turkey oak leaves are also attacked by a vast array of free-feeding herbivores, such as the eastern buck moth *H. maia* and the white tussock moth *Orgyia leucostigma* (Lepidoptera: Lymantriidae). Other common leaf miners are undetermined species of *Stigmella* (Lepidoptera: Nepticulidae) and *Cameraria* (Lepidoptera: Gracillariidae). Eyespot galls are the most common leaf galls observed in turkey oak leaves in our field sites and no stem galls have ever been recorded.

Methods

Data collection

This study was conducted between February and November of 2003 at the University of South Florida

Botanical Garden. To examine the effects of plant phenology and nutritional quality on the abundance of leaf miners and other herbivores, 20 individuals of *Q. geminata* and 20 individuals of *Q. laevis* were marked in February, just before budbreak and leaf flush. *Q. geminata* trees ranged from 1.9 to 2.5 m in height and *Q. laevis* trees ranged from 2.5 to 3.2 m in height. On each individual plant, 5 upper-canopy (above 2.0 m) and 5 lower-canopy (below 1.5 m) branches were selected and tagged. Selected branches had similar number of leaves per branch (*Q. geminata*: range 28–45 leaves, mean number \pm SE of leaves per branch: 36.4 ± 3.58 ; *Q. laevis* range: range 9–15 leaves, mean number \pm SE of leaves per branch: 11.21 ± 1.69) and all leaves on each branch were individually numbered using a permanent marker. A total of 6489 marked leaves on *Q. geminata* and 2243 marked leaves on *Q. laevis* were followed over the season. Leaves were marked at the time of leaf flush when all leaves were still intact, and examined monthly for the appearance of leaf miners, gall formers and damage by chewers. For *Q. geminata*, we suggest that most chewing damage was caused by *H. maia* at the beginning of the season, and for *Q. laevis* chewing damage was mainly caused by *H. maia* and *O. leucostigma*, although we cannot rule out the possibility that other chewing caterpillars also caused some damage to the leaves of both oak species.

To assess variation in host plant quality among trees, water content, foliar nitrogen concentration, tannin concentration and leaf toughness were evaluated monthly for each individual plant. On each collection date, 10 undamaged leaves (5 from the upper-canopy and 5 from the lower-canopy) were sampled from each tree, placed immediately on ice, and leaf toughness was evaluated using an Effegi FT-011 penetrometer (International Ripening Co, Italy). Water content was quantified by the difference between leaf wet and dry weights and leaves were further oven-dried and milled to a fine powder. Tannins were extracted from 50 mg of dry tissue, and tannin concentration was quantified using the radial diffusion assay with three replicates per leaf (for details see Hagerman, 1987). Nitrogen content was determined using a CE Instruments NC2100 CN Analyzer (CE Elantech, Incorporated, Lakewood, New Jersey, USA).

To assess the effects of conspecifics and other herbivores on the survivorship of the most common leaf miners on both oak species, on each individual plant, we noted leaves that exhibited each of the following combinations: *Q. geminata*: (1) one *Stilbosis* mine, (2) two *Stilbosis* mines, (3) three or more *Stilbosis* mines, (4) one *Stilbosis*, one *Brachys* mine, (5) one *Stilbosis* and one or more eyespot galls, (6) one *Stilbosis* mine and leaf damage by chewers. Although *Brachys* mines occur mainly on *Q. laevis*, some mines have been observed on *Q. geminata* where both plant species

co-occur (T. Cornelissen, pers. obs). *Acrocercops* was never recorded in *Q. geminata* in our field sites. For *Q. laevis* the following combinations were investigated: (1) one *Acrocercops* mine, (2) two or more *Acrocercops* mines, (3) one *Brachys* mine, (4) two or more *Brachys* mines, (5) one or more *Acrocercops* and one or more *Brachys* mine, (6) one or more *Acrocercops* mine and damage by chewers, (7) one or more *Brachys* mine and damage by chewers. The fate of leaf miners on each combination was followed over the season, with observations conducted at bi-weekly intervals ($n = 598$ marked leaves for *Q. geminata* and 708 leaves for *Q. laevis*) and leaf-miner survivorship and mortality were scored for each leaf combination throughout the season. Survival rates were determined as number of emerged adults. Leaf miners offer a great opportunity to assess population mortality factors since a record of the miner success is clearly observed on the leaves: parasitized mines have parasitoids inside, tiny circular exit holes on mine's surface and/or parasitoid pupae attached to the larva. Mines that were preyed upon are usually found ripped open and the larva is missing. Successfully emerged larvae of *Acrocercops* cut open the mines and pupate usually on the same leaf where the mine developed. *Brachys* larvae pupate inside mines and/or cut circular holes on the underside of the leaf. *Stilbosis* mines cut semi-circular holes at the mine edge and larvae exit to pupate in the soil.

Data analysis

To examine the spatial distribution of leaf miners both between and within individual plants, the distribution of leaf miners was compared to random (Poisson) and clumped (negative binomial) distributions using a χ^2 analysis (Ludwig & Reynolds, 1988), with individual plants and branches within plants used as sampling units. We also calculated indexes of dispersion (variance-to-mean ratio) and Green's modified index of clumping (Green, 1966) for each data set. The variance and mean are equal in a theoretical Poisson distribution and the index of dispersion was calculated as the ratio between the mean and the variance as $ID = s^2/\bar{x}$, where s^2 and \bar{x} are the variance and sample mean, respectively. Significant departures of ID from the value of 1.0 were tested using an approximation of a χ^2 statistic as

$$\chi^2 = \frac{\left(\sum_{i=1}^N (x_i - \bar{x})^2\right)}{\bar{x}},$$

where x_i is the number of individuals in the i th sampling unit and N is the total number of sampling units. For small sample sizes ($N < 30$), the calculated χ^2 is a good approximation to a χ^2 with $N-1$ degrees of freedom. Green's index of clumping was calculated as $GI = [(s^2/\bar{x}) - 1/n - 1]$, where $n =$ sample size. GI

varies between 0 (for random) and 1 (for maximum clumping).

To test for differences in nutritional quality among plants and between leaves at different canopy positions, we averaged toughness, water, nitrogen and tannin contents for each plant over the season and regressed these values with the abundance of the most common leaf miners and other herbivores, summed over the season. Previous studies on these oak trees demonstrated that there was no tree vs. season interaction (see Cornelissen & Stiling, 2006), i.e., individual trees were consistent in quality throughout the season, allowing the use of tree average as a measure of individual plant quality. Differences in leaf quality and herbivore abundance between low and high canopy were tested using a one-way ANOVA. All the variables analyzed were first submitted to Lilliefors's test for data normality and log transformations (nitrogen concentration, tannin concentration, water content) and arcsine root transformations (proportional survivorship among leaves with different herbivore combinations) were employed to stabilize variances and normalize the data. However, for the sake of clarity, figure axes and means (+1SEM) show untransformed data.

To examine the community effects of herbivores on oaks, we first analyzed data on mine survivorship among all the leaf categories as described in the methods. Single ($n = 457$ leaves) and double mines ($n = 377$) were the most frequent combinations, followed by mines and damage by chewers ($n = 228$), and mines and galls ($n = 149$). Differential survivorship among categories was tested using a χ^2 test and proportional survivorship among classes was tested using an ANOVA with individual plants as replicates. Because previous studies have shown non-random distribution of leaf miners and other herbivores on oaks, we also asked whether clumped distributions among individual plants differed among herbivores and among guilds, i.e., if a plant already heavily occupied by a herbivore in particular is avoided or preferred by other herbivores. To test whether herbivores on oaks co-occur significantly more or less than expected at the plant scale we used a null models analysis. Here, observed patterns are randomly generated and a null model is then used to randomize the occurrence of species and to compare the patterns in these “artificial” communities with those in real communities (Ribas & Schoereder, 2002). Data on species distribution among the oak plants were transformed into presence/absence matrices, in which columns are individual plants and rows are herbivore species. Analyses were conducted separately for *Q. geminata* and *Q. laevis*. We used the *C*-score index (Stone & Roberts, 1990) as a metric to quantify the pattern of co-occurrence of leaf miners and gall formers within a presence–absence matrix, as follows: $C = (r_i - S)(r_j - S)$, where r_i and r_j are the row totals, and

S is the number of sites occupied by both species. The *C*-score measures the average number of “checkerboard units” (Gotelli & Entsminger, 1999) and is an index negatively correlated to species co-occurrence. The null hypothesis in this case is that the presence of a given herbivore species does not influence the occurrence of other species and if the index of co-occurrence falls within the 95% frequency distribution of the randomized matrices, the null hypothesis is accepted and the hypothesis of biological mechanisms conditioning the species co-occurrence is rejected (Ribas & Schoereder, 2002). To test these distributions against randomized matrices, we used a fixed–fixed model, with 5000 iterations, in which the row and column sums of the original matrix are preserved. This algorithm was chosen for the fact that it has a low frequency of Type I and Type II errors (Gotelli & Ellison, 2002) and random matrices were created using a swapping algorithm, in which the original matrix is shuffled through repeated swapping of random submatrices. All analyses were conducted using EcoSim (Gotelli & Entsminger, 1999). Analyses were conducted only for leaf miners and gall formers due to their sessile habit and for the fact that leaf chewers move freely among plants and should be less influenced by the clumped distribution of endophagous herbivores.

Results

Testing the data against the null hypothesis of a Poisson (random) distribution indicated that all leaf-miner species studied were not randomly distributed among plants (*Acrocercops*: $\chi^2 = 52.68$, $df = 11$, $P \geq 0.05$; *Brachys*: $\chi^2 = 43.86$, $df = 10$, $P \geq 0.05$; *Stilbosis*: $\chi^2 = 68.45$, $df = 13$, $P \geq 0.05$) and within branches on individual plants (*Acrocercops*: $\chi^2 = 711.72$, $df = 5$, $P \geq 0.05$; *Brachys*: $\chi^2 = 135.11$, $df = 5$, $P \geq 0.05$; *Stilbosis*: $\chi^2 = 744.3$, $df = 6$, $P \geq 0.05$). The distribution of all leaf miners, however, did fit a negative binomial distribution, suggesting clumped distributions both among plants (*Acrocercops*: $\chi^2 = 11.47$, $df = 10$, $P \leq 0.05$; *Brachys*: $\chi^2 = 5.187$, $df = 8$, $P \leq 0.05$; *Stilbosis*: $\chi^2 = 7.713$, $df = 9$, $P \leq 0.05$) and within branches on individual plants (*Acrocercops*: $\chi^2 = 14.88$, $df = 14$, $P \leq 0.05$; *Brachys*: $\chi^2 = 7.11$, $df = 9$, $P \leq 0.05$; *Stilbosis*: $\chi^2 = 9.37$, $df = 13$, $P \leq 0.05$). Indices of dispersion and Green's index corroborated the clumped distribution of leaf miners on both *Q. laevis* (*Acrocercops*: $ID = 8.03$, $\chi^2 = 152.58$, $P \geq 0.05$, $GI = 0.741$; *Brachys*: $ID = 4.40$, $\chi^2 = 83.62$, $P \geq 0.05$, $GI = 0.853$) and *Q. geminata* (*Stilbosis*: $ID = 5.19$, $\chi^2 = 1008.3$, $P \geq 0.05$, $GI = 0.727$).

No significant differences in nutritional quality were observed between leaves from the lower- and upper-canopy for both plant species (all $P > 0.05$), although *Q. geminata* leaves tended to be softer in the lower

canopy (average toughness lower-canopy leaves: 0.71 ± 0.023 , average toughness high-canopy leaves: 0.84 ± 0.072), and *Q. laevis* upper-canopy leaves tended to exhibit higher tannin concentration (average tannin concentration 0.286 ± 0.091) than lower-canopy leaves (average tannin concentration 0.223 ± 0.11). Although leaf-miner abundance tended to be higher in lower-canopy leaves compared to upper-canopy leaves for both oak species, these differences were not statistically significant (all $P > 0.05$).

For both oak species, we observed seasonal trends in plant quality, with a decrease in nitrogen concentration over the season, as well as an increase in toughness and tannin concentration (Fig. 1). For *Q. geminata*, we observed higher *Stilbosis* density in plants with more nitrogen and softer leaves (Fig. 2), whereas *Q. laevis* plants with higher nitrogen and lower tannin concentration supported significantly higher densities of the first generation of *Brachys* (Fig. 3). None of the plant quality variables analyzed in this study affected the abundance of *Acrocercops* mines on *Q. laevis* (all $P > 0.05$), as well as eyespot galls, *Andricus* galls and chewed leaves on *Q. geminata*. For *Q. laevis*, however, a higher percentage of damage by chewers was observed in individual plants with softer leaves ($r^2 = 0.21$, $P = 0.04$), but no effects of variation in plant quality affected the variation in eyespot galls among plants (all $P > 0.05$) or the variation of other leaf miners among individual turkey oaks.

Survivorship of leaf miners was highest when mines were single on leaves, compared to double mines or mines occurring on leaves that were also chewed

($\chi^2 = 14.69 - 19.55$, all $P < 0.05$). Lowest survivorship rates were observed for *Stilbosis* mines occurring on *Q. geminata* leaves with one or more eyespot galls ($F_{1,18} = 16.44$, $P = 0.032$). For the community of herbivores on both oak species, null models indicated that both leaf miners and gall formers co-occurred at the plant scale (Fig. 4) and there was no evidence for competitive exclusion between and within guilds, as indicated by *C*-score indices falling within the 95% limits of frequency distribution of the randomized matrices (Table 1).

Discussion

Our data showed that leaf miners on both oak species were not randomly distributed, but clumped among host plants and within branches on individual plants. Several factors could potentially select for non-random distribution of leaf miners. Bottom-up factors such as foliar nitrogen concentration and secondary chemistry have been frequently invoked as potential factors affecting the distribution, abundance and survivorship of phytophagous insects and leaf miners in particular (e.g., Eber, 2004; Faeth, 1991; Stiling et al., 1982). Our data corroborates the hypothesis that leaf miners are, to some extent, affected by bottom-up factors, as plants with higher nitrogen concentration, lower leaf toughness and lower tannin concentration exhibited significantly more mines than plants with lower nutritional quality. It is important to point out, however, that although

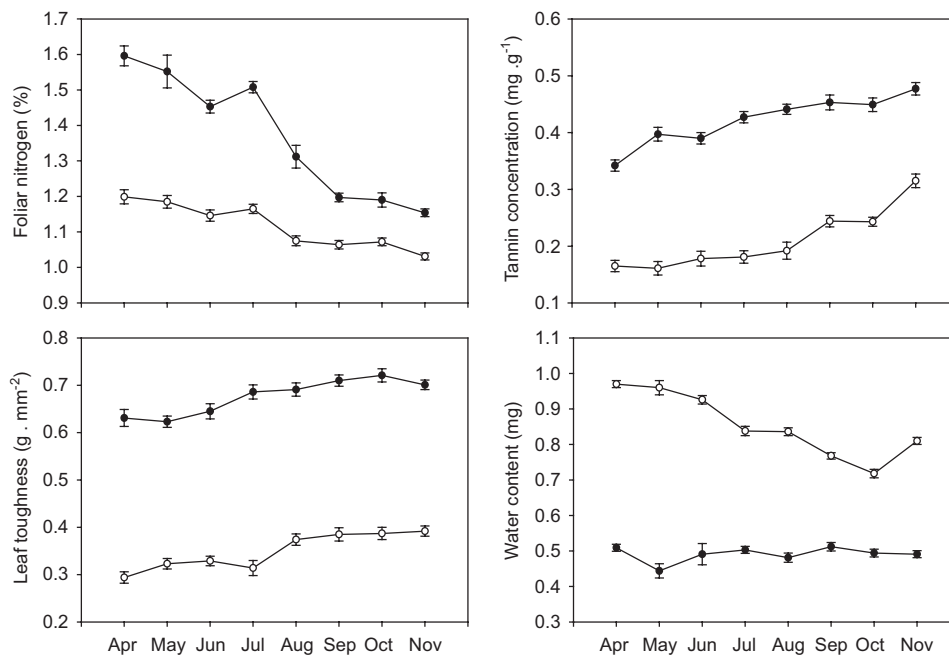


Fig. 1. Temporal variation on the concentration of foliar nitrogen, tannins, leaf toughness and leaf water content for *Q. geminata* (solid circles) and *Q. laevis* (open circles). Data are means (+1SE) of 10 undamaged leaves per individual tree, with lower- and upper-canopy leaves combined.

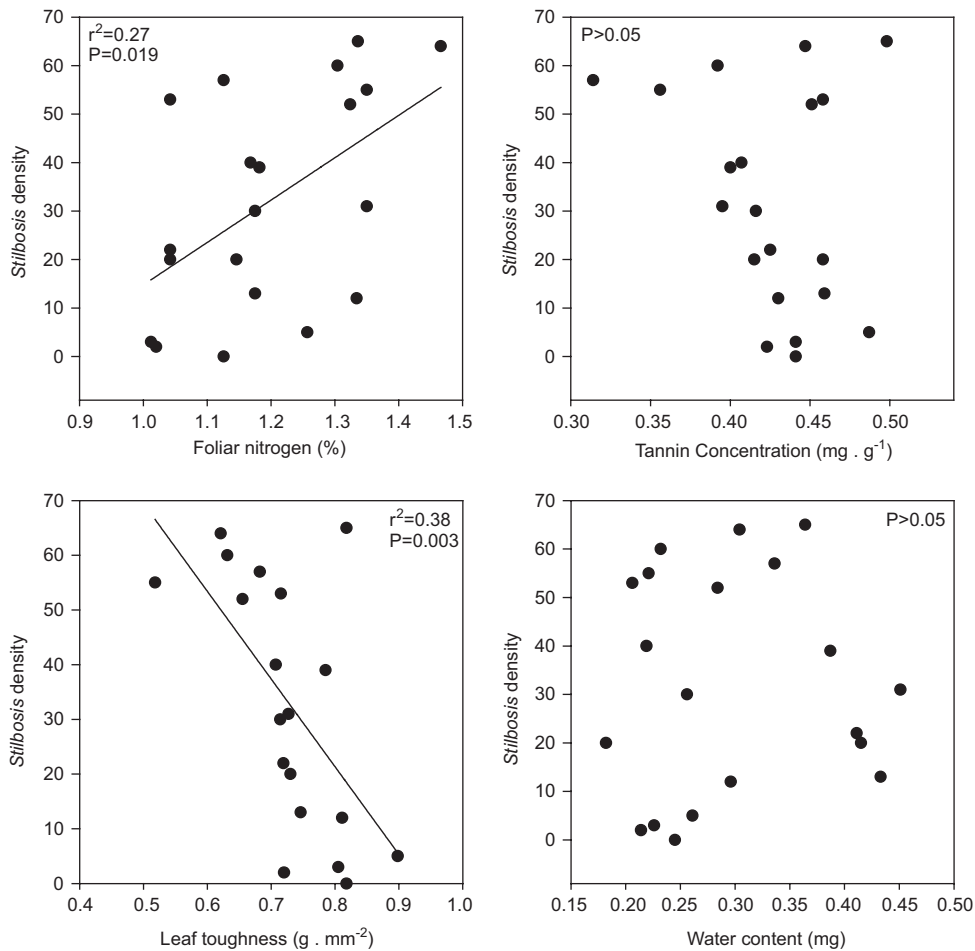


Fig. 2. Relationship between the abundance of *Stilbosis* mines and variation in *Q. geminata* nutritional quality. Data on *Stilbosis* abundance are summed over the season for each individual plant and data on plant nutritional quality are averages over the season.

significant, variations in foliar nitrogen and tannins explained less than 30% of the variation in the number of mines among plants. Also, studies addressing variation in individual phenolic compounds among oak leaves sampled throughout the season (e.g., Feeny, 1970; Salminen et al., 2004) have shown that responses of individual compounds tend to be variable and different seasonal patterns emerge among different phenolic compounds. Feeny (1970) firstly demonstrated a general increase in tannin concentration of *Q. robur* leaves over the summer and he suggested that the levels of hydrolyzable tannins remained approximately constant over the season, whereas condensed tannins tended to increase. Salminen et al. (2004), on the other hand, showed that hydrolyzable tannins decrease in concentration over the season, whereas condensed tannins tend to increase. These results suggest that the significant inverse relationship found between the abundance of *Brachys* mines and tannin concentration found on *Q. laevis* leaves should be interpreted with caution, as we provide only a very gross estimate of phenolic compounds on *Q. laevis* and *Q. geminata*, as detected by

the radial diffusion assay, and we did not provide any quantification of specific phenolic compounds and variation of these over the season in our oak leaves. However, it has been suggested that herbivore performance cannot be determined by phenolic content alone and the impact of phenolics should be interpreted against the background of changes in other nutrients and other leaf features over the season (Haukioja, 2003; Salminen et al., 2004). Our results demonstrated that besides variation in tannins over the season, nitrogen levels tended to decrease over the season, whereas leaf toughness tended to be higher by the end of the season, when water content was relatively low. Variation in nitrogen levels, especially, both among and within plants has been demonstrated to affect leaf miners choice for oviposition sites and larval development (Mattson, 1980; Scheirs, DeBruyn, & Verhagen, 2001, 2002). Faeth (1990), on the other hand, observed that larvae of the leaf-miner *Cameraria* sp. on *Quercus emoryi* were highly clumped at various spatial scales among and within trees, but his study did not support the hypothesis that leaf miners cluster because of variation in plant

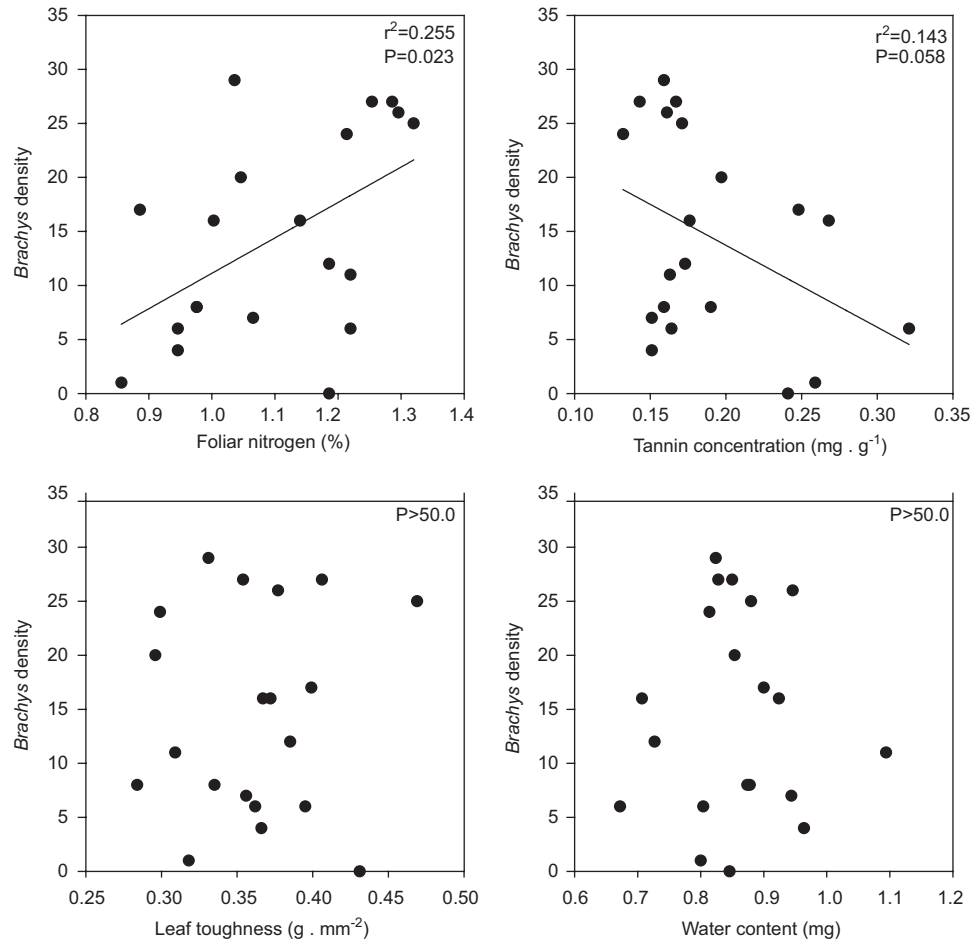


Fig. 3. Relationship between the abundance of *Brachys* mines and variation in *Q. laevis* nutritional quality. Data on *Brachys* abundance are summed over the season for each individual plant and data on plant nutritional quality are averages over the season.

nutritional quality. Although our study showed that leaf miners respond to bottom-up factors, responses to plant quality varied among the leaf miners studied. *Stilbosis* mines were mainly affected by foliar nitrogen concentration and leaf toughness, whereas *Brachys* abundance was influenced by nitrogen and tannin concentration, and none of the plant quality variables affected the abundance of *Acrocercops*. These differential responses might be explained by differences in life-history traits of these leaf miners. *Acrocercops albinatella* causes relatively small linear-blotch superficial mines just under the leaf epidermis of turkey oak leaves and development times do not exceed 10 days. These mines are unlikely to be strongly affected by variations in plant quality due to their fast development rates and the fact that they create limited depth mines in young leaves with higher nitrogen content and lower concentrations of defensive chemicals. *Brachys* and *Stilbosis* mines, on the other hand, are more likely to be affected by host quality, having longer developmental times and full depth mines.

Another factor operating at the leaf scale that potentially selects for non-random distribution of leaf

miners is resource or interference competition. Insects that are relatively immobile seem particularly susceptible to competitive influences because they cannot easily escape from neighboring individuals (Stiling et al., 1987) and results of competitive interactions should then be manifested in their distribution patterns. In a previous study of the distribution of *Stilbosis* mines on *Q. geminata* and *Q. nigra*, Stiling et al. (1987) have shown that fewer mines were found together on the same side of the leaf mid-vein than expected by chance. Intra-specific competition among leaf miners has mostly been neglected as a regulatory mechanism on their population dynamics (Eber, 2004), even though leaf miners are restricted to small “resource islands” represented by individual leaves (Janzen, 1968). Our results have shown that mines usually occurred singly on leaves, but lower survivorship was observed on leaves with double or triple mines. In these categories, 34% of the leaf mines dissected exhibited dead and dry larvae inside the mine. Similar results have been found for other systems (e.g., Auerbach & Simberloff, 1989; Bultman & Faeth, 1986) and interference competition

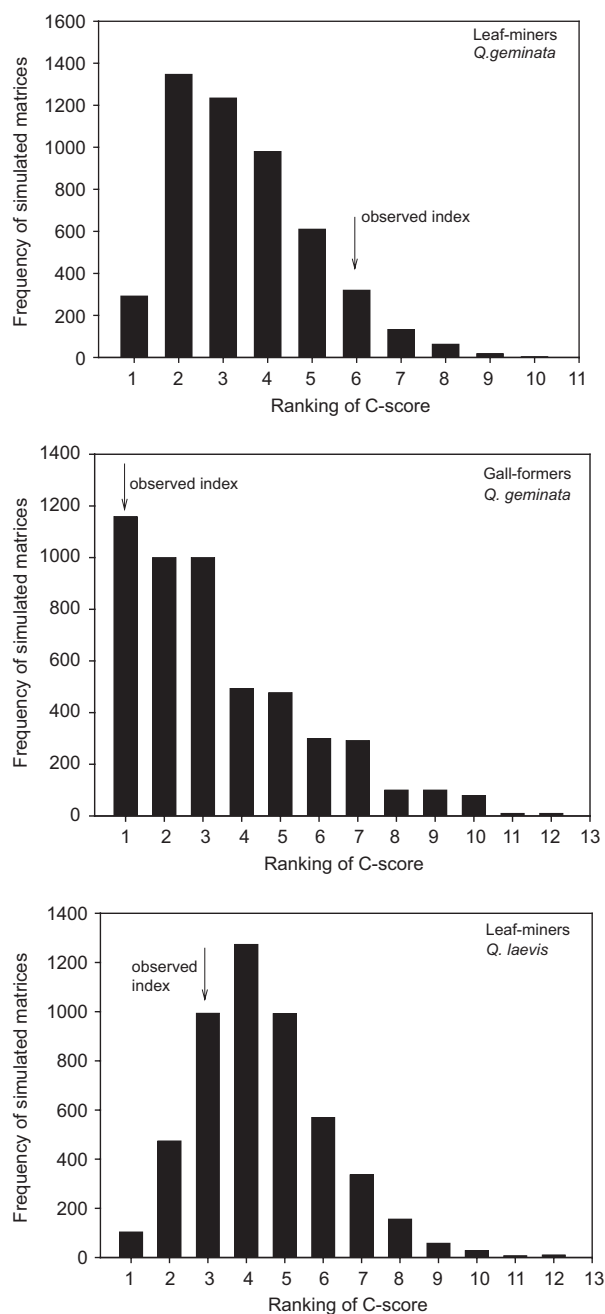


Fig. 4. Co-occurrence patterns of leaf miners and gall formers at the plant scale. The histograms give the frequencies of simulated *C*-scores using a fixed–fixed model. Ranking of *C*-scores are frequency classes of randomized matrices represented by numbers to facilitate scaling. Refer to Table 1 to minimum and maximum numbers of scale. Arrows represent the observed *C*-score indices for each data set. Indices falling within the 95% limits of frequency distribution of randomized matrices indicate co-occurrence, whereas higher *C*-scores represent smaller co-occurrence than expected by chance and lower *C*-scores indicate higher co-occurrence than expected by chance.

or indirect exploitative competition with conspecifics has been suggested as a dominant source of mortality for many leaf-miner species (reviewed by Auerbach, Connor, & Mopper, 1995). We also observed that leaf miners developing on leaves that were also damaged by chewers experienced lower survivorship than mines developing on intact leaves. Previous or concurrent feeding by other phytophages may alter physical and chemical aspects of the leaf or reduce leaf size so that insufficient area remains for development, especially for sessile herbivores such as leaf miners and gall formers. Faeth (1985), for example, observed that *Stilbosis juvantis* mines developing on leaves that were artificially and/or naturally damaged by chewer herbivores also experienced significantly lower survivorship than did miners on intact leaves due to increased parasitism levels. The higher attack rate of parasitoids on mines that were developing on damaged leaves was probably attributable to physical and/or chemical alterations caused by chewing herbivores. The exact mechanism by which damage and intensified parasitism levels interact is unclear, although physical, visual, and chemical cues associated with damaged leaves may influence parasitoid searching behavior and oviposition preferences (Faeth, 1985).

Variation and changes in resource quality can lead to different patterns of distribution of insect damage, and herbivores might become positively or negatively associated, both intra- and inter-specifically (Fisher, Hartley, & Young, 1999). For the silver birch, *Betula pendula*, several studies have demonstrated a negative association between generalist herbivores and the leaf miner *Eriocrania*, possibly due to direct interference and resource removal (e.g., Bylund & Tenow, 1994; Valladares & Hartley, 1994). In our systems, although intra-specific competition was an important source of mortality for the leaf miners studied, we did not find evidence suggesting that inter-specific competition structured distributional patterns on individual host trees. Although leaf miners were clumped among host trees, and at the leaf scale competition might reduce leaf-mine survivorship, at the plant scale both leaf miners and gall formers co-occur. These results might be explained by the fact that although leaf miners were clumped among plants, they occurred in low densities in our field sites (mean \pm 1SE per 200 leaves in 35 plants: *Acrocercops*: 36.7 ± 3.49 ; *Brachys* first generation: 21.1 ± 2.03 ; *Brachys* second generation: 8.55 ± 1.91 ; *Stilbosis*: 35.64 ± 3.25) and patterns of inter-specific repulsion might be detected only during outbreak seasons.

In conclusion, our results indicated that leaf miners show non-random patterns of distribution both among and within plants and this differential distribution might be determined by variation in several aspects of plant quality. Intra-specific competition is suggested as a regulatory mechanism in the population dynamics of the

Table 1. C-score indices of the randomised and observed matrices for leaf miners on *Q. laevis* and *Q. geminata* and gall formers on *Q. geminata*

Guild	Randomized matrix		Obs. matrix	P-values	
	Minimum	Maximum		Obs. > Exp.	Obs. < Exp.
Leaf miners <i>Q. geminata</i>	11.10	14.70	12.70	0.107	0.921
Gall formers <i>Q. geminata</i>	7.59	10.16	7.50	0.987	0.631
Leaf miners <i>Q. laevis</i>	10.46	12.86	11.01	0.759	0.314

The table shows the minimum and maximum values of the indices calculated for 5000 randomized matrices per data set, together with the observed index and P-values in two-tailed tests (Obs., observed; Exp., expected).

species studied at the leaf scale, although at the plant scale inter-specific associations do not seem to be important mechanisms determining the community structure of these oak herbivores.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.baae.2006.08.007](https://doi.org/10.1016/j.baae.2006.08.007).

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