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Larval Performance and Association Within and Between Two Species of Hackberry Nipple Gall Insects, Pachypsylla spp. (Homoptera: Psyllidae)

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ABSTRACT—We studied patterns of association within and between two species of *Pachypsylla* gall insects (*P. celtidismamma* and *P.* sp. A) on hackberry (*Celtis occidentalis* L.) trees in eastern Iowa. *Pachypsylla* is a recently radiating clade of jumping plant lice (Homoptera: Psyllidae). Exploitative competition has been invoked to account for niche diversification in *Pachypsylla* as new lineages switched from leaves to feeding on different plant parts. Our results suggest that competition did not drive diversification, but could have reinforced it. Focal *P. celtidismamma* gallmakers grew larger when they shared a leaf with more conspecific gallmakers, which is consistent with intraspecific facilitation. Facilitation may result because gallmaker aggregations act as physiological sinks for photosynthate originating elsewhere in the plant. *Pachypsylla celtidismamma* gallmakers were smaller when they shared their gall with more *Pachypsylla* sp. A, which live as inquilines inside *P. celtidismamma* galls; this is consistent with the occurrence of interspecific competition. At spatial scales larger than a single leaf, we detected no significant relationships between larval densities and performance.

INTRODUCTION

The ecology and evolution of phytophagous insects has attracted much attention, in part because of the astounding diversity of many of the clades that have adopted the phytophagous habit. A common prediction is that specialized phytophagous insects should have high diversification rates compared to more generalized or nonphytophagous clades (Price, 1980; Futuyma and Moreno, 1988; Mitter *et al.*, 1988). Several mechanisms for diversification have been suggested, including reciprocal bursts of radiation in plant defensive chemistry and insect ability to exploit defended hosts (Ehrlich and Raven, 1964), sympatric speciation via host race formation (Bush, 1969; Craig *et al.*, 1993), and shifts onto different plant parts or hosts driven by intraspecific competition (*e.g.*, Yang and Mitter, 1994). While there is support for a connection between phytophagy and diversification in general (Mitter *et al.*, 1988; Farrell and Mitter, 1993), little is known of the ecological mechanisms for phytophage diversification in any particular clade (Mitter *et al.*, 1988; Farrell and Mitter, 1993; Heard and Hauser, 1995).

Yang and Mitter (1994) recently examined the systematics of hackberry-galling psyllids (Homoptera: Psyllidae), a recently diversifying clade of specialist phytophagous insects with up to 14 species exploiting hackberry trees. Yang and Mitter (1994) suggested a role for exploitative competition in driving diversification of hackberry gallmakers currently exploiting different plant parts. Under this scenario, reduction in competition favored mutants exploiting plant parts other than leaves (leaf galling being considered ancestral; Yang and Mitter, 1994). Incipient species, presumably, later became isolated because mate choice was connected either ecologically or genetically to gall location (as with host races; Craig *et al.*, 1993). An implicit prediction of this model for hackberry psyllid diversification is that both intraspecific and interspecific competition should be detectable among leaf gallmak-

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ers. However, the frequency and importance of competition among phytophagous insects have been sources of contention (Lawton and Hassell, 1984; Strong, 1984; Strong *et al.*, 1984; Denno *et al.*, 1995), and no ecological data bearing on the existence or strength of competition within or among hackberry psyllid species have been available. We studied patterns of association within and between two species of the most conspicuous and (at our site) most common leaf gallmakers: nipple gall psyllids. We tested the hypothesis that *Pachypsylla celtidismamma* nipple gallmakers experience intraspecific competition and also interspecific competition (from an inquiline *Pachypsylla* species infesting their galls), and that competition would be reflected in negative relationships between gallmaker densities and individual performance.

METHODS

We studied populations of the two hackberry nipple gall insects (*Pachypsylla* spp.) occurring on hackberry (*Celtis occidentalis* L.) trees in Iowa City, Iowa (41°20′N, 91°32′W). Galls of the major hackberry nipple gallmaker, *Pachypsylla celtidismamma* Riley, are initiated on leaves by first instar nymphs in early spring (Beisler and Baker, 1992). Nymphs feed on phloem sap, possibly via a nutritive tissue derived from leaf parenchyma (Beisler and Baker, 1992), until adult emergence in autumn (Tuthill, 1943). The galls protrude from the underside of the leaf, are more or less truncate-conic, and can be large (6 mm diam, 10 mm high). Many *Pachypsylla cetidismamma* galls are multi-chambered, with an easily identified center cell and one to many side cells each inhabited by a single *Pachypsylla* nymph. Yang and Mitter (1994) showed that side-cell individuals belong to a distinct (undescribed) species that is unable to initiate gall formation on its own, living instead as an inquiline inside galls initiated by *P. celtidismamma.* We refer to these two species here as center-cell and side-cell gallmakers, or *Pachypsylla celtidismamma* and *Pachypsylla* sp. A, respectively.

We studied patterns in larval performance and association within and between species of *Pachypsylla* on five hackberry trees in Iowa City. Trees were separated by 1 to 6 km. Hackberries are common street, park and forest trees in eastern Iowa, and galls inhabited by the two *Pachypsylla* gallmakers are abundant (we have observed as many as 28 galls on a single leaf).

We collected hackberry leaves in late September 1996, just before adult emergence. We collected branchlets haphazardly, within 20 feet of ground level, from among available branchlets bearing galled leaves. We defined a branchlet as the twig and all its leaves from the tip back to the junction with the previous year's growth (whether or not this junction was a fork with another twig).

For each branchlet, we recorded the number of leaves and total number of galls, and then chose one galled leaf at random as the "focal" leaf. We recorded leaf size (length in mm) and number of galls for the focal leaf and the four leaves closest to it (two leaves stemward and two tipward). We then calculated measures of gall load per unit leaf on two different spatial scales: "neighborhood" (total number of galls on the nearest two leaves stemward and the nearest two leaves tipward, divided by the sum of their lengths), and "branch" (total number of galls on the branchlet, divided by the number of leaves). The focal leaf was preserved in 70% ethanol for later dissection. We collected approximately 30 focal leaves from each of five trees.

In the laboratory we chose one focal gall at random on each preserved leaf. We then dissected the leaf and measured dry mass (after drying for 48 h at 55 C) separately for each of the following components: the center-cell gallmaker from the focal gall, any side-cell gallmakers in the focal gall, the focal gall tissue, gallmakers of either type in other galls on the same leaf, gall tissue from other galls, and leaf tissue.

TABLE 1.—Variables used in multiple regression predicting dry mass of center-cell galler in the focal gall

Variable	Contribution to model*
Side-cell gallmaker mass in focal gall (mg)	yes
Gallmaker mass from other galls on focal leaf (mg)	yes
Focal gall tissue mass (mg)	yes
Gall tissue mass, other galls on focal leaf (mg)	no
Leaf tissue mass excluding galls (mg)	no
Length of focal leaf (mm)	yes
Number of galls on focal leaf	no
Gall density in 4-leaf neighborhood (galls/mm)	no
Gall density on branchlet (galls/leaf)	no

* Variables contributing to the model are those remaining after backward elimination ($\alpha = 0.10$)

We looked for patterns predicted under the competition hypothesis by seeking predictors among our gallmaker and leaf variables (Table 1) for dry mass of the center-cell gallmaker in the focal gall. We were ultimately interested in intraspecific and interspecific effects on center-cell gallmakers, but we included the other variables in the analyses so they would not confound our results. As a first step, we used multiple regression with backwards elimination (setting α for elimination at 0.10) to winnow the list of possible predictor variables. The four variables retained in the multiple regression model (Table 1) were then used in analysis of covariance (ANCOVA), along with a categorical variable for the five study trees. We were not explicitly interested in differences among trees, but wished to control for such differences in our analysis. We began with an analysis including main effects and all possible interactions, and then used a sequential pooling procedure to improve estimates of error variance. At each step, we tested the highest-order interaction(s) remaining in the model; if they were not significant ($\alpha = 0.05$), we reanalyzed the data pooling those interactions with the error. This new analysis was used to check the next level of interactions, and so on until only significant interactions remained. We report only the final analysis. This procedure is equivalent to the standard treatment of interactions in simple one-way ANCOVAs (Zar, 1984).

In July 1997, we collected a sample of leaves from two of our trees to test for aggregation among gallmakers at a leaf-by-leaf scale. We collected 150 leaves from each tree, haphazardly and without regard for gall load. We counted galls on each leaf and used G tests (Sokal and Rohlf, 1981) to compare the frequency distribution of gall numbers to Poisson distribution with the same means.

All statistical analyses (except the G tests) were conducted with SAS Version 6.04 (SAS Institute Inc., Cary, N.C.) using type III sums of squares.

RESULTS

For the gallmaker performance data set, four of our nine variables were retained by backward-elimination multiple regression (Table 1). These four variables were subsequently included as covariates in ANCOVAs, along with the categorical "tree" variable. The "tree" effect, all four covariates, and a tree-by-gall size interaction all explained significant amounts of gallmaker-size variation (Table 2; model $r^2 = 53.1\%$). No other interactions were significant.

After controlling for tree and leaf effects, center-cell gallmakers were larger in larger galls and in galls sharing a leaf with more gallmakers in other galls; they were smaller in galls

TABLE 2.—ANCOVA results for dry mass of center-cell gallers in focal galls. "Tree" is a categorial variable; all others are continuous (units mm for focal leaf length; mg dry mass for others)

Effect	df	MS	P	Slope	Partial r^2
Tree	4	0.21	0.0001	—	4.1%[1]
Gall size	1	5.76	0.0001	0.018[2]	28.5%
Tree* Gall size	4	0.24	0.012	—	4.8%
Side-cell gallmakers	1	0.98	0.0003	−0.15	4.8%
Gallers in other galls	1	0.41	0.018	0.0160	2.0%
Focal leaf length	1	0.32	0.036	−0.0026	1.6%
Error	132	0.072	—	—	—

[1] Total model r^2 = 53.1%. Partial r^2s sum to only 45.8% because they are based on type III sums of squares and thus exclude variation that can be attributed to more than one independent variable

[2] Estimate of slope pooled across trees; however, test of tree*focal gall tissue interaction reveals significant slope heterogeneity. Table 3 lists slopes estimated for each tree

with more side-cell gallmakers and (surprisingly) in galls on larger leaves (Table 2). However, only ca. 7% of the total variance in focal gallmaker mass was explained by the gallmaker density variables. The relationship between center-cell gallmaker mass and gall size varied significantly among trees (tree by gall size interaction, Table 2), but was always positive (Table 3).

Hackberry nipple galls (and therefore gallmakers of both species) were significantly aggregated at a leaf-by-leaf scale (tree "WP": $G = 7.8$, $P < 0.025$; tree "BB": $G = 20.6$, $P < 0.001$).

DISCUSSION

We found no evidence for interactions among nipple gallmakers on different leaves (dry mass of our focal gallmakers was not predicted by gall load at either neighborhood or branchlet scales). However, we cannot rule out interactions among gallmakers at even larger spatial scales. Interactions among herbivores can be mediated by changes in tissue chemistry induced by herbivory (Denno *et al.*, 1995), and these effects need not be spatially restricted within the plant (*e.g.*, Baldwin and Schultz, 1983; Masters and Brown, 1992).

At a smaller spatial scale, we detected weak ($r^2 < 5\%$) but significant intra- and interspecific effects on performance of center-cell gallmakers. We begin with the intraspecific pattern. Focal center-cell gallmakers were larger when they shared a leaf with more (dry mass) gallmakers in other galls (mostly center-cell gallers, but sometimes including a few side-cell

TABLE 3.—Slopes for dry mass of center-cell gallmaker in focal gall vs. gall size, calculated separately for each tree. Slopes are from multiple regressions including all variables (except "tree") from Table 2

Tree	Slope	P
BB	0.026	<0.0001
BD	0.0075	0.10
CS	0.018	0.0006
LM	0.0128	0.008
WP	0.030	<0.0001

gallers). This positive response suggests intraspecific facilitation. Facilitation of feeding by membership in a local aggregation is known for other phloem-feeding homopterans (Forrest, 1971; Shearer, 1976; Kidd *et al.,* 1985; Freese and Zwölfer, 1996), and probably occurs because aggregations of phloem-feeders can act as physiological sinks (Harris, 1980; Larson and Whitham, 1991; Inbar *et al.,* 1995).

Since center-cell gallmakers show intraspecific facilitation, their strong aggregation is consistent with an adaptive oviposition response by females. However, aggregated distributions are common among insects (review in Heard and Remer, 1997), and can also result from oviposition site preferences (Ives, 1991) or the avoidance of travel costs (Heard, 1998). Our data do not allow us to distinguish among these possibilities.

In contrast to the intraspecific pattern, the interaction between the two *Pachypsylla* species is negative: center-cell gallmakers were smaller when they shared their gall with more side-cell gallmakers. Exploitative competition is the most compelling explanation for this pattern. Galls with more side-cell gallmakers were no larger ($r = 0.03$, $P = 0.76$), and so we suspect that side-cell gallmakers reduce resource availability to the center-cell gallmaker (and/or vice versa). The direct dependence of center-cell galler mass on gall size is consistent with this interpretation, although the very weak ($r^2 = 1.6\%$) negative effect of leaf size is puzzling. Interference competition is less likely because each gallmaker inhabits a separate chamber within the gall (but *see* Riemann, 1961). While the same negative association could arise from side-cell females preferring to oviposit next to small center-cell gallmakers, this seems unlikely and probably maladaptive.

For three reasons, our results (implying competition between *Pachypsylla* species, but facilitation within *P. celtidismamma*) are important to our general understanding of community structure and diversification in phytophagous insects. First, our results add to the volume of evidence that interspecific competition should not be neglected in the study of phytophage communities (Denno *et al.,* 1995). Second, some authors have held that intraspecific competition is a necessary condition for the occurrence of interspecific competition (*e.g.,* Strong *et al.,* 1984: 121), but our *Pachypsylla* results indicate that this is not so. Karban (1989) reported a similar result: interspecific competition between spittlebugs and plume moths on fleabane, but facilitation among spittlebugs. Finally, *Pachypsylla* gallmakers have been used as a model system for studying the relative importance of specialization and the galling habit in driving diversification among phytophagous insects. Yang and Mitter (1994) suggested that competition may have been involved in diversification within hackberry *Pachypsylla.* Our data are consistent with competition between center-cell and side-cell gallmakers, but not with the intraspecific competition necessary for competition alone to have driven diversification. We cannot exclude, however, a reinforcing role for competition after divergence achieved by other means.

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