

## HOST PLANT QUALITY AND LOCAL ADAPTATION DETERMINE THE DISTRIBUTION OF A GALL-FORMING HERBIVORE

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**Abstract.** Herein we report results of transplant experiments that link variation in host plant quality to herbivore fitness at the local scale (among adjacent plants) with the process of local (demic) adaptation at the landscape scale to explain the observed distribution of the specialist gall former *Belonocnema treatae* (Hymenoptera: Cynipidae) within populations of its host plant, *Quercus fusiformis*. Field surveys show that leaf gall densities vary by orders of magnitude among adjacent trees and that high-gall-density trees are both rare (<5%) and patchily distributed. *B. treatae* from each of five high-gall-density trees were reared on (1) the four nearest low-gall-density trees, (2) the four alternative high-gall-density trees, and (3) their natal trees (control). Each treatment (source × rearing site) was replicated three times. Nine components of performance that sequentially contribute to fitness were evaluated with over 21 000 galls censused across the 25 experimental trees. When reared on their natal trees and compared with low-gall-density neighbors, transplanted gall formers had higher gall initiation success ( $P < 0.05$ ), produced more ( $P < 0.001$ ) and larger galls ( $P < 0.001$ ), and produced a higher proportion of galls that exceeded the threshold size for natural enemy avoidance ( $P < 0.05$ ). Comparison of gall-former performance on natal vs. alternative high-gall-density trees demonstrated significant ( $P < 0.001$ ) differences in six performance measures with five differing in the direction predicted by the hypothesis of local adaptation. Overall, these linked experiments document direct and indirect effects of host plant variation on gall-former performance and demonstrate convincingly that (1) high-gall-density trees equate to high-quality trees that are surrounded by trees of relatively lower quality to the herbivore and (2) gall-former populations have become locally adapted to individual trees.

**Key words:** *Belonocnema treatae*; Cynipidae; deme formation; gall former; host plant quality; local adaptation; mixed-effects ANOVA; *Quercus fusiformis*; spatial ecology; tri-trophic interactions.

### INTRODUCTION

Host-specific phytophagous insects exhibit a near-universal pattern of patchy distribution within populations of their host plants. Variation in host plants influence distribution and abundance (i.e., Larsson et al. 2000) and can lead to genetic substructuring of insect populations (Mopper and Strauss 1998). Host plant quality, measured by estimates of insect fitness, can be influenced by genotype (Carr and Eubanks 2002), age (Mopper et al. 2000), leaf phenology and abscission (Stiling et al. 1991, Mopper and Simberloff 1995), hypersensitivity (Fernandes 1998), and constitutive, induced, and structural defenses (Rosenthal and Berenbaum 1992, Bodnaryk 1996, Agrawal 1999). Given the range of interplant variation, high-quality plants or patches represent phenotypic or genotypic islands within a sea of lesser-quality hosts (Janzen 1968).

Edmunds and Alstad (1978) linked microgeographic variation among neighboring conspecific plants to herbivore fitness variation and proposed that insect

populations can become locally adapted to individual host plants. Strong selection and reduced gene flow can generate and maintain genetic substructure in insect populations (demes) at the scale of the individual plant (Boecklen and Mopper 1998). Evidence that selection can generate localized (demic) adaptation in insect herbivore populations at the scale of individual host plants has been documented in four orders (Mopper and Strauss 1998). However, although the evidence is taxonomically diverse, the meta-analyses by Van Zandt and Mopper (1998) and the review by Mopper (2005) of studies that employed reciprocal transplants and sought evidence of demic adaptation at the scale of individual plants showed that the evidence is not deep. Only six of 11 species studied and seven of the 12 experiments supported the hypothesis of demic adaptation. Together, these analyses suggest the need for studies of insect–plant interactions with features (both plant traits and insect life history attributes) that may promote demic adaptation via their effects on limiting gene flow and generating localized strong selection.

Selection from the host plant may be particularly strong for gall formers, as gall formers are restricted to feeding on highly modified nutritive tissue within individual host plants (Stone et al. 2002), and thus larval fates are dictated by maternal host selection. Gall

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formers require host tissues at specific developmental stages to initiate gall induction (Weis et al. 1988). As a consequence, selection may favor synchronization of adult emergence with the appearance of specific tissues when such tissues are restricted in time (e.g., phenology of bud break and leaf growth [Mopper and Simberloff 1995]) or as seen in some leaf galls that gall formers complete development prior to leaf abscission (Waddell et al. 2001). As a result, microgeographic variation in selection might be especially important when resource availability due to host plant phenology varies within populations (Mopper 2005).

Selection from natural enemies can further amplify differences among host plants when the interaction between the host plant and the insect affect natural enemy success (Price et al. 1980, Mopper et al. 1995). Gall formers in particular are under such selection, as gall size (Abrahamson and Weis 1997) and development time (Biggs and Latta 2001) are determined by the interaction of plant and insect genomes, and gall defensive traits directly influence mortality from parasitoids (Weis et al. 1992, Stone and Schönrogge 2003). Given that mortality from natural enemies is typically high for gall-forming cynipids (Stone et al. 2002), natural enemy pressure on gall traits may generate selection that further drives adaptation to individual host plants.

In the three-part analysis presented herein we (1) document the extreme variation among adjacent trees in leaf gall densities and the spatial pattern of high- and low-leaf-gall-density trees, and then investigate how (2) variation in plant quality at the local scale (Experiment 1) and (3) deme formation at the landscape scale (Experiment 2) influence the distribution and abundance of this phytophagous insect in two linked experiments. In Experiment 1, we used a one-way transplant to compare nine components of gall-former performance when reared on their high-gall-density (natal) trees and their low-gall-density neighbors to determine if gall density equates with host plant quality at the local scale. In Experiment 2, we used reciprocal transplants to compare performance measures for wasps reared on their natal and alternative high-gall-density trees to test for local adaptation. By simultaneously examining the effects of among-plant variation in plant quality at the local scale, and the consequences of demic adaptation on herbivore fitness at the patch scale, and by linking these two experiments via the shared high-gall-density trees, we are able to examine the individual and interactive effects of each on herbivore fitness and explain the highly variable distribution of the gall former.

## MATERIALS AND METHODS

### *Study system*

*Belonocnema treatae* exhibits a heterogonous life cycle typical of cynipids (Askew 1984) with temporally segregated sexual and asexual generations (Lund et al. 1998). Coincident with *Quercus fusiformis* bud break

and continuing through spring leaf flush, the short-lived sexual generation emerges from multilocular galls on the tree's roots. Females, who do not feed as adults and who emerge with eggs matured, mate immediately, fly to the canopy, and oviposit in the lateral veins on the undersides of immature leaves. Each ovipositor insertion leaves a permanent scar (i.e., a record of each oviposition attempt). Spherical, unilocular galls begin growth following leaf maturation in May and June. Gall growth proceeds until the gall former stops feeding and galls lignify (August to October). The asexual generation emerges from leaf galls from October to December and descends to the ground to oviposit into the tree's roots, inducing the multilocular root galls. *B. treatae* leaf gall density varies dramatically among trees within populations (Galusky 2000), a pattern common in the Cynipidae (Stone et al. 2002).

The distribution of terminal leaf gall sizes is bimodal in nature (Hall 2001, Reynolds 2001). Host plant variation directly or indirectly contributes to this bimodal distribution in three ways. By monitoring the emergence of *B. treatae* from galls individually isolated in gel capsules Lund (1998), Hall (2001), and Reynolds (2001) described the functional relationship between final gall size and the probability of *B. treatae* emergence from galls exposed to or protected from natural enemies throughout development. When protected from natural enemies, based on the weighted means of the combined results of the above studies (replicated across years and sites), the probability of emergence of a gall former as a function of gall size rises to  $\geq 5\%$  only when galls attain a size of  $\geq 2.62$  mm. Thus we define 2.62 mm as the "tree threshold" to partition plant phenotypic or genotypic effects that limit gall growth and/or extrinsic factors that act through the plant to limit gall growth (plant-mediated effects) from the effects of parasitoids on gall size. The percentage of galls that exceeds the tree threshold thus measures plant-mediated contributions to gall-former death and varies among trees. Second, the mean size of galls that produce adult gall formers varies among trees, and gall size influences the probability of *B. treatae* emergence from galls. Third, although *Q. fusiformis* retains the majority of annual leaves until early spring, leaf abscission occurs throughout the year. In this gall-former system, leaf abscission halts gall development, reduces gall size, results in gall-former death in early stage galls, and significantly reduces gall-former emergence from later stage galls.

*B. treatae* larvae are attacked directly or indirectly at all stages of gall growth per gall-former development by 24 species of parasitoids, hyperparasitoids, inquilines, and predators (Lund et al. 1998, Hall 2001). Adult *B. treatae* do not emerge from galls from which any of the above community members emerge (Hall 2001). Thus, all other insects that use the leaf gall are natural enemies. Mass rearing of leaf gall occupants from galls exposed to natural enemy attack demonstrates low emergence rates of the gall former across sites and years (e.g., 0.5% in

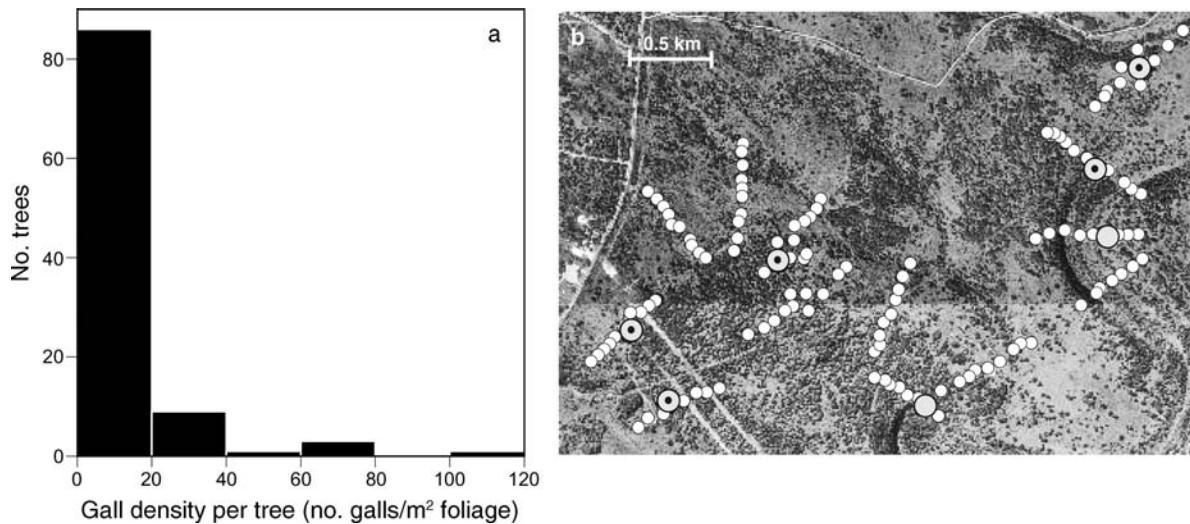


FIG. 1. (a) *Belonocnema treatae* leaf gall densities (galls/m<sup>2</sup> foliage) on 100 live oak trees sampled along 13 transects in 2001 at the study site. (b) Spatial distribution of high- and low-density trees mapped onto a satellite photo of the study site. Gray circles with black borders are high-density trees. Gray circles with a smaller black circle within are five high-density trees used in Experiments 1 and 2. Small white circles with black borders are low-density trees.

1996, 1.0% in 1997, 1.0% in 2000; Lund 1998, Hall 2001). Gall size at maturity affects the probability of gall former emergence (1 – susceptibility to parasitism) in tri-trophic systems (Abrahamson and Weis 1997) and within this system. Lund (1998), Hall (2001), and Reynolds (2001), working at the same site used for the studies reported herein and using the same gel capsule technique described earlier, showed that, when exposed to parasitism throughout development, 95% of *B. treatae* emerged from galls in the upper 50% of the gall size distribution and that the probability of gall-former emergence increased exponentially with increasing gall size. (The  $R^2$  and  $P$  values for all regressions of emergence on gall size were  $\geq 0.90$  and  $< 0.01$ , respectively.) Based on the weighted mean of the above studies, a gall diameter of  $\geq 5.82$  mm is defined as the *natural enemy size threshold*. This threshold value estimates the minimum size galls must attain to have a  $\geq 5\%$  probability of producing a gall former in the presence of natural enemies.

The host plant, plateau live oak *Q. fusiformis*, Small (Muller 1961), is a late-seral-stage tree reaching heights of 25 m, but more typically forming clonal clusters under 10 m in height. Some authors (e.g., Owens 1996) consider *Q. fusiformis* to be a subspecies of the more southeasterly distributed Virginia live oak *Quercus virginiana* (Nixon 1997). An expansive hybrid zone spanning southeast Texas is described between *Q. virginiana* and *Q. fusiformis* (Nixon 1997). *B. treatae* is found on both species and throughout the putative hybrid zone (J. Ott, *personal observation*). *Q. fusiformis* is generally restricted to the Edwards Plateau of central Texas (Nixon 1997). All research described herein was conducted on Texas State University's Freeman Ranch in Hays County, Texas, USA (29°55' N, 98°00' W).

Located on the eastern edge of the Edwards Plateau in central Texas, this 4000-acre ranch is dominated by oak–juniper savannas.

#### *Spatial distribution of the gall former*

To quantify the distribution and abundance of *B. treatae* within a representative population of its host plant, leaf gall densities were surveyed for 100 *Q. fusiformis* trees in Fall 2001. Thirteen transects were established and along each 400 m long transect, the nearest *Q. fusiformis* tree was chosen at 20-m intervals, and gall density was estimated by counting the number of leaf galls within a 0.25-m<sup>2</sup> grid placed in the lower canopy, one grid in each cardinal direction. Lower canopy foliage is typically “blanketed,” which allows a count of an essentially two-dimensional grid to estimate gall density quickly. The four density measurements were averaged to estimate galls/m<sup>2</sup> of foliage. For consistency, all sampling was confined to the lower canopy, as Galusky (2000) documented that *B. treatae* gall densities are typically highest in the lower one-third of *Q. fusiformis* canopies. GPS coordinates of each tree were recorded (Garmin GPS 12) and high- and low-density trees were mapped using GIS system software (ArcView).

#### *Experiment 1: Test of local host quality*

To test whether variation in gall density among neighboring trees at the local level correlates with variation in host plant quality we conducted a one-way transplant by transferring gall formers from high-density to low-density trees and measuring insect performance. We selected five high-leaf-gall-density trees (henceforth “natal” trees) identified in the survey (Fig. 1). For each natal tree, we selected the nearest tree in each cardinal

direction that exhibited low-leaf-gall densities (henceforth “neighbor” trees). The mean distance between natal and neighbor trees was  $60 \pm 10$  m (summary statistics are means  $\pm$  SE, unless otherwise noted), range 10–150 m. Natal and neighbor trees differed significantly in gall density ( $63.6 \pm 7.2$  and  $3.8 \pm 1.2$ , respectively;  $t = 14.51$ ,  $df = 23$ ,  $P < 0.0001$ ) but did not differ in diameter at breast height, a rough estimate of tree maturity ( $t = -0.156$ ,  $df = 23$ ,  $P = 0.88$ ).

In January 2002, two months prior to emergence of the *B. treatae* sexual generation, we placed screen enclosures around three branches on each of the five natal trees and on each of the 20 neighbor trees. Enclosures ( $60 \times 45$  cm) were constructed of Nytex screen (BioDesign, Carmel, New York, USA) sealed on three sides and fitted with Velcro strips on the open end to fit and seal over branches. In mid-February, following abscission of the 2001 leaf cohort, but prior to the 2002 bud break, enclosures were cleared of leaves and insects. Hundreds of newly flushed leaves subsequently provided unlimited oviposition sites for the five *B. treatae* females added to each enclosure. Enclosures screened out all other insect fauna including other *B. treatae*, predators, and parasitoids and thus allowed us to isolate individual host plant or plant-mediated effects on measures of gall-former performance.

During peak emergence of sexual-generation, *B. treatae* (April 2002) root galls were harvested from each natal tree and housed in 3-L chambers in the laboratory. Mated females were collected within two days of emergence. For each natal tree, five females from the natal tree were added to each of its three bagged branches. Five natal tree females were then added to each of the three bagged branches on each of the four respective neighbor trees. This totals to five trees, each with three bagged branches, with each bagged branch containing five females for a total of 75 females. Two weeks after transfer, all enclosures were surveyed for oviposition scars to verify that oviposition had occurred. Enclosures remained on trees until November to allow gall formers to complete development. Reciprocal transplants from neighbor to natal trees were not possible because neighbors did not have high enough root gall densities to fully stock the reciprocal replicates. Control enclosures were established on four natal trees, except that no *B. treatae* were added, to determine whether enclosures effectively precluded unwanted oviposition by wild *B. treatae*.

#### Sample processing and variable definitions

In November 2002, prior to the emergence of asexuals, all bagged branches were cut from trees and processed, as described here, to provide estimates of eight sequential components of gall-former performance on the host plant during that phase of the life cycle spent in leaf galls. The six variables that are ratios used nonoverlapping denominators and are independent in this sense. For each replicate, leaves with galls were

scored as having abscised or remained attached, and the number of galls per leaf per category was summed to estimate *gall production*. *Gall abscission* was then estimated as the percentage of galls per replicate on abscised leaves. *Gall initiation* was scored from samples of abscised leaves per replicate as the ratio of galls initiated (i.e., galls that developed to measurable three-dimensional form) to oviposition scars. Gall initiation was estimated from abscised leaves because attached leaves required more extensive processing. Subsequent analysis showed that gall initiation did not differ between abscised ( $43.6\% \pm 3\%$ ) and attached ( $43.7\% \pm 3\%$ ) leaves ( $t = -0.006$ ,  $df = 57$ ,  $P = 0.99$ ). Galls on attached leaves were further categorized as immature (fleshy) or mature (lignified). The proportion of galls that were mature was used to estimate the *development rate* of gall formers in each replicate. Galls with diameters that did not exceed the tree threshold were not used to estimate development rate. Galls on attached leaves were then removed, binned by replicate and developmental status, and housed in an incubator set to seasonally adjusted light and temperature to rear gall occupants. The percentage of galls from which *B. treatae* emerged was used to estimate *emergence success*. Subsequent analysis showed that emergence from immature galls ( $53\% \pm 2\%$ ) was significantly less than from mature galls ( $64\% \pm 1\%$ ;  $t = 3.2$ ,  $df = 311$ ,  $P < 0.01$ ) and that emergence from galls on abscised leaves ( $18\% \pm 0.5\%$ ) was significantly less ( $P < 0.001$ ) than that from attached leaves ( $62\% \pm 0.6\%$ ). Thus, to insure that the variables development rate and emergence success were independent, we used and report only emergence rates from mature galls on attached leaves. Similar to field observations and verifying that emergence was estimated under reasonable conditions, *B. treatae* emergence in the laboratory occurred from November to December. Following emergence, the diameter of all galls across all replicates and developmental fates was measured to 0.01 mm using digital calipers. Using galls on attached leaves (galls capable of developing to full size), we estimated the *mean gall size* per replicate and the percentage of galls exceeding the *tree threshold* ( $\geq 2.62$  mm). The percentage of galls exceeding the *natural enemy threshold* ( $\geq 5.82$  mm) was then computed from the subset of galls that exceeded the tree threshold.

Efficacy of enclosures was confirmed for exclusion of natural enemies by identifying emergents from all galls harvested (7088 *B. treatae*, 23 natural enemies; 0.3% parasitism) and exclusion of other ovipositing female *B. treatae* by searching for galls or oviposition scars in control enclosures where no wasps were added (4 enclosures, 361 leaves, 0 oviposition scars or galls, 0% intrusion by wild *B. treatae* females). In total, 7975 galls were examined from the 75 experimental enclosures distributed across five natal and 20 neighbor trees.

During post-harvest processing of enclosures, we observed that galls had failed to establish in some

replicates. Thus a ninth (and a posteriori) variable was measured: *establishment success* scored the percentage of replicates per treatment in which galls were established, and it was used to test two null hypotheses: (1) "zero gall" replicates were distributed randomly among treatments and (2) zero gall replicates were randomly distributed among trees within treatments. We interpret rejection of the null as indicating the presence of host plants unsuitable for gall initiation. This inference is drawn because when replicated sets of females were sampled from the same natal trees and bagged onto other trees, oviposition led to gall initiation.

#### *Experiment 2: Test of deme formation*

Using the same five trees employed in Experiment 1 as population sources, we conducted a fully balanced reciprocal transplant to test the deme-formation hypothesis. This design allowed us to examine simultaneously the performance of gall formers from each high-density tree on their own (natal) tree and across the four alternate high-density trees (henceforth novel trees). Natal trees differed significantly in gall density (range  $42.3 \pm 15.3$  to  $87.8 \pm 2.4$  galls/m<sup>2</sup>;  $F_{4,15} = 3.98$ ,  $P < 0.05$ ), but on all trees gall density was more than two standard deviations above the mean of  $7.36 \pm 15.3$  SD galls/m<sup>2</sup> observed in the survey of 100 trees described earlier. Mean distance between high-gall-density (natal) trees was  $1.40 \pm 0.2$  km, range 0.2–2.64 km. Procedures and variables followed Experiment 1. Five mated females were added to each of the three enclosures on their natal tree and to each of three enclosures on each novel tree. The nine variables each tested the general prediction that the mean performance of populations on their natal plants will be greater than on novel plants. Two specific predictions were examined for the summary variable emergence success to illustrate the contrasting manner in which evidence for deme formation can be viewed: hypothesis 1, the mean performance of the population from natal tree<sub>(i)</sub> reared on natal tree<sub>(i)</sub> will be greater than the mean performance of gall formers from novel trees reared on tree<sub>(i)</sub>; and hypothesis 2, the mean performance of gall formers from tree<sub>(i)</sub> reared on tree<sub>(i)</sub> will be greater than the mean performance of gall formers from tree<sub>(i)</sub> reared on all other novel trees. In total, 15 148 galls were scored from 75 experimental enclosures on the five trees.

#### *Statistical analysis*

A chi-square goodness-of-fit test was used to test the a posteriori null hypothesis that zero-gall replicates were distributed randomly among treatments. In Experiment 1 we compared natal vs. neighbor trees and for Experiment 2 we compared natal vs. novel trees.

Due to the repeated measures design, mixed-effects ANOVA was used to test the significance of each variable with tree (site of rearing) set as a fixed factor and insect population source (natal tree 1–5) set as a random factor (Horton et al. 1991, Downie 1999,

Pinheiro and Bates 2000). Significance of interaction terms was tested using log-likelihood tests that compared models with and without an interaction term. All analyses were performed using S-PLUS (Insightful, Seattle, Washington, USA).

Gall density per replicate was considered but rejected as a covariate as we found no significant effects for any variable. Prior to analysis, all percentage variables were transformed using the empirical logistic transformation (Cox 1970) so that treatments that differed widely in sample size could be compared (e.g., for the subset of replicates in which galls could be established, mean galls per replicate [ $\pm$ SE] =  $205 \pm 34$ , range 10–913 galls per bag). Means and SE values thus computed are not readily back-transformed; therefore, means  $\pm$  SE of untransformed percentages are presented. We considered transformations of gall size, such as  $\log_{10}$ , but found no difference in the results for any analysis; therefore we report results of untransformed analyses.

For Experiment 1, we compared insect performance of each a priori variable within each of the five natal tree–neighbor complexes. In each analysis, a significant tree effect indicates that natal trees differ from their low-density neighbors, and a nonsignificant interaction suggests a consistent pattern across all five complexes. Replicates on all neighbor trees surrounding each of the five natal trees were combined because wasps were unable to form galls on a number of neighbor trees. In Experiment 2, we compared the performance of gall formers when reared on their natal tree to their performance across novel trees. A significant tree effect indicates that performance differs among high-density trees and deme formation is indicated by a significant interaction between tree and insect population in the case where natal treatments outperform novel treatments. The order of results for the nine variables reflects the chronology of gall-former development.

## RESULTS

### *Variation in gall-former density among trees*

Leaf gall densities varied by three orders of magnitude among the 100 sampled trees, and this variation is apparent between adjacent trees. The distribution of gall densities per tree was leptokurtic (Fig. 1a). High-density trees ( $63.6 \pm 7.2$  galls/m<sup>2</sup>) were operationally defined as binned in the two right-most columns in Fig. 1a and were rare, constituting only 5% of sampled trees, and were widely dispersed among transects (Fig. 1b). Low-density trees ( $3.8 \pm 1.2$  galls/m<sup>2</sup>) were operationally defined as binned in the left-most two columns in Fig. 1a.

### *Confirmation of oviposition activity*

In both experiments, oviposition scars were present in all replicates of all treatments, confirming that wasps attempted to oviposit and ruling out the possibility that (1) no females survived transfers from the laboratory to

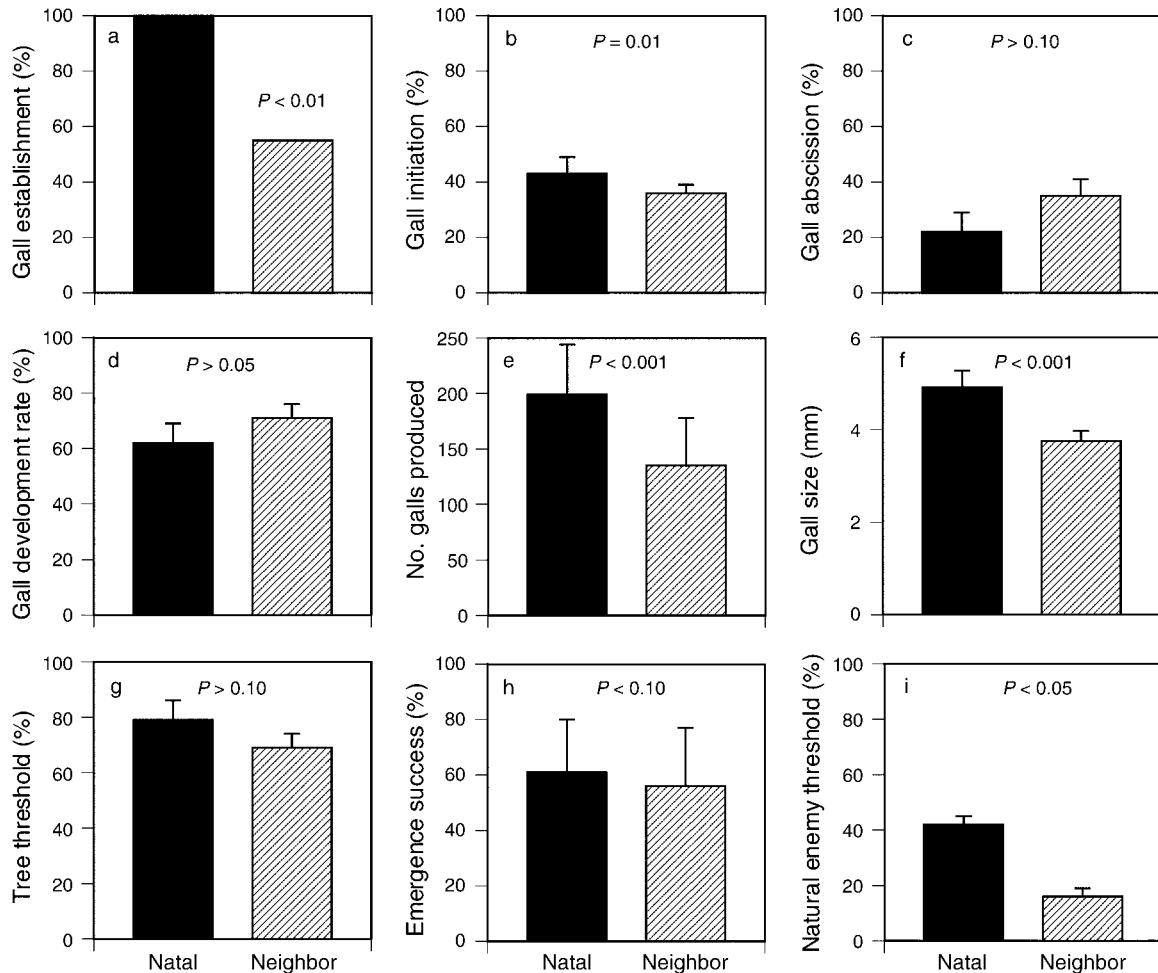


FIG. 2. Performance (mean + SE) for five *B. treatae* populations each reared on its natal and respective four neighbor trees; *P* values indicate significance levels for tests of the null hypothesis that mean performance on natal trees did not differ from neighbor trees. For variables b–i, the interaction of tree × rearing site was not significant, indicating that performance was consistent across all contrasts.

the field and (2) females refused to oviposit on certain trees.

#### Experiment 1: Test of local host quality

The proportion of enclosures per treatment in which galls failed to establish was nonrandom (natal trees = 0/15 enclosures, neighbor trees = 27/60 enclosures;  $\chi^2 = 6.75$ ,  $df = 1$ ,  $P < 0.01$ ; Fig. 2a). The mean number of replicates failing to produce galls on neighbor trees was  $1.35 \pm 1.26$  (mean  $\pm$  SD). However, 18 of the 27 enclosures in which galls failed to develop represented all three replicates on six of the 20 neighbor trees. A chi-square test was used to compare the observed to the expected distribution of the number of failures per neighbor tree based on a Poisson distribution with a mean of 1.35. This test revealed that trees for which all three replicates failed to produce galls were more frequent than expected by chance; i.e., failures were clumped in their distribution among neighbor trees ( $\chi^2 =$

9.84,  $df = 2$ ,  $P < 0.01$ ) indicating that some neighbor trees were likely of such poor quality that no galls could be established. In considering all subsequent results, it is important to note that, since wasps oviposited in all replicates on all trees, values for the eight a priori variables in those replicates in which galls failed to establish are technically 0, indicating extreme plant effects on all measures of gall-former performance. However, we omitted these 27 replicates and restricted our analysis to the replicates per tree capable of supporting gall development to provide decidedly conservative treatment estimates for the eight a priori variables (at the expense of a balanced design).

Detailed statistical results of the mixed-effects ANOVA from Experiment 1 are presented in Table A1 within Appendix A. In all eight tests of the local-quality hypothesis the interaction term was not significant, suggesting that the difference in performance on natal and neighbor trees was consistent across all five groups

of trees. Five of the eight a priori variables differed significantly in the direction predicted by the hypothesis that surveyed gall density per tree equates to host quality. Two others differed in the predicted direction but were not statistically significant.

Gall initiation on natal trees was significantly higher than on the 14 neighbor trees capable of supporting gall development (Fig. 2b). Gall abscission on natal trees and neighbor trees differed in the direction predicted by the host quality hypothesis, but this difference was not significant (Fig. 2c). As well, gall development rate did not differ between natal and neighbor trees (Fig. 2d). The 19% increase in gall initiation on natal trees translated into significantly more galls produced per enclosure on natal compared with neighbor trees (Fig. 2e). The mean size of mature galls on attached leaves was 31% larger on natal trees compared to neighboring trees, and this difference was significant (Fig. 2f). Comparison of gall size for just the subset of galls from which *B. treatae* emerged (i.e., galls that attained the maximum size for each insect–plant genome interaction on each host tree) showed decisively that natal trees produced larger galls than did neighbor trees ( $5.80 \pm 0.10$  and  $4.91 \pm 0.14$  mm respectively;  $F_{1,34} = 25.69$ ,  $P < 0.001$ ). A higher proportion of galls exceeded the tree threshold on natal trees, but this measure of performance did not differ significantly (Fig. 2g). *B. treatae* emergence from galls in the absence of parasitism, conservatively estimated from only those galls that were mature at harvest, was marginally higher on natal trees than on neighbor trees (Fig. 2h). When the comparison is restricted to the subset of mature galls that exceeded the tree threshold size this marginal difference disappeared ( $79\% \pm 21\%$  and  $76\% \pm 20\%$  for natal and neighbor trees, respectively;  $F_{1,41} = 1.54$ ,  $P > 0.10$ ). Thus, when protected from natural enemies, *B. treatae* that develop in galls that survive initial host plant effects or plant-mediated effects have equivalent probabilities of emerging from galls on natal and neighbor trees. However, the proportion of mature galls that exceeded the natural enemy size threshold on natal trees was more than double that on neighbor trees (Fig. 2i).

#### Experiment 2: Test of deme formation

The proportion of enclosures per treatment in which galls failed to develop was nonrandom (natal = 0/15 and novel = 11/60;  $\chi^2 = 2.75$ ,  $df = 1$ ,  $P < 0.10$ ; Fig. 3a), but in contrast to Experiment 1, replicates that failed to produce galls were not clustered on particular trees. Detailed statistical results of the mixed-effects ANOVA from Experiment 2 are available in Table A2 within Appendix A. Gall initiation differed significantly between natal and novel trees but in the opposite direction intuitively predicted by the hypothesis of deme formation (Fig. 3b). Gall abscission was 77% higher on novel trees relative to natal trees (Fig. 3c). Gall development rate differed slightly, but significantly, in the direction predicted by the hypothesis of deme formation with galls

on natal trees developing faster than did galls on other high-quality, but novel, trees (Fig. 3d). Gall production on natal and novel trees did not differ (Fig. 3e). However, for galls that remained attached throughout development and were mature at harvest, mean gall size was significantly larger on natal compared with novel trees (Fig. 3f). Restricting this comparison to those galls from which *B. treatae* emerged showed definitively that natal trees produced significantly larger galls than did novel trees ( $5.80 \pm 0.10$  mm and  $4.90 \pm 0.12$  mm, respectively;  $\chi^2 = 11.91$ ,  $df = 1$ ,  $P < 0.001$ ) and that a higher proportion of galls exceeded the tree threshold on natal trees than did on novel trees (Fig. 3g).

Gall-former emergence was significantly affected by both the tree on which development proceeded and the interaction of tree and source population (Appendix A: Table A2). The significant interaction confirms the general prediction of the deme formation hypothesis by demonstrating that on average, in the absence of parasitism, a greater percentage of gall wasps emerge when populations are reared on their natal tree than when reared on novel trees (Fig. 3h). As well, the significant tree effect demonstrates that although all high-density trees in this study were found to be high quality relative to each tree's nearest conspecific neighbors, they differed among themselves in quality based on gall wasp emergence. Emergence success among high-gall-density trees ranged from  $24\% \pm 5\%$  to  $70\% \pm 6\%$  (Fig. 4b, c).

The hypothesis, that the mean overall performance (measured by emergence success) of gall-former populations from each high-density tree<sub>(i)</sub> when reared on tree<sub>(i)</sub> will exceed the mean performance of gall formers from all novel trees reared on tree<sub>(i)</sub>, was supported (Fig. 4b). However, the hypothesis that the mean performance of gall formers from tree<sub>(i)</sub> when reared on tree<sub>(i)</sub> will exceed the mean performance of gall formers from tree<sub>(i)</sub> when reared on all other novel trees was not uniformly supported (Fig. 4c). Such an exception is shown by inspection and comparison of the specific tree and source population (highlighted in solid black circles in Fig. 4b, c). Fig. 4b shows a natal population that marginally outperformed all other populations on its natal tree ( $t = -1.69$ ,  $df = 17$ ,  $P = 0.096$ ), while Fig. 4c shows that the mean performance of this same natal population across all other novel trees exceeded its performance on its natal host plant ( $t = 3.513$ ,  $df = 53$ ,  $P < 0.0027$ ). Thus, it is likely that while wasps reared on their natal trees in general outperform experimental "immigrant wasps," that nevertheless some natal populations could perform higher on other trees given the opportunity. The proportion of galls that exceeded the natural enemy threshold size did not differ between natal and novel trees (Fig. 3i).

#### DISCUSSION

This study convincingly demonstrates that variation in local host-plant quality and deme formation at the

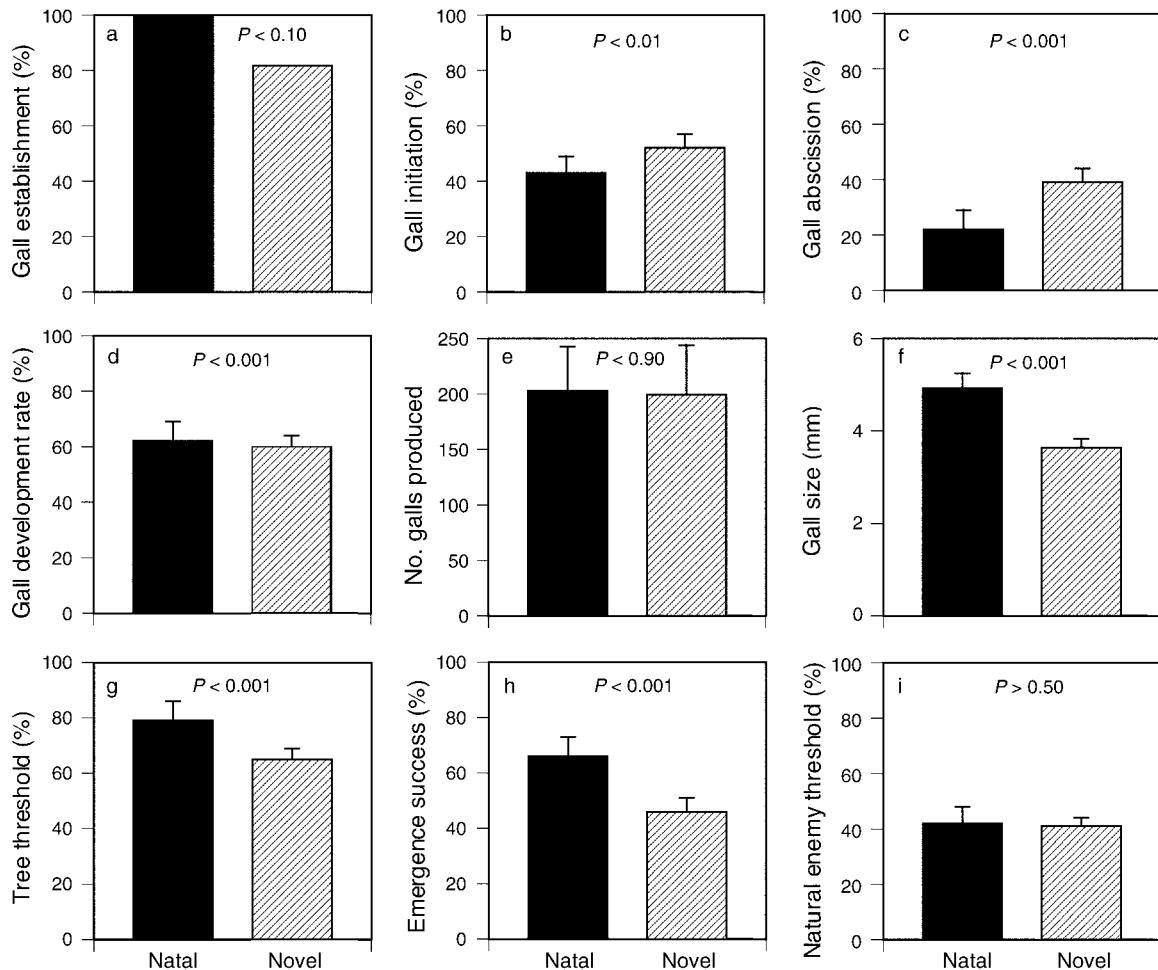


FIG. 3. Comparative performance (mean + SE) for five *B. treatae* populations, each reared on its natal high-density and the four novel high-density trees. For the variables b–i, a significant tree  $\times$  population interaction (denoted by the *P* value) indicates deme formation.

level of the individual host tree both contribute to the patchy distribution of a specialist herbivore within populations of its host plant. By testing a series of nine independent variables that act sequentially throughout the life cycle of *B. treatae*, these results specifically highlight the effects on individual life stage components of gall-former success in the absence of natural enemies and can be used to predict how host plant differences in quality and demic adaptation may be further reinforced in the presence of natural enemies.

#### *Spatial variation in gall-former density and host plant quality*

While patchy distributions of herbivorous insects are common in nature (Denno and McClure 1983, Mopper and Strauss 1998), deme formation is rarely observed in herbivorous insects, and its role in producing spatial variation in density is poorly understood. We have provided a comprehensive example of how among-plant variation in plant quality at the local scale acts together

with deme formation arising from variable plant quality at a larger scale to contribute to the patchy distribution of the specialist gall former *B. treatae* within populations of its host plant. We see evidence in our data that deme formation may both arise from, and contribute to, the patchy distribution of this gall former.

Our focus is on the effects of among-plant variation on herbivore fitness and how this translates into spatial variation in herbivore density within populations. While the environmental and genetic basis of among-tree variation in quality are important in understanding the origin and spatial context of among-plant variation, these key elements are as of yet unexplored in this cynipid–oak system. We observed that gall-former densities vary by orders of magnitude among trees within populations and even between immediately adjacent trees. In fact, by means of our sampling protocol, gall formers are undetectable on many trees immediately adjacent to high-density trees and remain unapparent even when protracted visual searches are

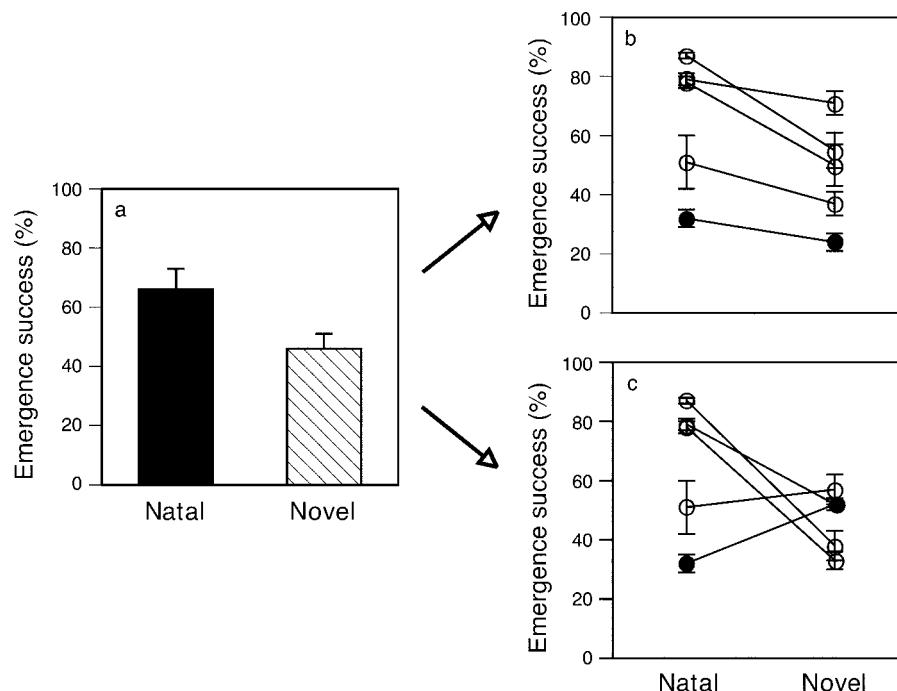


FIG. 4. Three ways to depict deme formation: (a) means comparison of emergence success across all trees and insect populations (used throughout Fig. 3); (b) within-tree dynamics, for which each line compares performance of natal and novel populations (mean  $\pm$  SE) of an insect population on its natal tree to its average performance across all novel hosts. The filled circles in panels (b) and (c) denote a population that could perform better on other high-quality trees but still outperforms novel populations on its natal tree.

conducted (J. Ott and S. Egan, *personal observation*). Trees that support high-gall-former densities are both rare and scattered across the landscape. Our tests of the hypothesis that observed leaf gall density per tree equates with tree quality (Experiment 1) demonstrate that observed gall densities equate with tree quality as indexed by multiple measures of gall-former performance. As well, our results demonstrate that high-gall-density “hotspots” evident in our field survey of live oaks represent islands of high-quality habitat and support studies implicating the importance of bottom-up effects in plant–herbivore systems (Hunter and Price 1992, Larsson et al. 2000). Ongoing longitudinal studies at our field site (J. Ott, *unpublished data*) suggest that, while gall densities vary across years, the classification of individual trees as having either low or high gall densities remains stable.

Experiment 1 revealed that variation in plant quality directly influenced gall-former density in two ways. First, among-tree variation was so great that 30% (6/20) of neighboring trees surrounding high-gall-density trees appeared unable to support gall establishment. Such resistant trees (Mopper et al. 1991) are a likely explanation for the finding that 28% of live oaks sampled in our field survey did not support *B. treatae* at detectable densities. Under the assumption that resistant trees are incapable of sustaining populations, we hypothesize that these trees absorb immigrants and

function as sinks (Pulliam 1988), thus effectively increasing the isolation of those trees that are suitable for colonization. This isolation in turn may facilitate demic adaptation of gall-former populations on high quality trees. Second, within the subset of neighbor trees that were capable of supporting gall initiation, herbivore fitness was reduced relative to high-density trees. Thus the expected corresponding reduction in population growth and production of migrants could serve to reinforce the discrepancy in gall densities among high- and low-quality trees.

By influencing *B. treatae* susceptibility to natural enemies variation in plant quality is also likely to have an indirect effect on gall-former densities. Based on the dramatic 2:1 difference between natal and neighbor trees in the percentage of galls exceeding the natural enemy threshold and the observation that gall size and susceptibility to natural enemies are negatively correlated in this (Reynolds 2001) and other gall-former systems (Weis et al. 1992, Stiling and Rossi 1996), we predict (*ceteris paribus*) that natural enemy mortality will be significantly greater on low-density trees. This prediction is further justified by the observation that mean gall size per tree and *B. treatae* emergence from galls is negatively correlated in natural populations (Reynolds 2001). If realized, the relationship between variation in tree quality and mortality due to natural enemies would further reinforce the observed orders-of-magnitude

difference in gall-former densities among trees at the local level. However, because both gall size and gall density differed among trees in both Reynolds' (2001) and the current study, the extent to which the predicted increased natural enemy impact on low-density trees under field conditions would be driven by size-associated probability of mortality vs. density dependence (Hails and Crawley 1992) needs to be investigated. In sum, direct effects via the presence of trees unable to support gall establishment and via low-quality tree effects on gall-former fitness and indirect enhancement of natural enemy effectiveness on low-quality trees may all serve to create and reinforce spatial variation in *B. treatae* densities.

Finally, the results of Experiment 1 are important because they diminish the possibility that untested alternative hypotheses explain the observed variation in density among trees at the local scale. For example, the hypothesis that all trees are equally suitable for *B. treatae*, but that only a subset of trees are colonized at any one point, is unlikely given our results. As well, the hypothesis that "centers of high-gall density" are mobile across the landscape, are of short duration, and that any two centers can be connected by simple colonization of intervening trees is less plausible.

#### *Host plant quality and demic adaptation*

The interaction of insect and plant genomes during gall induction (Ollerstam and Larsson 2003), in combination with the hypersensitive response of plants to gall formers (Fernandes and Negreiros 2001, Ollerstam et al. 2002), points to the fine-scale nature of the genetic interaction between host plants and their gall formers and suggests the potential for demic adaptation in this group of insect herbivores. In plateau live oak, variation in plant quality provides the template for local selection leading to deme formation. The reciprocal transplant (Experiment 2) mimicked immigration of *B. treatae* among high-quality trees at the patch scale. Natural selection on immigrants is known to be an important source of mortality between populations locally adapted to divergent habitats (Nosil et al. 2005). Experiment 2 showed that relative to offspring reared on natal trees, immigrant offspring are abscised at a higher rate, develop in galls that mature (lignify) more slowly, develop in galls of reduced size, succumb to early plant-mediated death at a greater rate, and emerge from galls at a lower rate, even in the absence of natural enemies. Each result documents direct and biologically meaningful negative effects on *B. treatae* fitness during sequential stages of development in novel environments and each result constitutes evidence of demic adaptation. Because leaf gall size and egg number in asexual *B. treatae* are correlated ( $r = 0.72$ ,  $P = 0.01$ ) (J. Ott, unpublished data), our finding that mean gall size was reduced on novel high-density plants also suggests that the fecundity of immigrant females may be reduced relative to resident females.

Herbivory can stimulate physiological processes that lead to early leaf abscission (Risley 1993) and reduce herbivore fitness (Waddell et al. 2001, but see Askew 1962). Thus abscission can be a selective force on phytophagous insects (Stiling and Simberloff 1989, Stiling et al. 1991). Our finding that rates of abscission were lower for natal in comparison to novel gall-former populations reared on the same tree (similar genetic background) suggests the intriguing possibility that natal populations of *B. treatae* are better able to avoid detection from their parent tree than are recent immigrants.

Parasitism of the asexual generation of *B. treatae* typically exceeds 99% in natural populations (Lund et al. 1998, Hall 2001). Given (1) this high level of parasitism, (2) the established relationship between gall size and the probability of emergence in this (Reynolds 2001) and other gall former systems (Abrahamson and Weis 1997, Ito and Hijii 2004), and (3) the fact that gall formation is a direct interaction between the insect and plant genotypes (Stone and Schönrogge 2003), we postulate that selective pressure from the third trophic level contributes to deme formation in this system. We applied the function developed by Reynolds (2001) that relates gall size and probability of emergence in this system to the distribution of gall sizes produced on novel and natal trees and found that in the presence of natural enemies that resident gall formers (mean gall size = 4.92 mm) would be 2.75 times more likely to emerge than immigrant gall formers (mean gall size = 3.64 mm). If confirmed by empirical studies this would be the first time that natural enemies have been implicated as contributing to deme formation (Mopper and Strauss 1998).

Deme formation at the scale of individual plants has been documented in a modest group of phytophagous insects including Homoptera (Edmunds and Alstad 1978, Hanks and Denno 1994, Komatsu and Akimoto 1995), Thysanoptera (Karban 1989), Lepidoptera (Mopper et al. 1995, 2000), and Diptera (Stiling and Rossi 1996). The evidence we provide here for deme formation in the phytophagous Hymenoptera adds to the suggestive findings of Eliason and Potter 2000. This is not surprising, however, since cynipid-oak systems share many similar characteristics that may facilitate deme formation, such as short-lived insects on long-lived host trees (Edmunds and Alstad 1978, Hanks and Denno 1994), endophagy (Mopper et al. 1995, Stiling and Rossi 1996), parthenogenesis (Karban 1989), haplodiploidy (Boecklen and Mopper 1998), and spatial isolation of host plants (Wainhouse and Howell 1983, Hanks and Denno 1994). The high parasitism rates, common in this and other cynipid gall-former systems (Stone et al. 2002), are implicated as another factor contributing to deme formation.

While the evidence for demic adaptation on the basis of performance comparisons appears compelling from our study, alternative interpretations of the patterns

revealed by our experiments are possible. For example, because inherited effects reflecting parental experience with the host plant were not controlled, maternal effects resulting in offspring that perform better on the natal host plant of the mother in theory could explain our results. However, results from Experiment 2 reveal that while natal populations uniformly outperformed novel populations on the natal background (Fig. 4b), no source population simultaneously exhibited the highest performance on its natal plant and the alternative host plants. This result diminishes the possibility of a general maternal effect that allowed progeny from some populations to outperform all other populations. In fact, as shown, performance of some populations actually increased on alternative high-density trees (Fig. 4c), illustrating that performance likely reflects the interaction of both insect and plant genome or at least insect genome and plant phenotypic state. Other sources of bias appear unlikely in our studies, given the biology of the gall former and our husbandry of adults during the setup of the experiments. Because adults do not feed, any variation in nutritional quality among plants is not meaningful during oviposition. Moreover, transplanted females developed in the field, which emerged from root galls under similar laboratory conditions, were of equivalent age when deployed, and were drawn at random from the root galls collected from each tree.

Overall, this study illustrates how both variation in host plant quality and deme formation at the level of the individual tree contribute to the patchy distribution of *B. treatae* within populations of its host plant. A complex multi-trophic-level community of at least 24 species of inquilines, parasitoids, and hyperparasitoids interact within *B. treatae* galls. Thus the spatial variation in *B. treatae* densities arising from plant quality variation and deme formation may determine the spatial structure of the associated community. Furthermore, because many gall parasitoids are typically generalists, effects may extend to other host-parasitoid systems associated with *Q. fusiformis* at this site.

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#### APPENDIX

Results of mixed-effects ANOVA for Experiments 1 and 2 (*Ecological Archives* E088-178-A1).