# HOST PLANT QUALITY AND DEME FORMATION AS DETERMINANTS OF THE DISTRIBUTION AND ABUNDANCE OF A GALL-FORMING HERBIVORE

THESIS

Presented to the Graduate Council of
Texas State University – San Marcos
in Partial Fulfillment of
the Requirements

For the Degree

Master of SCIENCE

By

Scott Patrick Egan, B.S.

San Marcos, Texas
December 2003

## ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Dr. James R. Ott, for his supportive mentorship. I would also like to thank the other members of my committee, Dr. Caitlin Gabor, Dr. Chris Nice, and Dr. Floyd Weckerly, for their guidance in this endeavor. Thanks to colleagues Greg Cryer, Richard Reynolds, and Lezlie Jones for their assistance in the development and execution of this project. The Texas State Biology Department, College of Science, and Graduate College, Freeman Ranch Advisory Council, and Sigma-Xi were extremely generous in their support of this work. I would like to thank my family for their constant support and my wife, Jen, for her unending encouragement and love.

## TABLE OF CONTENTS

Page

## ABSTRACT

### HOST PLANT QUALITY AND DEME FORMATION AS DETERMINANTS OF THE DISTRIBUTION AND ABUNDANCE OF A GALL-FORMING HERBIVORE

by

Scott Patrick Egan, B.S.
Texas State University – San Marcos
December 2003

Supervising Professor: James R. Ott

Here I link variation in host plant quality to herbivore fitness variation at the local scale (between adjacent plants) with the process of demic adaptation operating at the patch scale to provide a synthetic explanation to the patchy distribution of the specialist gall forming wasp *Belonocnema treatae* (Hymenoptera:Cynipidae) within populations of its host plant, *Quercus fusiformis*. In this gall former system gall densities vary by orders of magnitude between adjacent host trees; host trees supporting high gall densities are rare (<5%) and appear across the landscape as habitat islands within a sea of host plants supporting only low herbivore densities. To understand the relationship between herbivore fitness variation and variation in the abundance and distribution of the herbivore at the local and patch scales a manipulative transplant experiment was

performed. Mated sexual generation *B. treatae* females from each of five high gall density trees were placed on (a) their respective four nearest neighbor conspecifics that exhibited low gall density, (b) the four alternative high density trees, and (c) their natal trees (control). Each treatment (source population + rearing site) was replicated three times. Linear mixed effects ANOVA was used with the host tree, on which development took place, set as a fixed factor and the herbivore source population set as a random factor to test for effects of local variation in plant quality on herbivore fitness and deme formation. For each replicate, I recorded: (a) gall initiation success (# of galls established/# oviposition scars), (b) total number of galls produced, (c) leaf abscission rates, (d) gall developmental rate, (e) individual gall size, (f and g) percent of galls developing to established minimum emergent size thresholds for *B. treatae* in the absence and presence of natural enemies, and (h) overall emergence success (# of galls producing a *B. treatae*/total # galls). Results from the test of local quality (high density vs. respective near-neighbor low density trees) demonstrate that leaf gall density per tree equates with host quality. Transplanted populations of gall formers exhibited greater gall initiation success ($p < 0.05$), total number of galls produced ($p < 0.001$), average gall size ($p < 0.001$), and percent reaching natural enemy critical size threshold ($p = 0.05$) on focal trees compared to low density neighbors. Within this mosaic of local quality variation, comparison of herbivore performance on natal and novel high density trees demonstrate that the herbivore has undergone adaptation at the level of the individual host plant (deme formation) as shown by lower leaf abscission rate ($p < 0.001$), and higher developmental rate ($p < 0.001$), average gall size ($p < 0.001$), percent reaching tree critical threshold ($p < 0.001$), and overall emergence success ($p < 0.001$). Together these results from these

linked experiments demonstrate convincingly that (a) heavily galled focal trees are surrounded by host trees of relatively lower quality to the herbivore and (b) that deme formation has taken place. These results highlight the effects of host plant variation on individual life stage components of gall former success in the absence of natural enemies within and among demes and will be used to illustrate how differences in host plant quality are further amplified in the presence of natural enemies.

# INTRODUCTION

Host-specific phytophagous insects exhibit a near universal pattern of patchy distributions within populations of their host plants (Abrahamson and Weis 1997, Mopper and Strauss 1998). Bottom-up effects from the host plant influence this pattern of distribution and abundance (Hunter and Price 1992, Ylioja et al. 1999, Larsson et al. 2000) and can lead to genetic sub-structuring of insect populations (Mopper and Strauss 1998). Variation in host plant quality within a population, measured by insect performance, can vary due to plant genotype (hybridization - MacIntyre and Whitham 2003, inbreeding - Carr and Eubanks 2002), plant age (Mopper et al. 2000), plant sex (Strauss 1990), phenology of leaf growth and abscission (Stiling and Simberloff 1989, Stiling et al. 1991, Mopper and Simberloff 1995), leaf sensitivity to oviposition (Anderson et al. 1989, Fernandes 1998), chemical defenses (Rosenthal and Berenbaum 1991, Rosenthal and Berenbaum 1992), induced chemical defenses (Agrawal 1999), and structural defenses (Agren and Schemske 1993, Bodnaryk 1996).

Individual high quality host plants or patches of related individuals can then be viewed as phenotypically or genotypically heterogeneous islands existing among a sea of lesser quality hosts (Janzen 1968, Sork et al. 1993, Strauss and Karban 1994a, Strauss and Karban 1994b). The understanding of fine-scale micro-geographic variation in host

quality among neighboring conspecific plants led to the deme formation hypothesis (Edmunds and Alstad 1978). Given variation among individual plants due to genotype, phenotype, or genotype x environment interactions that influence their quality as host to phytophagous insects and genotypic variation among individual insects that results in some individuals realizing higher fitness than others on individual host plants, selection may favor particular insect genotypes on individual host plants. Given continued strong selection and reduced gene flow, genetic structure of insect populations (demes) at the scale of the individual host plant (or patch) can be generated and maintained (Mopper and Strauss 1998). Evidence that selection can generate localized demic adaptation in insect herbivore populations at the scale of individual host plant has been documented in a diverse group of insects including: Homoptera (Edmunds and Alstad 1978, Hanks and Denno 1994, Komatsu and Akimoto 1995), Thysanoptera (Karban 1989), Lepidoptera (Mopper et al. 1995, Mopper et al. 2000), and Diptera (Stiling and Rossi 1996, Stiling and Rossi 1998), but not in the Hymenoptera. Surprisingly, recent studies suggest that mobile insects compared with the low mobility insects, originally tested for deme formation, may be just as likely to exhibit population structure at the level of individual host trees (Van Zandt and Mopper 1998). This illuminates that other factors besides dispersal ability, such as selection from the host plant, can contribute to deme formation and furthermore, demonstrates that gene flow does not preclude the build up of local genetic structure when selection is strong (Wade and Goodnight 1998).

For endophagous insects, such as leaf miners and gall formers, specific host tissues at specific developmental stages are crucial to mine and gall initiation and success

(Weis et al. 1988). Thus variation in host plant phenology generating variation in tissue availability may be one of the strongest within-population forces responsible for producing the observed variation in abundance and distribution between host plants (Komatsu and Akimoto 1995, Van Dongen et al. 1997, Mopper and Simberloff 1995, Mopper et al. 2000), even facilitating reproductive isolation between trees (Mopper 1996). Endophagous insects also have an intimate relationship with the host plant, making them more succeptible to defensive chemicals (Mopper 1996, Stiling and Rossi 1998), which may also explain their propensity to adapt to individual host plants (Van Zandt and Mopper 1998). Reproductive strategy may also play an important role in deme formation. Wainhouse and Howell (1983) and Karban (1989) both provided evidence for parthenogenic insects adapted to individual host plants.

The magnitude and spatial scale among host plant variation in quality may be directly linked to the magnitude and spatial scale of genetic divergence in patchy insect populations. The deme formation hypothesis as proposed by Edmunds and Alstad (1978) imagined a world where each host plant offered herbivores the chance to colonize, establish populations, and adapt to the unique characteristics of individual plants. However, 25 years of subsequent insect-plant research has shown that individual host plants vary within their immediate locality, within the population and among populations such that established herbivore populations resident on a specific plants can be envisioned as existing within a matrix of possible hosts, of which only a limited few offer real possibilities for establishing new populations. Herein, I provide evidence that it is this variation in local quality in combination with the patchy distribution of suitable hosts

that may facilitate local adaptation at the level of the individual host tree and that it is imperative to understand the local variation in quality to understand the atmosphere where deme formation can occur and persist.

*Belonocnema treatae,* is a mobile, endophagous gall-forming wasp that shows variation in densities of up to three orders of magnitude between adjacent host trees. Longitudinal studies at our field sites show a long term pattern where high gall-former density trees are rare (Figure 1.A) and are spatially isolated across the landscape of available host trees (Figure 1.B). In the two-part analysis presented herein, I investigate how both variation in neighboring host plant quality at the local scale and deme formation on individual host trees at the landscape scale influence the distribution and abundance of this host specific gall-former, *B. treatae,* on its host, *Quercus fusiformis.* In **Experiment 1,** I use a one-way transplant experiment to compare measures of insect performance for gall-formers reared on their high density natal trees and their low density conspecific neighbors to determine if gall density equates with host plant quality at the local level. In **Experiment 2,** I use a reciprocal transplant experiment to measure performance on natal host trees and alternative high density host plants (putatively high quality hosts) to determine whether deme formation at the scale of individual host trees has occurred. By simultaneously examining both mechanisms that influence herbivore distribution and abundance in one study, and linking both studies by the shared high density focal trees, I am able to look at the individual effects of each and see how they interact to explain the pattern I observe in this plant-herbivore system.

## MATERIALS AND METHODS

### Study system

*Belonocnema treatae* Mayr (Hymenoptera: Cynipidae) is a host-specific gall former of the plateau live oak, *Quercus fusiformis*. *B. treatae* exhibit a heterogonous life cycle typical of cynipids (Askew 1984) in which temporally segregated sexual and asexual generations are required to complete the life cycle (Lund et al. 1998). Coincident with *Q. fusiformis* bud break and continuing through leaf flush in the Spring, the sexual generation emerges from multilocular galls located on the underground fibrous root tissue. Females mate immediately upon emergence, fly up to the canopy, and oviposit in the lateral veins on the undersides of immature leaves, leaving a permanent scar per oviposition attempt on each leaf. Spherical, unilocular galls initiate growth following leaf maturation (May). Gall growth proceeds until galls lignify in August and September. The asexual generation emerges from leaf galls in a narrow window from mid-October through mid-December and descends to the ground to oviposit into the shallow roots of *Q. fusiformis* inducing the multilocular root galls thereby completing the life cycle. Leaf gall density varies significantly between trees within populations and across years and sites (Galusky 2000; Reynolds 2001), a pattern common in the family Cynipidae (Askew1984; Stone et al. 2002).

The host plant, *Q. fusiformis*, is a late seral stage tree reaching heights of over 25m but more typically forming clonal clusters less than 10m in height. *Q. fusiformis* is generally restricted to the Edwards Plateau of central Texas (Nixon 1997) and is considered a wintergreen species, retaining annual leaves until early spring. Some authors consider *Q. fusiformis* a subspecies of the common live oak, *Quercus virginiana,* (e.g. Owens 1996).

*B. treatae* larvae developing within leaf galls are attacked at all stages of development by a diverse natural enemy community composed of parasitoids, hyper-parasitoids, and inquilines spanning 5 insect orders: Hymenoptera (n = 20 species); Coleoptera (n = 1 species); Lepidoptera (n =1 species); Diptera (n =1 species); and Orthoptera (n=1 unknown species) (Lund 1998; Hall 2001). Rearing of gall occupants in the lab in individually isolated gelatin capsules shows asexual *B. treatae* females do not emerge from galls from which any of the above community members emerge. As a consequence, regardless of life style (parasitoid or inquiline), all function as agents of mortality. Mass rearing of leaf gall occupants from galls exposed to natural enemy attack throughout the entire season of development across sites and years demonstrate extremely low emergence rates from leaf galls (eg. 0.5% in 1996; 1.0 % in 1997; 1.0 % in 2000; Lund et al. 1998; Hall et al. 2001). Lund et al. (1998) and Reynolds et al. (2001) working with the same population used for the studies described and reported herein showed that gall-former emergence success increased exponentially with increasing gall size with >95% of *B. treatae* emerging from galls in the upper 50% of the gall size distribution. Average gall size, differs significantly between trees (Reynolds et al. 2000)

and mean gall size per tree is correlated with *B. treatae* emergence success.

## Study site

Descriptive density surveys were performed in the Fall 2001 and transplant experiments ran from January to November 2002 on Texas State University's Freeman Ranch, Hays County, Texas, USA (29°55' N, 98°00' W). Located on the eastern edge of the Edwards Plateau in central Texas, the ranch is dominated by live oak-juniper savannas in the uplands that grade into closed-canopy woodlands in the intermittent drainages (Barnes et al. 2000).

## Spatial pattern of distribution

To quantify the distribution and abundance of *B. treatae* within populations of its host plant, leaf gall density was surveyed in the Fall 2001 across 100 *Q. fusiformis* trees at the study site. Trees were selected for study by first selecting 400m long transects that ran perpendicular to ranch roads through the study site. At each transect location, the nearest tree was chosen at 20m intervals. Leaf gall density per tree was estimated by counting the number of galls on leaves within a ¼ $m^2$ grid placed randomly throughout the canopy, one in each cardinal direction. Canopy foliage is layered and therefore this method provides a thorough index of gall densities. The four density measurements per

tree were averaged to estimate mean density per $m^2$ of tree foliage. Density estimates for all 100 trees were used to construct a frequency distribution of leaf gall densities at the study site. Each tree was marked using a Garmin GPS 12 hand-held unit and coordinates of each tree were used in conjunction with GIS system software (ArcView) to map the spatial distribution of high and low density host trees.

Experiment 1: Test of local host quality

To test whether variation in gall density among trees at the local level correlates with variation in host plant quality as measured by insect performance on high and low density trees I conducted a one-way transplant experiment. I selected five high leaf gall density trees identified in the above survey and for each high density (focal tree), I selected the nearest neighboring host tree in each cardinal direction that exhibited low densities of the gall former (mean ± SE; distance of neighbor trees to focal trees = 0.06 ± 0.01 km, range = 0.01 km to 0.15 km). Focal trees and neighboring trees differed significantly in leaf gall density (mean ± 1 SE, high density focal trees = 63.6 ± 7.2 galls/canopy grid, low density neighbor trees = 3.8 ± 1.2 galls/canopy grid, $t = 14.51$, $DF = 23$, $p < 0.0001$), but not differ in diameter at breast height, an estimate of tree maturity (mean ± 1 SE, high density focal trees = 172.1 ± 22.4 cm, low density neighbor trees = 176.3 ± 12.2 cm, $t = -0.156$, $DF = 23$, $p = 0.88$).

To establish the test of neighbor quality in January 2002 (two months prior to emergence of *B. treatae* sexual generation females), I placed screen enclosures around

three randomly selected branches of each of the five focal trees and the twenty near neighbor trees. Screen enclosures (60cm x 45cm) were constructed of 0.25 mm Nytex screen (Bio Design, Inc., New York, USA) sealed on three sides and fitted with 0.25 inch Velcro strips on the open end to fit and seal over branches. Bags contained hundreds of leaves and provided an unlimited number of oviposition sites for the relatively small number of females added to each bag. In mid-February, following abscission of the 2001 leaf cohort, but prior to bud break of the 2002 cohort, each bag was cleared of leaves and any residual insects (herbivores, natural enemies) and resealed to insure the integrity of the experiment. Thus deployed bags allowed us to isolate the effects of individual host plants on measures of gall former performance in the absence of natural enemies (see below).

Beginning in February 2002, root galls were harvested from rootlets under the crowns of each of the five focal trees to rear sexual generation wasps for experimental transplants. Root galls from each focal tree were housed separately in 3 liter screened chambers which were kept in the lab at room temperature (28°C) under a natural light schedule. Freshly emergent mated females were collected every 2 days. Within each of the five focal/neighbor clusters, five mated female *B. treatae* from the focal tree were added to the three replicate branches of each of the four respective low density nearest neighbors and the three replicate branches of the focal tree (see Figure 2). Two weeks following all transplants, wasp oviposition was inspected in all bags by looking for oviposition scars on the leaves. Bags remained on trees from April to early fall for galls to develop. Reciprocal transplants were not possible because neighboring trees did not

maintain high enough root gall densities to provide the requisite 75 females needed to establish full reciprocal replicates.

Just prior to peak emergence of asexual female *B. treatae* from leaf galls in early November (Lund et al. 1998, Hall 2001, Reynolds 2001) all bagged branches were cut from trees, returned to the lab, and processed as described below to provide estimates of seven measures of host plant quality that sequentially effect *B.treatae* during that phase of its life cycle spent in leaf galls. To estimate the percent leaf abscission (a potential direct host plant defense against galled leaves or an indirect defense if abscission rates are related to host stress/vigor), leaves in each replicate were separated into those that have abscised and those that remained attached. Galls on attached leaves were categorized as mature (fully lignified - reached full gall size) or immature (partially lignified or fleshy - not reaching full size). Lignified and fleshy galls are easily distinguished by differences in color and firmness. The proportion of mature galls at harvest gives an estimate of developmental timing of cohorts of galls for each replicate. For each replicate, all galls on attached leaves were removed, binned by treatment, replicate, and developmental status, and housed in an incubator set to natural light and temperature schedules to rear gall occupants to estimate percent emergence. Galls were separated by developmental status, (mature or immature at timing of peak emergence) to separately measure gall-former performance. *B. treatae* emergence from leaf galls occurs within two months following gall harvest. Galls on abscised leaves were left on leaves to later measure gall initiation success (# galls initiated / # oviposition scars or attempts per leaf), binned by treatment and replicate, and put into an incubator to assess emergence success. Galls were left on

abscised leaves to measure gall initiation success (# galls formed / # oviposition scars or attempts per leaf), another measure of insect performance for each tree x donor combination. Initiation success on abscised leaves was not different from leaves that remained on branches (mean ± 1 SE, abscised leaves = 0.436 ± 0.03 galls/oviposition attempt, attached leaves = 0.437 ± 0.03 galls/oviposition attempt, $t = -0.006$, $DF = 57$, $p = 0.99$) and therefore was used as a sub-sample to estimate initiation success.

For each replicate the following components of gall former performance were scored: (a) *oviposition success* - percent of galls established from the number of oviposition attempts from samples of abscised leaves, (b) *growth phenology* - percent of galls reaching final developmental state (lignified at time of harvest), (c) *gall abscission* – proportion of galls per replicate on abscised leaves, (d) *individual gall size* – diameter of gall (in mm) measured using digital calipers, (e and f) percent of galls developing to the minimum emergent size threshold in the absence (2.62 mm) and presence (5.82mm) of natural enemies (see below), and (g) *emergence success* – percent of mature galls producing a *B. treatae*. Only mature galls were used to estimate emergence success since immature galls had a lower overall emergence success rate (mean ± 1 SE; mature galls = 0.64 ± 0.01 wasps/gall; immature galls = 0.53 ± 0.02 wasps/gall; $t = 3.2$; $DF = 311$; $p < 0.01$). All variables measure insect performance.

Gall size is known to affect the probability of gall former emergence in tri-trophic systems (Abrahamson and Weis 1998) and does within this system as well (Reynold et al. 2000). I recognized two critical thresholds in the gall size distribution: a tree threshold and a natural enemy threshold. The tree threshold is the minimum size a gall must attain

in order for a gall-former to emerge from the gall in the absence of natural enemies. The percent of galls produced by individual trees that fail to attain this size is therefore a direct measure of the source of plant mediated early gall-former death that is common in this system. The natural enemy threshold is the minimum size galls must attain to have a no-zero probability of producing a gall-former in the presence of natural enemies. required to produce a wasp in the presence of natural enemies. Using data from previous parasitoid exclusion experiments and naturally harvested galls in this system (Lund et al. 1998, Hall et al. 2001, Reynolds et al. 2001), I calculated that in the absence of natural enemies, 95% of gall formers emerge from galls > 2.62 mm in diameter (plant threshold) and in the presence of natural enemies, 95% of gall formers emerge from galls >5.82 mm (natural enemy threshold).

Efficacy of screen bag enclosures was confirmed for: (a) exclusion of natural enemies – by identifying emergents from all galls harvested (7088 *B. treatae* and 23 natural enemies; 0.3 % parasitism) and (b) exclusion of other ovipositing female *B. treatae* - by looking for galls or oviposition scars in control bags where no wasps were added (4 bags; 361 leaves; 0 oviposition scars or galls; 0% intrusion by other *B. treatae* females). A total of 7,975 galls (mean per replicate ± SE; 153 ± 33 galls/bag) were indexed from 75 experimental bags across 5 high-density focal trees and 20 low-density neighbors in this test of local quality variation.

Experiment 2: Test of deme formation

Using the same five focal trees as population source trees, I transplanted sexual generation female *B. treatae* as before between all five focal trees in a fully balanced reciprocal transplant experiment to test the deme formation hypothesis. This design allowed us to examine performance for populations of gall-formers from each tree within and across all five focal trees and directly links to Experiment 1 by the shared focal/natal treatments. Focal trees differed between each other in leaf gall density (mean ± 1 SE, tree 1 = 87.8 ± 2.4 galls/canopy grid, tree 2 = 61.5 ± 2.0 galls/canopy grid, tree 3 = 62.0 ± 2.1 galls/canopy grid, tree 4 = 42.3 ± 15.3 galls/canopy grid, tree 5 = 64.3 ± 9.1 galls/canopy grid, $F_{4,15} = 3.98$, $p < 0.05$), but were all were greater than two standard deviations above the mean leaf gall density for all trees surveyed in our transect analysis of natural gall densities described earlier (mean ± 1 SD, 7.36 ± 15.3 galls/canopy grid). Following the methods described in Experiment 1, sets of five mated sexual generation females were added to each bag on the three replicate branches of their natal tree and each of three bags on each of the four novel high density focal trees (see Figure 2). The same procedures were used and the same variables (a – g) from Experiment 1 were measured in Experiment 2. A total of 15,148 galls (mean per replicate ± SE; 205 ± 34 galls/bag) were indexed from 75 experimental bags across 5 high density focal trees in this test of deme formation.

Statistical Analysis

To test distribution of bags where galls failed to initiate among treatments in this field study, I used a Chi-square goodness of fit test that compared observed and expected proportion of zero gall bags for each type of transfer in each experiment: neighbor vs. natal was compared in the test of local quality and natal vs. novel was compared in the test of deme formation.

To test for significant treatment effects on variables (a-g), ANOVA using a linear mixed effects model was fit to each response variable with tree (site of rearing) set as a fixed factor and source of insect population (focal tree 1-5) as a random factor. . When related herbivores can be gathered and subdivided (in this system – populations of leaf gall formers reared from root galls partitioned across multiple rearing conditions), a repeated measures design gives a more powerful test of the interaction term in studies of local adaptation (Horton et al. 1991; Boecklen and Mopper 1998), but has not been used extensively thus far (see Downie 1999). Linear mixed effects models are appropriate for these analyses for two reasons. First, because it give the best estimate for a repeated measures design with the source population over sets of alternative rearing sites and, second, this model is the most appropriate for an unbalanced design (Pinheiro and Bates 2000). While Experiments 1 and 2 were designed as fully balanced designs, failure of wasps to induce gall formation in 45% of bags rendered the design unbalanced for variables (a-g). I considered gall density within bags as a possible covariate, but found no effect on gall size ($R^2 = 0.007$; $p = 0.16$), abscission rate ($R^2 = 0.02$; $p = 0.21$), or

emergence success ($R^2 = 0.05$; $p = 0.13$), and therefore did not include it in the final analysis. Prior to analysis, all percent data was transformed using the empirical logistic transformation (Cox 1977). This transformation was applied because it provides a mechanism for computing both a variance and a weight for each replicate based on both the proportion (p) and the sample size (n). Thus treatments that differed widely in samples size (i.e. natal treatments, where wasps were likely to oviposit more often, versus novel and neighbor treatments) could be compared. Means and standard errors computed using empirical logistic transform are not readily back-transformed; therefore, the graphical presentation of results for these experiments display means and standard errors of the untransformed percentages. I considered transformations of gall size, such as $\log_{10}$, as suggested by Sokal and Rohlf (1995) for growth data, but found no difference in the results of the analysis, and therefore only report results of untransformed data on gall size. All analyses were performed using S-plus (Insightful Corporation, 2003, Seattle, Washington, USA).

For the test of Experiment 1 (neighbor quality), I compared insect performance using variables (a – g) within each of the five high density island/low density neighbor complexes. In the analysis, a significant tree effect indicates that high density islands differ from their nearest conspecific neighbors and a non-significant interaction term indicates a consistent pattern across all five high density island/low density neighbor complexes. Replicates on all neighbor trees in each complex were combined because wasps were unable to form galls on a number of neighbor trees, creating treatment level holes.

For the test of Experiment 2 (deme formation), I compared the performance of gall formers reared on heir own high density islands (natal tree) and the altenate four high density focal trees (novel tree). Here a significant tree effect indicates that these rare high density trees differ in quality to the herbivore. Deme formation is indicated by a significant interaction term between tree and insect population, where natal treatments outperform novel treatments.

## RESULTS

For both experiments, oviposition attempts were visually confirmed in 100% of bags two weeks after transplants (natal, novel, neighbor). This is evidence that wasps attempted to oviposit on all trees and in all treatments which rules out (a) that females did not survive transfers from the lab to bags in the field, (b) that host preference was a major issue so that females refused to oviposit on certain trees, and (c) that there was a mismatch between herbivores and individual host plant phonologies (Boecklen and Mopper 1998).

### Experiment 1: Test of local host quality

Although oviposition attempts were observed in all 75 bags in this experiment (15 bags across five focal trees; 60 bags across 20 neighbors), the proportion of bags in which gall-formers could not establish gall growth was nonrandom with respect to treatment (27/60 bags with no gall growth on neighbor trees; 0/15 bags with no gall growth on natal trees; $X^2_{df=1} = 6.75$; $P < 0.01$). The majority (67%) of failed bags clustered on six low density neighbor trees that could not produce a single gall given five mated female *B. treatae* added into each of three experimental bags (6/20 neighbor trees produced zero galls; resulting in 18/27 bags with do gall growth). This suggests that certain neighboring trees standing close to high density focal trees could not support gall growth whatsoever.

In all following analyses, tests of the interaction term were not significant and are therefore not reported for each insect performance variable (see all $X^2$ values, degrees of freedom, and p-values in Table 1). This means that when there is a significant tree effect, the relationship of insect performance on focal trees and neighbors is consistent across all five groups of host trees. To estimate gall initiation success, I did not include the 27 bags with 0% oviposition success and only used bags that produced galls, to give us an independent, conservative estimate of performance. Gall initiation success on focal trees (mean ± SE, 0.43 ± 0.06 galls/oviposition scar; range, 0.20 – 0.75 galls/oviposition scar) was significantly higher ($F_{1,28} = 7.52$; $p = 0.01$) than on the subset of neighbor trees (14/20 neighbors) that were capable of supporting gall initiation (mean ± SE, 0.36 ± 0.03 gall/oviposition scar; range, 0.08 – 0.61 galls/oviposition scar; Figure 3.A). This 19% increase in gall initiation success on focal trees, translated into considerably more galls produced per bag (per five mated females) on high density focal trees (mean ± SE, 199 ± 45 galls/bag and 135 ± 43 galls/bag for focal trees and neighbor trees, respectively; $F_{1,41} = 6.22$; $p < 0.001$; Figure 3.B).

Gall size, for lignified galls on attached leaves, was 31% larger on focal trees (mean ± SE, 4.92 ± 0.36 mm; range, 1.4 – 6.1 mm) than on neighboring trees (mean ± SE, 3.75 ± 0.22 mm; range, 1.1 –5.5 mm) and means were significantly different ($F_{1,34} = 18.50$; $p < 0.001$; Figure 3.C). Focusing on galls that completed development and produced a *B. treatae* to give us our most conservative estimate of final gall size, focal trees still produced significantly larger galls than neighbor trees (mean ± SE, 5.80 ± 0.10 and 4.91 ± 0.14 mm for focal and neighbor trees, respectively; $F_{1,34} = 25.69$; $p < 0.001$).

*Belonocnema treatae* emergence success in the absence of parasitism, estimated from galls that were mature at harvest, was marginally higher ($F_{1,41} = 6.83$; $p < 0.10$) on focal trees (mean ± SE, 0.61 ± 0.19 *B. treatae*/gall) than on neighbor trees (mean ± SE, 0.56 ± 0.21 *B. treatae*/gall). Refining this analysis to only mature galls that exceeded the critical tree threshold, thus having a probability greater than zero to produce an insect, this marginal difference disappears (mean ± SE, 0.79 ± 0.21 and 0.76 ± 0.20 *B. treatae*/gall for focal and neighbor trees, respectively; $F_{1,41} = 1.54$; $p > 0.10$). This means that the marginal difference was driven by tree effects on early gall former death and when you remove these early deaths, emergence does not differ between focal and neighbor trees for galls that mature quickly and reach the tree size threshold.

High density focal trees doubled the proportion of galls produced that exceeded the natural enemy size threshold of 5.82 mm (mean ± SE, 0.42 ± 0.03) compared to neighbor trees (mean ± SE, 0.16 ± 0.03; $F_{1,41} = 7.08$; $p < 0.05$; Figure 3.D). Thus, under natural conditions in the field where the third trophic level would be present I expect emergence success on focal trees to be twice that on neighbor trees.

More galls were abscised on neighbor trees that harbored low gall densities (mean ± SE, 0.35 ± 0.06; range, 0.00 – 1.00) than on focal trees (mean ± SE, 0.22 ± 0.07; range, 0.00 – 1.00), however this difference was not significant ($F_{1,46} = 1.65$; $p > 0.10$). A higher proportion of galls exceeded the tree threshold on focal trees versus neighbors, but this measure of performance was also not significant (mean ± SE, 0.79 ± 0.07 and 0.69 ± 0.05; range 0.01 – 1.00 and 0.00 – 1.00 for focal and neighbor trees, respectively; $F_{1,41} = 0.82$; $p > 0.10$). The proportion of mature galls at harvest was not different between focal trees (mean ± SE, 0.62 ± 0.07; range, 0.06 – 1.00) and neighbor trees (mean ± SE, 0.71 ±

0.05; range, 0.16 – 1.00; $F_{1,38} = 0.05$; $p > 0.50$). Results of all analyses for the test of neighboring host plant quality are summarized in Table 1.

Experiment 2: Test of deme formation

In the test of deme formation, a total of 15 replicates tested insect performance on their natal tree and 60 replicates tested performance on novel, high density trees. Although oviposition attempts were observed in all bags in this experiment as well, the proportion of bags where galls failed to establish was nonrandom with respect to treatment (11/60 failed bags from novel treatment; 0/15 failed bags from natal treatment; $X^2_{df=1} = 2.75$; $P<0.10$). This is a statistically marginal difference, however surprising, since all of these trees were found to be high quality host trees in experiment 1 (see Results from Experiment 1, Figure 3, and Table 1). In this study, failed bags did not cluster around any one tree or population as they did in Experiment 1 and may represent evidence for localized poor quality areas for herbivores within individual trees.

Again, to estimate gall initiation success, I did not include failed bags (0% oviposition success) and only used bags that produced galls, to give us a conservative and independent estimate of performance. Gall initiation success was significantly different, but in the opposite pattern predicted by deme formation (mean ± SE, 0.43 ± 0.06 and 0.52 ± 0.05 galls/oviposition scar; range 0.20 – 0.75 and 0.12 – 1.00 for natal and novel combinations, respectively; $X^2_{df=1} = 10.80$; $p < 0.01$; Figure 4.A). However, this 21% difference in initiation success did not translate into more galls produced per bag for five female *B. treatae* on novel trees (mean ± SE, 199.1 ± 45.0 galls; range, 0 – 1272) versus

their natal tree (mean ± SE, 202.7 ± 40.1 galls; range, 4 – 631; $X^2_{df=1} = 0.01$; $p > 0.90$; Figure 4.B). Thus, oviposition intensity (# of oviposition attempts per 5 females) or egg commitment per oviposition attempt must have been higher on natal trees than novel trees to compensate for this difference.

The proportion of galls on leaves that were abscised was significantly higher on novel trees (mean ± SE, 0.39 ± 0.05; range, 0.00 – 1.00) than natal trees (mean ± SE, 0.22 ± 0.07; range, 0.00 – 1.00; $X^2_{df=1} = 57.12$; $p < 0.001$; Figure 4.C). Gall abscission reduces emergence success and final gall size in our system (Egan, *unpublished data*). This suggests that wasps on their natal trees can reduce detection by the host tree or at least minimize whatever physiological change occurs in their specific leaf that causes them to abscise early.

There was a higher proportion of mature galls at harvest, just before peak emergence in nature (Lund et al. 1998), on their natal tree (mean ± SE, 0.62 ± 0.07; range, 0.06 – 1.00) than on other high quality novel trees (mean ± SE, 0.60 ± 0.04; range, 0.00 – 1.00; $X^2_{df=1} = 38.23$; $p < 0.001$). A higher proportion of mature galls on natal trees suggests that wasps can develop faster on natal trees than novel, high quality hosts (Figure 2.D).

For galls that remained attached throughout development and were mature at time of harvest, mean gall size was larger on natal trees (mean ± SE, 4.92 ± 0.33 mm; range, 3.11 – 6.08) than on other high quality novel trees (mean ± SE, 3.64 ± 0.19 mm; range, 1.95 – 5.29; $X^2_{df=1} = 13.67$; $p < 0.001$; Figure 2.E). Again, when I remove all galls from the analysis that did not produce a wasp for our most conservative estimate, gall size still differs between natal and novel treatments (mean ± SE, 5.80 ± 0.10 mm and 4.90 ± 0.12

mm; range, 5.04 – 6.34 and 3.32 -- 6.39 for natal and novel trees, respectively; $X^2_{df=1}$ = 11.91; $p < 0.001$). Looking within that distribution of gall sizes, a larger proportion of galls make it past the threshold of early plant-mediated gall death on natal trees (mean ± SE, 0.79 ± 0.07; range, 0.09 – 1.00) than on novel trees (mean ± SE, 0.65 ± 0.04; range, 0.00 – 1.00; $X^2_{df=1} = 33.16$; $p < 0.001$; Figure 2.F). The proportion reaching the natural enemy size threshold was not significantly different between treatments (mean ± SE, 0.42 ± 0.06 and 0.14 ± 0.03; range, 0.34 – 0.54 and 0.00 – 0.31 for natal and novel trees, respectively; $X^2_{df=1} = 0.10$; $p > 0.50$).

Using emergence success to test for deme formation, both the tree effect and the interaction were significant ($F_{5,16} = 2.54$; $p = 0.051$; $X^2_{df=1} = 20.20$; $p < 0.001$). This means that more wasps emerged from galls on their natal tree (mean ± SE, 0.66 ± 0.07 *B. treatae*/gall; range, 0.15 – 0.88) than on novel trees (mean ± SE, 0.46 ± 0.05 *B. treatae*/gall; range, 0.00 – 1.00) and demonstrates that although all trees in this study were found to be high quality relative their nearest conspecific neighbors, they also differ among themselves in quality based on gall wasp emergence (mean ± SE, 0.587 ± 0.10 *B. treatae*/gall, 0.70 ± 0.06 *B. treatae*/gall, 0.24 ± 0.05 *B. treatae*/gall, 0.60 ± 0.012 *B. treatae*/gall, 0.40 ± 0.08 *B. treatae*/gall for all five high quality trees; Figure 5.A and 5.C). Results of all analyses for the test of deme formation are summarized in Table 2.

## DISCUSSION

The results of this study demonstrate a clear and comprehensive example of how both the variation in local plant quality and deme formation within the context of variable local host quality can contribute to the patchy distribution of phytophagous insects within populations of its host plant. In this cynipid-oak system, a pattern exists where gall densities on average vary by an order of magnitude between adjacent host trees (high density focal trees = 63.6 ± 7.2 galls/canopy grid, low density neighbor trees = 3.8 ± 1.2 galls/canopy grid), the host trees supporting high gall densities are rare across the landscape (~5%) and appear as habitat islands within a sea of "available" hosts supporting low herbivore densities (Figure 1). When the sexual generation leaf gall inducing female *B. treatae* disperse locally on to historically low density conspecific neighbors, they immediately run a 30% risk of landing on the "zero-quality" trees (6 out of 20 of the neighbor trees) which failed to produce a single gall from all three bags that contained oviposition scars. If *B. treatae* avoid "zero-quality" trees and manage to land on the "low-quality" trees, they experience lower gall initiation success, lower overall number of galls produced, smaller gall size compared to focal trees, and a smaller proportion of galls that reach a size that provides relief from natural enemy attack (Figure 3, Table 1). The finding that local variation in gall densities equates with variation in host plant quality supports recent research implicating the importance of bottom-up effects in plant-herbivore systems (Hunter and Price 1992; Ylioja et al. 1999; Larsson 2000).

Natural enemies then reinforce or intensify present differences due to interactions solely between the herbivore and the host plant, regardless of whether insects are more apparent to natural enemies, which has been found in demes of a leaf miner on oak (Mopper et al. 1995). In other studies involving tritrophic systems, gall size directly influences emergence success and this relationship is driven by natural enemies (Jones, 1983; Weis et al, 1985; Price, 1988; Zwolfer and Arnold-Rinehart, 1994; Stiling and Rossi, 1996). Among local hosts in this study, average gall size on high density island trees is 4.92 ± 0.36 mm whereas on neighboring trees it is only 3.81 ± 0.22 mm. Using a previously established survivorship curve, where emergence success increases exponentially as gall size increases (Reynolds et al. 2000), I calculated the probability of emergence in the presence of natural enemies. Galls developing on high density focal trees are 2.1 times more likely to produce an emergent *B. treatae* than galls on low density neighbors.

Due to the unexpected "zero-quality" trees, planned sample size was reduced by 45% (33 out of 60 bags produced galls that could be measured and reared in the lab) on neighboring trees. I believe that the 2/3 increase in proportion of galls on abscised leaves on natal trees and the 10% more galls exceeding the tree size threshold on focal trees were biologically significant differences that would argue further the differences in quality between focal and neighboring trees. However, I lacked the statistical power to detect these differences due to failed bags on these "zero-quality" trees.

Given this isolation of high density, high quality hosts by low density, low quality neighbors, these focal trees can be seen as habitat islands where adaptation to individual host plants is possible (Janzen 1968; Strauss and Mopper 1998). Additionally, within this matrix of high and low quality trees, neighbor trees may act as sponges for dispersing *B.*

*treatae* females, actually reducing gene flow between high density trees more than if there were no tree between them at all. If females manage to navigate this matrix to colonize other high density islands, they can face lower performance relative their natal host and/or lower performance within the novel high quality trees relative to the natal population (Figure 3.B & 3.C). This is in addition to the immediate potential costs to dispersal, such as the ineffective use of a short cynipid adult life span (<7 days; Askew 1984), breaking from behavior required by life cycle (emerge from root galls, oviposit in canopy; Lund et al. 1998), and their apparent weak flying ability (personal observation). Lower fitness on novel high quality trees is shown by higher abscission rate, slower developmental rate, smaller average gall size, a smaller proportion reaching critical tree threshold, and lower overall emergence success (Figure 2 & 3.A; Table 2) and is convincing evidence that the herbivore has undergone local adaptation at the level of the individual host tree (i.e., deme formation).

Deme formation has been documented on individual host trees in a diverse group of insects including: Homoptera (Edmunds and Alstad 1978, Hanks and Denno 1994, Komatsu and Akimoto 1995), Thysanoptera (Karban 1989), Lepidoptera (Mopper et al. 1995, Mopper et al. 2000), and Diptera (Stiling and Rossi 1996, Stiling and Rossi 1998), but this is the first evidence from the phytophagous Hymenoptera. However this is not surprising, since cynipid-oak systems share many similar characteristics that may predispose deme formation to occur, such as short lived insects on long lived host trees (Edmunds and Alstad 1978; Hanks and Denno 1994), strong selection from the host plant (Strauss and Karban 1998), endophagous mode of feeding (Mopper et al. 1995; Stiling and Rossi 1996,1998), parthenogenic reproduction (Karban 1989; Strauss and Karban

1994), haplodiploidy (Boecklen and Mopper 1998; however, see Van Zandt and Mopper 1998), and host plant isolation (Wainhouse and Howell 1983; Hanks and Denno 1994). However members of the Cynipidae are unique to other insects tested for deme formation in that they are characterized by a cyclically parthenogenetic life cycle, with the ability to induce a wide diversity of generation specific galls on generation specific locations of their host trees (Stone et al. 2002). In our system, only certain host trees offer the phenotype of a carpet of root shoots surrounding the central trunk and the underlying fibrous root tissue required by the root galling generation. This requirement may restrict *B. treatae* to these high quality focal trees.

I made every effort to assure that our variables were independent of each other to give us the most comprehensive evidence of local quality and deme formation. By testing a series of independent variables, sequential across the process of oviposition to emergence, I can see "into" where the effects lie and how bottom-up effects interact with top-down effects from natural enemies making our study unique to other tests of deme formation. Although previously not used to test for deme formation, leaf abscission is known to reduce herbivore fitness (Waddell et al. 2001) and can be a major selective force on phytophagous insects (Stiling and Simberloff 1989; Stiling et al. 1991; Mopper et al. 2000). Yet, in this study, differential abscission rates between natal and novel insects on the same tree (similar genotypic background), suggest adaptations by natal insects to avoid or reduce abscission by the host tree (Figure 2.C). Developmental rate is also rarely used to test for deme formation (see Komatsu and Akimoto 1995 and Strauss 1997), but has been proposed as a plant defensive mechanism that reduces herbivore fitness indirectly through an increased vulnerability to both natural enemies (Clancy and

Price 1987; Williams 1999) and weather (Fordyce and Shapiro 2003). In this study, a smaller proportion of galls reached maturity (lignification) on novel high quality hosts (Figure 2.D), indicating faster development through this "window of vulnerability" by natal insects (Biggs and Latto 2001). This is an important effect in this system where *B. treatae* is continuously attacked by a diverse and abundant natural enemy community (N=30+) from oviposition to gall lignification (Hall et al. 2002) and because galls reach maturity in late summer to early fall where extremely high and low temperatures are possible.

Mopper (1998) criticizes previous tests of deme formation for (a) overlooking interactions between host plants and natural enemies and (b) not identifying the specific source of mortality. For the latter, I isolated interactions between host tree and gall former from oviposition through emergence to get a clear picture of host tree effects and therefore a clear picture of the agent of mortality (the host tree). In addition, I was able to decompose plant mediated mortality at different stages of gall development: (1) inability of egg to induce gall growth, (2) proportion reaching tree threshold, and (3) the proportion reaching the natural enemy threshold (indirect host tree effect on mortality). To think more directly about the effects of natural enemies on the present performance differences between natal versus novel gall growth, I can look at the average gall size (Figure 2.E) on natal trees (4.92 ± 0.36 mm) and novel trees (3.64 ± 0.19 mm) and using previously established survivorship curve, calculate the probability of emergence in the presence of natural enemies across tree (Reynolds et al. 2000). Based on the survivorship curve, gall formers on their natal trees are 2.75 times more likely to produce an emergent *B. treatae* than on novel trees. Gall size was a highly likely variable to detect deme

formation due to heavy parasitism rates in nature (>95%) and the adaptive significance of large gall size to thwart attacks from parasitoids (Weis and Abrahamson 1985; Price and Clancy 1986). Because gall growth is an interaction between the insect and plant genotypes, and large gall size increase emergence success in the presence of natural enemies, deme formation in this system may, in part, be driven by heavy selective pressure from the third trophic level.

Previous experiments testing deme formation analyzed data used log-linear models (Mopper et al. 1995, Stiling and Rossi 1998) when response variable were discrete and used factorial analysis of variance (Karban 1989, Hanks and Denno 1994, Strauss 1997) for continuous response variables. Data analysis in this study differs from most previous studies of local genetic structure because I employed a repeated measures design, which provides a powerful test of the host x population interaction by anticipating the large levels of interindividual variation within populations of phytophagous insects (Futuyma and Peterson 1985; Horton et al. 1991). Previous studies of deme formation offer similar experimental designs (fully reciprocal transplants), but do not analyze their data as such (Karban 1989). One drawback of repeated measures ANOVA is the assumption of a balanced design, which is fairly unlikely in field experiments. Our full design included 135 bags across 25 trees and I ended up with 38 bags dropped from the study due to the inability of gall induction on certain neighbor and novel trees. Our solution was to use a linear mixed effects model because it provides a powerful and flexible estimate of the covariance structure with fewer inherent assumptions (Pinheiro and Bates 2000; S-PLUS 2003). Low statistical power has been a common problem in detecting local adaptation (Boecklen and Mopper 1998; Van Zandt and Mopper 1998),

but a repeated measures design analyzed using linear mixed effects models offers increased experimental efficiency in the face of experimental (life-history or size of focal organisms) or logistic (space and time) constraints.

One possible criticism of this study would be that I did not control for possible inherited parental effects, which can affect results in studies of deme formation (Rossiter 1998). The only way I saw to control for maternal effects would be to control for the size of the females I added to each bags, which can effect the number eggs per female and the egg size (Rossiter 1996). There was not a perfect solution to this problem, because females are small (4-5 mm) and measurement techniques require manipulation of the insect (specifically the tibia length or head width), which can damage the insect. In the neighbor quality study, all females tested within each focal-neighbor complex were from the same host tree, and, in the test of deme formation, females from all trees were repeated across all other trees, to remove any host tree driven maternal effects. In addition, females used in transplants were reared in root galls under the exact same lab conditions.

This was a field experiment were no common garden experiment was used, so another possible criticism of this study could be that all effects were environmental effects on host tree (microsite differences). For the test of local quality, I would argue that neighbor and focal trees were close enough that this was not a problem (mean ± SE, 0.08 ± 0.02 km). For the test of deme formation, environmental effects are not an issue due to the complete reciprocal transplant design. All insect genotypes (natal vs. novel) are compared against the same genotypic background in each host tree (see Figure 3.C).

Furthermore, I find that intratree variation is not driving these differences because I dispersed all bags throughout tree canopy (Whitham et al. 1983).

Using both an understanding of local host plant quality with deme formation to explain the patchy distribution of a herbivore within populations of its host plant may be applicable to a more broad pattern with 805 species of cynipids in N. America and 1300 world-wide (Dreger-Jauffret and Shorthouse. 1992), over 80% feeding on *Quercus* (Askew 1984), and most cynipids exhibiting a patchy distribution within populations of their host plant (Stone et al 2002).This study convincingly demonstrates that variation in local host plant quality and deme formation at the level of the individual host tree both contribute to the patchy distribution of a specialist herbivore within populations of its host plant. These results specifically highlight the effects on individual life stage components of gall former success in the absence of natural enemies and how host plant differences in quality and demic adaptation are further reinforced in the presence of natural enemies.

## APPENDIX I:

## LIST OF TABLES

## LIST OF TABLES

Page

**Table 1.** Linear mixed effects model fit to response variables[a] in the test of neighboring host plant quality, where tree was set as a fixed effect and insect population set as a random effect. Significant tree effect indicates high-density focal trees are locally the highest quality tree; a non-significant interaction term indicates that this relationship is consistent across each focal/neighbor complex.

| RESPONSE VARIABLE | TEST OF TREE EFFECT | | | | TEST OF INTERACTION | | |
|---|---|---|---|---|---|---|---|
| | numDF | denDF | F-value | p-value | DF | $X^2$ | p-value |
| Initiation success | 1 | 28 | 7.52 | **0.01** | 1 | 0.77 | 0.38 |
| Galls produced | 1 | 41 | 6.22 | **<0.001** | 1 | 0.01 | 0.99 |
| Abscision rate | 1 | 46 | 1.65 | 0.21 | 1 | 0.01 | 0.99 |
| Development rate | 1 | 38 | 0.05 | 0.82 | 1 | 0.01 | 0.99 |
| Mean gall size | 1 | 34 | 18.50 | **<0.001** | 1 | 1.36 | 0.99 |
| Tree threshold | 1 | 41 | 0.82 | 0.37 | 1 | 3.26 | 0.07 |
| N.E. Threshold | 1 | 41 | 7.08 | **0.05** | 1 | 0.08 | 0.78 |
| Emergence success | 1 | 41 | 6.83 | 0.09 | 1 | .01 | 0.99 |

[a] All percent data was transformed prior to analysis using the empirical logistic transform

**Table 2.** Linear mixed effects model fit to response variables[a] in the test of deme formation on individual host plants, where tree was set as a fixed effect and insect population as a random effect. Interaction term is tested by comparing the fit of model with and without interaction term included. Significant tree effect indicates difference in quality between high-density focal trees; a significant interaction term indicates possible deme formation at the scale of the individual host tree.

| RESPONSE VARIABLE | TEST OF TREE EFFECT | | | | TEST OF INTERACTION | | |
|---|---|---|---|---|---|---|---|
| | numDF | denDF | F-value | p-value | DF | $X^2$ | p-value |
| Initiation success | 5 | 16 | 1.73 | 0.19 | 1 | 10.80 | **0.001** |
| Galls produced | 5 | 25 | 1.42 | 0.29 | 1 | 0.01 | 0.99 |
| Abscision rate | 5 | 25 | 1.39 | 0.26 | 1 | 57.12 | **<0.001** |
| Development rate | 5 | 19 | 1.63 | 0.19 | 1 | 38.23 | **<0.001** |
| Mean gall size | 5 | 24 | 0.22 | 0.95 | 1 | 13.67 | **<0.001** |
| Tree threshold | 5 | 16 | 0.97 | 0.44 | 1 | 33.16 | **<0.001** |
| N.E. Threshold | 5 | 16 | 0.84 | 0.51 | 1 | 0.10 | 0.76 |
| Emergence success | 5 | 16 | 2.54 | **0.05** | 1 | 20.20 | **<0.001** |

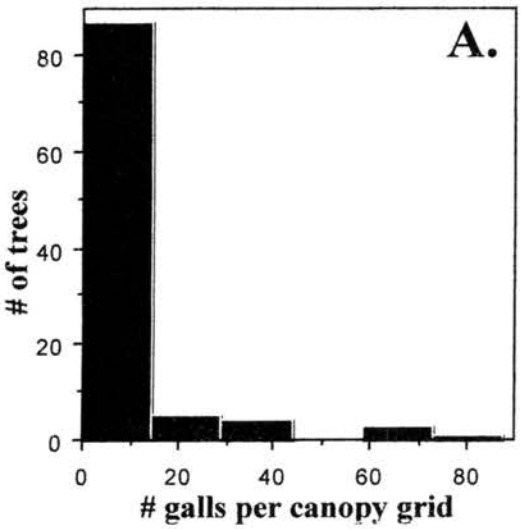
[a] All percent data was transformed prior to analysis using the empirical logistic transform

## APPENDIX II:

## LIST OF FIGURES

## LIST OF FIGURES

Page



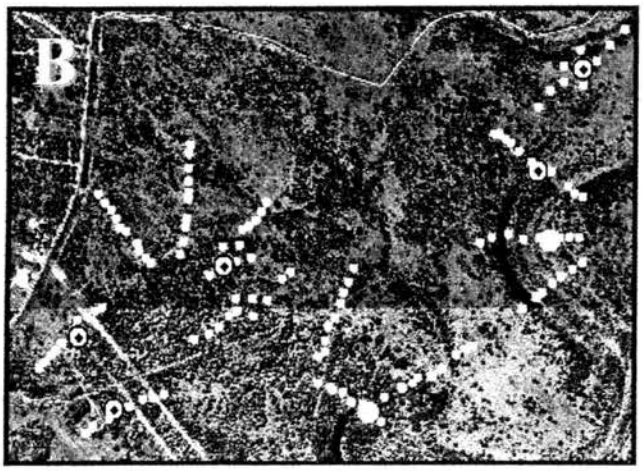




**FIGURE 1.** (A.) Frequency distribution of *B. treatae* leaf gall densities for 100 host trees (*Q. fusiformis*) along thirteen transects sampled across the study site in central Texas. Gall density is measured as total number of leaf galls counted within four 1/4 $m^2$ grid samples of canopy foliage. Notice rare trees that exhibit high gall density, (B.) Distribution of high and low density trees (mean ± 1 SE, high density trees = 63.6 ± 7.2 galls/canopy grid, low density trees = 3.8 ± 1.2 galls/canopy grid) mapped onto satellite photo of study site. High-density focal trees appear as larger highlighted circles and the five used in Experiments 1 and 2 herein are marked with dots at the center; low-density trees are represented by smaller open circles. Note: there are no high density trees found along six of the thirteen transects

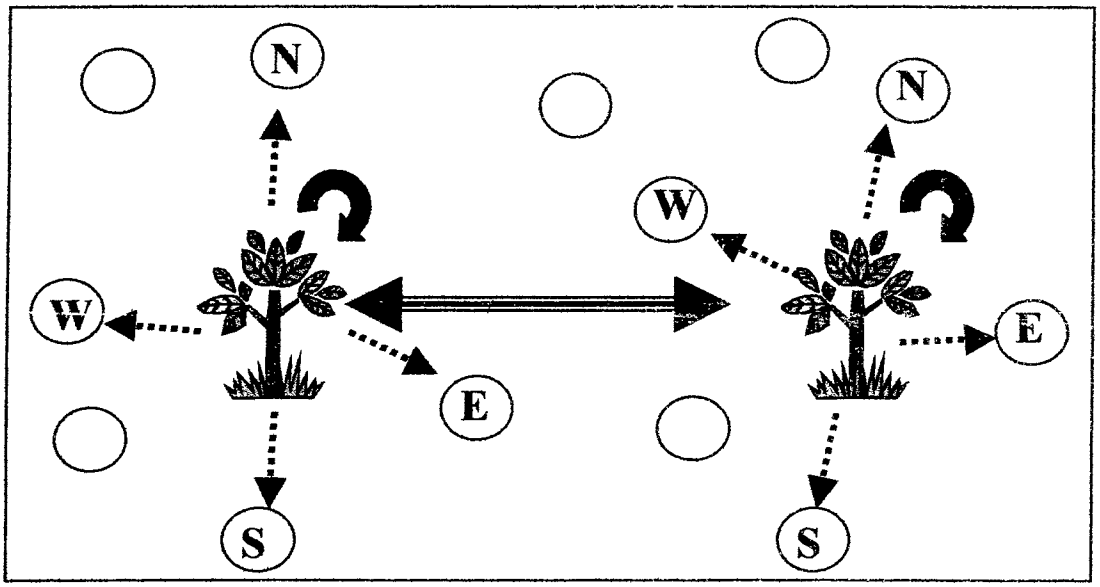



**FIGURE 2.** Diagram of the full experimental design to test both local quality and deme formation. Tree figures represent high-density focal trees, circles represent surrounding low-density trees, and those with directional symbols in the center represent nearest neighbors used in experiments. Thin dotted arrows are one-way transplants from focal trees onto low density near neighbor trees, thick striped arrow shows reciprocal transplants between high-density focal trees, and thick solid arrows are transplants back onto natal tree. Each source/rearing site combination was replicated 3 times in this experiment by adding five mated *B. treatae* into each of the three replicate bags per tree.

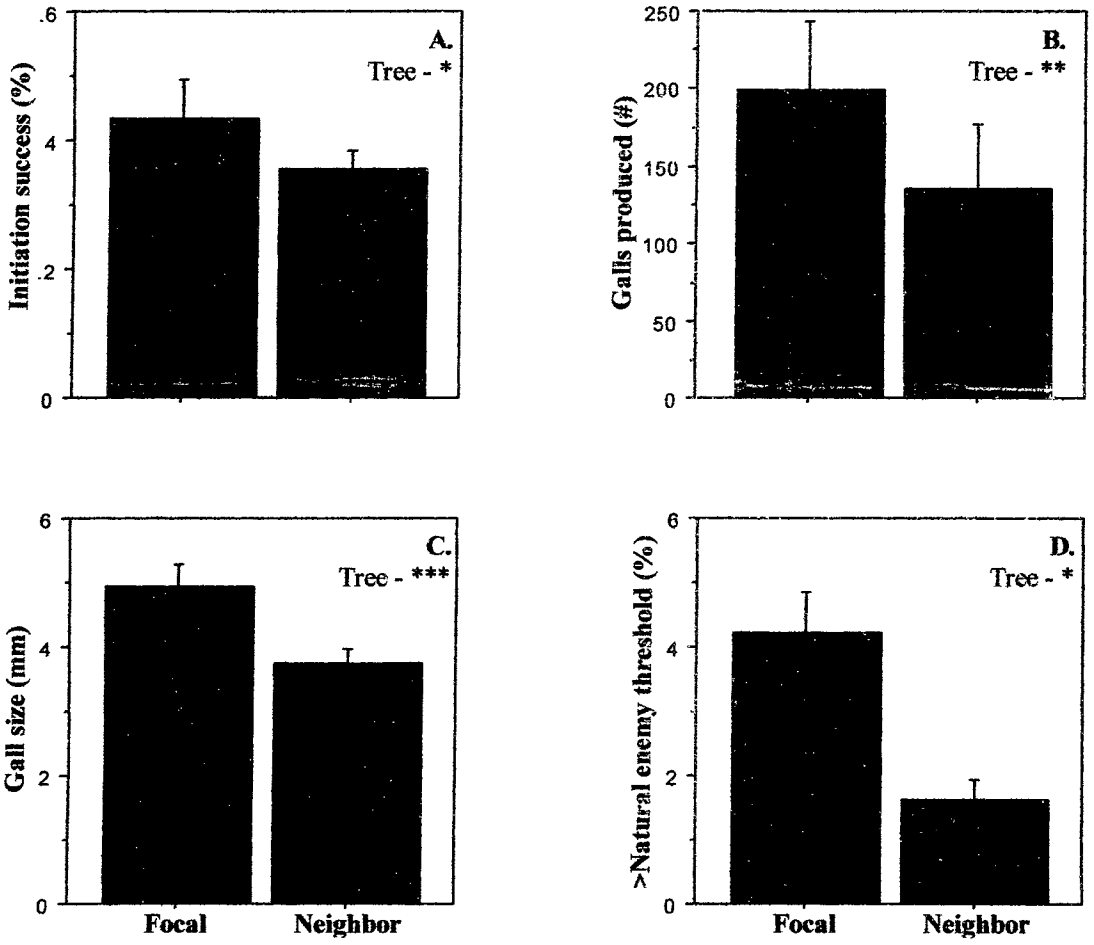



**FIGURE 3.** Components of performance for *B. treatae* leaf galling generation reared on high-density focal trees and low-density neighboring trees. Mean initiation success (A), galls produced (B), gall size (C), and proportion reaching critical natural enemy threshold (D) for five *B. treatae* populations on high-density focal trees and neighboring conspecifics. Data was analyzed using a linear mixed effects model with tree as a fixed effect and insect population as random effect. For analysis of all variables, interaction was not significant indicating a consistent relationship between each high-density island and its neighbors for insect performance. Significant tree effects are noted (* = $p < 0.05$; ** = $p < 0.01$; ** = $p < 0.001$). All percent data were transformed using empirical logistic transform prior to analysis. Shown here are untransformed means ± 1 SE.

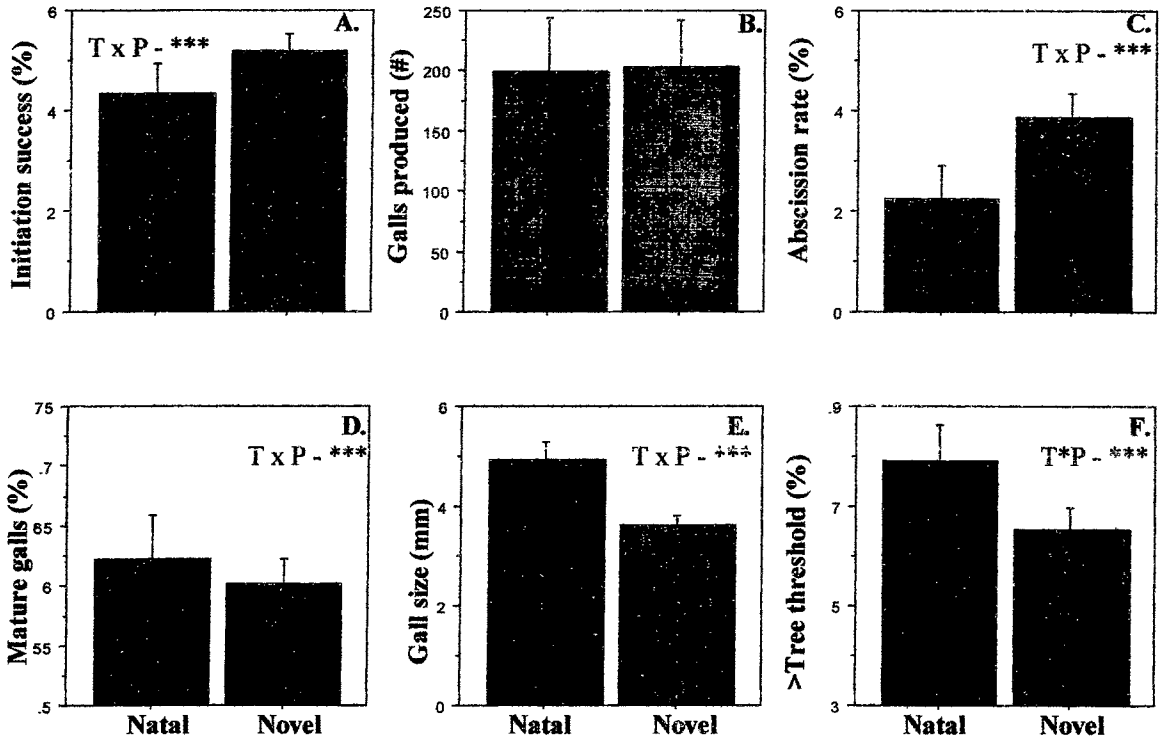



**FIGURE 4.** Components of performance for *B. treatae* leaf galling generation reared on natal trees and novel trees. Mean initiation success (A), galls produced (B), abscission rate (C), proportion fully developed (D), gall size (E), and proportion reaching critical tree threshold (F) for five wasp populations on natal and novel high density host trees. Data was analyzed using a linear mixed effects model with tree as a fixed effect and insect population as a random effect. For analysis of all variables shown here, tree effect was not significant indicating that mean insect performance did not differ between high-density trees. Significant interaction terms are noted (*** = $p<0.001$). All percent data were transformed using empirical logistic transform prior to analysis. Shown here are untransformed means ± 1 SE.

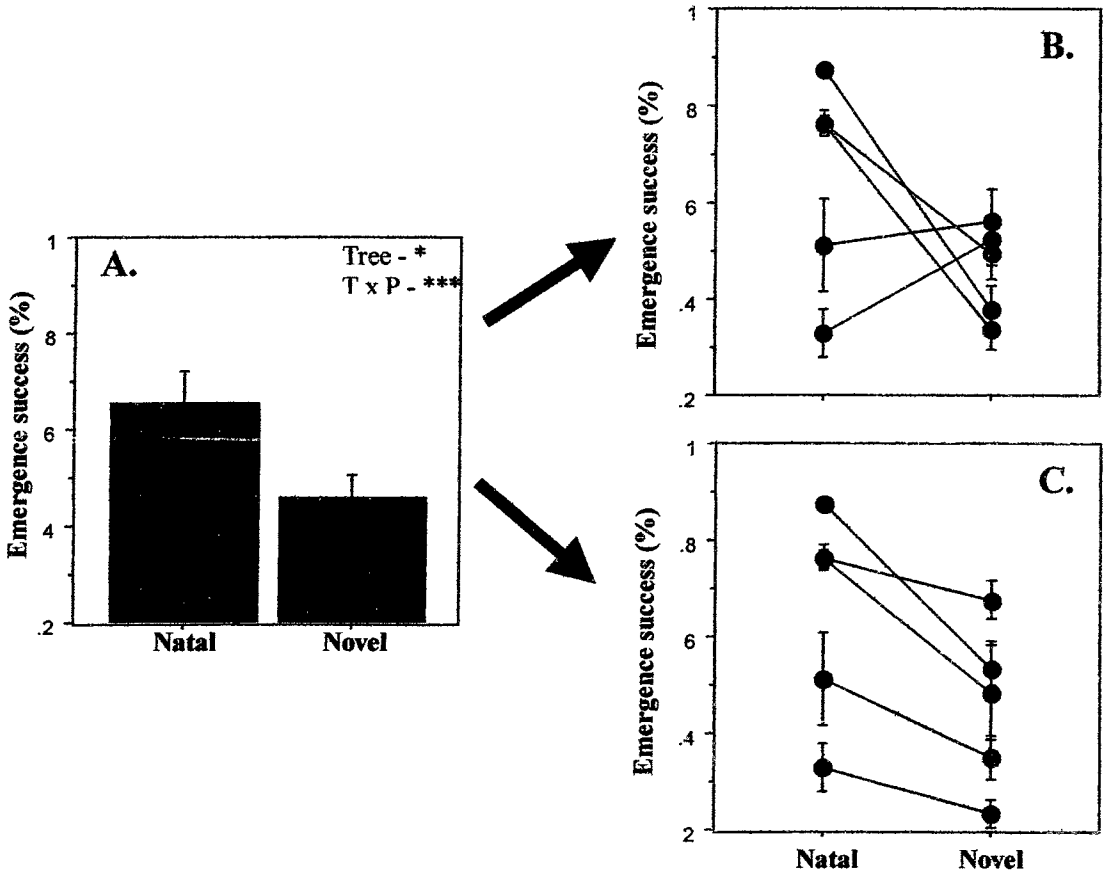



**FIGURE 5.** Mean emergence success on natal and novel trees summarized in three different approaches. (A) General, across all trees and insect populations, (B) Intertree dynamics summary, where each line connects performance (mean ± 1 SE) of an insect population on its natal tree and alternate novel hosts, and (C) Intratree dynamics summary, where each line compares natal and novel insect performance (mean ± 1 SE) within the same tree. Note that different conclusions might be made from different methods of representing the same data

## REFERENCES

Abrahamson, W.G., and A.E. Weis. 1997. Evolutionary ecology across three trophic levels. Monographs in Biology 29. Princeton University press, N.J., U.S.A.

Agrawal, A.A. 1999. Induced plant Defense: Evolution of induction and adaptive phenotypic plasticity. In: Agrawal, A.A., S. Tuzun, E. Bent. Induced Plant Defenses Against Pathogens and Herbivores: Biochemistry, Ecology, and Agriculture.

Agren, J., and D.W. Schemske. 1993. The cost of defense against herbivores: an experimental study of trichome production in *Brassica rapa*. American Naturalist 141:338-350.

Askew, R.R. 1984. The Biology of Gall Wasps. In: Ananthakrishnan, T.N. (ed.) The Biology of Gall Insects. Oxford and IBH Publishing Co. New Delhi, India.

Barnes, P.W., S.-Y. Liang, K.E. Jessup, L.E. Ruiseco, P.L. Phillips, and S.J. Reagan. 2000. Soils, topography and vegetation of the Freeman Ranch. Freeman Ranch Publication Series No. 1-2000. Southwest Texas State University Press, San Marcos, TX.

Berenbaum, M.R., and A.R. Zangerl. 1992. Genetics of secondary metabolism and herbivore resistance in plants. In: G. Rosenthal and M. Berenbaum, (Eds.), Herbivores: Their interactions with secondary plant metabolites, Vol. 2, 2nd ed. Academic Press, San Diego, CA.

Biggs, C.J. and J. Latto. 2001. Interactions between the egg and larval parasitoids of a

gall-forming midge and their impact on the host. Ecological Entomology 26:109-115.

Boecklen, W.J. and S. Mopper. 1998. Local adaptation in specialist herbivores: Theory and evidence. In: Mopper, S. and S.Y. Strauss (eds). Genetic Structure and Local Adaptation in Natural Insect Populations. Effects of Ecology, Life History, and Behavior. Chapman and Hall, New York.

Bodnaryk, R.P. 1996. Physical and chemical defences of pods and seeds of white mustard (*Sinapis alba* L.) against tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois) (Heteroptera: Miridae). Canadian Journal of Plant Science 76:33-36.

Carr, D.E., and M. D. Eubanks. 2002. Inbreeding alters resistance to insect herbivory and host plant quality in *Mimulus guttatus* (Scrophulariaceae). Evolution 56:22-30.

Clancy, K.M., and P.W. Price. 1987. Rapid herbivore growth enhances enemy attack: sublethal plant defenses remain a paradox. Ecology 68:733-737.

Cox, D.R. 1970. The analysis of binary data. Methuen, London.

Downie, D.A. 1999. Performance of native grape phylloxera on host plants within and among terrestrial islands in Arizona, USA. Oecologia 121:527-536.

Dreger-Jauffret, F. and J.D. Shorthouse. 1992. In: Shorthouse and Rohfritsch (eds.) Biology of Insect-Induced Galls. Oxford Univ. Press. New York. p.8-33.

Edmunds, G.F., and D.N. Alstad. 1978. Coevolution in insect herbivores and conifers. Science 199: 941-945.

Fernandes, G.W. 1998. Hypersensitivity as a phenotypic basis of plant induced resistance against a galling insect (Diptera: Cecidomyiidae). Environmental Entomology 27:260-267.

Fordyce, J.A. and A.M. Shapiro. 2003. Another perspective on the slow-growth/high-mortality hypothesis: Chilling effects on swallowtail larvae. Ecology 84:263-268.

Galusky, P. 2000. Implications of the within canopy oviposition preference, abundance, and larval performance patterns of a host specific cynipid gall former. Master's Thesis. Southwest Texas State University, San Marcos, Texas.

Hall. C. 2001. Community structure of parasitoids attacking leaf galls of Belonocnema treatae on Quercus fusiformis. Master's Thesis. Southwest Texas State University. San Marcos, TX, U.S.A.

Hanks, L.M. and R.F. Denno. 1994. Local adaptation in the armored scale insect Pseudaulacaspis pentagona (Homopteral: Diaspididae). Ecology 75:2301-2310.

Horton, D.R., P.L.Chapman, and J.L. Capinera. 1991. Detecting local adaptation in phytophagous insects using repeated measures designs. Environmental Entomology 20:410-418.

Hunter, M. D., and P. W. Price. 1992. Playing chutes and ladders: Heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. Ecology 73:724-732.

Janzen, D. 1968. Host plants as islands in evolutionary and contemporary time. American Naturalist 102:592-595.

Karban, R. 1989. Fine-scale adaptation of herbivorous thrips to individual host plants. Nature 340:60-61.

Komatsu, T., and S. Akimoto. 1995. Genetic differentiation as a result of adaptation to the phenologies of individual host trees in the galling aphid *Kaltenbachiella japonica*. Ecological Entomology 20: 33-42.

Larsson, S., B.Ekbom, and B. Bjorkman. 2000. Influence of plant quality on pine sawfly population dynamics. Oikos 89:440-450.

Lund, J.N., J.R. Ott, and R. Lyons. 1998. Heterogony in *Belonocnema treatae* Mayr (Hymenoptera: Cynipidae), Proc. Entomol. Soc. Wash. 100: 755-76.

MacIntyre, P.J., and T.G. Whitham. 2003. Plant genotype affects long-term herbivore population cynamics and extinction: Conservation Implications. Ecology 84:311-322.

Mopper, S. 1996. Adaptive genetic structure in phytophagous insect populations. Trends in Ecology and Evolution 11:235-238.

Mopper, S. 1998. Local adaptation and stochastic events in an oak leaf-miner population. In: Mopper, S. and S.Y. Strauss (eds). Genetic Structure and Local Adaptation in Natural Insect Populations. Effects of Ecology, Life History, and Behavior. Chapman and Hall, New York.

Mopper, S. and D. Simberloff. 1995. Differential herbivory in an oak population: The role of plant phenology and insect performance. Ecology 76:1233-1241.

Mopper, S., M. Beck, D. Simberloff, and P. Stiling. 1995. Local adaptation and agents of selection in a mobile insect. Evolution 49:810-815.

Mopper, S. and S.Y. Strauss (eds). 1998. Genetic Structure and Local Adaptation in Natural Insect Populations. Effects of Ecology, Life History, and Behavior. Chapman and Hall, New York.

Mopper, S., P. Stiling, K. Landau, et al. 2000. Spatiotemoral variation in leafminer population structure and adaptation to individual oak trees. Ecology 81:1577-1587.

Nixon, K.C. 1997. Fagaceae. In: Flora of North America Editorial Committee, eds, Flora of North America North of Mexico. Vol. 3. New York: Oxford University Press; 436-437.

Pinheiro, J.C. and D.M. Bates. 2000. Mixed-Effects Models in S and S-PLUS. Springer-Verlag New York, Inc., New York, USA.

Price, P.W. 1997. Insect Ecology. 3rd ed. John Wiley and Sons, New York.

Price, P. W., C. E. Bouton, P. Gross, B. A. McPheron, J. N. Thompson, and A. E. Weis. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. Annual Review of Ecology and Systematics 11:41-65.

Reynolds, R.J. 2001. The role of natural enemies in determining the relationship between gall size and emergence success of a host-specific cynipid. Master's thesis. Southwest Texas State University, San Marcos, TX, U.S.A.

Ronquist, F., and J. Liljeblad. 2001. Evolution of gall wasp-host plant association. Evolution 55:2503-2522.

Rosenthal, G. and M.R. Berenbaum. 1991. Herbivores: Their interactions with secondary plant metabolites,Vol. 1. Academic Press, San Diego, CA.

Rosenthal, G. and M.R. Berenbaum. 1992. Herbivores: Their interactions with secondary plant metabolites, Vol. 2. Academic Press, San Diego, CA.

Rossiter, M. 1996. Incidence and consequences of inherited environmental effects. Annual Review of Ecology and Systematics 27:451-476.

Rossiter, M. 1998. Assessment of genetic variation in the presence of maternal or paternal effects in herbivorous insects. In: Mopper, S. and S.Y. Strauss (eds).

Genetic Structure and Local Adaptation in Natural Insect Populations. Effects of Ecology, Life History, and Behavior. Chapman and Hall, New York.

Sokal, R.R., and F.J. Rohlf. 1995. Biometry, 3rd. ed. W.H. Freeman, San Francisco, USA.

Sork, V.L., K.A. Stowe, and C. Hochwender. 1993. Evidence for local adaptation in closely adjacent subpopulations of northern red oak (*Quercus rubra* L.) expressed as resistance to leaf herbivores. Evolution 142:928-936.

Stiling, P. and A.M. Rossi. 1996. Complex effects of genotype and environment on insect herbivores and their enemies. Ecology 77:2212-2218.

Stiling, P. and A.M. Rossi. 1998. Deme formation in a dispersive gall-forming midge. In: Mopper, S. and S.Y. Strauss (eds). Genetic Structure and Local Adaptation in Natural Insect Populations. Effects of Ecology, Life History, and Behavior. Chapman and Hall, New York.

Stiling, P., and D. Simberloff. 1989. Leaf abscission: induced defense against pests or response to damage? Oikos 55:43-49.

Stiling, P., D. Simberloff, and B. Brodbeck. 1991. Variation in rates of leaf abscission between plants may affect the distribution patterns of sessile insects. Oecologia 88:367-370.

S-PLUS. 2003. Insightful Corporation. Seattle, Washington, USA.

Strauss, S.Y. 1990. The role of plant genotype, environment and gender in resistance to a specialist chrysomelid herbivore. Oecologia 84: 111-116.

Strauss, S.Y. and R. Karban. 1994. The significance of outcrossing in an intimate plant/herbivore relationship. I. Does outcrossing provide an escape for progeny from herbivores adapted to the parental plant? Evolution 48:454-464.

Strauss, S.Y. and R. Karban. 1994. The significance of outcrossing in an intimate plant/herbivore relationship. II. Does outcrossing pose a problem for thrips adapted to the host-plant clone? Evolution 48:465-476.

Strauss, S.Y. and R. Karban. 1998. The strength of selection: Intraspecific variation in host-plant quality and the fitness of herbivores. In: Mopper, S. and S.Y. Strauss (eds). Genetic Structure and Local Adaptation in Natural Insect Populations. Effects of Ecology, Life History, and Behavior. Chapman and Hall, New York.

Stone, G. N., K. Schonrogge, R. J. Attkinson, D. Bellido, and J. Pujade-Villar. 2002. The population biology of oak gall wasps (Hymenopterra:Cynipidae). Annual Review of Entomology 47:633-668.

Van Dongen, S., T. Backeljau, T. Matthysen, and A.A. Dhondt. 1997. Synchronization of hatching date with budburst of individual host trees (*Quercus robur*) in the winter moth (*Operophtera brumata*) and its fitness consequences. Journal of Animal Ecology 66:113-121.

Van Zandt, P.A. and S. Mopper. 1998. A meta-analysis of adaptive deme formation in phytophgous insect populations. American Naturalist 152:595-604.

Wade, M.J., and C.J. Goodnight. 1998. Perspective: the theories of Fisher and Wright in the context of metapopulations: where nature does many small experiments. Evolution 52:1537-1553.

Waddell, K.J., C.W. Fox, K.D. White, and T.A. Mousseau. 2001. Leaf abscission phenology of a scrub oak: consequences for growth and survivorship of a leaf mining beetle. Oecologia 127:251-258.

Wainhouse, D., and R.S. Howell. 1983. Intraspecific variation in beech scale populations

and in susceptibility of their host *Fagus sylvatica*. Eco. Entomology 8:351-59.

Williams, I.S. 1999. Slow-growth, high-mortality – a general hypothesis, or is it? Ecological Entomology 24:490-495.

Ylioja,T., H. Roininen, M.P. Ayres, et al. 1999. Host-driven population dynamics in an herbivorous insect. PNAS 96:10735-10740.

# VITA

Scott Patrick Egan was born in Newton, New Jersey, on June 7, 1977, the son of John Patrick and Patricia Scott Egan. After graduating from Humble High School in 1995, he entered the University of Texas at Austin and received a degree of Bachelor of Science in August 1999. He entered the Graduate College at Texas State University - San Marcos in January 2001 to pursue a Master of Science degree in Biology. During his tenure at Texas State, Scott was employed as an I.A. for the Organismal Biology labs.

Permanent address: 7614 Hurst Forest Dr.
Humble, TX 77346

This thesis was typed by Scott Patrick Egan.