NATURAL SELECTION ON GALL SIZE: VARIABLE CONTRIBUTIONS OF INDIVIDUAL HOST PLANTS TO POPULATION-WIDE PATTERNS

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Studies that provide estimates of the form and magnitude of selection on herbivore traits at the level of individual plants in natural populations represent a vital step in understanding the interaction of selection and gene flow among host-affiliated insect populations when individual plants equate to differing selective regimes. We analyzed phenotypic selection on the trait gall size for a host-specific gall former at both the individual host plant and population level (across host plants) in each of two years. Linear and nonlinear selection and the fitness function relating gall size to the probability of survivorship in the absence of natural enemies were estimated for each level and year. Selection imposed by the host plant was observed in 19 of the 22 subpopulations monitored. At the population level, linear and nonlinear selection were evident each year. However, population-level estimates masked the significant heterogeneity in the form and direction of selection evident among plants each year. Heterogeneity among gall-former subpopulations is emphasized by our findings that selection varied from directional to stabilizing among plants and the majority of selection gradients estimated for individual plants did not fall within the 95% CIs of the population-level estimates.

KEY WORDS: Cynipidae, gall former, local adaptation, *Quercus*, selection gradients, spatial and temporal variation.

The maintenance of genetic variation within natural populations given selection is a core and long-standing issue in evolutionary biology (Fisher 1930; Wright 1932; Levene 1953; Felsenstein 1976; Wade and Goodknight 1998; Johnson and Barton 2005). Empirical studies that can provide estimates of spatial and (or) temporal variation in the form and magnitude of selection within populations (reviewed by Hoekstra et al. 2001; Kingsolver et al. 2001; Hereford et al. 2004; Siepielski et al. 2009) inform theory

(Johnson and Barton 2005). However, regardless of spatial scale, assessing heterogeneity in selection within populations necessitates correctly delimiting environments that potentially constitute differing selective regimes and then assigning individuals whose fitness is assessed to the appropriate selective regime. A priori recognition of potentially differing selective environments is facilitated when discontinuities among environments/habitats are clearly evident, as in the case of distinct habitat patches (Barton and Whitlock 1997), readily discernable boundaries among spatially distinct environmental states (Caruso 2001), and marked variation in biotic and (or) abiotic factors between episodes of selection (Grant and Grant 1995). When populations are

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recognized as comprising multiple environments/selective regimes, analysis of selection can yield estimates of the magnitude and form of selection at both the population level (across all individuals and environments) and local (subpopulation) level. Inspection of the relationship between variation in environment and variation in the form, direction, and strength of selection at the local level can generate insights into the causes of fitness variation (Wade and Kalisz 1990). The local level can represent distinct or graduated environments measured at the scale of meters to kilometers (Kalisz 1986; Gosden and Svensson 2008), neighborhoods (Svensson and Sinervo 2004), or, for host-specific phytophagous insects, individual host plants (Egan and Ott 2007). Viewed in reverse perspective, when within-population heterogeneity in selective regimes goes unnoticed and samples collected from within the population are pooled across regimes to estimate selection, the resultant parameter estimates, while population specific, by definition apply to no specific environment within the population; further, they may be biased in both magnitude and direction due to unequal sampling among environments (Svennsson and Sinervo 2004). Moreover, estimates of selection generated under such conditions may be biased upward by unrecognized environmentally induced covariance between fitness and trait values (Rausher 1992; Stinchcombe et al. 2002.)

For phytophagous insect species whose larvae develop on a single plant, the borders of individual host plants, intraplant variation notwithstanding (Roslin et al. 2006), delimit discrete environments. Thus to the degree that individual plants differ in nutritional state (Ruhnke et al. 2009), defensive chemistry (Osier et al. 2000), phenology (Mopper 2005), suite or impacts of associated natural enemies (Clancy and Price 1987; Hare 1992), and biotic and abiotic setting (Bach 1980; Henriksson et al. 2003), individual plants potentially represent different selective environments. Host plant dependent variation in fitness surrogates such as performance and survival are hallmark observations of insect–plant interactions for polyphagous insect species at the level of among species of host plants (Ferrari et al. 2008) and among populations of the same host plant species for host-specific insects (Craig et al. 2007). The few cases of demic adaptation of host-specific phytophagous insects (Boecklen and Mopper 1998; Egan and Ott 2007) stand as evidence for variation in selective regimes at the finest level—among individual plants within populations. Although many studies attest to the central role of variation among individual plants within populations in determining the ecological and evolutionary dynamics of phytophagous insect populations (Denno and McClure 1983; Karban 1992; Koricheva et al. 1998), to our knowledge no studies to date have estimated the form and magnitude of selection on insect herbivore traits at the level of the individual plant in natural populations.

Assessing heterogeneity in selection within populations also necessitates securing samples of sufficient size from within each putative selective regime to ensure that tests of significance have adequate power to detect the estimated effects sizes reliably (Hersch and Phillips 2004). However, constraints on sample size imposed by the limiting relationship between plant size and insect population size in combination with natural among-plant variation in herbivore density, difficulty in delimiting the boundaries of individual plants or differentiating genets from ramets, and the movement of insects among plants, all stand as impediments to quantifying selection on insect traits at the level of individual plants within natural populations.

The abundance and availability of gall-forming insects, particularly on large plants, in conjunction with their biology and ecology facilitate studies of selection at the individual plant level. Gall formers are typically both host and tissue specific (Stone et al. 2002), sessile during development, and easily located. Moreover, the fates of individuals within galls are readily monitored and associated with gall characteristics (Price and Clancy 1986; Craig et al. 2007; Hood and Ott 2010). Galls, which house developing larvae, represent extended phenotypes of the gall former and thus the trait, maximum gall size attained (henceforth gall size), reflects the interaction of insect and plant genome and the environment (Weis and Abrahamson 1985; 1986; Weis and Gorman 1990). Gall size is of known ecological importance, including natural enemy avoidance (Stone and Schönrogge 2003), adult female size, and potential fecundity (Ito and Hijii 2004, G.R. Hood and J. R. Ott, unpubl. data). In many species of gall formers, including the species studied here, gall growth is maintained by active larval feeding (Stone et al. 2002), thus gall size records the size attained at the completion of active feeding prior to pupation or the size attained by the time larvae succumb to the effects of natural enemies, host plant defenses, or pathogens. Consequently, gall size can be tested for association with the probability of survival (adult emergence) in the presence (Craig et al. 2007) or absence of natural enemies (present study).

Herein we provide estimates of linear and nonlinear selection on the size of galls produced by the host-specific gall-former *Belonocnema treatae* on the leaves of its host plant plateau live oak, *Quercus fusiformis*. We assess selection at the level of the individual plant (subpopulation level) within a natural population of plateau live oaks and across plants (population level) in each of two years. We treated the gall formers on each plant as a separate unit (i.e., a subpopulation) in estimating phenotypic selection for five reasons. First, the cohort of *B. treatae* larvae that develops on each tree is restricted to the same tree during development. Second and third, the trees on which gall-former populations were monitored varied significantly in the mean and range of gall sizes produced, and gall-former survivorship varied among trees (Egan and Ott 2007; Hood and Ott 2010). Fourth, the size of the host plant in conjunction with the abundance of the gall former developing on the leaves of each tree allowed us to collect gall samples of

sufficient size to conduct analyses centered on individual trees. Fifth, individual live oaks have been shown to support locally adapted gall-former populations, which suggests that individual trees can equate to different selective regimes (Egan and Ott 2007).

We assessed heterogeneity in selection among plants by first estimating both linear and nonlinear selection at each level by means of selection gradient analysis (Lande and Arnold 1983) and then tested for within-population heterogeneity in selection gradients each year (Svennsson and Sinervo 2004). We further described the fitness function that describes the relationship between variation in leaf gall size and the probability of emergence of the gall former at both the population and individual plant levels by fitting cubic splines (Schluter 1988). Our analyses demonstrate that population-level estimates of selection on gall size mask the significant heterogeneity present among subpopulations of gall formers on individual trees.

Methods
STUDY SYSTEM AND DATASETS

Belonocnema treatae (Hymenoptera: Cynipidae) exhibits a life cycle typical of cynipid gall formers in which sexual and asexual generations are temporally segregated (Lund et al. 1998). The asexual generation develops within leaf galls following oviposition by sexual-generation females into immature leaves of plateau live oak, *Q. fusiformis* Small (subgenus: *Quercus*; section: *Quercus*; series: *Virentes*; Muller 1961). Asexuals develop singly within leaf galls throughout spring and summer, emerge during fall and winter, and then oviposit into the roots of plateau live oak, giving rise to the sexual generation (Lund et al. 1998). In central Texas, both generations develop exclusively on plateau live oak, which is generally restricted to the Edwards Plateau (Nixon 1997). Several field aspects of *B. treatae* natural history suggest limited but likely gene flow at the scale of the present study. Females mate and begin ovipositing on the natal host immediately upon emergence, males lek in the areas of female emergence, adults are poor fliers, and adults have a short life span (4–10 days; Lund et al. 1998). Fuller details of the life cycle and ecology of *B. treatae* are presented in Lund et al. (1998) and Egan and Ott (2007), including documentation of local adaptation at the spatial scale of the current study. Central to the current study is the fact that the asexual generation of *B. treatae* experiences strong plant-mediated mortality (e.g., $\bar{x} \pm$ SE, $30\% \pm 4$, Egan and Ott [2007]; $42\% \pm 3$, Hood and Ott [2010]). The goal of the research presented here is to determine whether this plant-mediated mortality results in selection on gall size and whether the pattern of selection differs among plants within a population.

The data analyzed herein were produced from studies of *B. treatae* performance conducted in 2002 (Egan and Ott 2007) and 2008 (Hood and Ott 2010). Salient features needed to understand the analysis of selection are presented here. In each study, the naturally occurring mature live oak trees sampled for *B. treatae* were located at Texas State University's Freeman Ranch in Hays County, Texas (29°55′N, 98°00′W). A map depicting the distribution of previously sampled live oaks containing the subset of 19 trees sampled here appears in Egan and Ott (2007). The average distance between trees included in the current study was 0.57 km, with a minimum distance of 0.1 km and a maximum distance of 2.64 km. Study trees exist in a matrix of other live oaks and plant species that typify the juniper–oak savanna of the eastern edge of the Edwards Plateau in central Texas. Both studies involved rearing the progeny of sexual-generation females within enclosures placed on the branches of live oaks. Enclosures effectively protected the gall formers from natural enemies (parasitism rates for the 2002 and 2008 studies were 0.03% and 0.01%, respectively). Thus enclosures allowed us to isolate the intrinsic (plant-mediated) relationship between the sizes of leaf galls produced by asexual-generation larvae and adult survivorship (i.e., probability of emergence from the leaf gall). We estimated the functional relationship between gall size and the probability of survivorship of the gall former for individual trees (i.e., the subpopulation level) and across all trees (i.e., the population level) in each of the two years.

The 2002 dataset comprised the galls produced on 17 of the 25 live oak trees examined in Egan and Ott (2007); the six trees that did not support gall establishment and the two trees that did not produce sufficient galls ($N < 39$) to estimate and test selection coefficients were dropped. In total, gall size and survivorship data for 7496 gall formers distributed across the 17 trees ($\bar{x} \pm$ SE galls/tree $= 441 \pm 125$; range $= 39$–1769) are analyzed. The number of enclosures per tree varied from two to 12. Galls were harvested prior to peak *B. treatae* emergence, housed by tree within collection traps, and incubated at seasonally adjusted light and temperature regimes to capture emerging *B. treatae*. Only mature galls (fully developed and lignified) at the time of harvest are included in the analyses.

In 2008, gall size and survivorship data were collected for 6787 gall formers distributed across the five trees ($\bar{x} \pm$ SE galls/tree $= 1357 \pm 251$; range $= 638$–2090) studied by Hood and Ott (2010). The number of enclosures per tree varied from 15 to 20. Three of these trees were examined in 2002, allowing for comparisons of selection on these trees across years. Methods followed those employed in 2002 with three exceptions. First, in 2008 two cohorts of leaf gallers, separated by six to eight weeks, were initiated on each tree; however, cohorts were pooled because the timing of initiation did not influence either gall size or survival (Hood and Ott 2010). Second, on each tree one-half of enclosures were later opened to natural enemies. Galls from these enclosures were excluded from analysis. Third, for each tree, subsets of galls were collected at the onset of *B. treatae* emergence and

gall maturation (mid-November 2008); during peak emergence, by which time virtually all galls were mature (mid-December 2008); and following cessation of emergence (February 2009). Importantly, this collection scheme provided a test of whether the patterns of survivorship observed in 2002 and again in 2008 (and in each case attributed to host plant effects) were artifacts of the timing of gall harvest via an interaction of timing of harvest and survivorship. Harvest time did not affect gall-former survivorship or gall size (Hood and Ott 2010) or the magnitude of selection as demonstrated by preliminary analyses. Thus this potential artifact was ruled out, and galls were pooled within trees.

Based on the numbers of sexual generation females initiating the asexual generation within each enclosure and the number of enclosures per tree, up to 490 females contributed to the 7496 galls produced in 2002 and up to 430 females contributed to the 6787 galls produced in 2008. The number of females added per enclosure ranged from 3–5 and the number of eggs per female is highly variable (range: 128–1138; G. R. Hood and J. R. Ott, unpubl. data). Following the cessation of *B. treatae* emergence each year, the diameter of each spherical gall was measured to 0.01 mm by use of digital calipers, and each gall was scored for the presence or absence of an emergence hole (evidence that an adult *B. treatae* had emerged). Thus for each gall on every tree, we had a measurement of gall size and *B. treatae* survivorship. These data on the fates of 14,283 individuals form the basis for our analysis.

GALL SIZE AND SURVIVORSHIP PER TREE ACROSS THE POPULATION

To begin our inspection of selection, we first established the degree to which individual trees varied in the distribution of gall sizes produced and in *B. treatae* survivorship. To compare gall size among trees and years, a nested ANOVA was conducted with year considered as a fixed effect and tree nested within year as a random effect. A blocking factor to account for any differences among the multiple exclosures per tree was included as a nested factor within tree within each year. For the three trees monitored in both 2002 and 2008, *t*-tests of the hypothesis of no difference in mean gall size between years were also conducted. Contingency table analysis based on log-likelihood ratio tests was used to test for differences in survivorship among trees and years, again with year as a fixed effect and tree nested within year as a random effect. Similarly, a blocking factor for bag was included as a nested factor within tree within year. Finally, to determine whether the gall formers that developed on trees producing larger galls had higher survivorship, we pooled trees from both years and computed the nonparametric Spearman's rank correlation (r_s) between mean gall size per tree ($N = 22$) and survivorship.

VARIATION IN GALL SIZE AND GALL-FORMER FITNESS

The fitness functions of interest here are the relationships between variation in individual gall size (x) and the probability of *B. treatae* survival (y) at the level of the individual tree (subpopulation) and population (i.e., all galls from all trees pooled within each year). Characterizing the linear and nonlinear regression components of each fitness function provides information on the form and magnitude of selection at each level (Lande and Arnold 1983). The linear term provides information on the magnitude of directional selection for either reduced (-β) or increased (+β) gall size, whereas the nonlinear term (γ) provides information on the magnitude and form of variance selection: either nonlinear directional or stabilizing selection (γ < 0) or disruptive selection (γ > 0). Although Lande and Arnold (1983) considered significant negative nonlinear regression terms as evidence of stabilizing selection, we follow Schluter (1988) and reserve the term to indicate the condition in which a significant nonlinear regression term is accompanied by evidence indicating the presence of a fitness maximum associated with an intermediate phenotype within the range of observed phenotypes (Mitchell-Olds and Shaw 1987). Because fitness is categorical (*B. treatae* either emerged or failed to emerge) we used logistic regression followed by log-likelihood tests to estimate and test the significance of regression terms (Brodie et al. 1995; Janzen and Stern 1998). Gall size was standardized to a mean of 0 and a standard deviation of 1 prior to analysis so that regression coefficients when appropriately transformed (see below) would equate to standard selection gradients. Although logistic regression provides appropriate tests of significance for categorical data (Brodie and Janzen 1996), the resultant linear and nonlinear coefficients cannot be directly compared with standard-derived selection gradients (Janzen & Stern 1998) or used to predict evolutionary responses to selection (Lande 1979). Thus we used an SAS script (http://www.public.iastate.edu/~fjanzen/homepage.html) to transform logistically derived regression coefficients into approximate selection gradients readily comparable with traditional approaches to measuring selection (Janzen and Stern 1998). Transformed nonlinear selection gradients (γ) and associated standard errors were then doubled to estimate the magnitude and associated standard error of γ (Stinchcombe et al. 2008).

SPATIAL VARIATION IN SELECTION WITHIN THE POPULATION

Using logistic regression we first assessed linear and nonlinear selection at the population level. We then tested for spatial variation among trees in β and γ within each year by fitting a more complex logistic regression model to *B. treatae* survivorship. The model included gall size, gall size^2, tree, gall size × tree, and gall size^2 × tree. Significant interaction terms provide information about spatial variation in selection across the population

(Svensson and Sinervo 2004). Based on significant interaction terms presented in Table 2, we then estimated logistic regression coefficients and tested for linear and nonlinear selection at the level of the individual tree within each year. To describe central tendencies and variation in selection among trees, the mean, median, and standard deviation (SD) of the 22 estimates of $|\beta|$ and $|\gamma|$ were computed.

THE SHAPE OF SELECTION

To visualize the shape of selection at the population level and for individual trees within that population, we fit a nonparametric cubic spline (Schluter 1988) to the relationship between gall size and *B. treatae* survivorship by means of the program *glms*40 (http://www.zoology.ubc.ca/~schluter/software.html). To estimate the shape of selection at the population level each year, we fit a cubic spline with 1000 bootstrap replicates and included individual tree as a covariate. We then visualized the shape of selection on each tree within each year, again using a cubic spline with 1000 bootstrap replicates to describe variation among trees that make up the population-wide pattern of selection. The resultant fitness functions, with SEs, allowed us to predict the survival probability of gall formers as a function of gall size and compare probability of survival functions among subpopulations. Uncertainty in estimated shapes of selection, generated by sample size variation, is captured in the standard errors generated by the bootstrap resampling method. When the quadratic term from the logistic regression of survival on gall size was negative, we relied on the presence of an apparent intermediate survivorship maxima predicted by the cubic spline to distinguish between a nonlinear but monotonic fitness function (indicative of directional selection) and evidence for a true quadratic relationship between fitness and phenotype (indicative of stabilizing selection). Although both Mitchell-Olds and Shaw (1987) and Murtaugh (2003) present formal techniques to delineate internal optima of nonlinear functions for continuously distributed data, neither is appropriate for binomial data.

PATTERNS OF SELECTION IN RELATION TO GALL SIZE AND SURVIVORSHIP

Using the variation observed in the average size gall produced by each of the 22 gall-former subpopulations and the associated estimates of $|\beta|$ and $|\gamma|$, we tested the hypothesis that the incidence of selection (i.e., the probability of detecting statistically significant selection) and the magnitude of selection were functionally related to mean gall size per tree. To assess the incidence of selection, we first scored linear and nonlinear selection as present or absent for each tree based on tests of significance presented in Table 3. For each type of selection gradient, we then used this binomial response variable as the dependent variable in a logistic regression with mean gall size per subpopulation as the independent variable.

To test whether $|\beta|$ and $|\gamma|$ were functions of the mean gall size per subpopulation, we used linear regression. Finally, to test the hypothesis that variation in observed survivorship per tree was related to the presence and mode of selection we first used the results in Table 3 to bin the 22 subpopulations into three categories: no evidence of selection, evidence of directional selection, and evidence of stabilizing selection. We then compared survivorship among the three categories using arcsine-square-root-transformed estimates of survivorship per subpopulation in a one-way ANOVA followed by means comparison using Tukey's post-hoc test ($\alpha = 0.05$).

SAMPLE SIZE AND POWER

Following each logistic regression used to estimate β and γ, a post-hoc calculation of achieved power was performed using the program G*Power 3.1.0 (http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3), which required values for the effect size (estimated within JMP by the odds ratio), α-value (0.05), and sample size. We then used these power analyses to examine the relationship between effect size, sample size, and power across the range of sample sizes per tree present in our study ($N = 39$–2090). All analyses were performed in JMP 5.0.1a (SAS 2002). Results are presented throughout as $\bar{x} \pm$ SE unless noted; *N*s refer to the number of galls examined at the indicated level.

Results

VARIATION IN GALL SIZE AND SURVIVORSHIP

Variation in the size of galls produced by the interaction of the gall former and the host plant is manifestly evident at the level of within trees, among trees, and among years (Table 1). A wide range of gall sizes were produced on each tree each year with the coefficient of variation (CV) for each tree and for the data pooled within years ranging from 0.17 to 0.33. However, even against this within-plant variation, average gall size varied significantly among trees within years ($F_{20,105} = 40.6$, $P < 0.0001$). For example, in 2002 average gall size per tree varied from a minimum of 3.96 ± 0.09 mm to a maximum of 6.01 ± 0.05 mm. Gall size varied between years as well ($F_{1,20} = 16.2$, $P < 0.0001$), with the average size of galls produced in 2002 (5.10 mm ± 0.02; $N = 7496$) exceeding the size of galls produced in 2008 (4.72 ± 0.01 mm; $N = 6787$). All three trees monitored in both 2002 and 2008 produced smaller galls in 2008 ($P < 0.001$, Table 1). The observed variation in gall size among trees and years suggests that individual trees potentially represent differing selective environments. Similarly, *B. treatae* survivorship differed significantly among individual trees within years ($\chi^2_{df=20} = 595.4$, $P < 0.0001$) but not between years ($\chi^2_{df=1} = 0.01$, $P = 0.93$; Table 1). In 2002, average survivorship ranged from a low of 34% to a high of 94% among trees. Gall-former

Table 1. Descriptive statistics for gall size and survival of subpopulations of *B. treatae* on each of 17 trees in 2002, five trees in 2008, across all trees within each year (All), and in both years for trees 1, 4, and 12.

Pop_{yr}	GS range	CV	GS-All ($\pm$SE)	GS-Surv ($\pm$SE)	GS-Dead ($\pm$SE)	Opt-GS	%Surv ()
$T1_{02}$	0.86–8.36	0.285	5.02$\pm$0.03	4.82$\pm$0.05	5.12$\pm$0.04	4.22	34
$T2_{02}$	0.32–8.00	0.218	5.14$\pm$0.03	5.19$\pm$0.03	4.93$\pm$0.08	8.00	81
$T3_{02}$	0.87–8.80	0.334	4.63$\pm$0.05	4.81$\pm$0.06	4.46$\pm$0.08	4.48	53
$T4_{02}$	0.40–8.44	0.201	5.67$\pm$0.04	5.75$\pm$0.04	5.23$\pm$0.13	8.44	85
$T5_{02}$	1.76–8.30	0.189	5.73$\pm$0.05	5.79$\pm$0.05	5.58$\pm$0.11	5.48	71
$T6_{02}$	0.99–8.32	0.201	6.01$\pm$0.05	6.09$\pm$0.05	5.76$\pm$0.13	5.91	74
$T7_{02}$	0.85–8.28	0.309	4.34$\pm$0.06	4.58$\pm$0.07	3.89$\pm$0.11	4.80	64
$T8_{02}$	1.55–6.04	0.172	4.28$\pm$0.05	4.36$\pm$0.06	4.08$\pm$0.12	6.04	72
$T9_{02}$	1.40–8.66	0.314	4.90$\pm$0.11	5.31$\pm$0.16	4.71$\pm$0.15	5.73	32
$T10_{02}$	1.41–7.18	0.305	3.96$\pm$0.09	4.21$\pm$0.10	3.40$\pm$0.17	5.10	69
$T11_{02}$	3.17–8.38	0.199	5.62$\pm$0.10	5.59$\pm$0.11	5.72$\pm$0.19	8.38	78
$T12_{02}$	2.01–8.07	0.222	5.53$\pm$0.14	5.52$\pm$0.15	5.53$\pm$0.26	5.40	55
$T13_{02}$	0.63–9.71	0.319	4.58$\pm$0.19	5.02$\pm$0.16	3.23$\pm$0.44	9.71	75
$T14_{02}$	2.61–7.81	0.268	4.92$\pm$0.18	5.00$\pm$0.19	3.75$\pm$0.71	7.81	94
$T15_{02}$	3.44–7.33	0.172	5.71$\pm$0.14	5.91$\pm$0.15	4.85$\pm$0.24	7.33	81
$T16_{02}$	2.62–7.29	0.227	4.97$\pm$0.18	5.08$\pm$0.17	4.58$\pm$0.52	5.53	78
$T17_{02}$	2.78–7.40	0.224	4.99$\pm$0.18	4.98$\pm$0.16	5.05$\pm$0.97	5.03	87
All_{02}	0.32–9.71	0.271	5.10$\pm$0.02	5.23$\pm$0.02	4.90$\pm$0.03	5.03	62
$T1_{08}$	1.90–8.15	0.267	4.50$\pm$0.03	4.70$\pm$0.03	4.28$\pm$0.04	4.36	52
$T4_{08}$	1.68–8.40	0.241	4.82$\pm$0.03	5.01$\pm$0.03	4.54$\pm$0.06	4.52	59
$T12_{08}$	1.99–8.61	0.205	5.01$\pm$0.03	5.08$\pm$0.4	4.91$\pm$0.0.6	4.26	61
$T18_{08}$	0.89–8.35	0.276	4.60$\pm$0.03	4.72$\pm$0.04	4.48$\pm$0.05	4.39	50
$T19_{08}$	1.92–8.91	0.249	4.99$\pm$0.05	5.06$\pm$0.05	4.86$\pm$0.10	4.59	65
All_{08}	0.89–8.91	0.255	4.72$\pm$0.01	4.88$\pm$0.02	4.51$\pm$0.03	4.36	56

Pop_{yr} = subpopulation (i.e., tree) and year of sampling; GS = gall size (diameter in mm); CV = coefficient of variation of gall size; GS-All = mean size of all galls monitored; GS-Surv = mean size of galls that produced a *B. treatae*; GS-Dead = mean size of galls that did not produce a *B. treatae*; Opt-GS = predicted optimal gall size from cubic spline; %Surv = observed survival.

survivorship in 2008, although differing significantly among trees, was less variable, ranging from 50% to 65%. The blocking factor "exclosure" included in these nested analyses also explained a significant amount of the variation in both gall size and survivorship ($P < 0.05$). Across the 22 subpopulations mean gall size and survivorship per tree were not correlated ($r_s = 0.27$, $P = 0.20$; Fig. 1). This result demonstrates that trees producing the largest galls do not necessarily have the highest *B. treatae* survival and suggests that the gall size that maximizes survivorship might differ among trees.

SELECTION AT THE POPULATION LEVEL

When all galls from all trees were pooled to estimate selection at the population level, both the linear and nonlinear terms from the logistic regression describing the relationship between gall size and the probability of *B. treatae* survivorship were significant in both 2002 and 2008 ($P < 0.0001$ for all four log-likelihood ratio tests; Table 3). In both years, the transformed linear selection gradients (2002: $\beta = 0.05 \pm 0.01$; 2008: $\beta = 0.07 \pm 0.01$) indicated weak directional selection at the population level for increased

gall size. In contrast, the sign and magnitude of γ in 2002 and 2008 ($\gamma = -0.30 \pm 0.02$, and $\gamma = -0.63 \pm 0.03$, respectively), in conjunction with the shape of the cubic spline describing the fitness function for each year (Fig. 2), indicated that gall formers experienced moderate stabilizing selection for intermediate gall size at the population level. Thus in both years *B. treatae* experienced a mixture of directional and stabilizing selection at the population level. In 2008, however, the magnitude of stabilizing selection was twice that observed in 2002, and the shape of the quadratic fitness function differed substantially between the years. In 2002, the fitness function was characterized by a larger optimal gall size (5.03 mm) and a broader fitness peak (Table 1 and Fig. 2).

SELECTION AT THE SUBPOPULATION LEVEL

In both 2002 and 2008, significant gall size $\times$ tree and gall size^2 $\times$ tree interactions (Table 2) indicated significant heterogeneity in the magnitude of β and γ among trees. Thus we proceeded to examine selection at the subpopulation level. Phenotypic selection on gall size was commonly observed on individual trees

Table 2. Spatial variation in host plant-mediated linear and nonlinear selection on the size of galls produced by *B. treatae* in each of two years. All effects were tested using likelihood ratio tests. Significant interaction terms indicate heterogeneity in directional and stabilizing selection among subpopulations of gall formers on individual trees each year

Year	Effect	df	χ^2	*P*
2002	Gall size	1	0.57	0.4504
	Gall size^2	1	18.26	<0.0001
	Tree	18	600.47	<0.0001
	Gall size×tree	18	133.09	<0.0001
	Gall size^2×tree	18	56.01	<0.0001
2008	Gall size	1	21.63	<0.0001
	Gall size^2	1	575.61	<0.0001
	Tree	4	24.26	0.0001
	Gall size×tree	4	26.37	<0.0001
	Gall size^2×tree	4	96.02	<0.0001

in both years as 14 of 17 trees in 2002 and five of five trees in 2008 showed evidence of some form of selection (Table 3). Patterns of selection were complex, with individual trees exhibiting evidence of either linear, that is, pure directional selection ($N = 2$), nonlinear directional selection ($N = 2$), stabilizing selection ($N = 9$), or a combination of directional and stabilizing selection ($N = 6$) (Table 3 and Fig. 3). The importance of an individual tree-level analysis is further highlighted by the fact that for both β and γ only 6 of 22 estimates fell within the respective 95% CIs of the population level estimates (Table 3).

Statistically significant estimates of β were observed for subpopulations of gall formers on 10 of 22 trees (45%) with marginal significance ($P < 0.09$) observed for an additional tree. Directional selection was present in both years, with nine of 17 trees in 2002 and one of five trees in 2008 exhibiting significant βs. For the 10 subpopulations for which β was found significant, the mean $|\beta|$ was 0.192 ± 0.034. Significant βs ranged from –0.25 to 0.35, indicating that directional selection on gall size varied in both direction and strength among trees; however, directional selection was predominantly for increased gall size as nine trees exhibited β's ranging from 0.03 to 0.35, whereas selection was estimated to favor reduced gall size for only a single tree. Overall variation in $|\beta|$ among the 22 subpopulations (SD $|\beta| = 0.106$) rivaled the mean (0.109) and exceeded the median (0.055) value of $|\beta|$.

Subpopulations of gall formers on two trees (e.g., T13 and T15) experienced linear directional selection only ($\beta = 0.22$ and 0.33, respectively), whereas gall formers on eight trees experienced both linear and nonlinear selection (Table 3). For two of these eight trees (i.e., T2 and T4 [2002]), the shapes of the fit-

ness functions (Fig. 3) suggested that gall-former fitness was a nonlinear but monotonically increasing function of gall size. We interpreted these relationships to indicate nonlinear directional selection. The remaining six trees showing evidence of linear selection (i.e., T1, 3, 7, 9, and 10 in 2002 and T1 in 2008) also exhibited evidence of nonlinear selection. For each of these trees, the optimal gall size predicted by the cubic spline fell within the range of observed gall sizes (Table 1). We interpreted these relationships to indicate that while gall formers on these trees were subjected to directional selection favoring increased gall size, stabilizing selection favoring an intermediate gall size was the primary mode of selection.

Overall, we observed statistically significant nonlinear regression coefficients for 17 of 22 subpopulations (77%), including subpopulations on 12 of 17 trees in 2002 and on all five trees in 2008 (Table 3). Variation in $|\gamma|$ (SD = 0.28) for the 22 subpopulations was roughly equivalent to the average (0.37) and median (0.29) value of $|\gamma|$. Significant γs ranged from –0.06 to –0.76 in 2002 and from 0.22 to –0.99 in 2008. The average value ($\pm$SE) of significant estimates of γ for 2002, 2008, and overall were –0.36 $\pm$ 0.07 ($N = 12$), –0.60 $\pm$ 0.14 ($N = 5$), and –0.43 $\pm$ 0.07 ($N = 17$), respectively. Comparison of the coefficients of variation for $|\beta|$ (CV = 0.76) and $|\gamma|$ (CV = 0.97) suggests that the strength of linear selection was slightly less variable than the strength of nonlinear selection among trees.

The negative sign of all significant γs in conjunction with inspection of cubic splines indicated that for these 17 trees fitness functions were concave–down, tending toward quadratic to true quadratic (Fig. 3). Inspection of β and γ for these trees in conjunction with estimates of optimal gall sizes suggested that gall formers experienced only stabilizing selection for gall size (i.e., β = not significant and γ = significant) on nine of the 17 trees with significant estimates of γ (i.e., T5, 6, 12, 16, and 17 in 2002 and T4, 12, 18, and 19 in 2008). Gall formers experienced a mix of directional and stabilizing selection on the remaining six trees. In sum, statistically significant stabilizing selection on gall size was observed on 15 of 22 trees (68%), and thus stabilizing selection for gall size is frequent in the *B. treatae* subpopulations studied. When significant stabilizing selection was detected, the mean $\gamma = -0.48 \pm 0.07$ ($N = 15$) indicated moderate selection.

WITHIN-TREE VARIATION IN SELECTION BETWEEN YEARS

The three trees for which selection was assessed in both years document variability in the form and magnitude of selection encountered by gall-former subpopulations resident on individual trees through time (Table 3 and Fig. 3). For two trees (T4 and T12), the predicted βs for 2002 and 2008 were in close agreement, indicating weak and (or) not statistically detectable positive directional selection in each year. However, for T1,

Table 3. Estimates of linear (β) and nonlinear (γ) selection on the diameter of leaf galls produced by *B. treatae* on live oak trees accompanied by results of power analyses. Estimates of β and γ are shown for each of 17 trees in 2002, five trees in 2008, across all trees within each year (population level = All) and in both years for trees 1, 4, and 12 (Pop_{yr} = subpopulation [i.e., tree] and year of sampling). Tests of significance were conducted on logistic regression coefficients by means of log-likelihood ratio tests (*$P < 0.05$, **$P < 0.01$, *** $P < 0.001$, ****$P < 0.0001$). Logistic regression coefficients (not shown) were then transformed to yield the equivalent linear and nonlinear selection gradients that are shown. Selection gradients with *P*-values < 0.05 are in bold. The 95% CI for (β) and (γ) at the population level for each year is italicized and subtends the respective rows labeled All_{02} and All_{08}

Pop_{yr}	N	Linear selection			Nonlinear selection		
		β ± SE	χ^2	Power	γ ± SE	χ^2	Power
$T1_{02}$	1769	**−0.25±0.04**	40.99****	>0.99	**−0.68±0.08**	66.93****	>0.99
$T2_{02}$	1315	**0.03±0.01**	5.80*	>0.99	**−0.06±0.02**	6.24*	>0.99
$T3_{02}$	1047	**0.12±0.03**	16.16****	>0.99	**−0.29±0.06**	23.17****	>0.99
$T4_{02}$	808	**0.04±0.02**	6.95**	>0.99	**−0.08±0.03**	6.92**	>0.99
$T5_{02}$	557	−0.02±0.03	0.45	0.97	**−0.33±0.06**	27.65****	>0.99
$T6_{02}$	528	0.01±0.03	0.20	0.79	**−0.21±0.06**	12.01***	>0.99
$T7_{02}$	469	**0.20±0.03**	36.41****	>0.99	**−0.31±0.07**	20.86****	>0.99
$T8_{02}$	192	0.07±0.05	2.64	>0.99	−0.14±0.10	2.10	>0.99
$T9_{02}$	184	**0.35±0.13**	8.34**	>0.99	**−0.75±0.27**	9.70**	>0.99
$T10_{02}$	174	**0.21±0.05**	21.29****	>0.99	**−0.29±0.10**	8.28**	>0.99
$T11_{02}$	138	−0.03±0.05	0.44	0.89	0.07±0.10	0.48	0.95
$T12_{02}$	75	−0.03±0.11	0.05	0.21	**−0.76±0.31**	9.12**	>0.99
$T13_{02}$	61	**0.22±0.07**	15.30***	>0.99	−0.23±0.15	1.33	>0.99
$T14_{02}$	51	0.06±0.05	2.63	>0.99	−0.02±0.09	0.07	0.24
$T15_{02}$	48	**0.33±0.16**	10.23**	>0.99	0.24±0.20	1.78	>0.99
$T16_{02}$	41	0.08±0.07	1.30	0.81	**−0.29±0.14**	4.44*	0.99
$T17_{02}$	39	0.04±0.04	1.13	0.23	**−0.26±0.10**	11.74***	0.99
All_{02}	7496	**0.05±0.01**	39.47****	>0.99	**−0.30±0.02**	239.11****	>0.99
		95% CI=0.03–0.07			*−0.34 to 0.26*		
$T1_{08}$	2090	**0.17±0.02**	68.86****	>0.99	**−0.76±0.05**	279.19****	>0.99
$T4_{08}$	1564	0.02±0.02	1.04	>0.99	**−0.71±0.06**	211.89****	>0.99
$T12_{08}$	987	0.03±0.03	1.44	>0.99	**−0.22±0.05**	17.64****	>0.99
$T18_{08}$	1508	0.03±0.03	1.01	>0.99	**−0.99±0.08**	249.35****	>0.99
$T19_{08}$	638	0.05±0.03	2.87	>0.99	**−0.34±0.06**	36.39****	>0.99
All_{08}	6787	**0.07±0.01**	42.79****	>0.99	**−0.63±0.03**	692.47****	>0.99
		95% CI=0.05–0.09			*−0.69 to 0.57*		

directional selection was evident in both years, but the direction of selection changed between years. The general pattern of nonlinear selection was consistent across years, with T1 and T12 showing evidence of stabilizing selection each year and selection on T4 morphing from nonlinear directional selection (γ = –0.08 ± 0.03) in 2002 to stabilizing selection (γ = –0.71 ± 0.06) in 2008. Although the form of selection stayed constant, the magnitude of γ changed dramatically between years for T12 (Table 3).

SELECTION, GALL SIZE, AND SURVIVORSHIP AMONG TREES

Both the incidence of directional selection and the magnitude of |β| were independent of the average gall size per tree ($\chi^2_{df=1}$ =

1.45, $P = 0.23$, $N = 22$; and $F_{1,20} = 1.83$, $P = 0.19$ as shown by logistic and linear regression, respectively). Similarly, the incidence of nonlinear selection and the magnitude of |γ| could not be predicted on the basis of average gall size per tree ($\chi^2_{df=1}$ = 0.01, $P = 0.90$, $N = 22$; and $F_{1,20} = 0.86$, $P = 0.36$). Comparison of observed survivorship for subpopulations of gallers on trees where selection was not observed (82.5% ± 0.9, $N = 3$) with trees where either directional (80.6% ± 0.14, $N = 4$) or stabilizing selection (60.7% ± 0.20, $N = 15$) was observed showed that survivorship differed among outcomes ($F_{2,19} = 5.79$, $P = 0.01$). Survivorship of gallers was lower on trees exhibiting stabilizing selection compared with trees exhibiting no evidence of selection or evidence of directional selection (Tukey's post hoc test $P <$ 0.05).
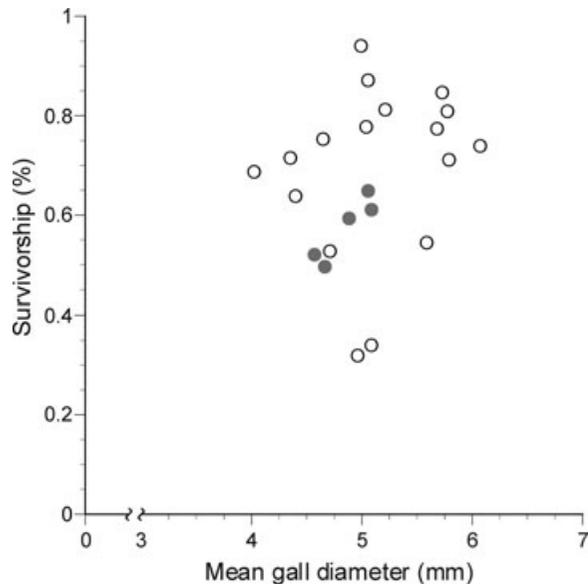
Figure 1. Correlation of survivorship of *B. treatae* per tree and mean gall size per tree based on 17 trees in 2002 (open circles) and five trees in 2008 (shaded circles); ($r_s = 0.27$, $P = 0.20$).

TEST OF SELECTION AND POWER

For the 27 selection gradients found to be significant the power to detect selection was >0.99 in each case. The tests of significance for seven of the 12 estimates of β and four of the five estimates of γ declared not significant had sufficient power to detect selection of the estimated magnitude (power > 0.97 and > 0.95, respectively). Thus only six of 44 tests of significance lacked the power (< 0.89) to detect selection of the estimated magnitude.

Discussion

A growing number of studies have examined selection operating at fine scales (Kalisz 1986; Kelly 1992; Linhart and Grant 1996; Svensson and Sinervo 2004; Gosden and Svensson 2008). When complemented with investigations of the ecological basis of fitness variation, such analyses help understand the interplay of selection and gene flow and hence the maintenance of phenotypic variability within and among populations (Johnson and Barton 2005). Analyses of selection on the gall former *Eurosta solidaginis* by Weis et al. (1992) and Craig et al. (2007) have demonstrated variation in the form and magnitude of selection on gall size among biomes, among populations within biomes, and among years. Herein we have further sharpened the focus to examine variability in the form and magnitude of selection among subpopulations of a host-specific gall former inhabiting individual trees within a live oak population. Despite the long-standing interest in detecting divergence among host-specific insect populations affiliated with individual plants initiated by Edmunds and

Alstad (1978), ours is the first study to provide estimates of selection for a phytophagous insect species at the level of individual plants in natural populations.

Our overall goals have been (1) to determine whether the plant-mediated mortality results in natural selection on gall size in the asexual generation of *B. treatae* and, if so, to characterize selection at the population level in each of two years; (2) to assess the incidence of, and the degree to which, selection differs among subpopulations of gall formers on individual trees; and (3) to determine whether the form and magnitude of selection assessed at the population level adequately predicts patterns of selection on individual trees. The significant heterogeneity in selection among subpopulations documented via ANOVA and the range of estimates of linear and nonlinear selection demonstrate that analysis of selection at the individual plant level is warranted. Our results demonstrate that plant-mediated mortality of *B. treatae* is typically nonrandom with respect to gall size and that patterns of mortality translate into phenotypic selection on gall size. At the population level, stabilizing selection of moderate magnitude and directional selection of weak magnitude on gall size is evident in each year. At the subpopulation level, selection was commonly observed. Our results provide evidence of significant variation in the incidence, form, and magnitude of selection among subpopulations of gall formers on individual trees—including among subpopulations inhabiting adjacent trees. Heterogeneity in selection among subpopulations of *B. treatae* is emphasized by our findings that (1) only 27% of estimates of linear and nonlinear selection for individual plants fell within the respective 95% CI of the population-level estimates, (2) variation in linear selection among plants was greater than, and variation in nonlinear selection equivalent to, the respective median values of selection averaged across all 22 subpopulations, and (3) when present, selection varied from directional to stabilizing.

Two issues of general importance when measuring selection at fine scales and one issue specific to assessing selection on gall phenotypes for gall-forming insects are raised by our results. First, to characterize selection by a single population-level estimate when multiple samples have been gathered, samples are assumed to estimate a common selection gradient (i.e., differences in estimates of selection among samples represent sampling error only). The heterogeneity we documented violates this assumption. Moreover, the relationships between both the incidence and the magnitude of linear and nonlinear selection on gall size in relation to sample size show that sampling error is not the explanation for differences in estimates of selection among sampled trees (J. R. Ott, and S. P. Egan, unpubl. data). As noted by Hersch and Phillips (2004) limited sample sizes in conjunction with small effect sizes often result in insufficient power to detect selection of the estimated magnitude. However, the effect sizes and sample
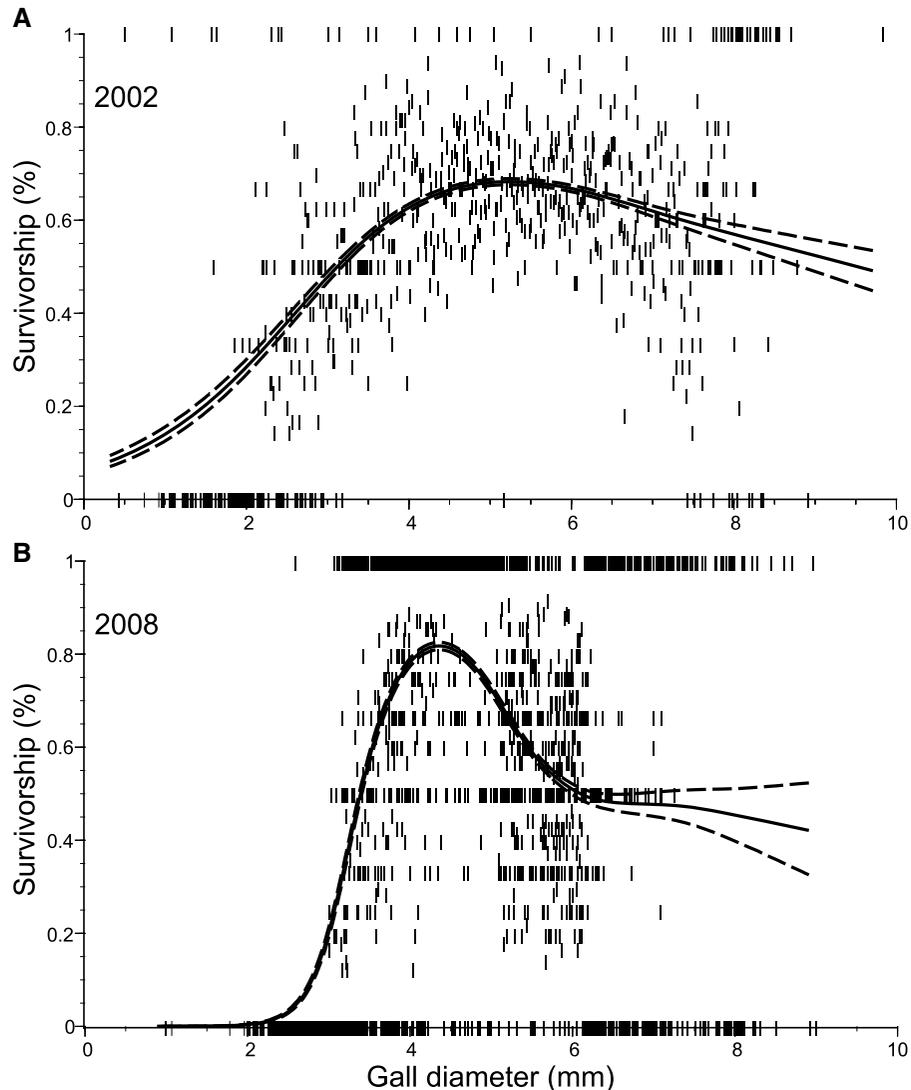
Figure 2. The shape of selection at the population level on the diameter of galls produced by *B. treatae* on the leaves of plateau live oak. For both (A) 2002, *N* = 7496 and (B) 2008, *N* = 6787, survivorship in the absence of natural enemies was modeled by fitting a cubic spline (solid line) ±SE (dashed line) to the relationship of survival to gall size. Although individuals survive or fail to survive, to illustrate the shape of selection survivorship is averaged when multiple measurements exist for the same gall diameter value (measured to 0.01 mm).

sizes for the sampled subpopulations were such that we had sufficient power to detect selection reliably for 86% of our estimates of β and γ (Table 3). Thus, the heterogeneity we observed among subpopulations of gall formers, sampled from individual trees, stands as a cautionary reminder for researchers who by design or necessity pool samples prior to estimating selection—a reemphasis of the concern voiced by Endler (1986). Had we simply pooled galls each year to form a composite estimate of selection at the population level and stopped there, our estimate, while specific to gall formers resident on the population of live oaks studied, may not have applied to the gall formers found on any given individual tree. Moreover, given the heterogeneity in selection we observed, the pooled estimate is clearly influenced by the disproportionate

contribution of individual subpopulations due to variation in sample size. Finally, relying on a single estimate to describe selection would have missed the rich variation in selection present in the population. It is this variation that is likely related at a core level to understanding within-population phenotypic variation in gall size and, by extension, selection on traits correlated to gall size. The second general issue raised by the observed heterogeneity in selection among subpopulations is the appropriate scale at which to standardize trait values and fitness prior to estimating selection (i.e., in our case whether to standardize trait values and fitness relative to each tree's observed distribution of gall sizes and mean fitness or relative to the distribution of gall sizes and mean fitness estimated by pooling across trees). Finally, our results suggest the

need for natural-enemy-free treatments to assess and thus control for host-plant mediated mortality for studies that seek to describe selection on gall phenotypes, including size (Stone et al. 2002; Stone and Schönrogge 2003).

SELECTIVE MORTALITY AND THE FORM AND INCIDENCE OF SELECTION

Plant-mediated mortality in this system occurs between gall formation and adult emergence in the absence of natural enemies (Hood and Ott 2010). Our analyses demonstrate that the plant-mediated mortality reported by Egan and Ott (2007) and Hood and Ott (2010) is nonrandom with respect to gall size within trees, and differences in mortality among trees are related to the form of selection. Both mean survivorship among trees and the magnitudes of both linear and nonlinear selection were independent of average gall size per tree. Instead, survivorship on trees characterized by stabilizing selection was significantly lower than was survivorship on trees where selection did not occur or where directional selection only was observed. The complex functional relationships within individual host plants between survivorship and gall size gave rise to a diversity of fitness functions, and thus modes of selection varied among plants from linear to nonlinear directional selection to combinations of directional and stabilizing selection to stabilizing selection. Gall formers on 86% of trees exhibited evidence of linear and (or) nonlinear selection. Tests of significance of β and γ at both the population and individual tree level demonstrated that stabilizing selection was the predominant mode of selection. Our results raise a host of questions in this study system regarding the measurement and spatial dynamics of phenotypic selection and causation.

SELECTION ON GALL SIZE

As a generalization, gall-former species experience high mortality from natural enemies (Stone and Schönrogge 2003), and enemy avoidance is the only hypothesis of those seeking to explain the adaptive value of gall morphology (Price et al. 1987) that can account for the diversity and distribution of gall defensive traits (Stone and Cook 1998; Stone and Schönrogge 2003). For example, exploration of the role of natural enemies in the evolution of the size of galls produced by *E. solidaginis* on *Solidago altissima* has produced a textbook example of stabilizing selection (Weis et al. 1992). In the *Eurosta–Solidago* system, stabilizing selection arises from opposing directional selection by arthropod natural enemies for increased gall size and directional selection by avian predators for reduced gall size. Geographic variation in selection is driven by the complement of natural enemies present (Craig et al. 2007). In contrast, in the *B. treatae–Q. fusiformis* system, stabilizing selection on gall size in the asexual generation arises in the absence of natural enemies as a result of mortality imposed by the host plant. Future research will examine total selection on gall size arising from both host plant mediated and natural enemy mediated selection.

SPATIAL SCALE AND RESPONSE TO SELECTION

The fitness functions estimated at the subpopulation and population level are critical to understanding the spatial and temporal dynamics of selection on leaf gall size in this system. Which fitness function best represents the form and magnitude of selection in considering the response to selection (ceteris paribus) of the gall formers resident on individual trees? Resolution of this question is also intimately related to determining the appropriate level at which to standardize both trait values and the fitness of gall-former populations on each tree prior to phenotypic selection analysis. Although defining the gall formers resident on individual trees to be the appropriate unit at which to standardize variables and estimate phenotypic selection within a generation (as we did) is supported by the analyses presented (i.e., only 12 of 44 tree-level estimates of selection gradients fell within the 95% CIs of gradients estimated at the population level), whether the gall formers developing on individual trees represent distinct populations in an evolutionary sense (i.e., with respect to the response to selection) is unknown. In this heterogonic species, selection on the size of galls housing the asexual generation and the response to this selection are separated by the intervening sexual phase of the life cycle. Thus, two bouts of dispersal separate selection on leaf gall size and any response: postselection dispersal of surviving asexuals and the subsequent dispersal of the sexual generation prior to induction of leaf galls. The extent of dispersal will determine whether the selective regime experienced by subpopulations of asexual gall formers developing on individual trees in generation$_{(n)}$ differs from that experienced by the asexual descendents at generation$_{(n+1)}$. At one extreme, if gall formers are essentially panmictic within host plant populations at the spatial scale we studied, then the population-derived fitness function may apply to gall formers on each tree. Hence, both gall size and gall-former fitness should be standardized at the population scale. In contrast, given complete natal philopatry, the fitness function estimated for each tree applies only to that tree and thus gall size and relative fitness should be standardized relative to each tree. Further complicating the issue, the sexual generation of *B. treatae* develops within multichambered root galls. Whether the same genes are involved in determining gall size in the alternate generation is unanswered in cynipids. Detailed studies of gene dispersal, population substructure, and the genetic basis of gall size in both generations are needed to understand better the spatial dynamics of selection on leaf gall size, the response to selection, and constraints on the response. Previous work on *B. treatae* and the present study provide initial clues to the spatial and temporal dynamics of selection on leaf gall size. The significant blocking effect of the enclosures found in the present study
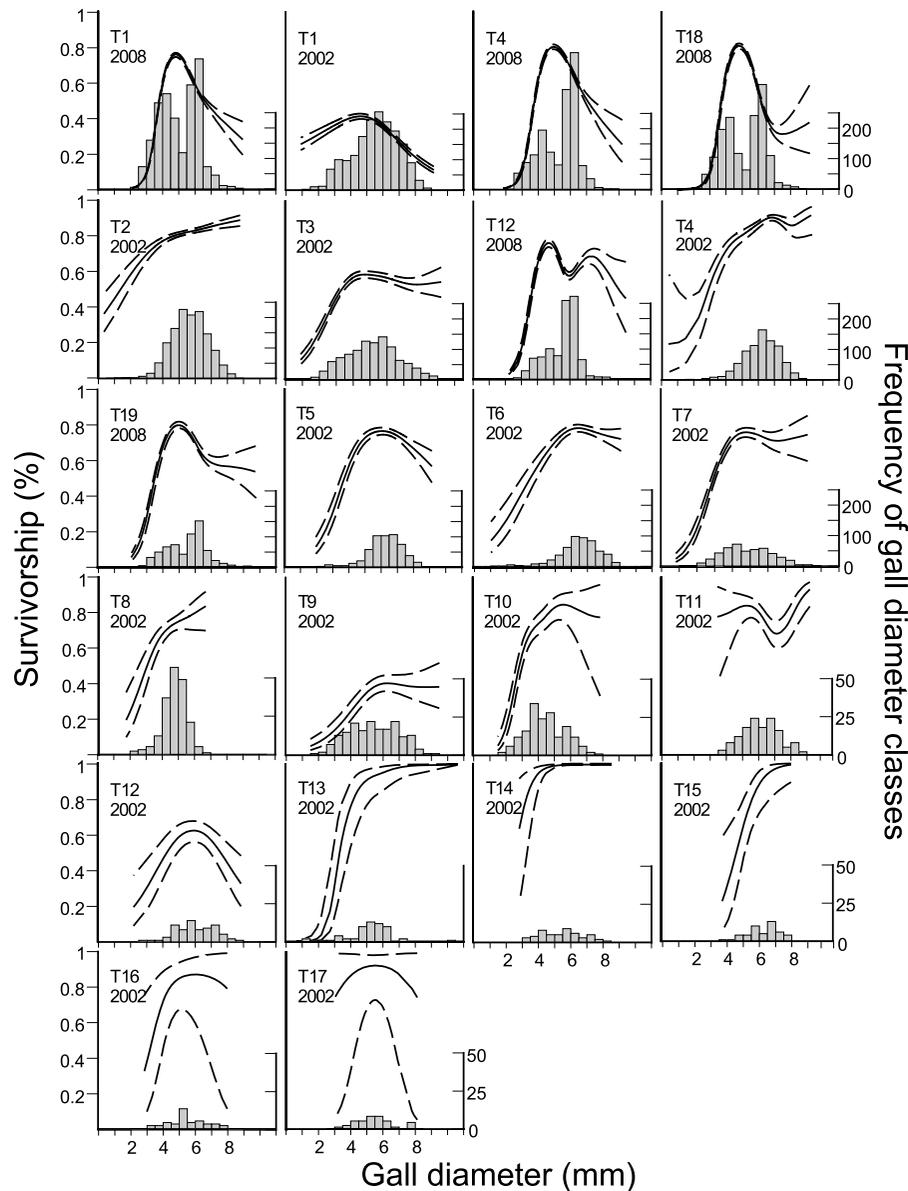
Figure 3. Cubic spline (solid line) ± SE (dashed line) fit to survivorship as a function of gall diameter for subpopulations of *B. treatae* on each of 22 plateau live oak trees. The gall size–survivorship relationship was estimated on 17 trees in 2002 and five trees in 2008. Subpopulations on trees 1, 4, and 12 were monitored in both years. Trees are arranged by sample size from top left to bottom right. The shaded histograms depict the observed distribution of gall diameters for each tree. Note the change in the right *y*-axis scale for the frequency data to accommodate variable sample sizes. See Table 1 for descriptive statistics and Table 3 for estimates of linear and nonlinear selection and results of tests of significance.

suggests the possibility of differences within trees (c.f., Roslin et al. 2006), differences among females, or female by tree interactions (c.f., Egan and Ott 2007), which all warrant further investigation.

Egan and Ott (2007) analyzed a suite of fitness components (including gall size) for asexual generation gall formers and found evidence for local adaptation on individual trees. Support for the hypothesis of local adaptation suggests that selection and the response to selection are likely linked within individual trees. By

extension, the among-tree differences in selection on gall size observed in the current study may be accompanied by individual tree-based responses to selection. In this event *B. treatae* subpopulations distributed across oaks could be viewed as a series of semiautonomous units each scaling its own fitness peak, with the fitness function estimated for each subpopulation applying only to the tree from which it was estimated. Both gall size and fitness should then be standardized relative to each tree. Egan and Ott (2007) also showed that a high percentage of hosts

exhibited low or undetectable densities of gall formers and that many trees do not support gall development. Taken together, these lines of evidence suggest that the spatial ecology of the gall former–host plant interaction favors local adaptation of leaf gall size. However, the observations that (1) both the mean gall size produced and the predicted optimal size differed between years for each of the three trees followed for two years and (2) the direction of selection reversed for one of these trees suggests that optimal gall size may be a moving target that changes among years.

Lastly, in regard to the general response to selection by each member of the interaction, we note that the conflicting interests of the host plant and insect may lead to the evolution of the cynipid subpopulations, the population of oaks, or both. Bottom-up effects of the host plant on the evolution of cynipid gall-formers have been clearly documented (e.g., Egan and Ott 2007). In contrast, even though cynipids can damage and kill their host plants, no study to date has demonstrated top-down effects of gall wasps on the evolution of their host plants (Stone et al. 2002). The variation in outcomes of this cynipid-oak interaction exhibited among trees (and among bags within trees) documented in Egan and Ott (2007) and the present study suggests the opportunity for a response in either, assuming a partial genetic basis. However, individual host plants may live hundreds of years with overlapping generations, whereas the cynipid undergoes two discrete and temporally segregated generations per year, thus the time course of a response may differ among each member of the interaction.

SPATIAL AND TEMPORAL VARIATION IN SELECTION GRADIENTS

We observed directional (linear) selection, nonlinear directional selection, stabilizing selection only, and the combination of stabilizing and directional selection. Siepielski et al. (2009) compared interannual estimates of phenotypic selection and showed that the SDs of $|\beta|$ and $|\gamma|$ rivaled the median values of both forms of selection. Following Siepielski et al. (2009), we compared the SDs of $|\beta|$ and $|\gamma|$ to the median values of $|\beta|$ and $|\gamma|$ for the 17 trees tracked in 2002 to assess spatial variability in the magnitude of selection. Our results showed that variation in $|\beta|$ and $|\gamma|$ (SD = 0.11 and 0.23 respectively), approximates the median values of $|\beta|$ (0.07) and $|\gamma|$ (0.26). We conclude that spatial variability in selection in this system is as rugged as the temporal variation catalogued by Siepielski et al. 2009. It is not surprising that selection varied across years as the number and identity of trees sampled in each year only partially overlapped. However, even when just those three trees sampled in both years were compared, selection was shown to be highly variable between years.

SELECTION AND CAUSATION

When the relationship between variation in a gall trait and gall-former survival arises as a consequence of selective mortality imposed by natural enemies (Zwölfer and Arnold-Rinehart 1994; Abrahamson and Weis 1997), the gall trait (in these examples, size) is identified as the target of selection and natural enemies as agents of selection: causation can be inferred (Endler 1986). We documented selection on the size of leaf galls produced by *B. treatae* in the absence of natural enemies and, in fact, in the absence of any recognized agent(s) of selection external to, or associated with, the environment of the gall created by the gall former–host plant interaction. We have learned that gall size (or unstudied traits associated with gall size) can be related to variation in gall-former survival. The mechanism underlying selective mortality, and hence the causal basis of selection, is unknown. Moreover, gall size, treated as a single continuously distributed trait and analyzed as the target of selection, must represent traits interacting throughout development. Further complicating the matter, variation in the expression of gall traits likely arises from the three-way interaction of the gall-former genome, plant genome, and the environment (Weis and Gorman 1990; Stone and Schönrogge 2003). In fact, previous work in gall-former systems has documented nongenetic influences on gall former performance, including the role of host plant soil moisture and nutrients, age, developmental status, and gall location within the canopy (reviewed in Stone et al. 2002). Thus, the actual trait(s) being optimized by selection among the suite of traits involved are unknown (Travis 1989). In the simplest sense the trait that may be the actual target of selection is the ability to produce a gall of size *x* given the plant genome and environmental variation experienced. Because galls induced by cynipids grow only during active feeding, the size of galls from which gall formers emerge estimates the maximum size attainable given the insect, plant genome, and environment interaction. For gall formers that failed to emerge the galler either died prior to the programmed cessation of feeding, during metamorphosis, or following metamorphosis but prior to emergence. In the first case, gall size attained reflects only a portion of the maximal attainable size whereas in the latter two cases gall size again reflects maximal attainable size. To gain insight into the causal basis of selection, the relationship between gall size (and traits associated with gall size) and the probability of survival through each of the above stages must be explored as the relationship between gall traits and the probability of survival may differ among the classes of outcomes.

CONCLUSIONS

In this study we measured the fate of 14,283 individual asexual generation *B. treatae* distributed across 22 putative selective environments. We analyzed phenotypic selection on the trait leaf gall size at the level of the individual host plant (subpopulation level)

and across host plants within the population in each of two years. At the population level, moderate stabilizing and weak directional selection were evident each year. However, population-level estimates of selection masked the heterogeneity present in the form, magnitude, and direction of selection among subpopulations resident on individual trees. As shown in this study, failure to recognize the correct scale in which selection varies can produce estimates of linear and nonlinear selection that while specific to pooled samples do not apply to subpopulations.

ACKNOWLEDGMENTS

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