The influence of an host-plant hybrid zone on the distribution and success of the leaf-galling wasp, Beloncnema treatae.

Introduction: Hybridization is recognized as an important factor of speciation within the plant kingdom and has been recorded extensively in numerous plant groups (2,3). Recently, ecologists have begun exploring the role of plant hybridization on the dynamics of plant-insect interactions by recording abundance and distribution of species, guilds, and communities of herbivores across hybrid zones (4-17). Results indicate that plant hybrid zones may support 1) higher (8,10,11,16,17) 2) intermediate (5,7,9-12,15) or 3) lower (6,7,11) levels of herbivory than either parental host plant or 4) herbivory levels equivalent to a single parental host plant (10,11,13). Particular phytophage response patterns, in addition, may vary between years (11). The relative frequencies of the above patterns across hybrid zones, however, are based on a limited number of studies, and mechanisms underlying phytophage response to hybrids remain obscured by varying methodologies (4). As a consequence, the implications of plant hybridization on the evolution of plant-insect interactions are generally underdeveloped.

The hybrid bridge hypothesis predicts that plant hybrid zones facilitate the colonization of novel host plants by host specific phytophagous insects (9). Coupled with subsequent divergent selection pressures associated with the parental and novel host, such colonization may promote race formation and subsequent speciation (9). According to this hypothesis, hybrid zones facilitate phytophage range expansion by presenting herbivores with a series of hybrids that are morphological and genetic intermediates between parental and potential host plants. In essence, phytophages are exposed to the recognition cues and/or defense mechanisms of both plants and thus require lesser degrees of pre-adaptation or adaptation to colonize a hybrid than a genetically distinct potential host. As pre-adaptations are expressed and adaptations accumulate, an accelerated host shift across the hybrid zone can result (9). In the process of such range expansion across hybrid zones, herbivore densities and success should decrease as hybrid genomes more closely resemble that of the novel host (9). The recorded abundance and distribution patterns of herbivores across hybrid zones, however, cast doubt on the significance of this prediction.
Phenology effects, environmental gradients, and inheritance of plant defense properties have been proposed both individually and in combination as mechanisms to explain observed distributions of phytophagous insects across plant hybrid zones (4,5,8-11,13,17). Difficulties in controlling for each of the above predictors prevents direct comparison between studies and thus precludes corroborative mechanistic conclusions (4). It has also been suggested that introgression dynamics of the hybrid zone significantly influence phytophage response (4,9). First generation hybrids may be sterile, capable of backcrossing to a single parent, or capable of two-way introgression (18,19) and thus create unique genetic clines across plant hybrid zones with varying hybridization patterns. Not surprisingly then, herbivory levels have been shown to vary between categories of hybrids (F1, backcross, etc.) within the same hybrid zone (13,17). To establish the validity of the hybrid bridge hypothesis and weight the significance of plant hybridization on patterns of host plant use by phytophagous insects, the relative frequencies of these patterns across hybrid zones and their underlying mechanisms require substantiation and clarification via additional studies and study systems.

**Objectives:**

1A) To record the response pattern of a specialized herbivore across a naturally occurring host plant hybrid zone by comparing herbivore densities between pure parental and hybrid zones. 
1B) To examine the role of inherited plant resistance properties in determining the observed phytophage response pattern. 
2A) To test the hybrid bridge hypothesis by estimating herbivore density as a function of plant relatedness to the original host plant within the hybrid zone. 
2B) To examine the role of inherited plant resistance properties in determining that function.

**Study System and Methods:** The study system is comprised of a leaf-galling cynipid wasp, *Belonocnema treatae*, and its host plant, *Quercus fusiformis* (Fagaceae). (20). *Quercus fusiformis* occurs in the Edwards Plateau of central Texas but forms hybrids with *Q. virginiana* at the eastern edge of its range (21). Both plants are sub-evergreen trees. I have established nineteen sampling sites along a three-hundred mile transect spanning the *Q. fusiformis x virginiana* hybrid zone and parts of both pure parental ranges (21). Ten trees have been randomly selected at each site. Ovipuncture marks and successfully initiated galls will be scored for one thousand leaves collected from eight locations at each of three strata per tree. Data will be used to estimate host plant defense (percent successful gall initiation) and herbivore density (galls per leaf) for each tree, site, and region. Differences in parameters will be tested with a nested (region(site(tree))) ANOVA. An implicit test of the relationship between herbivory levels and the genetic resemblance of hybrids to the original host plant will be conducted by regressing herbivore density and gall initiation success on site distance from the pure *Q. fusiformis* range. Two years of data will be collected.

**Objective 1A:** Comparing herbivore densities between plant hybrid and pure parental zones. Differences in herbivore densities (galls per leaf) between regions of hybridization and pure parental plants will be indicated by a significant region effect in the ANOVA. Post-hoc tests between estimated densities will indicate where and in what direction the differences occur so that definitive response patterns of the herbivore across the plant hybrid zone can be recorded within and between years.
Objective 1B: Examining the role of plant resistance properties in determining phytophage density variation between plant hybrid and pure parental zones. Differences in gall initiation success between plant regions (indicated by significant region effect in ANOVA) will indicate differences in plant resistance properties. Positively correlated herbivore densities and gall initiation success between regions will implicate plant defense properties in moderating herbivore densities across plant hybrid zones.

Objective 2A: Testing the hybrid bridge hypothesis. A negative correlation between gall density and sampling site distance from the pure Q. fusiformis zone will satisfy the prediction of the hybrid bridge hypothesis that, during phytophage range expansion, herbivory levels decrease as the genetic resemblance of hybrid plants to the original host plant decreases. For objectives 2A and 2B, I am assuming that, as reported by Nixon (21), hybrid plants closer to the pure Q. fusiformis zone are more closely related to Q. fusiformis.

Objective 2B: Testing the influence of plant resistance properties on the relationship between gall density and the degree of plant hybridity. Positive correlation between gall density and gall initiation success within sites across the hybrid zone will implicate inherited plant resistance properties as a mechanism underlying distribution patterns within hybrid zones.

Preliminary Study: In order to evaluate the validity of the sampling scheme employed above, I am currently mapping within tree distribution of the gall-former. Differential allocation of resources has been shown to occur within the crown of Q. fusiformis individuals. In particular, trees unimpeded by external shading tend to be more vigorous in the upper stratum of the northeast direction (22). Cynipid gall-formers rely on newly formed and undifferentiated plant tissue for nutrition (23). Directional resource partitioning in the host plant may thus subject the gall-former to correlated pressures on oviposition behavior. To determine if gall-former distribution within trees is random, herbivore density and gall initiation success will be estimated according to direction and strata. Sixteen trees have been identified as having moderate to high relative densities of B. treatae galls from sites within the range of pure Q. fusiformis. Leaves have been collected from eight cardinal directions at each of three strata (<6 ft, 6-12 ft, and 12-18 ft) per tree. Ovipuncture marks and successfully initiated galls have been scored from 100 leaves per sample for a total of 1200 leaves per tree. Estimations of directional, stratified, and directional x stratified herbivore density and gall initiation success will be compared via ANOVA within and between trees. Patterns discerned from this analysis will be evaluated with post-hoc tests and adjustments to the sampling scheme will be made if better estimates of parameters are possible.

Research and Graduate School Goals: As a student of evolutionary ecology, my goal is to obtain a university level faculty position involving both research and teaching. Receiving a masters degree in ecology while completing a worthy thesis project represents a necessary step toward eventually competing for such a position. Exposure to primary literature and evolutionary and ecological theory will promote critical thinking skills and familiarization with the parlance necessary to communicate my own ideas. Research and data analysis experience
will facilitate development of the skills necessary to test these ideas. My immediate goal is thus
to obtain full and balanced preparation for a career in science.