

HOST PLANT INFLUENCES ON PERFORMANCE AND HAPLOTYPE  
DIVERSITY OF *DALBULUS MAIDIS*, A SPECIALIST HERBIVORE OF *ZEA*

A Thesis

by

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## ABSTRACT

In one study, a suite of plants from the maize genus *Zea* L. (Poaceae) and the specialist herbivore *Dalbulus maidis* (DeLong and Wolcott, 1923) (Hemiptera: Cicadellidae) were used to test the hypotheses that anti-herbivore defenses are affected by plant life-history evolution and human intervention through domestication and breeding for high yield. The suite of *Zea* host plants included one Mexican commercial hybrid maize *Zea mays* ssp. *mays* L., a landrace variety of maize, two populations of Balsas teosinte (*Zea mays* ssp. *parviglumis* Iltis & Doebley), and perennial teosinte (*Z. diploperennis* Iltis, Doebley & Guzman). This suite of host plants includes three transitions evident within the genus *Zea*: life history form perennial to annual life cycle evident between perennial teosinte and Balsas teosinte, domestication transition from wild annual to domesticated annual evident between Balsas teosinte and landrace maize, and; breeding transition from landrace cultivar to a hybrid cultivar. The transitions were correlated with differences in plant defenses, as indicated by corn leafhopper performance. Results showed a performance gradient, suggesting a pattern in which plant defense is stronger in perennial than annual plants, Balsas teosinte than landrace maize, and in landraces than in hybrid maize. Furthermore, results suggested that domesticated maize would be the least defended, most suitable host for corn leafhopper.

In a second study, haplotype diversity was assessed to address structuring and interconnectedness among samples of corn leafhopper collected in the southwestern region of Mexico to address whether different hosts represent different templates for

microevolution. The geographic focus of the study was maintained within an area encompassing the presumed centers of radiation of *Dalbulus* and its host genus *Zea*, and of maize domestication. Samples were complemented with samples of corn leafhopper sequences available at GenBank. Results revealed seven haplotypes from three host plants within *Zea*: perennial teosinte, Balsas teosinte, and maize. Furthermore, genetic differentiation was present and haplotype diversity appears to correlate with differences in genetic structure between perennial teosinte and maize. One haplotype was found to be present throughout all sites, which appears to parallel the spread of maize cultivation. As maize cultivation spread beyond its area of domestication, corn leafhoppers colonized perennial teosinte, further suggesting that subsequent decreases in maize cultivation in perennial teosinte habitat created a refuge where perennial teosinte- adapted haplotypes could persist. Altogether, my research suggests that the combination of historical expansion of maize cultivation expansion and the weaker anti-herbivore defenses associated within maize domestication appears to have favored genotypes particularly adapted for exploiting maize.

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# CHAPTER I

## INTRODUCTION

The relationships between plants and insects can have effects at all organizational levels, from biochemistry of individuals to species. Some of these relationships can be mutually beneficial, such as pollination or seed dispersal, while others can be antagonistic, such as insect herbivory of plants, and conversely, plant defense against herbivorous insects.

Plant strategies for resisting or avoiding insects have diversified in the course of evolution. Some plant species employ biochemical defenses by accumulating high levels of toxic compounds that repel, poison and even reduce digestibility, or interfere with insect behavior and/or physiology (Gatehouse 2002). Other species make use of physical traits, such as trichomes, spines or thorns. Even within a plant species, different populations or races may adopt subtly different strategies for coexisting with their herbivores (Anstead et al. 2002; Dres and Mallet 2002; Rosas-García et al. 2010).

Whether or not insects coevolve with their host plants, they must cope with plant defenses, and plant chemistry is one among many selection pressures that will influence an insect's host plant range (Thorsteinson, 1960; Thompson 1988). Plant domestication and agronomic selection are processes that are likely to alter a plant's chemistry. Within maize and its wild relatives, Rozenhal and Dirzo (1997) demonstrated that plants selected to produce high yields were less defended against insects because they allocated more resources to growth and reproduction than to defense. A pattern of weaker insect



defenses in domesticated relative to wild taxa can also be seen in other crop plants, such as in American cranberry (Rodríguez-Saona et al. 2011), domesticated sunflower (Chen and Welter 2005, 2007), and domesticated olive (Wang et al. 2009).

Plant defense can also be altered by the ability of phytophagous insects to specialize on a narrow range of host plants. Because specialization on one type of plant requires the capacity to overcome plant defenses, exploitation of a particular plant taxa as a source of food involves metabolic adjustments on the part of an insect (Ehrlich and Raven 1964). Thus, insect herbivores may influence host plant defense evolution, and plants may influence insect host range evolution.

Formation of host-associated insect populations, or insect-adapted plant populations are examples of microevolutionary paths that affect plant defense and insect host range evolution. Human activities can further mediate microevolution, for example through movement of insects or plants beyond their endemic ranges which may expose them to novel selective forces in the form of novel host plants or herbivores (Anstead et al. 2002; Oliver 2006; Alvarez et al. 2007). The weakening of plant defenses in crop species due to domestication and breeding for yield also may promote insect herbivore microevolution (Medina et al. 2012).

This study addresses how evolutionary forces associated with domestication in the plant genus *Zea* L. may affect performance and microevolution in the specialist insect herbivore *Dalbulus maidis* (DeLong and Wolcott). In particular, chapter II considers how transitions in life history evolution (from a perennial to an annual life cycle), domestication (from a wild, annual grass to a domesticated, annual grass), and breeding (from an outbreeding, landrace maize to an inbred, hybrid maize) within the

maize genus *Zea* affect performance of *D. maidis*. Chapter III assesses haplotype diversity to address whether a wild, perennial host (*Zea diploperennis* Iltis, Doebley & Guzman) and maize (*Zea mays mays* L.) represent different templates for microevolution of *D. maidis* and to infer whether maize domestication and spread beginning ~9,000 YBP affected genetic diversity of this insect. Overall, my study provides insight to whether host-plant evolution and domestication affect performance and microevolution of a specialized herbivore.

CHAPTER II  
EFFECTS OF LIFE HISTORY, DOMESTICATION, AND BREEDING OF *ZEA* ON  
*DALBULUS MAIDIS*

**Introduction**

Crop plants have been shown to be poorly defended against herbivore insects compared to their ancestors and wild relatives. For example, Rosenthal and Dirzo (1997) found that relative injury by a diverse assemblage of folivorous and stem-boring insects to various taxa of *Zea* L. (Poaceae) was predicted by the hypothesis that a gradient of herbivore defense, from strongest to weakest, would be evident between the “least domesticated” and “most domesticated” *Zea* taxa in their study. In particular, they found that a wild, perennial species of *Zea* was most defended, followed by a wild annual subspecies, a landrace maize (*Zea mays* ssp. *mays* L.), and a modern, high-yielding maize, which was the least defended. In some cases, differences in the strength of herbivore defenses between wild and domesticated plants are due to differences in the quality and/or quantity of chemical defenses, and are evident as direct effects on herbivore performance or on recruitment of natural enemies of herbivores. Thus, Benrey et al. (1998) found that the performances of the herbivores *Pieris rapae* L. and *Zabrotes subfasciatus* (Boheman) were higher on cultivated versus wild crucifers (*Lunaria* L., *Brassica* L.) and beans (*Phaseolus* L.), respectively; several studies revealed significant variation among maize cultivars and teosintes (the wild taxa of *Zea*) in production of herbivore-induced volatiles that attract the natural enemies of herbivores (Gouinguene et al. 2001; Fritzsche-Hoballah

et al. 2002; Degen et al. 2004); the performance of *Lymantria dispar* (L.) was highest on a high-yielding, American cranberry (*Vaccinium macrocarpon* Ait.) cultivar compared to ancestral cultivars, and chemical defenses, in the forms of volatile sesquiterpenes and the defense-related hormone cis-jasmonic acid (JA), were reduced in the high yielding cultivar (Rodríguez-Saona et al. 2011), and; Takahashi et al. (in press) found more injury in the field and higher performance of *Spodoptera frugiperda* (J.E. Smith) on a maize landrace compared to Balsas teosinte (*Zea mays* ssp. *parviglumis* Iltis & Doebley), the immediate ancestor of maize. In other cases, domestication seemed to have weakened plant defenses because it created a refuge from natural enemies for herbivorous insects. For example, Chen and Welter (2005, 2007) found that the harder and larger seeds of domesticated sunflower (*Helianthus annuus* L.) compared to wild sunflower partially protected the larvae of *Homoeosoma electellum* Hulst from parasitoids, which suffered less parasitism on domesticated sunflower, and; similarly, Wang et al. (2009) found that larvae of *Bactrocera oleae* (Rossi) suffered less parasitism on domesticated olive (*Olea europaea* L.) than on wild olive because the larger fruits of the former provided larvae a structural refuge against parasitism.

Following crop domestication, directed breeding may lead to differences in plant defense between modern and ancestral crop cultivars, such as landraces. Modern crop cultivars (e.g., hybrid and other high-yielding varieties) are the products of systematic (science-based) breeding efforts emphasizing high productivity (reproductive and/or vegetative) and typically have narrow, inbred genetic backgrounds. In contrast, ancestral crop cultivars, such as landraces, are genetically diverse and have been selected by local environments and farmers over many generations to satisfy particular dietary, culinary,

and other needs, while maintaining moderate productivity under variable environmental conditions. Thus, Rosenthal and Dirzo (1997) found differences in insect injury between landrace maize and modern, high-yielding maize, and Rodríguez-Saona et al. (2011) found differences between high-yielding cranberry cultivars and their ancestral cultivars in herbivore performance and production of defensive chemicals, as noted above. Other studies showed differences in emission of volatiles that recruit natural enemies and recruitment of natural enemies among maize cultivars, including maize landraces and modern, high-yielding cultivars (Rassman et al. 2005; Tamiru et al. 2011).

In addition to effects of directed human selection through domestication and breeding, natural selection and evolutionary processes, such as life history evolution, may affect plant defense against herbivory. Presumably, plant life histories reflect adaptations to environments with differing environmental pressures, including herbivore pressures. Thus, perennial species generally allocate more resources to their root systems and defenses because they must survive environmental extremes across growing seasons and are more likely to suffer injury from herbivores and pathogens over their lifetime, while annual species generally allocate more to growth and reproduction because their reproductive opportunities are limited to a single growing season. While few studies have compared the defense strategies of closely related perennial and annual plant species, some predictions can be made concerning the strength or nature of defenses on the bases of life history theory. For example, the plant apparency hypothesis predicts that apparent plants, such as perennial species, will invest in broadly effective anti-herbivore defenses, including compounds that are effective against specialists and generalist herbivores, while unapparent plants, such as annual species, will invest in qualitative toxins that are

effective against non-adapted specialists and generalist herbivores (Feeny 1976). Other predictions can be made on the bases of resource allocation strategies. Thus, iteroparous perennial species, such as perennial grasses, may be minimally impacted by herbivory compared to woody perennials because they do not invest in permanent structures vulnerable to attack by herbivores, and may evade herbivores by "moving" in space through stolons, rhizomes, or other reproductive tissues (Crawley, 1986). Mutkainen and Walls (1995) directly compared the defense strategies of closely- related annual and iteroparous perennial plants under variable levels of resource availability, and hypothesized that perennials should show stronger induced responses to herbivory than annual plants, which tend to invest more in reproduction than defense regardless of the level of available resources. Rosenthal et al. found that both defense against and tolerance of herbivory by caterpillars were higher in perennial teosinte (*Zea diploperennis* Iltis, Doebley, & Guzmán) compared to annual, Balsas teosinte (Rosenthal and Welter 1995; Rosenthal and Dirzo 1997). Nault and Madden (1985) compared the performances of insects (*Dalbulus* DeLong) specializing on perennial grasses (on both *Tripsacum* L. and *Zea*), annual grasses (*Zea*), and non-specialists (on both *Tripsacum* and *Zea*) and found that the specialist insect performance generally suffered on perennial compared to annual hosts, independently of the insect's host specialization, which suggests that perennial species are better defended than closely related annual species.

In this study, I compared the performance of a specialist herbivore, corn leafhopper [*Dalbulus maidis* (DeLong & Wolcott)] (Hemiptera: Cicadellidae)] on a suite of host plants (*Zea* spp.) representing three transitions evident in the host plant's genus: first, a life history transition, from perennial to annual life cycle; second, a domestication

transition from wild annual to domesticated annual, and; third, a breeding transition, from an ancestral, landrace cultivar to a modern, hybrid cultivar. The objective of the study was to determine whether such transitions in *Zea* are correlated with differences in plant defenses, as indicated by corn leafhopper performance. I met my objective through a “forced colonization” approach: Corn leafhopper females were forced to colonize one of five known host plants, and their offspring’s performance was monitored from birth to reproduction on the newly colonized host. The five host plants considered in my study were: (i) perennial teosinte (*Z. diploperennis*); (ii) and (iii) two “geographic populations” of Balsas teosinte, i.e. maize’s ancestor (*Z. m. parviglumis*), and; (iv) and (v) two maize (*Z. mays mays*) cultivars, a landrace and a modern hybrid. Correspondingly for each transition, viz. life history, domestication, and breeding, I hypothesized that performance would be higher (i.e. plant defense weaker) on Balsas teosinte versus perennial teosinte, on landrace maize versus Balsas teosinte, and on hybrid maize versus landrace maize. Overall, the study’s results will help us understand how natural (life history) and artificial (domestication, breeding) selection acting on plants may influence herbivore performance, microevolution and host-race formation, and the emergence and evolution of agricultural pests.

## **Methods**

### *Insect and host plant natural history*

Corn leafhopper feeds and reproduces almost exclusively on members of the genus *Zea* (Nault 1990; Pitre 1970). Although it is endemic in low- to mid-elevations (<1,000 m a.s.l.) in subtropical and tropical areas, its distribution ranges from northern

Argentina to California and the USA Gulf states (Heady et al. 1985; Triplehorn and Nault, 1985). The genus *Dalbulus* is believed to have evolved in western-central Mexico on the all-perennial *Tripsacum* and *Zea* (Nault and DeLong 1980; Dietrich et al. 1998). The genus then expanded its host range to the perennial *Zea* and eventually to the annual *Zea* species, including maize (Triplehorn and Nault, 1985; Triplehorn et al. 1990; Dietrich et al. 1998). Unlike most of its congeners, corn leafhopper overwinters as an active, non-reproducing adult (Larsen et al. 1992; Summers et al. 2004; Moya-Raygoza et al. 2007), and can feed on a variety of hosts that are known associates of maize and wild *Zea* or teosintes (Pitre 1970).

The grass genus *Zea* includes five species: *Z. diploperennis* (perennial teosinte), *Zea perennis* (Hitchc.) Reeves & Manglesdorf, *Zea luxurians* (Durieu & Asch.) Bird, *Zea nicaraguensis* Iltis & Benz, and *Z. mays*. The last species is further divided into four subspecies: *Z. mays huehuetenangensis* (Iltis and Doebley) Doebley, *Zea mays mexicana* (Schrader) Iltis, *Z. mays parviglumis* (Balsas teosinte), and *Z. mays mays* (maize) (Buckler and Stevens 2005). Maize is the only domesticated taxon in the genus *Zea*, and it is the most widely distributed and cultivated host taxa considered in my study. Studies have shown that maize was domesticated in western-southern Mexico from Balsas teosinte, from where it subsequently spread throughout the Americas and worldwide (Matsuoka et al. 2002; Fukunaga et al. 2005; Vigouroux et al. 2008; van Heerwaarden et al. 2011). Balsas teosinte occurs in south western-central Mexico at low- to mid-elevations (ca. 480–1,360 m a.s.l.), whereas the perennial teosinte site considered in my study occurs in mid- to high-elevation (ca. 1,350–2,250 m a.s.l.) in a small mountain range in the southern portion of the Mexican state of Jalisco (Benz et al. 1990; Sanchez-



Gonzalez et al. 1998; Buckler and Stevens 2005). In this study, I compared the performance of corn leafhopper on perennial teosinte, the annual Balsas teosinte, a landrace maize cultivar and a hybrid maize cultivar.

Three transitions relevant to plant defense against herbivores are evident in *Zea*: (i) life history, (ii) domestication, and (iii) breeding. Because of its close relationship with *Zea*, corn leafhopper is an ideal herbivore for testing the hypotheses that life history (annual v. perennial), domestication (wild v. domesticated), and breeding (genetically diverse landrace v. genetically homogeneous, high-yielding hybrid variety) influence the strength of herbivore defenses in plants. Perennial teosinte, annual teosinte, landrace maize, and hybrid maize would represent different microevolutionary templates for corn leafhopper. Insect specialists, such as the corn leafhopper, may develop specific adaptations in order to overcome host plant defenses. This makes specialists ideal for measuring performance vis-à-vis plant defense variation because they should be more sensitive to any changes caused by placing them on novel hosts.

#### *Experimental insects and host plants*

All corn leafhoppers used in this study were obtained from a colony initiated with individuals collected from landrace maize in the vicinity of El Grullo (Jalisco state, Mexico; 19°48' N, 104°13' W) in the summer of 2008. The colony was kept in a plastic frame mesh cage (BugDorm-44545F, Megaview Science Co., Ltd., Taichung 40762, Taiwan) on seedlings of a Mexican landrace of maize (Elotes Occidentales), usually in their 4-6 leaf stage, in a room with a photoperiod of 12:12 (L:D) and a temperature of 24-28 °C.

Five different host plants were grown from seed in a greenhouse: (i) perennial teosinte, (ii) Balsas teosinte 1, (iii) Balsas teosinte 2, (iv) landrace maize, and (v) hybrid maize. Seed of perennial teosinte was collected in the location of Corralitos (19°36'48.78"N, 104°18'23.49"W), within the Sierra de Manantlán Biosphere Reserve (Jalisco state, Mexico) (UNESCO, 2011). Balsas teosinte 1 and Balsas teosinte 2 were collected in San Lorenzo (Ejutla, Jalisco, Mexico; 19°56'60"N, 103°59'0" W) and El Cuyotomate (Ejutla, Jalisco, Mexico; 19°58'10.39"N, 104°4'3.00"W), respectively. Seed of Tuxpeño landrace maize were obtained from USDA NPGS (GRIN accession PI 511649), and seed of hybrid maize were purchased from a commercial seed provider (variety NB2, Híbridos NOVASEM, S.A., Zapopan, Jalisco, Mexico).

All plant seedlings were grown on BACCTO Premium Potting soil (85-15-10) (Michigan Peat Company, Houston, TX, USA), without additional fertilizer. The perennial and Balsas teosinte 1 and 2 plants were grown from seeds that were germinated in Petri dishes after they were extracted from their fruitcases with the aid of nail clippers. Seedlings were used in the experiment when they were in the V3-V5 stage (3-5 collared leaves).

### *Experiment*

Uniformly-aged (1-5 d old  $\pm$  1.5 d) corn leafhopper females were obtained by creating cohorts by placing a potted maize seedling (Elotes occidentales landrace) inside the colony cage for 3 days so that females from the colony would oviposit on the seedling. The seedling was removed from the colony cage, all adult and immature corn leafhoppers

were removed, and the seedling was held in a separate cage, free of corn leafhoppers. Corn leafhoppers were allowed to emerge, mature and mate. Adult females were then removed with the aid of an aspirator for use in the experiment. This process was repeated weekly to ensure a constant supply of corn leafhopper females of a known age. To promote oviposition during the experiment, corn leafhopper females used in experiments were isolated, without access to host plant substrate, in 50-ml centrifuge tubes (with a fine-mesh window on the cap) containing a moist paper towel for 24 h immediately prior to initiating a replicate trial.

For each replicate trial, individual corn leafhopper females (hereafter “F<sub>0</sub> females”) were forced to colonize one of five host plant seedlings: (i) perennial teosinte, (ii) Balsas teosinte 1, (iii) Balsas teosinte 2, (iv) landrace maize, or (v) hybrid maize. Forced colonization consisted of caging an individual F<sub>0</sub> female for 48 h on a leaf (youngest leaf after the whorl leaf) of a seedling. Each cage consisted of two frames (13 × 8 × 1 cm) cut from 10 mm-thick Cellfoam 88 (Midwest Products, Indiana, USA) lined with a soft-foam gasket between the frames (internal dimensions 11 × 6 × 1.5 cm), which sandwiched the leaf and contained the corn leafhopper female. After 48 h, each F<sub>0</sub> female and cage were removed, and the pot holding each seedling was fitted with a plastic cage to enclose the seedling and incubated (25 °C ± 2 °C, ~70 relative humidity, 14 light: 10 dark cycle). Thereafter, seedlings were examined daily to record the following performance variables: (i) F<sub>0</sub> fecundity (= number of eggs laid by F<sub>0</sub> females in 48 h); (ii) F<sub>0</sub> egg survival (= proportion eggs hatching based on F<sub>0</sub> fecundity); (iii) F<sub>0</sub> egg development time (= days from oviposition to appearance of each 1<sup>st</sup>-instar nymph); (iv) F<sub>1</sub> nymph development time (= days between appearances of F<sub>1</sub> 1st-instar nymphs and

adult); (v) F<sub>1</sub> nymph survival (= proportion of F<sub>1</sub> adults based on number of 1<sup>st</sup>- instar nymphs); (vi) F<sub>1</sub> female mass (= mass of adult F<sub>1</sub> females); (vii) F<sub>1</sub> male mass (= mass of adult F<sub>1</sub> males), and; (viii) F<sub>1</sub> fecundity (= eggs laid by F<sub>1</sub> females in 48 h on the host on which they developed). Leaves were excised from plants exposed to F<sub>0</sub> females in order to measure F<sub>0</sub> fecundity and estimate F<sub>0</sub> egg survival. This was done by staining the leaves (and eggs within) following an optimized McBride technique (Backus et al. 1988), then counting the number of F<sub>0</sub> eggs laid in each plant, while noting the number of eggs that showed evidence of hatching. Similarly, measurement of F<sub>1</sub> fecundity relied on excising then staining leaves in order to record F<sub>1</sub> eggs. When emergence of F<sub>1</sub> adults ceased, they were grouped by gender, and all males and a subsample of females were sacrificed by freezing then dried to constant weight (3 d at 80 °C) to measure their mass; the remaining females were individually caged on a new plant of the same host on which they had developed (i.e. the host that their female parent was forced to colonize), and allowed to oviposit for 48 h in order to assess F<sub>1</sub> fecundity.

### *Statistical analysis*

I applied MANOVA (Y = performance variables, except F<sub>0</sub> fecundity which was used as a weight; X = host plants) to address whether the performance of corn leafhopper varied among the five host plants, followed by planned, *a priori* contrasts (with Sidak's correction; Abdi and Williams 2010) to evaluate whether corn leafhopper overall performance was affected by transitions in *Zea*: (i) life history, i.e. perennial teosinte vs. Balsas teosinte 1 and 2; (ii) domestication, i.e. Balsas teosinte 1 and 2 vs. landrace maize; and (iii) breeding, i.e. landrace maize vs. hybrid maize. Additionally, I generated variable

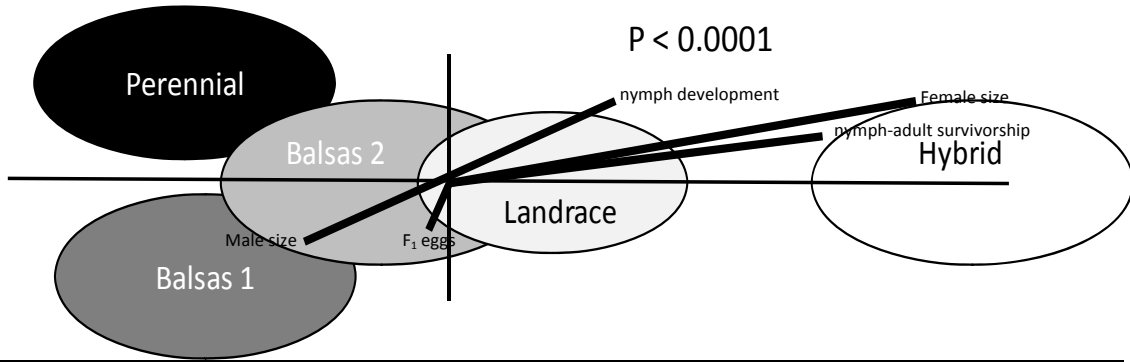
maps to visualize how each variable contributed differentiation between two groups within each *a priori* contrast. The X-axis of the Variable maps indicates the average ratio between two groups, while the Y-axis indicates  $-\text{Log}(\text{ratio})$ ; variables in the upper-right and lower-left panels most distant from origin are most significant, and those near origin contribute least to differentiation.

Univariate, fully-randomized ANOVA was applied to each of the performance variables indicated above, except F1 fecundity. In the case of F1 fecundity, I applied univariate ANCOVA utilizing F0 eggs as a covariable to guard against ovipositional preference for maize, the host plant used to maintain the laboratory colony, in the F1 females, although a prior study showed that (F0) females did not show an ovipositional preference for any of the host plants if denied a choice of host plant (Bellota-Villafuerte 2012). When warranted by the ANOVA ( $P < 0.05$ ), I applied planned, *a priori* contrasts (with Sidak's correction; Abdi and Williams 2010) to evaluate whether individual performance variables were affected by transitions in *Zea* (i) life history, (ii) domestication, and (iii) breeding, as described above.

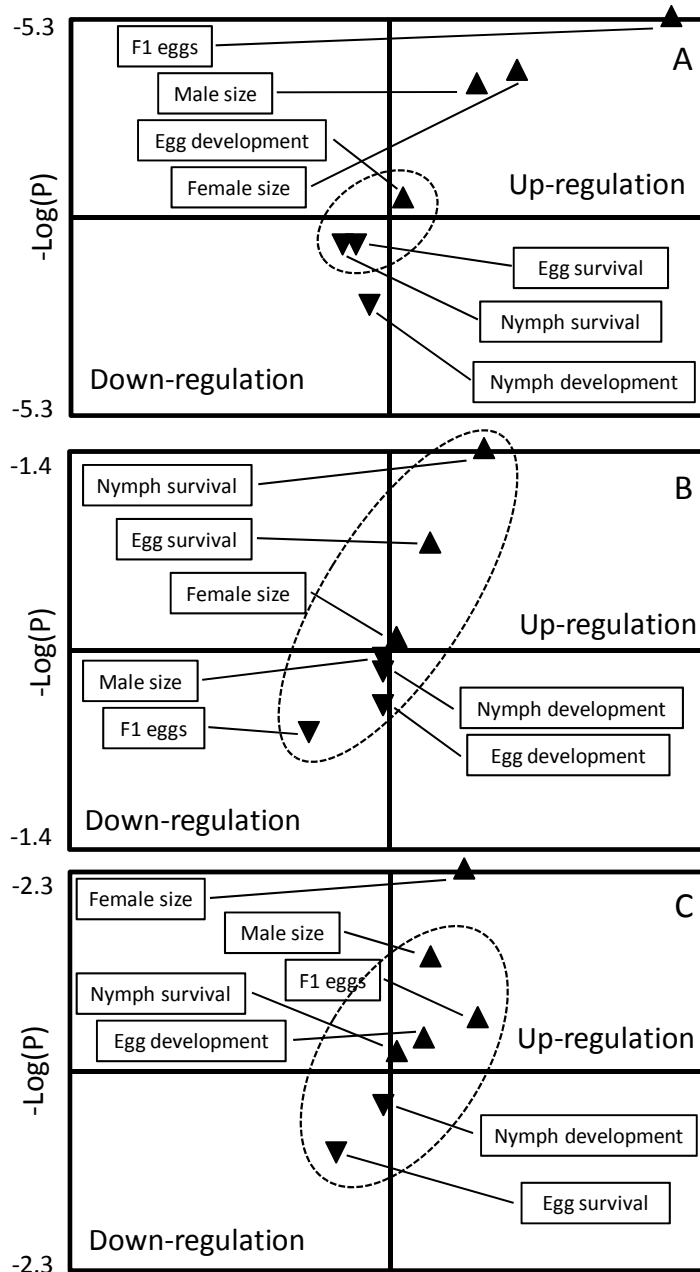
I used mean F1 and corresponding F0 female fecundity on each of the host plants to assess whether colonization of the different hosts affected the (48 h) fecundity of (F1) females relative to that of the corresponding (F0) female parents. Based on the mean F1 and F0 female fecundity values I calculated relative fecundity of F1 to F0 females ( $= \text{mean F1 fecundity} / \text{F0 female fecundity}$ ), and tested whether F1 fecundity changed relative to F0 fecundity via a one-sample *t*-test test on the differences between F1 and corresponding F0 fecundity values ( $= \text{mean F1 fecundity} - \text{F0 fecundity}$ , per replicate) under the hypothesis that the difference was nil.

## Results

One-way MANOVA confirmed a significant multivariate main effect of host plant on corn leafhopper performance (Wilks'  $\lambda = 0.068$ ,  $P < 0.0001$ ,  $F = 4.18$ ,  $df = 28$ , 106) (Figure 2.1), and *a priori* contrast comparisons showed that the transitions in life history ( $P = 0.004$ ,  $F = 3.94$ ,  $df = 7, 29$ ), domestication ( $P = 0.013$ ,  $F = 3.15$ ,  $df = 7, 29$ ), and breeding ( $P = 0.001$ ,  $F = 5.03$ ,  $df = 7, 29$ ) significantly affected corn leafhopper performance. The Variable maps suggested that performance variables associated with F1 individuals contributed most to the effects of the three transitions that were evaluated. Thus, F1 fecundity, F1 female mass, and F1 male mass contributed most to the effect of life history transition (Fig. 2.2a), F1 nymph survival contributed most to the effect of domestication transition (Fig. 2.2b), and F1 female mass and F1 male mass contributed most to the effect of breeding transition (Fig. 2.2c). Similarly, univariate ANOVA (details below) confirmed that all performance variables associated with F1 individuals (nymph development time, nymph survival, female and male mass, and fecundity), but none with F0 individuals (fecundity, egg survival, egg development time), significantly affected corn leafhopper performance. Overall, MANOVA suggested that corn leafhopper performance was best on hybrid maize, poorest on perennial teosinte, and intermediate on Balsas teosintes 1 and 2 and landrace maize (Fig. 2.1).



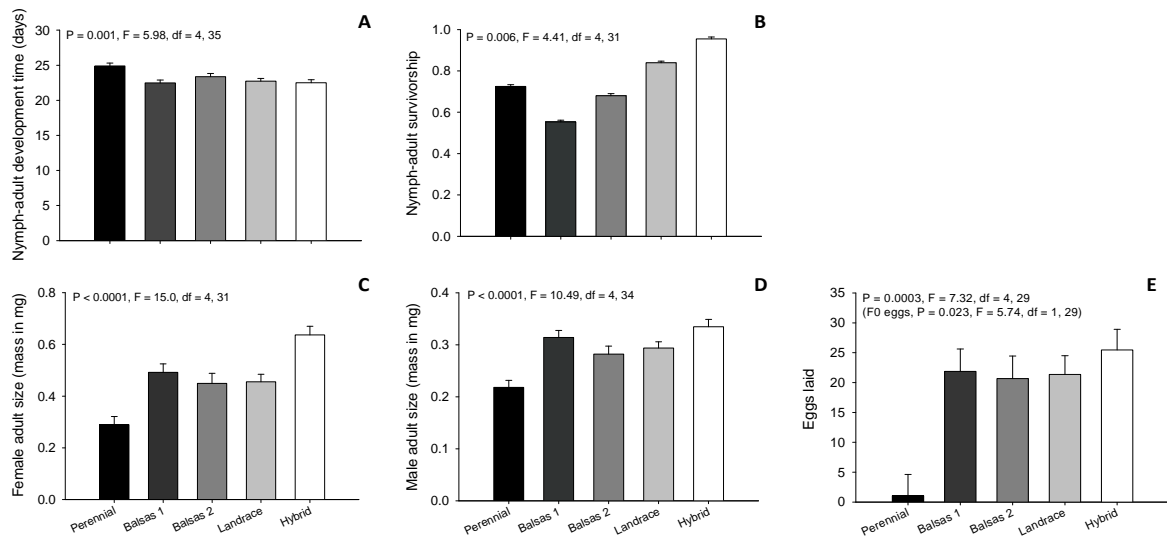
**Figure 2.1:** MANOVA group centroid plot; Perennial= *Zea diploperennis*; Balsas 1= *Zea mays parviglumis* San Lorenzo variety 1; Balsas 2= *Zea mays parviglumis* El Cuyotomate variety; Landrace= *Zea mays mays* Tuxpeño variety; Hybrid= *Zea mays mays* hybrid variety. Biplot rays indicate the direction of the variables. MANOVA suggested that corn leafhopper performance was best on hybrid maize, poorest on perennial teosinte, and intermediate on Balsas teosintes 1 and 2 and landrace maize.



**Figure 2.2** Variable map showing the contributions of each of seven performance variables to the effects of three *Zea* transitions on overall performance of *D. maidis* (A) Life history transition; (B) Domestication transition; (C) Breeding transition. Triangles contained within dotted ellipses did not contribute significantly to overall performance differences (ANOVA,  $P > 0.05$ ). Up-regulation= variable means increase with transition; Down-regulation= variable decrease with transition.



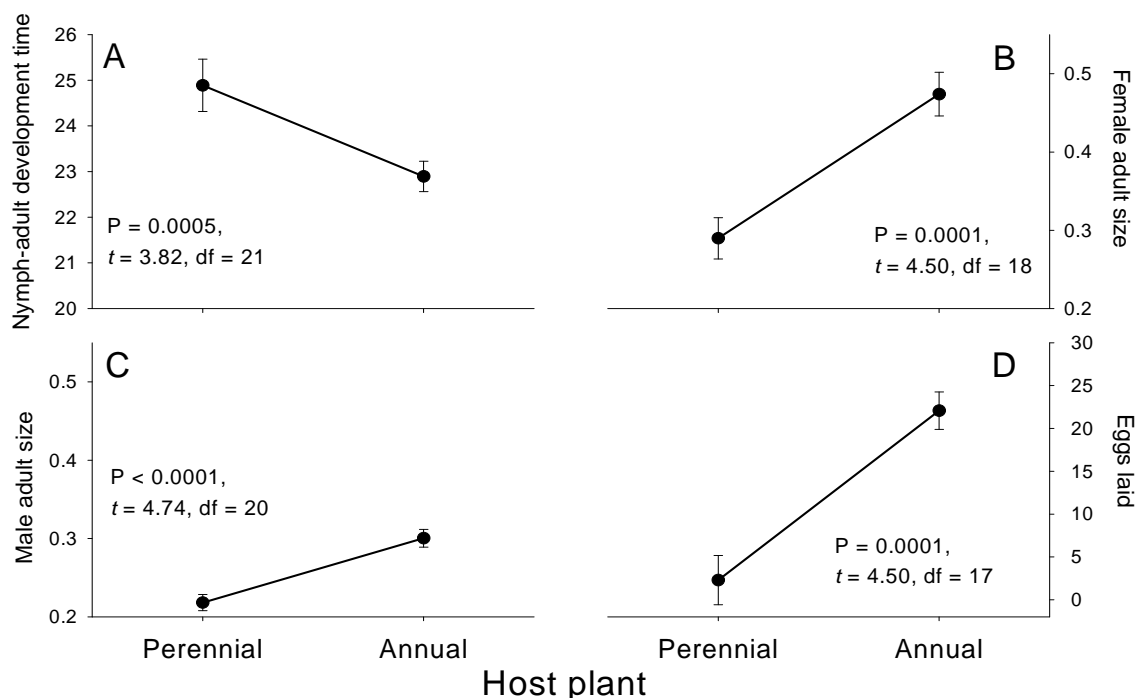
Univariate ANOVA indicated that corn leafhopper  $F_0$  fecundity,  $F_0$  egg survival, and  $F_0$  egg development time were not affected by the host plant colonized by the parental female. Across the five *Zea* host plants, corn leafhopper females oviposited  $29.5 \pm 10.5$  eggs (mean  $\pm$  SD) ( $P = 0.56$ ,  $F = 0.76$ ,  $df = 4, 35$ ), their eggs hatched successfully at a rate of  $73.2 \pm 6.3\%$  ( $P = 0.43$ ,  $F = 0.99$ ,  $df = 4, 35$ ) after  $8.3 \pm 0.7$  d ( $P = 0.88$ ,  $F = 0.30$ ,  $df = 4, 35$ ).



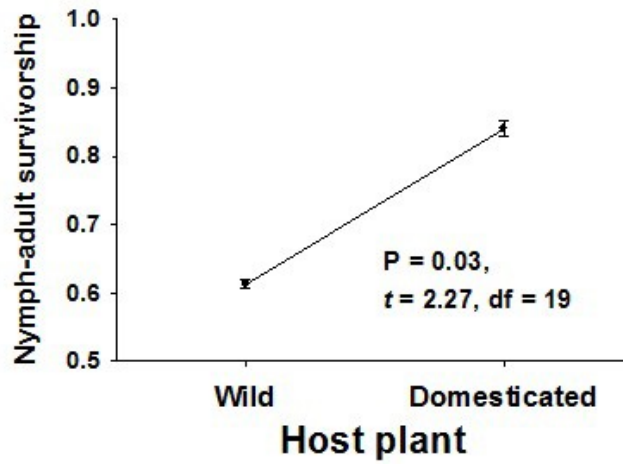
**Figure 2.3 A-E:** Effects of host plant on individual *D. maidis* performance variables; **(A)**  $F_1$  nymph to adult development time; **(B)** survivorship of nymphs; **(C)** mass of females; **(D)** mass of males; **(E)** fecundity of  $F_1$  females.  $F_0$  fecundity,  $F_0$  egg survival,  $F_0$  egg development time were not affected by host plant ( $p > 0.05$ ).

In contrast to  $F_0$  fecundity, survival, and egg development time, the nymph development time, nymph survival, female and male masses, and fecundity of  $F_1$  corn leafhoppers were affected by the host plant that was colonized by the parental female (Fig. 2.3A-E). The  $F_1$  nymph to adult development time ( $P = 0.001$ ) (Fig. 2.3A), survivorship of nymphs ( $P = 0.006$ ) (Fig. 2.3B), mass of females ( $P < 0.0001$ ) and

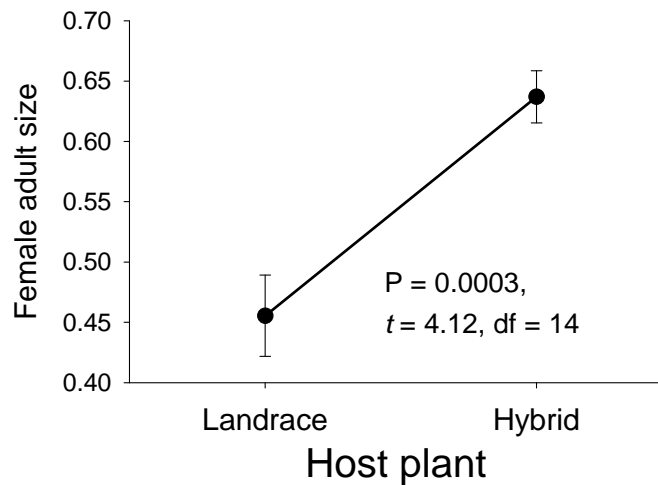
males ( $P < 0.0001$ ) (Fig. 2.3C, D), and fecundity of F1 females ( $P < 0.0003$ ) (Fig. 2.3E) were all affected by the host plant that was colonized by the female parent. Of the three relevant transitions, the life history transition had the most effect on multiple corn leafhopper performance variables (Fig 2.4A-D). Thus, nymph to adult development time decreased ( $P = 0.0005$ ) (Fig. 2.4A), female and male adult mass increased ( $P \leq 0.0001$ ) (Fig. 2.4B, D), and fecundity increased ( $P = 0.0001$ ) (Fig. 2.4C) on (annual) Balsas teosintes compared to perennial teosinte. Nymph to adult development time decreased from Balsas teosinte to landrace maize, as affected by the domestication transition ( $P = 0.03$ ) (Fig. 2.5), while the breeding transition, from landrace maize to hybrid maize, positively affected the mass of adult females ( $P = 0.0003$ ) (Fig. 2.6).



**Figure 2.4 A-D:** Effects of *Zea* life history transitions (from perennial to annual life cycle) on individual *D. maidis* performance variables; (A) nymph to adult development time decreased; (B,D) female and male adult mass increased; (C) fecundity increased.



**Figure 2.5:** Effect of maize domestication (Balsas teosinte versus hybrid maize) on *D. maidis* showing increase in nymph to adult survivorship.



**Figure 2.6:** Effect of maize breeding for improvement (landrace versus hybrid maize) on *D. maidis* showing increase in mass of adult females.

For each host plant, the fecundity of F1 females seemed to suffer relative to that of their (F0) female parent's fecundity, from a low of ~10% loss in females whose parents colonized Balsas teosinte 1 to a high of ~89% loss in females whose parents

colonized perennial teosinte (Table 2.1). However, only the fecundity loss of females whose parents colonized perennial teosinte was statistically significant ( $P < 0.01$ ).

**Table 2.1** The fecundity of *Dalbulus maidis* females ( $F_1$ ) relative to the fecundity of their female parents ( $F_0$ ) is influenced by the *Zea* host that was colonized by the parent (critical P per Bonferroni correction is 0.01)

Ratio offspring to parent fecundity			$P(F_0 \text{ fecundity} - F_1 \text{ fecundity} = 0)$
Perennial teosinte	2.8/26.1	0.11	$< 0.01$ ( $t = -5.08$ , $df = 7$ )
Balsas teosinte 1	26.6/29.5	0.90	0.73 ( $t = -0.36$ , $df = 7$ )
Balsas teosinte 2	20.7/26.1	0.79	0.34 ( $t = -1.04$ , $df = 6$ )
Landrace maize	17.8/33.5	0.53	0.05 ( $t = -2.33$ , $df = 9$ )
Hybrid maize	25.0/31.0	0.81	0.23 ( $t = -1.34$ , $df = 6$ )

## Discussion

The results of this experiment suggested that the performance of the specialist herbivore *D. maidis* is affected by three host plant transitions, life history, breeding, and domestication. My overall hypothesis was that corn leafhopper performance would be highest on plants strongly selected for high productivity over herbivore defenses, such as the maizes, and lowest on plants selected strongly for herbivore defense as a means of achieving sufficient productivity, such as the teosintes. Thus, corn leafhopper performance was expected to be higher on Balsas teosinte than on perennial teosinte, on landrace maize than on Balsa teosinte, and on hybrid maize than on landrace maize.

The effects of *Zea* life history transition on corn leafhopper were evaluated by

contrasting perennial teosinte with the annual Balsas teosinte. Performance was higher on Balsas teosinte compared to perennial teosinte, consistent with my prediction.

Furthermore, while MANOVA showed effects on overall performance of all three transitions, subsequent univariate analyses showed that life-history transition affected the most number of performance parameters. Perennials and annuals not only occupy contrasting temporal growing seasons, but they have different metabolic resource allocation strategies. Perennial teosinte is an iteroparous, rhizomatous grass with clonal propagation characteristics (Masarovicova et al. 2000). It can persist for many growing seasons, which allows for numerous opportunities to reproduce, and metabolic resource allocation is mostly to the roots. Clonal colonies tend to peak during the spring of the second year (Sánchez-Velásquez et al. 2001). There is little information on the biology and ecology of Balsas teosinte (e.g., Mondragon and Vibrams 2005), however, it is an annual grass that completes its entire life cycle within a single growing season (~July-November), having only one opportunity to reproduce and allocating much of its resources to reproduction. The different resource allocation strategies corresponding to the two life histories should affect how the plants interact with their environments, including insect herbivores.

The effects of the domestication transition on corn leafhopper performance were evaluated by comparing between Balsas teosinte and the maize landrace, Tuxpeno. My results show a significant effect of one performance parameter, nymph to adult survivorship. Based on the resource allocation hypothesis, landrace maize should allocate more of its metabolic resources to productivity, and conversely, Balsas teosinte should allocate more of its resources to defense. This is evident in the striking difference

in morphologies between the two species. For example, the teosinte “ear” consists of 5-12 seeds enclosed in fruitcases, whereas the landrace maize ear consists of hundreds of naked seeds. Although there is such an extreme difference in morphology between species, maize retains ca. 90% of the gene diversity and  $\sim 3/4$  of the alleles found in its progenitor Balsas teosinte (Vigorous et al. 2005; Buckler and Stevens 2005) and their phylogenetical and ecological proximity allow them to easily cross-fertilize and form fully fertile F<sub>1</sub> hybrids in the field (Doebly 2004). This suggests that plant defenses at the chemical level may not be acting directly on the adults, since plant chemistry across Balsas teosinte and maize appear to be similar in composition. The negative effect on nymph to adult survivorship suggests that defenses might be acting at the nymphal level and becoming manifest at the adult stage. No study has looked at the nutrient intake efficiency of nymphs. Physical defenses could be at play. For example, it is possible that Balsas teosinte is a tougher plant to penetrate by nymphs, thus reducing the ability to efficiently develop into adults.

How the breeding transition affected corn leafhopper performance was evaluated by contrasting the maize landrace against the modern cultivar. My results were consistent with my prediction that corn leafhopper would perform better on the modern cultivar than on the landrace. Genetic variability within cultivars of maize may be weighing strongly on the breeding effect. Mexican maize races rank among the most genetically variable groups of organisms (Doebly et al. 1985). In the process of domestication, plants undergo artificial selection which alters the allelochemical and nutritional qualities of cultivars within domesticated species (Van Emden, 1987). Breeding can intensify the variability among varieties by selecting for specific traits such

as adaptation to local weather, soil, and photoperiod. In return, insect specialists, such as corn leafhopper, are forced to adapt to such plant variability. Breeding effects have been observed in other studies. Moreau et al. (2006) found that a variety of grape, Gewurztraminer, induced a slower larval development of the European grapevine moth *Lobesia botrana* (Denis & Schiffmüller) (Lepidoptera: Tortricidae) than other cultivars, suggesting that this cultivar is of lower nutritional value for the moth's larvae. Also, Broekgaardeon et al. (2007, 2008) demonstrated that two cultivars of *Brassica oleracea*, Rivera and Christmas Drumhead, differed dramatically in transcriptional responses to herbivory by a specialist caterpillar (*Pieris rapae* L.), a generalist caterpillar (*Mamestra brassicae* L.), and the cabbage aphid *Brevicoryne brassicae* L. Lower numbers of both the specialist and generalist larvae were found on Rivera than on Christmas Drumhead, suggesting differences in larval performance and/or oviposition preference between the cultivars. Other studies showed similar results under greenhouse conditions (Poelman et al., 2009; Broekgaarden et al., 2007). Furthermore, Broekgaarden et al. (2009) found that the herbivore community composition of two *B. oleracea* cultivars developed clear differences as the season progressed. Several defense-related genes showed higher levels of expression in the cultivar that harbored the lowest numbers of herbivores. It was suggested that the differences could be related to differences in gene expression between the cultivars. My null hypothesis was that the performance of corn leafhopper is similar across cultivars, landrace or hybrid. However, no study to date has tested corn leafhopper performance on a wide range of maize cultivars. Performance differences may be evident at a landscape level in that insect population growth rates are depressed by resistant plant genotypes, while susceptible genotypes serve as reservoirs for growing

insect populations (Agrawal et al. 2006). It is possible that performance of corn leafhopper may be poorer on some landrace or hybrid cultivars than on others, as found in this study, due to variation among maize cultivars in defense responses (e.g., Szczepaniec et al. 2012) and/or among corn leafhopper genotypes (Medina et al. 2012; Chapter 3). However, such differences may not be apparent for corn leafhopper because of the widespread distribution of maize and the insect's ability to disperse among crop fields and across large areas.

Adult male and female mass and F1 fecundity were the performance parameters most notably affected by the transitions, specifically by increments of 1.5-, 2.2-, and 23.9-fold from perennial teosinte to hybrid maize. Egg production requires large amounts of accumulated resources. Since females are responsible for egg production, larger females would be better equipped to accumulate more energy reserves and of better quality. Therefore, a larger body size would be more advantageous since a larger size would allow for high probability of survival and would allow for the storage of more eggs. However, adult size and fecundity can be affected by several factors, including genetics, developmental conditions and environmental conditions during oviposition (Honek 1993). Even though predicting the number of eggs that will be laid from the size of the female is not possible, under constant environmental conditions, fecundity is positively correlated with female size (Wang et al. 2006; Salavert et al. 2011; Yee et al. 2011). In my study, F1 fecundity and female mass are correlated across all host plants (Pearson's  $r = 0.751$ ,  $P < 0.0001$ , data not shown). On the other hand, few studies have tested whether male mass has any effect on survival and fitness of individuals. Increased male size in my results could simply reflect better nutrient quality



provided by hybrid maize (Salavert et al. 2001). It is possible that the size difference may affect a male's contribution to the production of fertile eggs (Delisle and Hardy 1997), however, this has not been tested.

With regards to performance parameters associated with the (F<sub>0</sub>) founding generation (F<sub>0</sub> fecundity, egg development time, egg survival), my results showed no differences among host plants. This suggests that defense against corn leafhopper involved antibiosis, and that antixenosis and any defenses targeting eggs are either not relevant or are weak. This agrees with findings by Bellota-Villafuerte (2012) where, in a no-choice context as in my study, females oviposited with equal frequencies on the host plants evaluated. A similar pattern was observed by Pitre (1970), who found that females were able to oviposit in up to 20 different plants species, but only achieve complete development on maize. Bellota-Villafuerte (2012) suggested that in the context of initial oviposition choice made by corn leafhopper females, life history, domestication, and breeding transitions in *Zea* may not be relevant to defense against this specialist herbivore.

Offspring (F<sub>1</sub>) fecundity suffered significantly only for forced colonization of perennial teosinte. These results would suggest that antiherbivore defenses are stronger in wild relatives compared to the domesticated crop plants. Chemical defenses can affect several performance parameters, including nymphal developmental time, total developmental time, nymphal survival, total survival, fecundity and female longevity (Cordona et al. 1989; Beland and Hatchett 1976; Jindal et al. 2008). However, it was unclear whether performance was affected at the adult stage or the nymph. Furthermore, this difference in performance between wild host versus domesticated host can help

explain how corn leafhopper exists as two subpopulations (Medina et al. 2012; Chapter 3). Results from Chapter 3 suggests that certain haplotypes of corn leafhopper may be better equipped to persist on wild perennials.

Physical defenses such as pubescence, texture, or color can affect herbivore performance (Jindal and Dhaliwal 2011; Le Roux et al. 2008; Bellota-Villafuerte (2012). For example, Bellota-Villafuerte (2012) assessed whether two putative physical defenses, pubescence and leaf toughness, against corn leafhopper were affected by life history, domestication, and breeding transitions in the *Zea* taxa I evaluated in my study. His results showed that across-taxa differences in these physical defenses were not consistent with a pattern of weaker defenses in lesser-domesticated hosts. In contrast, this study showed that corn leafhopper's ovipositional preferences were consistent with a pattern of weaker defenses in lesser-domesticated hosts, when females were allowed a choice of hosts. Overall, he concluded that if oviposition preferences were not correlated with physical defenses, then preference may be driven by differences in chemical defenses. My results reflect the latter conclusion, showing poorer performance on lesser-domesticated hosts.

Evidence from Medina et al. (2012) and results from Chapter III suggest that corn leafhopper consists of genetically discrete subpopulations, including an annual *Zea* (maize and Balsas teosinte) and a perennial teosinte population. All the corn leafhopper individuals bioassayed in this study belonged to the annual *Zea*-associated subpopulation. Thus, my results concerning performance may have been biased against perennial teosinte. Nevertheless, my results offer a glimpse into how performance may suffer if individuals of the corn leafhopper annual *Zea*-associated subpopulation colonize a non-

associated host. F1 females on perennial teosinte produced ~3% of the eggs that were produced overall by F1 females on the annual *Zea* (Balsas teosintes 1 and 2 and Tuxpeno and hybrid maizes). This suggests a mechanism maintaining subpopulation identity between annual *Zea*-associated corn leafhopper and wild perennial teosinte-associated corn leafhopper (Medina et al. 2012). In particular, my study suggests that the performance of corn leafhopper individuals of the annual *Zea* subpopulation colonizing perennial teosinte suffers significantly.

In conclusion, my findings agree with those of previous studies suggesting that plant defenses are stronger in perennial than annual plants, in crop ancestors than crops, and in landraces than modern varieties (e.g., Rosenthal and Dirzo 1997; Benrey et al. 1998; Rodriguez-Sanoa et al. 2011; Szczepaniec et al. 2012; Takahashi et al. 2012). Moreover, my results showed that the life-history transition had the most consistent effects on corn leafhopper performance parameters, which is consistent with the results of studies indicating stronger herbivore defenses in perennial compared to annual plant species (Rosenthal and Dirzo 1997; Benrey et al. 1998; Rodriguez-Sanoa et al. 2011; Szczepaniec et al. 2012; Takahashi et al. 2012). Adult mass (female and male) and fecundity of F1 females were the performance parameters most significantly affected among the parameters that I evaluated. The gains in adult mass and fecundity from the perennial host to the hybrid maize were consistent with predictions of the resource allocation hypothesis, viz. that domesticated maize would be the least defended, most suitable host for corn leafhopper (Rosenthal and Dirzo 1997). The relevance of lower adult mass to male fitness is unclear, though other studies show that nutrient quality, and contribution to the production of fertile eggs may be playing a role (Delisle and Hardy

1997; Ritchie et al. 1998; Salavert et al. 2001). The performance parameters associated with F0 individuals (F0 fecundity, egg development time and survival) were not affected by the transitions I evaluated nor by the host plants colonized by corn leafhopper females. Thus the life history, domestication, and breeding transitions in *Zea* and the differences among host plants seemed particularly relevant to the nymphal stage and become manifest during the adult stage, when differences in adult mass and female fecundity are especially notable. My study leaves open the question of whether important *Zea* defenses against corn leafhopper are chemical or physical, or both. Further studies should address potential differences in nutrient intake efficiency among corn leafhoppers on different host plants, which could result in performance differences. Lastly, my results suggested that the performance of the corn leafhopper individuals associated with annual *Zea* suffers when they colonize perennial teosinte, suggesting a mechanism for maintaining genetic differentiation between subpopulations (Medina et al. 2012).

## CHAPTER III

### HAPLOTYPE ANALYSIS OF *DALBULUS MAIDIS*

#### **Introduction**

Molecular genetics techniques are powerful tools for addressing questions concerning genetic variation in populations, and especially for analyses of microevolutionary patterns and processes (Zhao et al. 2003). In particular, haplotype network analyses can be used for assessing genetic differentiation among populations and reconstructing their historical interconnectedness (Ahern et al. 2009; Ballman et al. 2012; Yuan et al. 2010). For example, where herbivore populations are genetically structured by host plant (i.e. host-associated differentiation), haplotype network analyses can be used to infer which population is ancestral on the basis of haplotype diversity, and may provide an estimate of time since divergence between populations (Havill et al. 2006). Haplotype networks can also provide insight into population genetic structures where insect populations are geographically separated by examining questions dealing with population-structuring events, such as glaciations (Ahern et al. 2009). Similarly, in the context of agriculture, haplotype network analyses may be used to gain insight to historical processes behind contemporary distributions and genetic differentiation of crop pests.

A recent study based on AFLP analyses showed population genetic structuring in corn leafhopper [*Dalbulus maidis* (DeLong & Wolcott)], an economically important pest of maize in the Neotropics (Nault 1990; Medina et al. 2012). Specifically, corn leafhopper in western-central Mexico was found to be divided in two genetically distinct

subpopulations: one widespread subpopulation associated with cultivated maize (*Z. mays* ssp. *mays* L.) and maize's wild ancestor Balsas teosinte (*Z. mays* ssp. *parviglumis* Iltis & Doebley), and another associated with perennial teosinte (*Z. diploperennis* Iltis, Doebley, & Guzmán) and confined to a biosphere reserve in the southern portion of the Mexican state of Jalisco. Medina et al. (2012) hypothesized that the subpopulation associated with perennial teosinte (hereafter "Perennial teosinte subpopulation") originated from the subpopulation associated with maize and Balsas teosinte (hereafter "Zea mays subpopulation") subsequent to maize's domestication and spread into perennial teosinte's habitat. Their hypothesis was based on (i) habitat difference between the area of endemicity of perennial teosinte (mesophyllous mountain forest >1800 m asl) and the area in which Balsas teosinte is endemic and maize was domesticated (tropical deciduous forest at ~1000 m asl), and (ii) the upland and general spread of maize cultivation following the crop's domestication. An understanding of the filial relationship (i.e., who was the parental population) between the two corn leafhopper subpopulations would provide insight to origin and spread of the *Zea mays* subpopulation, i.e. the subpopulation that is currently an economically important pest of maize.

Haplotype analysis using mitochondrial DNA (mtDNA) gene sequences have proven to be informative for analyses of genetic structure and gene flow studies (Anstead et al. 2002; Boykin et al. 2007; Giordano et al. 2005; Shufran et al. 2000; Zhao et al. 2003). Several properties make mtDNA sequences choice markers for studies of genetic structure and gene flow. For example, mtDNA is easy to obtain and amplify because it is present in high copy numbers in animal cells, and it is strongly conserved across animals, with very little recombination (Gissi et al. 2008). The

latter property can be particularly valuable for tracing related lineages back through time. Additionally, mtDNA is highly variable within natural populations because of its elevated mutation rate, which can help generate information about population history over short time frames (Galtier et al. 2009). Thus, haplotype analyses may provide insight into the level and directionality of gene flow across corn leafhopper's distribution, which would further our understanding of processes leading to corn leafhopper's emergence and evolution as a maize pest in particular, as well as processes leading to emergence and evolution of agricultural pests in general.

MtDNA markers have already been used to assess genetic diversity in Mexican and Argentinean samples of corn leafhopper. Palomera et al. (2012) looked for variation among corn leafhopper samples collected in four Mexican localities from maize and maize wild relatives [Balsas teosinte and *Zea perennis* (Hitchc.) Reeves & Manglesdorf] and seven Argentinean localities from maize. They sequenced and analyzed regions of the mitochondrial gene coding for the cytochrome oxidase subunit I (COI) and the ribosomal internal transcribed spacer (ITS2). Their analyses did not uncover genetic variation (i.e. a single haplotype was found) among Mexican and Argentinean populations of corn leafhopper in the ribosomal region, and found a single COI haplotype each within their Mexican and Argentinian samples, though their sample sizes were very small (17 and 22 sequences for ITS2 and COI, respectively).

The goal of this study was to assess haplotype diversity, structuring and interconnectedness among corn leafhoppers collected in their putative center of origin in Mexico. I focused on samples from within an area encompassing the presumed centers of radiation of both *Dalbulus* and its host genus *Zea* and of maize domestication (Nault

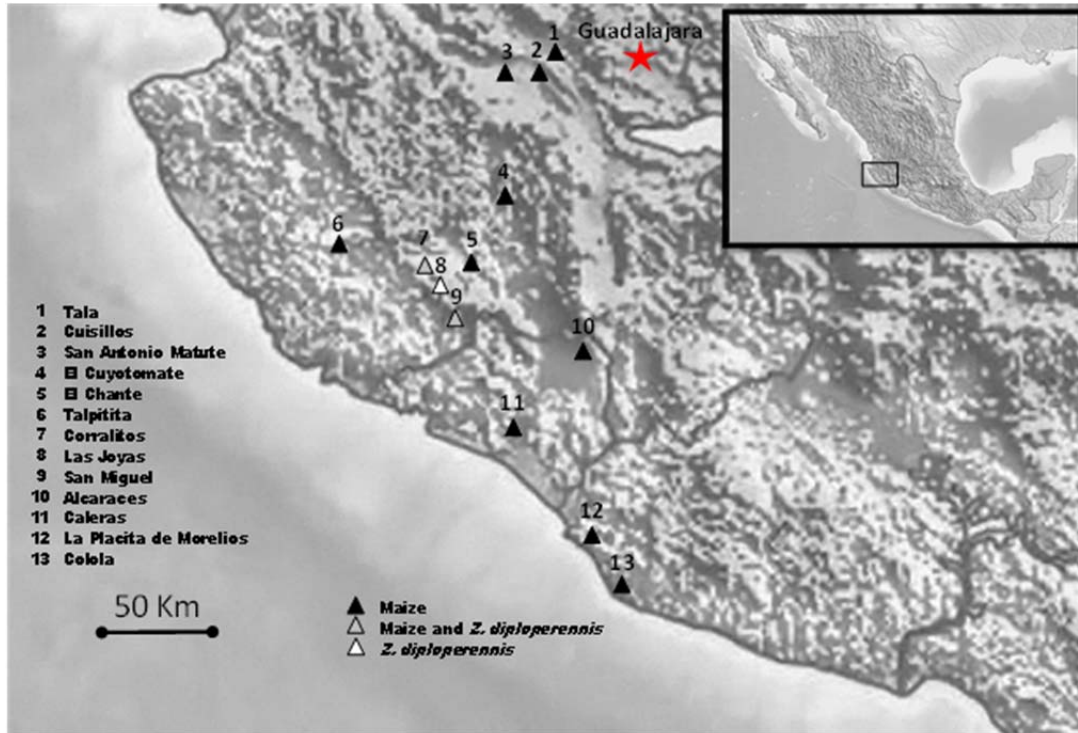
1990; Buckler & Stevens 2005) so that I could begin inferring on structuring and historical gene flow between the Perennial teosinte and *Zea mays* subpopulations of corn leafhopper. I complemented my samples with corn leafhopper COI sequences available at GenBank (Palomera et al. 2012) in order to broaden the scope of my study. Following prior studies (Medina et al. 2012) I expected that genetic differentiation (structuring) would be evident between corn leafhopper samples from maize and perennial teosinte.

## **Methods**

### *Insect collection*

Individuals of corn leafhopper were collected from 13 sites in Jalisco, Colima, and Michoacan states, Mexico and stored in 95% EtOH (Table 3.1). Specimens were collected by sweep net and aspirator. Leafhoppers from maize were collected from twelve locations and from perennial teosinte from three locations (Figure 3.1 and Table 3.1). Two of the three perennial teosinte locations, overlapped with two maize collection sites where maize and perennial teosinte grow side by side (San Miguel, Corralitos). The remaining perennial teosinte site (Las Joyas), is a confined site of perennial teosinte with no maize in the immediate vicinity.





**Figure 3.1:** Collection sites of *D. maidis* in central western Mexico. Black triangles are maize collection sites, grey triangles are maize and *Z. diploperennis* collection sites, white triangles are *Z. diploperennis* collection sites.

Table 3. 1: Collection sites of adult *D. maidis*.

Site	Distance from Las Joyas (Km)	Elevation (m asl)	Latitude	Longitude
Las Joyas	0	1860	19 35 32.06 N	104 16 52.29 W
Corralitos *	4	1810	19 36 48.78 N	104 18 23.49 W
San Miguel *	13	1540	19 29 59.1 N	104 12 28.60 W
El Chante	15	912	19 42 27.64 N	104 12 14.20 W
El Cuyotomate	48	1270	19 58 10.39 N	104 04 01 W
Talpitita	55	370	19 42 46.48 N	104 47 16.66 W
Caleras	78	80	18 59 51.06 N	103 52 56.82 W
Los Alcaraces	80	1110	19 21 48.02 N	103 33 34.85 W
San Antonio Matute	114	1255	20 33 47.58 N	103 57 12.79 W
Cuisillos	125	1280	20 36 31.86 N	103 46 33.16 W
Tala	132	1320	20 39 05.29 N	103 42 26.85 W
La Placita de Morelios	140	15	18 32 14.94 N	103 35 32.36 W
Colola	173	20	18 17 39.44 N	103 24 10.98 W

\* sites with both maize and perennial teosinte

### *DNA isolation*

Genomic DNA was extracted from random whole individual specimens (i.e., adult fleahoppers) using DNeasy kit following the manufacturer's protocols (Qiagen, Valencia, CA, USA). The average number of samples per site was 13 individuals. DNA concentration and quality were measured for each specimen using a spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA.)

### *DNA amplification*

A fragment of the mitochondrial cytochrome oxidase I gene (mtCOI) was amplified with a primer pair specific for *D. maidis* mtCOI region (Palomera et al (2012), dalCOI fwd (5'TAG CTC AAC CTG GGT CGT TT), and dalCOI rev (5'TGGTAT AGG ATT GGGTCA CCA). PCR reactions were performed in a 25  $\mu$ L final volume containing 1.5ul of DNA template (minimum 50ng), 14ul of 2 X Terra PCR Direct Buffer (with MgCl<sub>2</sub> and dNTPs), 1.5ul of 100  $\mu$ M of each primer and 0.7ul of 1.5 U/ $\mu$ L Terra PCR Direct Polymerase Mix. PCRs were run with a hot start of 94 °C for 5 min, followed by 25 cycles each of 30 s at 94 °C, 60 s at 58 °C, 60 s at 72 °C, and a hold step at 4°C.

### *Electrophoresis and sequencing*

PCR products were visualized on 1% agarose gels to confirm amplification of samples and non amplification of negative controls. PCR product was then sent via FedEx on dry ice to University of Florida Interdisciplinary Center for Biotechnology Research (ICBR) for PCR purification and sequencing.

### *Analytical methods*

Partial COI sequences were aligned manually in Sequencher 4.8 (GeneCodes, Ann Harbor, USA) based on published sequences by Palomera et al. (2012). Consensus sequences were trimmed to 350bp. Chromatograms were visually inspected and only sequences with unambiguous basecalls were included in the analyses. Additional mtDNA sequences published by Palomera et al (2012) (GenBank accession numbers, JN411693, JN411694, JN411695, JN411696, JN411697, JN411698, JN411699, JN411700, JN411701, JN411702 and JN411703) were included to construct a haplotype network. Relationships between mtDNA haplotypes were examined using a statistical parsimony haplotype network generated at the 95% connection limit with TCS v1.21 (Clement et al. 2000). Statistical analyses were carried out using several functions within DnaSP (Librado and Rozas 2009). Genetic diversity for each site was assessed by calculating haplotype diversity ( $H_d$ ), average number of nucleotide differences ( $K$ ), and nucleotide diversity ( $\pi$ ). Genetic differentiation between samples collected from maize or perennial teosinte was assessed using haplotype-based statistics,  $H_s$  and  $H_{st}$ , and a  $\chi^2$ -test (Nei 1987; Hudson et al. 1992a). Parameter estimates included sequences from my sites as well as from Palomera et al. (2012), unless indicated otherwise.

### **Results**

I obtained 350 bp of the COI gene from each of 188 corn leafhopper specimens from 13 sites and two host plants, maize and perennial teosinte, in western-central Mexico (Table 3.1, Figure 3.1); 11 additional corn leafhopper (COI) sequences were obtained

from GenBank (see above for GenBank accession numbers), and corresponded to specimens collected from maize and Balsas teosinte in Mexico, and maize in Argentina (Palomera et al. 2012). Six different mitochondrial haplotypes (hereafter labeled “A” through “F”) were found among the 188 specimens from my 13 sampling sites in Mexico (Figure 3.2; Figure 3.3; Table 3.2). The 11 corn leafhopper sequences obtained from GenBank yielded two haplotypes: the Mexican sequences yielded one haplotype, which corresponded to my haplotype E, while the Argentinean sequences yielded two haplotypes, corresponding to my haplotype E and an additional haplotype G (Figure 3.2; Table 3.2). Haplotype E was found in all my 13 sampling sites and on both maize and perennial teosinte, and in both the Mexican and Argentinean samples of Palomera et al. (2012), and was shared by 182 specimens, including 4 sequences of Palomera et al. (2012). Haplotypes A and F were unique to a single sampling site, Los Alcaraces, and each was found in single individuals collected from maize. Haplotype B was found in two localities, at Colola on maize and Las Joyas on perennial teosinte. Haplotype C was collected only at San Miguel from perennial teosinte, and was found in a single individual. Similarly, haplotype D was collected only at Corralitos from perennial teosinte, and was found in a single individual. Haplotype G was found only among the 7 GenBank sequences derived from samples taken from maize in Argentina (Palomera et al. 2012).

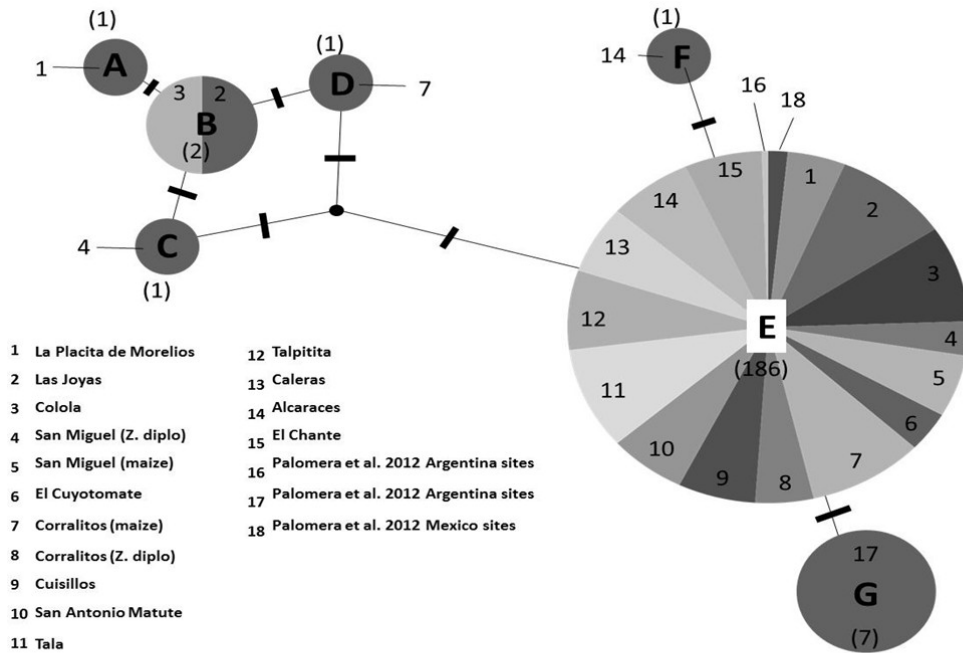


Figure 3.2: Haplotype map showing relationships among haplotypes. Upper case letters indicate haplotype; number in parenthesis indicate number of sequences. Tick marks between haplotypes indicate one base-pair change.

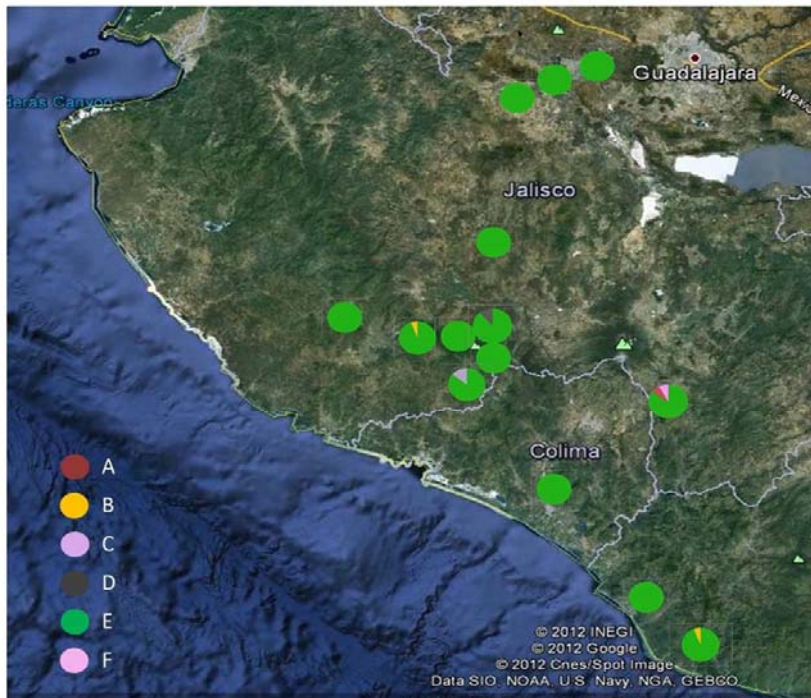


Figure 3.3 Map showing proportion of haplotypes within each site. Individual pie charts correspond to a collection site. Haplotype E is dominant across all sites.

Haplotype diversity (Hd) varied between 0.111 and 0.333 among samples collected from perennial teosinte at three sites, while it was most frequently nil (i.e. a single haplotype was detected) (11/14 samples) but as high a 0.294 among samples collected from maize or Balsas teosinte at 14 sites (Table 3.2). Nucleotide differences (K) varied between 0.333 and 0.667 among samples collected from perennial teosinte at three sites, while it was most frequently nil (11/14 samples) but as high a 0.769 among samples collected from maize or Balsas teosinte at 14 sites (Table 3.2). Nucleotide diversity ( $\pi$ ) varied between 0.001 and 0.002 among samples collected from perennial

**Table 3.2** : Overall mtDNA analyses parameters of *D. maidis*

Sample	Host	Haplotype	No. of sequences (n)	No. of haplotypes (h)	Haplotype diversity (Hd)	Avg. nucleotide differences (K)	Nucleotide diversity ( $\pi$ )
Las Joyas	Perennial teosinte	B, E	18	2	0.11111	0.33333	0.00095
Corralitos	Maize	E	18	1	0	0	0
Corralitos	Perennial teosinte	D, E	9	2	0.22222	0.44444	0.00127
San Miguel	Maize	E	11	1	0	0	0
San Miguel	Perennial teosinte	C, E	6	2	0.33333	0.66667	0.00190
El Chante	Maize	E	12	1	0	0	0
El Cuyotomate	Maize	E	7	1	0	0	0
Talpitita	Maize	E	14	1	0	0	0
Caleras	Maize	E	12	1	0	0	0
Los Alcaraces	Maize	A, E, F	13	3	0.29487	0.76923	0.00220
San Antonio Matute	Maize	E	12	1	0	0	0
Cuisillos	Maize	E	12	1	0	0	0
Tala	Maize	E	18	1	0	0	0
Colola	Maize	B, E	17	2	0.11765	0.35294	0.00101
La Placita de Morelios	Maize	E	9	1	0	0	0
Palomera et al. Mexico sites	<i>Zea mays</i> *	E	3	1	0	0	0
Palomera et al. Argentina sites	Maize	E, G	8	2	0.25000	0.25000	0.00071

\*two sites from maize and one site from *Zea mays parviglumis*

teosinte at three sites, while it was most frequently nil (11/14 samples) but as high as 0.002 among samples collected from maize or Balsas teosinte at 14 sites (Table 3.2). Overall, four haplotypes, B, C, D, E, were found among 33 individuals collected from perennial teosinte at three sampling sites, and five haplotypes, A, B, E, F, G, were found among 155 individuals collected from maize at 12 sites in Mexico and 11 sequences from GenBank (Palomera et al. 2012) (Table 3.2). Haplotype diversity (Hd) was ~1.5- fold greater, and average nucleotide differences (K) and nucleotide diversity ( $\pi$ ) were ~2.3-fold greater among individuals collected from perennial teosinte relative to individuals from maize, indicating significant genetic differentiation between samples from these host plants ( $P = 0.036$ ) (Table 3.3).

**Table 3.3:** Genetic differentiation parameters comparing the *Zea mays* and Perennial teosinte subpopulations of *D. maidis*.

Subpopulation	No. of sequences (n)	No. of haplotypes(h)	Haplotype diversity (Hd)	Avg. nucleotide differences (K)	Nucleotide diversity ( $\pi$ )
Zea mays	166	5	0.116	0.177	0.0005
Perennial teosinte	33	4	0.176	0.405	0.0012
$\chi^2$ test	$\chi^2 = 13.492$ , $P = 0.036$ , $df = 6$				

## Discussion

Overall, my results revealed the existence of seven mitochondrial haplotypes in corn leafhopper specimens from 13 sites in Mexico and Argentina. My samples included previously reported haplotypes from Mexico and Argentina (Palomera et al 2012). One haplotype (haplotype E) was present in all sites, including in Argentina, and on both maize and perennial teosinte, while the remaining haplotypes were relatively rare, being represented in single to few specimens per site. The

ubiquitousness of haplotype E seems to parallel the spread of maize cultivation. Such ubiquitousness of haplotype E coupled with the greater haplotype diversity in corn leafhopper samples from perennial teosinte suggest that: (i) haplotype E has largely displaced other haplotypes from maize, and; (ii) the greater haplotype diversity evident among samples from perennial teosinte compared to maize may represent past diversity. This interpretation is coincident with an earlier hypothesis proposing that corn leafhopper was pre-adapted for successfully colonizing and exploiting maize upon the crop's domestication (Nault 1990). Thus, expanding maize cultivation likely provided a novel and increasingly abundant host which allowed corn leafhopper (especially through pre- adapted genotypes) to expand its host range, distribution, and abundance. Moreover, such expansions may have been facilitated by weaker anti-herbivore defenses in maize compared to wild hosts, which has been shown previously for other herbivores (Rosenthal & Dirzo, 1997; Takahashi et al. 2012; Szczepaniec et al. 2012; Chapter 2). Thus, genotypes particularly suited for exploiting maize may have become particularly abundant as maize cultivation expanded. Moreover, increasingly larger populations of corn leafhopper and strong selection for particular haplotypes in cultivated maize may have resulted in the emergence of a dominant and pestiferous maize-adapted haplotype, such as haplotype E.

The scenario described above presumes that prior to maize domestication, corn leafhopper (genotypes) may have been pre-adapted to colonize and exploit maize (Nault 1990), and that haplotype diversity was higher than at present. It is plausible that as maize cultivation became widespread, maize provided a novel and particularly suitable host for a pre-adapted genotype, which could thus outcompete most other genotypes,



causing a significant loss of genetic diversity. Human-driven plant range expansions, such as that of maize following its domestication, have been associated with decreasing genetic diversity and weaker population genetic differentiation in other insect species (Oliver 2006). As maize cultivation spread beyond its area of domestication, in the Pacific, central Mexican lowlands, corn leafhopper may have colonized yet another novel host, perennial teosinte in high elevation, temperate forest (Medina et al. 2012). Subsequent decreases in maize cultivation around perennial teosinte habitat (with Spanish colonization first, and later the creation of the Sierra de Manantlan Biosphere Reserve) may have created a partial refuge where perennial teosinte-adapted genotypes now persist (Medina et al. 2012). Thus where perennial teosinte coexists with maize, such as in the San Miguel and Corralitos sites, haplotype E may be displacing other haplotypes, while haplotypes exclusive to perennial teosinte, such as C and D, subsist at low frequency where perennial teosinte does not immediately coexist with maize. My results also revealed that the frequency of unique haplotypes is higher on perennial teosinte than on maize (when one corrects for the differences in the number of individuals collected). . Thus, haplotype diversity is greater among samples from perennial teosinte compared to samples from maize, even though considerably fewer individuals from perennial teosinte were sampled. This further supports my hypothesis that perennial teosinte may be acting as a refuge for haplotype diversity.

My overall expectations were that genetic structuring would be evident between corn leafhopper samples from maize and perennial teosinte, and that haplotype diversity would be correlated with genetic structuring. My results paralleled prior results showing genetic structuring between samples collected from perennial teosinte and from maize or

Balsas teosinte, thus confirming the occurrence of two corn leafhopper subpopulations, i.e. the Perennial teosinte and *Zea mays* subpopulations. Moreover, my results suggested that haplotype diversity is correlated with genetic structuring so that the Perennial teosinte corn leafhopper subpopulation (viz. samples collected from perennial teosinte) contains four haplotypes, including two haplotypes (C, D) seemingly exclusive to this subpopulation, while the *Zea mays* subpopulation (viz. maize samples, including Palomera et al. 2012 sequences) contains 5 haplotypes, including three haplotypes (A, F, G) seemingly exclusive to this subpopulation.

It is interesting that my survey of genetic structure within the small portion of the COI mitochondrial gene region of corn leafhopper uncovered a relatively high level of haplotype diversity and significant population structuring. My results provide some insight into the extent that host and geographic range expansions may have influenced corn leafhopper microevolution. Previously, it was hypothesized that corn leafhopper expanded its host range from Balsas teosinte to maize, and subsequently to perennial teosinte (Nault and DeLong, 1980, Triplehorn and Nault, 1985, Nault, 1990, Triplehorn et al., 1990, Dietrich et al., 1998; Medina et al. 2012). According to the demic expansion model, genetic diversity is expected to be higher at the center of origin and to decrease with distance (Cavalli-Sforza et al. 1993). Genetic diversity should also be greater within historical host ranges and lower in recently colonized ranges (Oliver 2006). My results show high haplotype diversity within the sampled area, which overlaps the presumed origin of *Zea* diversification and maize domestication (Nault 1990). This supports hypotheses pointing to this area as the center of radiation of *Dalbulus*, though studies with a broader geographical focus are required.

In conclusion, my results revealed the presence of at least seven mitochondrial haplotypes in corn leafhopper specimens from Mexico and Argentina, one of which was present and dominant at all sites. I suggest that the combination of historical expansion of maize cultivation and the weaker anti-herbivore defenses associated within maize domestication, favored genotypes particularly adapted for exploiting maize so that they became particularly abundant and thus pestiferous. As maize cultivation spread beyond its area of domestication, corn leafhoppers colonized yet another novel host, perennial teosinte. I further suggest that subsequent decreases in maize cultivation in perennial teosinte habitat created a refuge where perennial teosinte-adapted haplotypes could persist. This is supported by my results showing that relative to the number of individuals, the frequency of unique haplotypes is higher on perennial teosinte than on maize. Finally, my overall expectations were that genetic structuring would be evident between corn leafhopper samples from maize and perennial teosinte, and that genetic structuring would be correlated with genetic differentiation. My results indeed confirmed genetic structuring between samples collected from perennial teosinte or from maize or Balsas teosinte, confirming the occurrence of two corn leafhopper subpopulations, and that haplotype diversity is correlated with the genetic structuring. Furthermore, my results support hypotheses predicting that the center of radiation of *Dalbulus* overlaps the presumed areas of endemism of Balsas teosinte and of maize domestication.

## CHAPTER IV

### CONCLUSION

Overall, the results from chapter II agreed with previous studies suggesting that perennials show stronger induced responses to herbivory than annual plants (Mutkainen and Walls 1995; Rosenthal and Walter 1995; Rosenthal and Dirzo 1997). The plant life history, domestication, and breeding transitions evident in *Zea* affected the performance of the specialist herbivore *D. maidis*. I argued that the effect observed by contrasting the two life histories correspond to perennials and annuals having different metabolic resource allocation strategies. Concerning the domestication transition, I suggested that the effect on overall performance might be acting at the nymphal level and becoming manifest at the adult stage. I further suggested that the effect observed by contrasting the breeding transition is due to intraspecific genetic variability within cultivars of *Zea mays*, where breeding can intensify the variability among varieties by selecting for specific traits (i.e. adaptation to local weather, soil, and photoperiod). Life-history transition also had the strongest effect on performance parameters related to adult mass (female and male) and fecundity of F1 females. The gains in adult mass and fecundity were consistent with predictions of the resource allocation hypothesis (Rosenthal and Dirzo 1997), where domesticated maize would be the least defended, most suitable host for corn leafhopper. My results support that antiherbivore defenses are stronger in wild relatives compared to the domesticated crop plants. However, I were unable to determine whether defenses are chemical or physical and whether they are acting on nymphs or adults. I suggested that further studies should be done to specifically test for feeding nutrient intake efficiency on

nymphs. I also found that performance of corn leafhopper associated with annual *Zea* suffers when they colonize a perennial teosinte. However, my results do not support the host associated differentiation hypothesis laid out by Medina et al. (2012). Therefore, I suggest that performance studies should be carried out using individuals of the perennial teosinte associated *D. maidis* population.

Results from Chapter III revealed the presence of seven mitochondrial haplotypes in *D. maidis* specimens from Mexico and Argentina with one of the haplotypes dominant at all sites. I suggest that this was a result of the combination of historical expansion of maize cultivation and weaker anti-herbivore defenses in maize. This would have favored genotypes (haplotypes) particularly adapted for exploiting maize, becoming abundant and pestiferous. As maize cultivation spread, corn leafhoppers colonized perennial teosinte. My results showed that relative to the number of individuals, the frequency of unique haplotypes is higher on perennial teosinte than on maize. Thus, I suggested that subsequent decreases in maize cultivation in perennial teosinte habitat created a refuge where perennial teosinte-adapted haplotypes could persist. I also found genetic structuring between corn leafhoppers on perennial teosinte and maize or Balsas teosinte, confirming the occurrence of two corn leafhopper subpopulations.

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