


ORIGINAL ARTICLE

Do assortative mating and immigrant inviability help maintain population genetic structuring of an herbivore on a crop and a wild relative?

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Abstract Population genetic structuring is common among herbivorous insects and frequently is associated with divergent host plants, such as crops and their wild relatives. Previous studies showed population genetic structuring in corn leafhopper *Dalbulus maidis* in Mexico, such that the species consists of two sympatric, host plant-associated populations: an abundant and widespread “pestiferous” population on maize (*Zea mays mays*), and a small and localized “wild” population on perennial teosinte (*Zea diploperennis*), a maize wild relative with a limited distribution. This study addressed whether assortative mating and immigrant inviability mediate genetic structuring of corn leafhopper by comparing the mating and reproductive successes of pestiferous and wild females that colonize their nonassociated host plants against the successes of females colonizing their associated host plants. Assortative mating was assessed by comparing mating frequencies and pre-mating and mating times among females of each population on each host plant; immigrant inviability was assessed by comparing, across two generations, the fecundity, survival, development time, sex ratio, and population growth rate among leafhopper populations and host plants. Our results showed that on maize, and compared to resident, pestiferous females, wild females were more likely to mate, and greater proportions of their offspring survived to adult stage and were daughters; consequently, the per-generation population growth rate on maize was greater for immigrant, wild leafhoppers compared to resident, pestiferous leafhoppers. Our results suggested that wild leafhoppers emigrating to maize have a fitness advantage over resident, pestiferous leafhoppers, while immigrant pestiferous and resident wild leafhoppers on teosinte have similar fitnesses.

Key words crop domestication; *Dalbulus maidis*; ecological speciation; host associated differentiation; *Zea diploperennis*; *Zea mays mays*

Introduction

Population genetic structuring may be mediated by many factors, including migration, divergent selection, mutation, genetic drift, and limited gene flow among popula-

tions (Roderick, 1996; Vekemans & Hardy, 2004). Among such mechanisms, assortative mating and immigrant inviability are known to contribute significantly to restricting gene flow within species (Nosil *et al.*, 2005; Andersson & Iwasa, 2006). Assortative mating occurs when individuals of one population mate preferentially with individuals of the same population (Abrahamson *et al.*, 2001; Drès & Mallet, 2002; Andersson & Iwasa, 2006). For example, females of the fly *Eurosa solidiaginis* (Fitch) copulate more frequently with males that developed on the

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same host plant than with males that developed on an alternative host plant (Craig *et al.*, 1993). Immigrant inviability refers to reductions in offspring viability due to higher mortality, lower fecundity, or generally, lack of adaptation of individuals that migrate to habitats (e.g., alternate host plants) other than those on which their parents developed (Nosil *et al.*, 2005). An example of immigrant inviability involves the beetle *Neochlamisus bebbianae* (Brown), whose offspring migrate to alternate host plants where they suffer higher larval mortality, reach lower body masses, and have a lower likelihood of reaching the pupal stage (Funk, 1998).

Population genetic structuring is common among insect populations, especially in insects with widespread distributions. While genetic structuring may result from a variety of isolating mechanisms that limit gene flow, as noted above, one recurring feature among documented cases of structuring is a close affinity between populations of herbivorous insects and particular host plants. In herbivorous insects, such isolating mechanisms may consist of landscape features (e.g., mountain ranges, deserts, or oceans) that lead to reproductive isolation in space, or ecological barriers that lead to reproductive isolation in habitat or time, such as divergent host plant populations, including crop plants and their wild relatives (Medina, 2012; Medina *et al.*, 2012). Crop domestication and agriculture may generate ecological and geographic barriers to gene flow between populations of herbivorous insects in various ways. For example, due to human intervention crop distributions may cross geographical barriers insurmountable by crop wild ancestors, agricultural fields and wild habitats may represent differently suitable habitats, or herbivore defenses may have been weakened by domestication in crops relative to their wild ancestors (e.g., Dávila-Flores *et al.*, 2013; Chen *et al.*, 2015).

Recent studies using AFLP genetic markers and mtDNA (COI) sequences showed distinct population structuring in corn leafhopper [*Dalbulus maidis* (DeLong & Wolcott)] (Hemiptera: Cicadellidae) (Dávila-Flores, 2012; Medina *et al.*, 2012). In those studies, corn leafhopper in Mexico was found to be structured into two genetically distinct populations: an abundant and widespread “pestiferous” population associated with maize (*Z. mays* ssp. *mays* L.), and a rare and geographically restricted “wild” population associated with perennial teosinte (*Z. diploperennis* Iltis, Doebley, & Guzmán). While those studies’ results were strongly suggestive of host plant affinity as the basis for genetic structuring of corn leafhopper, the authors questioned whether such affinity alone was sufficient to maintain structuring given the contiguous distributions of the two leafhopper populations (<5 km separation between host plants) on one

hand, and on the other hand, the uninterrupted gene flow within the pestiferous population, even across hundreds of kilometers and geographical barriers. The authors of those studies proposed several nonexclusive mechanisms that may help maintain genetic structuring of corn leafhopper, such as habitat isolation (agricultural vs. mesophyllous forest), habitat persistence (ephemeral vs. persistent), assortative mating, and immigrant inviability.

In this study, we addressed whether assortative mating and immigrant inviability may help maintain population genetic structuring in corn leafhopper by assessing whether these mechanisms operate in leafhoppers that colonize the host plant that is not associated to their population. Thus, we focused specifically on two scenarios: pestiferous corn leafhoppers colonizing perennial teosinte, and wild leafhoppers colonizing maize. If assortative mating and immigrant viability are relevant to maintaining corn leafhopper genetic structuring, we expected to find that on their associated host plant: (i) resident leafhoppers would more readily mate between themselves than with immigrant leafhoppers of the other population and (ii) resident leafhoppers would have greater survivorship and reproduction compared to immigrant leafhoppers of the other population. A better understanding of mechanisms underlying population genetic structuring in corn leafhopper, whether host plant affinity, assortative mating, immigrant inviability, or a combination of mechanisms that restrict gene flow, would shed light on processes relevant to the insect’s evolution to a pest.

Materials and methods

Natural history of corn leafhopper and Zea

Because detailed accounts of the natural histories of both corn leafhopper and *Zea* are available elsewhere, only brief descriptions of relevant aspects of those histories are presented here (Benz *et al.*, 1990; Nault, 1990; Medina *et al.*, 2012; Dávila-Flores *et al.*, 2013; Chinchilla-Ramírez *et al.*, 2017). Corn leafhopper is a specialized herbivore on species of *Zea* that is endemic to lowland, subtropical and tropical western Mexico, and a widespread pest of maize. On maize, it is known from northern Argentina to California and the USA Gulf states, and the Caribbean. It maintains year-round populations in lowland, tropical and subtropical areas, and is a seasonal migratory pest in highland and temperate areas. All 13 members of the genus *Dalbulus* are specialists on grasses, particularly in the genera *Zea* and *Tripsacum*,

and are believed to have evolved in the lowlands along the Pacific coast in central, western Mexico, an area that broadly overlaps the centers of origin of their host plants and of maize domestication. Corn leafhopper in particular is presumed to have evolved on Balsas teosinte (*Zea mays parviglumis* Iltis & Doebley), a lowland grass and maize's immediate ancestor, and maize. Upon domestication of maize, corn leafhopper is believed to have expanded its host range to include the crop, and with the spread of maize agriculture is believed to have further expanded its host range to include perennial teosinte.

In the field, corn leafhopper adults disperse away from senescing maize or teosinte hosts as the dry season begins in the fall in search of overwintering hosts. Overwintering adults will feed (but not reproduce) on a variety of host plants, including dicotyledonous species. As the rainy season begins the following cycle, corn leafhopper adults will disperse away from overwintering hosts to colonize maize or teosintes, where they will reproduce for two generations. In contrast, in areas where soil moisture and warm winter temperatures allow dry-season (winter-spring) maize crops, corn leafhopper disperses locally between summer-fall and winter-spring maize fields. Similarly, corn leafhoppers on perennial teosinte probably disperse locally within the small, mesophyllous forest clearings where perennial teosinte grows, moving to and from the teosinte and a variety of overwintering hosts growing in their immediate vicinity. In Mexico, corn leafhopper is structured into two populations, a “wild” population on perennial teosinte and a “pestiferous” population on maize, as noted above.

Zea L. and *Tripsacum* L., the host genera of *Dalbulus*, evolved in present-day Mexico. *Zea* is the sole host for corn leafhopper, as noted above, and all species of *Zea* are commonly known as teosintes, except *Z. mays mays* or maize. Perennial teosinte is the basal species of *Zea*, and together with its sister species *Zea perennis* (Hitc.) Reeves and Manglesdorf are the genus' only perennial species. Perennial teosinte is a rare, protected species known from as few as three permanent populations, all above 1350 m a.s.l., and all within the Sierra de Manantlán Biosphere Reserve. It grows mainly as secondary vegetation in clearings within cloud and mesophyllous mountain forest. The perennial teosinte population host to the wild corn leafhopper population grows isolated from maize, the nearest maize fields occurring within ~5 km. Maize was domesticated from Balsas teosinte ~9200 years ago in lowland, central, western Mexico. It is a widespread crop in Mexico, and may be grown year-round where winter temperatures and irrigation or soil moisture permit, but is mostly

a rainfed crop, growing within the summer-fall rainy season.

Experimental insects and plants

Cultures of corn leafhopper corresponding to “pestiferous” and “wild” corn leafhopper populations, as described above and earlier (Dávila-Flores, 2012; Medina *et al.*, 2012), were maintained in the Biological Control Laboratory, CUCBA, Universidad de Guadalajara, Mexico. The culture of pestiferous leafhoppers was started from individuals collected from maize in the vicinity of El Grullo, Jalisco state, México (19°50'N, 104°16'W) (Dávila-Flores *et al.*, 2013). This culture was refreshed yearly by adding individuals collected at the original locality. The culture of wild corn leafhopper was started from individuals collected from perennial teosinte within the Sierra de Manantlán Biosphere Reserve (19°36'N, 104°18'W, Jalisco, Mexico) (UNESCO, 2011) in October 2011. Both cultures were maintained as previously described, the pestiferous culture on maize seedlings, the wild culture on perennial teosinte seedlings (Ramirez-Romero *et al.*, 2014). Briefly, the cultures were maintained isolated from one another, each in a cage holding six healthy maize or teosinte seedlings, which were replaced as necessary. The cages were held under constant temperature (25 ± 3°C), relative humidity (60% ± 10%), and day length (10 : 14 light : dark photoperiod).

Maize seedlings were of the improved landrace “Tuxpeño Sequía,” germinated from seed provided by the International Maize and Wheat Improvement Center (CIMMYT, El Batán, Mexico state, Mexico), while perennial teosinte seedlings were germinated from seeds provided by the Instituto de Mejoramiento y Aprovechamiento de Recursos Fitogenéticos, Universidad de Guadalajara, Zapopan (Jalisco, Mexico). Teosinte seeds were removed from their fruit cases with the aid of nail clippers prior to germination (Dávila-Flores *et al.*, 2013), and allowed to germinate on a coconut fiber substrate in a Petri dish; once germinated the seedlings were transplanted into pots. Maize seeds were germinated directly in pots. Both maize and teosinte seedlings were grown in a greenhouse in pots (14 cm × 18 cm diam.) filled with Nutrigarden® soil (Sulfatos y Derivados, S.A. de C.V., El Marqués, Querétaro, Mexico), and watered thrice weekly with Triple 18 solution (SQM Comercial de México, Guadalajara, Jalisco, Mexico); all seedlings were maintained in cages covered with insect-proof screening to exclude all herbivores. Seedlings were used when they had —four to six collared leaves (Albarracin *et al.*, 2006).

Assortative mating

The goal of this experiment was to compare for each host plant, maize and perennial teosinte, the mating success of resident females (i.e., on their corresponding host plant) of each corn leafhopper population against the success of immigrant females of the other population. Thus, we simulated four scenarios in which individual virgin females encountered a male: (i) immigrant, wild females colonizing maize and encountering resident, pestiferous males; (ii) resident, pestiferous females on maize encountering resident, pestiferous males; (iii) immigrant, pestiferous females colonizing teosinte and encountering a resident, wild male; and (iv) resident, wild females on teosinte encountering resident, wild males.

Virgin leafhopper females were obtained following a previously described procedure (Ramirez-Romero *et al.*, 2014). Briefly, ~200 adult leafhoppers were given access to four caged seedlings for 72 h so that females could lay eggs, after which period all adults were removed from the cage; pestiferous leafhoppers were given access to maize seedlings, and wild leafhoppers to teosinte seedlings. The seedlings bearing leafhopper eggs remained in their corresponding cage to allow eggs to hatch and nymphs to develop. After 25 days, late-instar nymphs (3rd and 4th instars) were collected with an oral aspirator and placed singly into “clip cages” (15 mm diam. × 18 mm height) that were attached to a leaf of a corresponding seedling to allow their development to continue. The “clip cages” were checked daily to examine for emergence of adult leafhoppers. Upon emergence as adults, leafhoppers were separated by gender and population (pestiferous or wild) into plastic boxes (50 × 40 × 20 mm), which were attached to leaves of the corresponding seedling. The leafhopper adults grouped by gender and population were maintained in their respective boxes for 5–9 d, after which time they were used in trials.

Trials consisted of pairing within an arena (described below) a resident male, pestiferous or wild, on its corresponding host plant, with either a resident virgin female (i.e., belonging to the same population as the male) or an immigrant virgin female (i.e., not belonging to the same population as the male) and observing for at least 5 h. The response variables that were recorded included: (i) the premating time (= the time in minutes from placing a leafhopper pair inside an arena and the beginning of a mating event, indicated by coupling of the leafhoppers); (ii) the mating time (= the time in seconds from the beginning of a mating event to its conclusion, indicated by decoupling of the leafhoppers); and (iii) the mating frequency (= the number of mating events observed during

the 5 h period). All trials were conducted under constant temperature (25 ± 2 °C), humidity ($60\% \pm 10\%$) and light (150 lux) conditions. A plastic box (90 mm high × 55 mm long × 40 mm wide) was used as an arena for observations; the arena contained a piece of moist filter paper to provide humidity and an excised leaf segment to serve as a food source for the leafhoppers; the leaf segment was of the host plant corresponding to the resident male and female leafhoppers (Ramirez-Romero *et al.*, 2014). All observations began at 9:00 am, and continued for at least 5 h, as noted above. A total of 104 trials were conducted, 22–29 per each mating scenario described above. Trials were conducted over 10 dates, with equal replicates of each scenario represented on each date; all trials were considered for analysis of mating frequencies, while trials in which mating was not observed during the 5 h observation period were excluded from analyses of premating and mating times.

Statistical analyses for premating and mating times consisted of two-way least-squares means ANOVA by blocks (= trial dates), with host plant (maize, perennial teosinte) and corn leafhopper population (pestiferous, wild), nested within host plant, as main effects; consequently, all comparisons were paired within main effects. Premating and mating times were converted to their cube-root values to comply with normality and homoscedasticity requirements. Statistical analyses for mating frequencies consisted of *G*-tests of independence within each of the two host plants, maize and perennial teosinte. Analyses were conducted using JMP Pro 13.1.0 (SAS Institute Inc., 2016) and PopTools V 3.2.5 (Hood, 2010) software.

Immigrant inviability

The goal of this experiment was to compare within each host plant, maize, and perennial teosinte, the viability of immigrant leafhoppers (i.e., on their noncorresponding host plant) against the viability of resident leafhoppers (i.e., on their corresponding host plant). Viability was assessed across two generations in terms of indices of leafhopper fecundity, development time, survivorship, and sex ratio, and a composite index of per-generation population growth rate. Thus, we simulated four scenarios in which individual females colonized a host plant, and their F_0 and F_1 offspring were allowed to develop to adulthood: (i) immigrant, wild females colonizing maize; (ii) resident, pestiferous females on maize; (iii) immigrant, pestiferous females colonizing teosinte; and (iv) resident, wild females on teosinte. The procedure described below was repeated on 13–19 seedlings for each scenario for F_0 and F_1 generations.

F₀ generation Maize or perennial teosinte seedlings confined in cages were exposed to ~200 adults of each corn leafhopper population for 72 h, after which period all adults were removed, as described above. New adult leafhoppers began appearing after ~28 d, and were allowed to mate, feed, and oviposit for two weeks; seedlings were replaced as necessary during these time periods. After two weeks, females (presumed mated) were taken from each cage and placed individually in clip-cages (55 × 20 × 45 mm) attached to a maize or teosinte seedling where they were confined for 5 d; the leaf section covered by the clip-cage and exposed to ovipositing females was marked with a permanent marker (Moya-Raygoza & Garcia-Medina, 2010; Dávila-Flores *et al.*, 2013). Females were removed after 5 d, and discarded, and seedlings were placed inside a cage, and leafhopper eggs allowed to hatch into nymphs. Fifteen days later, the number of nymphs per seedling was recorded, and the leaf section exposed to ovipositing females was excised from the seedling for subsequent recording of oviposition (below); the timing and spread of egg hatch and adult emergence times were known from prior studies (Dávila-Flores *et al.*, 2013; Bellota *et al.*, 2017). Beginning at this time, cages were examined daily to record the emergence of adult leafhoppers. Adults were removed as they appeared, and the time (days) to emergence and gender were recorded. The excised leaf section, exposed to oviposition, was stained per McBryde (1936) to facilitate counting the number of eggs that were originally laid on a seedling; this procedure as applied to corn leafhopper was successfully used in prior studies (Bellota *et al.*, 2013, 2017; Dávila-Flores *et al.*, 2013). The following data were recorded for each seedling: numbers of eggs, nymphs, and adults, gender of adults, and development times (= the period from oviposition to emergence of adults). These data were used to estimate the following response variables: (i) initial cohort size (= mean number of eggs per seedling), (ii) survivorship to adult stage (= number of adults/number of eggs per seedling), (iii) adult sex ratio (= adult females/total adults per seedling), and (iv) per-generation net reproductive rate R_0^* , following Krebs (2009). As estimated here, per-generation net reproductive rate is an approximation of the net reproductive rate, R_0 (Krebs, 2009): $R_0^* = (\text{survivorship to adult stage} \times \text{adult sex ratio} \times \text{fecundity of } F_1 \text{ females})/\text{initial cohort size}$ (see below, *F₁ Generation*, for explanation of fecundity of F_1 females).

F₁ generation Upon their emergence, adult leafhoppers of the F_0 generation were moved to a new cage holding a seedling of the host plant on which they had developed. Adults within a cage were no more than 5 d different in their age, and were kept together for two weeks

to allow mating (Nault, 1998). After this time, F_1 females were placed individually in clip-cages attached to leaves of the host plant on which they had developed, and were allowed to lay eggs for 5 d. Each seedling and leafhopper eggs, nymphs, and adults was processed as described above for the F_0 generation. Similarly, the response variables described above for the F_0 generation were recorded per seedling, except R_0^* ; R_0^* was not estimated because the experiment was terminated with the emergence of F_1 adults, and oviposition by F_1 females was not measured.

Statistical analyses for the response variables initial cohort size, survivorship to adult stage, and adult sex ratio consisted of least-squares means ANOVA with the following independent variables and interactions: (i) corn leafhopper population (pestiferous, wild) nested within host plant, (ii) host plant (maize, perennial teosinte), (iii) generation (F_0 , F_1), (iv) host plant × generation, and (v) corn leafhopper population nested within host plant × generation. Adult sex ratio and survivorship to adult stage data (proportions) were converted to arc-sine \sqrt{x} values for analyses. Statistical analysis for per-generation net reproductive rate consisted of ANOVA of rank-transformed R_0^* values. The model included corn leafhopper population (pestiferous, wild) nested within host plant, and host plant (maize, perennial teosinte) as independent variables. As warranted, *post hoc* comparisons were made via planned contrasts. All analyses were conducted using JMP Pro 13.1.0 (SAS Institute Inc., 2016) software.

Results

Assortative mating

Premating times did not differ between pestiferous and wild females on either of the host plants ($F_{2,97} = 0.96$, $P = 0.385$), nor between host plants ($F_{1,97} = 0.38$, $P = 0.540$); overall, premating time was 70.1 ± 4.8 min. Similarly, mating times did not differ between pestiferous and wild females on either of the host plants ($F_{2,97} = 1.30$, $P = 0.278$), though mating times were ~1.2 fold longer on maize (63.8 ± 0.8 min) compared to teosinte (54.8 ± 0.7 min) ($F_{1,97} = 3.95$, $P = 0.050$) across corn leafhopper populations. However, mating frequencies on maize were ~1.7 fold higher for immigrant, wild compared to resident, pestiferous females ($P < 0.01$) (Fig. 1A), but on teosinte did not differ between immigrant, wild and resident, pestiferous females ($P = 0.70$) (Fig. 1B). These results indicate that while premating times were similar, and mating times were longer on maize (independently of the leafhopper population), they were not mediated by interactions between the leafhopper population and the

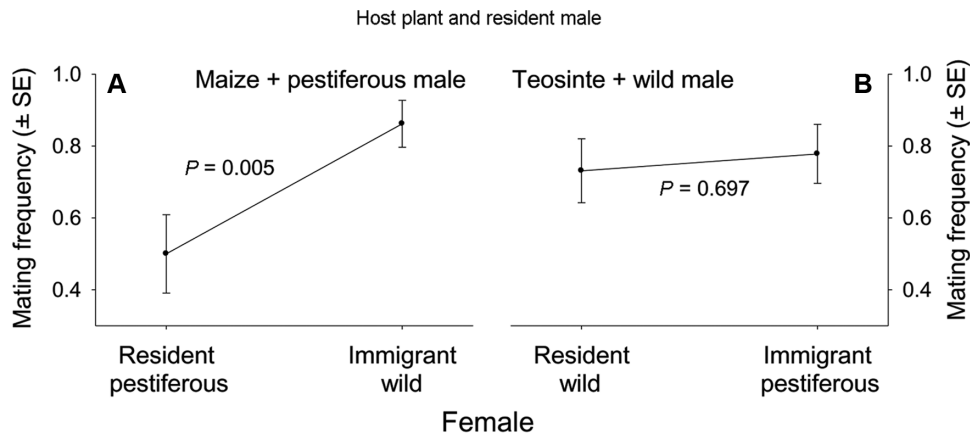


Fig. 1 The mating frequencies (trials with successful copulations relative to total number of trials) of immigrant and resident corn leafhoppers (*Dalbulus maidis*) of two populations, “pestiferous” or “wild,” on two host plants, maize (*Zea mays mays*) or perennial teosinte (*Zea diploperennis*). On maize (A), the mating frequency with resident, “pestiferous” males was significantly higher for immigrant, “wild” females than it was for resident, “pestiferous” females ($G = 7.727$), while on perennial teosinte (B), the mating frequency with resident, “wild” males was similar for immigrant, “wild” females and resident, “pestiferous” females ($G = 0.697$).

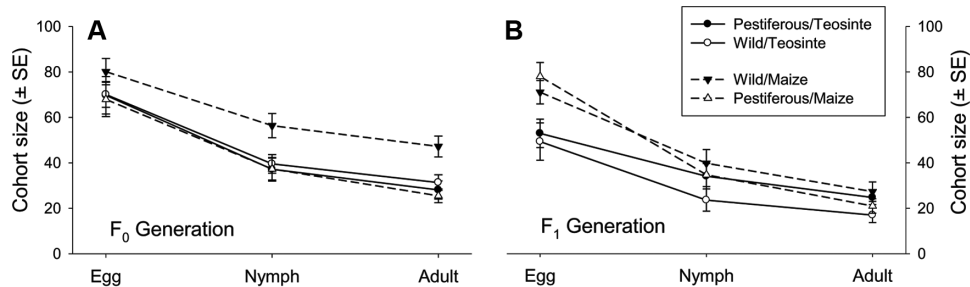


Fig. 2 Oviposition and survivorship dynamics of four corn leafhopper (*Dalbulus maidis*) × host plant combinations across two generations (F_0 , F_1): (i) Immigrant, “pestiferous” leafhoppers on perennial teosinte (*Zea diploperennis*) (filled circles), (ii) resident, wild leafhoppers on perennial teosinte (empty circles), (iii) immigrant, “wild” leafhoppers on maize (filled triangles), (iv) resident, pestiferous leafhoppers on maize (empty triangles). For each leafhopper × host plant combination, (A) individual females were allowed to oviposit on a host plant seedling and their F_0 offspring’s development and survival were monitored from the egg to the adult stage. Subsequently, (B) individual F_0 females of each leafhopper × host plant combination were allowed to oviposit and their F_1 offspring’s development and survival were monitored from the egg to the adult stage.

host plant. Importantly, however, on maize the mating frequency of immigrant, wild females was higher than that of resident, pestiferous females, while on teosinte mating frequencies did not differ between immigrant, pestiferous females and resident, wild females. These results suggest that on maize, immigrant, wild females have a mating advantage over resident, pestiferous females, while on teosinte, immigrant, pestiferous females have no mating advantage over resident, wild females.

Immigrant inviability

Cohort sizes were similar among the corn leafhopper population × host plant combinations at the beginning

of the experiment, $\sim 71.9 \pm 3.5$ eggs (Fig. 2A). Subsequently, cohort sizes within the first generation decreased at similar rates in all combinations, except for wild leafhoppers on maize in which survivorship to the nymphal and adult stages appeared to be higher than in the remaining combinations. Average cohort size at the beginning of the second generation ($\sim 62.8 \pm 3.7$ eggs) appeared lower relative to the first generation, and cohort sizes appeared to be lower on perennial teosinte than on maize, for both pestiferous and wild leafhoppers (Fig. 2B). However, cohort sizes in the second generation became increasingly similar among the corn leafhopper population × host plant combinations by the nymphal and adult stages.

Table 1 ANOVA statistics for response variables relevant to survival and reproduction of “pestiferous” and “wild” corn leafhopper (*Dalbulus maidis*) populations on maize (*Zea mays mays*) or perennial teosinte (*Zea diploperennis*) across two generations. Independent variables included in ANOVA were corn leafhopper population (pestiferous, wild) nested within host plant, host plant (maize, perennial teosinte), generation (F_0 , F_1), and the interaction terms host plant \times generation, and corn leafhopper population nested within host plant \times generation. Only independent variables with significant effects ($P \leq 0.05$) on response variables are shown.

Variable	Df _{num, den}	Sum of squares	F ratio	P
Cohort size (eggs)				
Plant	1, 96	4501.8	6.60	0.012
Development time				
Generation	1, 96	413.7	12.55	0.001
Adult sex ratio				
Population[Plant] [†]	2, 96	448.6	3.92	0.023
Plant	1, 96	407.5	7.12	0.009
Plant \times Generation	1, 96	321.7	5.62	0.020
Survivorship				
Population[Plant] [†]	2, 96	1257.4	4.67	0.012
Plant \times Generation	1, 96	882.1	6.55	0.012
Generation	1, 96	859.3	6.38	0.013

[†]Indicates nested factor, namely population nested within host plant.

Initial cohort size Of the main, nested, and interaction effects evaluated in ANOVA, only host plant significantly affected the initial cohort sizes (numbers of eggs) of corn leafhopper populations ($P \geq 0.075$ for all effects, except host plant) (Table 1). On average, 73.9 ± 3.7 eggs were laid on maize and 62.7 ± 3.8 eggs on teosinte, across the two leafhopper populations and two generations ($P = 0.01$). These results indicate that while leafhopper females laid ~ 1.2 fold more eggs on maize than on teosinte, the difference was independent of the female’s population of origin. This suggests that both pestiferous and wild leafhoppers have an ovipositional advantage when they colonize maize compared to teosinte, but that immigrant females of neither leafhopper population have an advantage over resident females.

Development time Of the main, nested, and interaction effects evaluated in ANOVA, only corn leafhopper generation significantly affected leafhopper development times (times-to-adult) ($P \geq 0.139$ for all effects, except generation) (Table 1). On average, time-to-adult was 21.0 ± 0.7 d in first-generation leafhoppers compared

to 25.3 ± 0.9 d in second-generation leafhoppers, across leafhopper population \times host plant combinations ($P < 0.001$). These results indicate that time-to-adult was neither mediated by leafhopper population nor host plant, though it varied across generations, and suggest that immigrant females of neither leafhopper population have an advantage over resident females.

Adult sex ratio The sex ratio of adult corn leafhoppers differed between pestiferous and wild leafhoppers on maize but not on teosinte ($P = 0.02$) (Table 1, Figs. 3A, B), between host plants ($P = 0.01$) (Table 1), and among host plants and generations ($P = 0.02$) (Table 1); adult sex ratios did not change between generations ($P = 0.39$), nor varied between generations among the corn leafhopper population \times host plant combinations ($P = 0.06$). In particular, on maize the adult sex ratio (across generations) of immigrant, wild leafhoppers was ~ 1.2 fold higher than that of resident, pestiferous leafhoppers (Fig. 3A), while on perennial teosinte the sex ratio did not differ between leafhopper populations, whether immigrant or resident (Fig. 3B). Overall, adult sex ratios were higher on teosinte ($56.1\% \pm 1.3\%$ females) than on maize ($50.1\% \pm 1.2\%$); moreover, while sex ratios were similar between host plants in the first generation ($54.1\% \pm 1.5\%$), they were higher on teosinte ($58.5\% \pm 2.0\%$) compared to maize in the second generation ($44.9\% \pm 1.8\%$). These results indicate that on maize, immigrant, wild leafhoppers had an adult-sex ratio advantage over resident, pestiferous leafhoppers, while on teosinte the sex ratios of immigrant, pestiferous and resident, wild leafhoppers were similar. Also, the results indicate that the adult sex ratio of leafhoppers was enhanced on teosinte compared to maize, suggesting a higher male mortality rate on teosinte relative to maize. Overall, these results suggest that on maize, immigrant, wild leafhoppers have a sex ratio advantage over resident, pestiferous leafhoppers, while on teosinte immigrant, pestiferous leafhoppers have no advantage over resident, wild leafhoppers.

Survivorship Corn leafhopper survivorship differed between pestiferous and wild leafhoppers on maize but not on teosinte ($P = 0.01$) (Table 1, Figs. 4A, B), between generations ($P = 0.01$) (Table 1), and among host plants and generations ($P = 0.01$) (Table 1); survivorship was not mediated by host plant ($P = 0.70$), nor varied between generations among the leafhopper population \times host plant combinations ($P = 0.16$). In particular, survivorship (across generations) on maize of immigrant, wild leafhoppers was higher than that of resident, pestiferous leafhoppers (Fig. 4A), while on teosinte survivorship

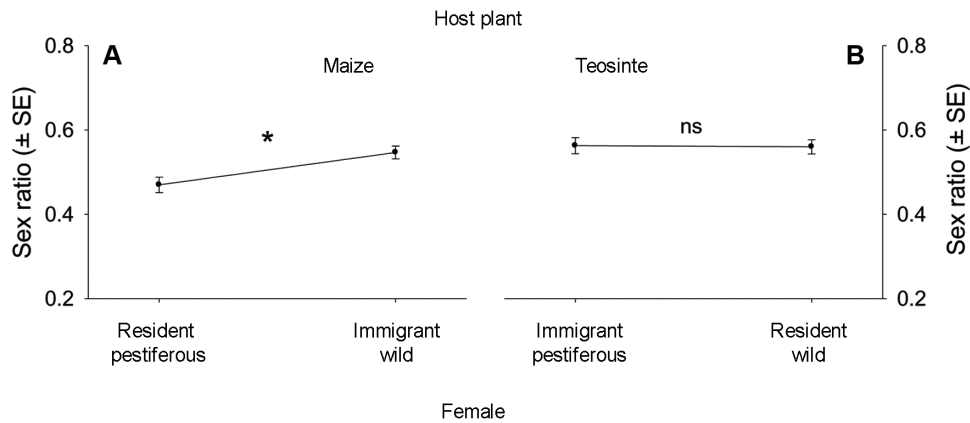


Fig. 3 The adult sex ratios (♀♀ relative to total adults) among offspring of immigrant and resident corn leafhoppers (*Dalbulus maidis*) of two populations, “pestiferous” or “wild,” on two host plants, maize (*Zea mays mays*) or perennial teosinte (*Zea diploperennis*) (host plant, $P = 0.009$; leafhopper population nested within host plant, $P = 0.023$). On maize (A), the sex ratio of immigrant, “wild” leafhoppers was higher than that of resident, “pestiferous” leafhoppers ($P = 0.007$), while on perennial teosinte (B), the difference between the sex ratios of immigrant, “pestiferous” and resident, “wild” leafhoppers was not significant ($P = 0.733$).

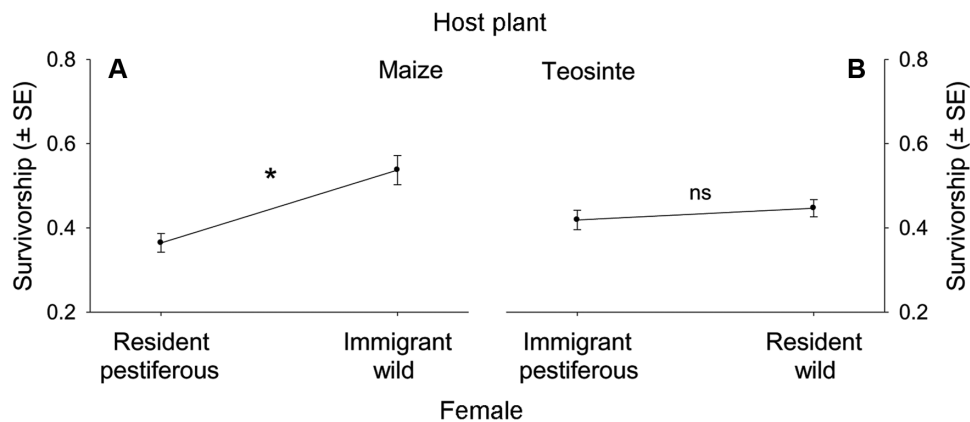


Fig. 4 The egg-to-adult survivorship of cohorts of offspring (individuals reaching the adult stage relative to initial numbers of eggs) of immigrant and resident corn leafhoppers (*Dalbulus maidis*) of two populations, “pestiferous” or “wild,” on two host plants, maize (*Zea mays mays*) or perennial teosinte (*Zea diploperennis*) (leafhopper population nested within host plant, $P = 0.012$, $F_{2,96} = 4.67$). On maize (A), the egg-to-adult survivorship of immigrant, “wild” leafhoppers was higher than that of resident, “pestiferous” leafhoppers ($P = 0.004$), while on perennial teosinte (B), the difference between the survivorship of immigrant, “pestiferous” and resident, “wild” leafhoppers was not significant ($P = 0.396$).

did not differ between leafhopper populations, whether immigrant or resident (Fig. 4B). Overall, survivorship was higher in the first generation ($47.9\% \pm 1.6\%$) compared to the second generation ($37.6\% \pm 1.9\%$); however, survivorship did not differ between host plants within each generation, and only differed between the first ($52.4\% \pm 2.2\%$) and second ($31.7\% \pm 2.4\%$) generations on maize. These results indicate that on maize, immigrant, wild leafhoppers have a survivorship advantage over resident, pestiferous leafhoppers, while on teosinte survivorship does not differ between leafhopper populations. Also, the results indicate that survivorship may vary across leafhop-

per generations. Overall, these results suggest that on maize, immigrant, wild leafhoppers have a survivorship advantage over resident, pestiferous leafhoppers, while on teosinte, immigrant, pestiferous leafhoppers have no advantage over resident, wild leafhoppers.

Per-generation net reproductive rate Corn leafhopper net reproductive rates differed between pestiferous and wild leafhoppers on maize (Fig. 5A), but not on teosinte (Fig. 5B) ($F_{2,65} = 8.11$, $P = 0.001$), and differed between host plants ($F_{1,65} = 11.55$, $P = 0.001$). In particular, on maize net reproductive rate of immigrant, wild

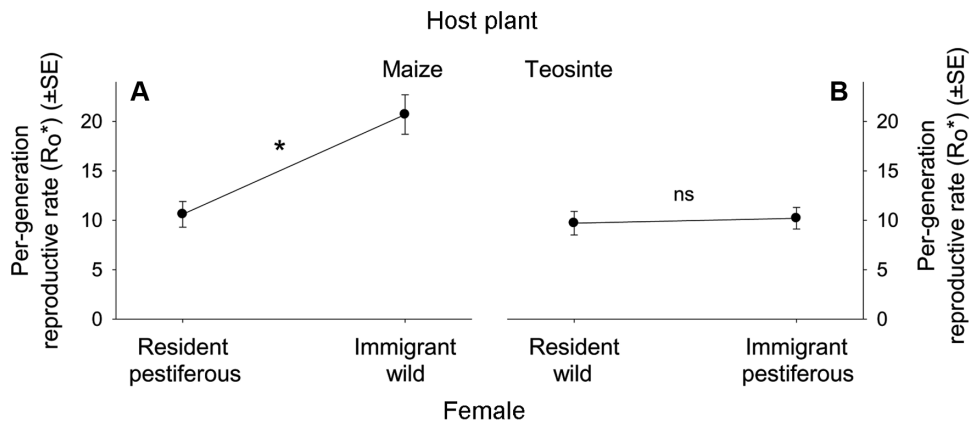


Fig. 5 The per-generation net reproductive rate R_0^* (an approximation of the net reproductive rate, R_0) of immigrant and resident corn leafhoppers (*Dalbulus maidis*) of two populations, “pestiferous” or “wild,” on two host plants, maize (*Zea mays mays*) or perennial teosinte (*Zea diploperennis*) (host plant, $P = 0.001$, $F_{1,62} = 11.55$; leafhopper population nested within host plant, $P = 0.001$, $F_{2,62} = 8.11$). On maize (A), the per-generation reproductive rate of immigrant, “wild” leafhoppers was significantly higher than that of resident, “pestiferous” leafhoppers ($P < 0.003$), while on perennial teosinte (B), the difference between the reproductive rates of immigrant, “pestiferous” and resident, “wild” leafhoppers was not significant ($P = 0.352$).

leafhoppers was ~ 2 fold higher than that of resident, pestiferous leafhoppers (Fig. 5A), while on teosinte net reproductive rates did not differ between leafhopper populations, whether immigrant or resident (Fig. 5B). Overall, net reproductive rate was ~ 1.5 fold higher on maize ($15.4\% \pm 1.4\%$) compared to teosinte ($10.0\% \pm 0.8\%$). These results indicate that net reproductive rate of corn leafhopper is higher on maize compared to teosinte, independently of leafhopper population. Also, the results suggest that wild leafhoppers colonizing maize have a net reproductive rate advantage over resident, pestiferous leafhoppers, while pestiferous leafhoppers colonizing teosinte have a net reproductive rate similar to that of resident, wild leafhoppers.

Discussion

Is corn leafhopper population structuring mediated by assortative mating and immigrant inviability?

Previous studies uncovered population genetic structuring in corn leafhopper in Mexico, such that the species consists of at least two populations, a “pestiferous” population on maize and a “wild” population on perennial teosinte, a wild relative of maize (Dávila-Flores, 2012; Medina *et al.*, 2012). Several mechanisms may underlie the structuring, including assortative mating and immigrant inviability, the two mechanisms that were hypothesized for this study. If assortative mating and immigrant inviability were significant mediators of genetic

structuring in corn leafhopper, we expected to find that on both maize and perennial teosinte: (i) resident leafhoppers would more frequently mate with other residents, rather than with immigrant leafhoppers (assortative mating) and (ii) immigrant leafhoppers would fare poorly compared to resident leafhoppers (immigrant inviability). Overall, our results suggested that assortative mating and immigrant inviability did not occur in the manners we expected. On maize, immigrant leafhoppers generally fared better than resident leafhoppers in terms of mating and net population growth, and on teosinte immigrants fared similarly to residents. Thus, cursory analysis would suggest that assortative mating and immigrant inviability would not help maintain corn leafhopper genetic structuring, and rather their combined effects on structuring would vary between weakening on maize and insignificant on perennial teosinte.

We believe that assortative mating and immigrant inviability would help maintain genetic structuring in corn leafhopper if they represented important barriers to successful colonization of perennial teosinte by pestiferous leafhoppers, while whether they represented or not barriers to colonization of maize by wild leafhoppers would be less consequential. This expectation stems from the likely multimagnitude difference in abundance between the widespread pestiferous population on maize, a ubiquitous crop in Mexico, and the localized wild population on perennial teosinte, an endangered, wild relative of maize. The population size of pestiferous leafhoppers is very likely several orders of magnitude larger than that of wild leafhoppers, given the areas planted with maize

relative to the known distribution of perennial teosinte. The area occupied by perennial teosinte is a small fraction of the (~140 000 ha) biological reserve where it grows, and does not exceed 400 ha (Benz *et al.*, 1990; UNESCO, 2011), while the area planted yearly to maize in Mexico is ~7.5 million hectares (SHCP, 2014), with scattered maize fields within <5 km of perennial teosinte and widespread maize within <15 km (Dávila-Flores, 2012; Medina *et al.*, 2012). Moreover, dispersal away from perennial teosinte may be less likely than dispersal from maize given the persistent (perennial) nature of the teosinte and its habitat (cloud and mesophyllous mountain forest) compared to the markedly seasonal (rainfed) nature of most maize in the vicinity of perennial teosinte, and in Mexico generally. In light of this plausible scenario—namely a pestiferous leafhopper population that is substantially larger and more prone to dispersal than a wild population—our results concerning assortative mating and immigrant inviability may help explain, though, the relative abundances of the two corn leafhopper populations on maize and perennial teosinte. While the research to date showed that corn leafhopper population structuring is clear and consistent across studies, it showed also that leafhopper samples from maize or perennial teosinte consist of mixtures of nominal pestiferous and wild individuals (Dávila-Flores, 2012; Medina *et al.*, 2012). Thus, a majority of leafhoppers collected from perennial teosinte were nominally pestiferous, and a small minority of leafhoppers collected from maize were nominally wild (Dávila-Flores, 2012; Medina *et al.*, 2012). Plausibly, the expectedly lower abundance and dispersal rate of wild leafhoppers may preclude their predominance on maize, despite their mating and reproductive advantages over resident, pestiferous leafhoppers. In contrast, pestiferous leafhoppers may become predominant on perennial teosinte by virtue of their likely higher abundance and dispersal rate, despite not having advantages over resident wild leafhoppers.

Overall, our study's results do not support attribution of either assortative mating or immigrant inviability as important mediators of population genetic structuring in corn leafhopper, though both mechanisms may help explain the relative abundances of nonassociated leafhoppers (i.e., wild leafhoppers on maize, pestiferous leafhoppers on perennial teosinte) in leafhopper samples taken from either maize or perennial teosinte. Future studies attempting to improve our understanding of how population genetic structuring is maintained in corn leafhopper should focus on additional mechanisms that may mediate gene flow between pestiferous and wild corn leafhoppers, and affect the viability of immigrant versus resident leafhoppers on maize and teosinte. For

example, studies that directly measure corn leafhopper dispersal away from and into maize and perennial teosinte would allow us to better gauge how much gene flow may occur between leafhopper populations, and studies that assess the fates of virgin leafhopper females emigrating to a nonassociated host, either maize or teosinte, would complement this study's results on immigrant inviability, which considered only previously mated females.

Assortative mating between corn leafhopper populations on maize and perennial teosinte

Our results were inconsistent with our expectations concerning assortative mating, and appeared to be mediated by host plant. On maize, immigrant females were more likely than resident females to mate with resident males, while on perennial teosinte, immigrant and resident females were equally likely to mate with resident males. The inconsistencies between our results and expectations under assortative mating may be explained in part through at least two plausible, nonexclusive, mechanisms. On one hand our results may reflect divergent premating, that is, courtship, communication between the two corn leafhopper populations, and on the other, they may be consequences of divergent morphologies between the populations (Chinchilla-Ramírez, 2014; Ramirez-Romero *et al.*, 2014).

Successful mating in corn leafhopper is contingent upon effective courtship communication between mating pairs, and stereotyped behaviors and signals are evident in both sexes during courtship and mating, though males seem to play a more active role compared to females (Ramirez-Romero *et al.*, 2014). The differences in mating frequencies we observed under two scenarios, immigrant wild females versus resident pestiferous females on maize, and immigrant pestiferous females versus resident wild females on teosinte, may reflect differences in male preference for females, as mediated by the host plant. By themselves, the host plant ($P = 0.58$), the male's population ($P = 0.26$), and the female's population ($P = 0.09$) did not seem to influence the mating frequency among the different male-female leafhopper pairs and host plants ($G = 0.31$ – 2.80 , data not shown). In contrast, on maize the mating frequency with resident males was higher for immigrant females than for resident females, while on teosinte the corresponding frequencies did not differ. This suggests that pestiferous males on maize preferred to mate with immigrant wild females over resident pestiferous females. Because only pestiferous (and not wild) males were assayed on maize, such preference could be associated to divergent courtship

signaling or morphologies between wild and pestiferous females, or both divergent signaling and morphologies. Prior studies documented stereotyped courtship signaling and behaviors in corn leafhopper, as well as divergent morphologies between pestiferous and wild leafhoppers, as noted above (Chinchilla-Ramírez, 2014; Ramírez-Romero *et al.*, 2014). In particular, one study showed that wild leafhoppers of both genders were generally larger than pestiferous leafhoppers (Chinchilla-Ramírez, 2014). Noteworthy from that study were the differences between leafhopper populations in the sizes of reproductive organs of both males and females, as well as the size of the thorax and the size and shape of the wings. Body size generally and reproductive organ features are known to underlie mating preferences in insects (e.g., Rowe & Arnqvist, 1996; Arnqvist *et al.*, 1997; Goulson *et al.*, 1999; Cocroft & Rodríguez, 2005; Simmons *et al.*, 2009; Hedrick & Kortet, 2012), and thorax and wing characteristics likely mediate acoustic courtship communication in species in which wing-fanning plays a role in courtship, such as in corn leafhopper and other leafhoppers (Heady *et al.*, 1986; Downham *et al.*, 1997; Ramírez-Romero *et al.*, 2014). A number of studies have shown male preference for larger over smaller females, though this is not consistently the case (Rowe & Arnqvist, 1996; Arnqvist *et al.*, 1997; Goulson *et al.*, 1999). Importantly, several studies have shown that host plants modulate acoustic courtship communication in insects (Cocroft *et al.*, 2006; Cokl *et al.*, 2007; Cokl, 2008; Zunik *et al.*, 2011), and that divergence in acoustic courtship signals may occur within a few generations (Winter, 1992; Claridge, 1995; Cocroft *et al.*, 2008; Bennett & O'Grady, 2012; Goodman *et al.*, 2015). Other studies have shown that strict reproductive isolation does not occur even in cases in which clear genetic structuring is evident, along with structuring in acoustic courtship signaling (Forbes *et al.*, 2017). Moreover, some studies showed that leafhoppers and other hemipterans tune their acoustic signaling accordingly with their host plant (Cocroft *et al.*, 2006; Cokl *et al.*, 2007; Cokl, 2008; Zunik *et al.*, 2011). Overall, our results suggested that divergent morphologies and courtship signaling, plausibly mediated by the host plants, between the two corn leafhopper populations may underlie the assortative mating evident between immigrant, wild females and resident, pestiferous males on maize. In other hemipterans, divergence in courtship signaling has been evident within a small number of generations (Winter, 1992; Claridge, 1995; Cocroft *et al.*, 2008; Bennett & O'Grady, 2012; Goodman *et al.*, 2015); the wild corn leafhopper population was partially isolated from the pestiferous population beginning ~35 years (~70 generations) ago when perennial teosinte's moun-

tain habitat was declared a biological reserve from which maize agriculture was proscribed (Benz *et al.*, 1990; Nault, 1990; Hernández-Vázquez *et al.*, 1992; Medina *et al.*, 2012). While it would seem plausible that assortative mating between wild females and pestiferous males on maize may tend to weaken genetic structuring in corn leafhopper, its impact on structuring may be negligible given the likely low abundance and dispersal rate to maize of wild leafhoppers. Future research contrasting courtship communication between pestiferous and wild leafhoppers, and any mediation by their associated host plants, would allow a better understanding of assortative mating between the corn leafhopper populations. Such research would be additionally important because divergence in courtship communication is a prelude to ecological speciation, as suggested for other leafhoppers (e.g., Claridge 1995; Bennett & O'Grady, 2012; Goodman *et al.*, 2015; Forbes *et al.*, 2017).

Immigrant inviability in corn leafhopper populations on maize and perennial teosinte

Our results were inconsistent with our expectations concerning immigrant inviability, and appeared to be mediated by host plant, similarly to our results for assortative mating. On maize immigrant leafhoppers fared better than residents, while on perennial teosinte there was no difference in how immigrant and resident leafhoppers fared. This result is generally surprising in that compared to residents, immigrants did not suffer on a nonassociated host plant, whether maize or teosinte, and particularly surprising because prior studies suggested that the performance of pestiferous leafhoppers would suffer significantly on perennial teosinte (Bellota *et al.*, 2013; Dávila-Flores *et al.*, 2013).

Population genetic structuring in corn leafhopper appears to be a clear case of host-associated differentiation in sympatry, especially given the uninterrupted gene flow within the pestiferous population across geographic barriers and hundreds of kilometers, and the reduced gene flow between the microsympatric wild and pestiferous populations (Dávila-Flores, 2012; Medina, 2012; Medina *et al.*, 2012). Genetic structuring in corn leafhopper may be the outcome of a host plant shift, from maize to perennial teosinte, facilitated by expansion of maize cultivation into and subsequent retraction from perennial teosinte's highland, mesophyllous forest habitat (Medina *et al.*, 2012). A recent review found that host plant shifting in herbivorous insects clearly can and does lead to ecological speciation (Forbes *et al.*, 2017). Moreover, the same review found that in addition to host shifting, genetic structuring plays prominent roles in ecological

speciation, which would point to the possibility of early ecological speciation in corn leafhopper. However, our results suggesting no immigrant inviability on perennial teosinte, and “immigrant advantage” on maize may imply that genetic structuring and host shifting may be incipient in corn leafhopper. As noted before, the wild leafhopper’s perennial teosinte habitat was isolated from maize beginning ~35 years (~70 generations) ago, though isolation of corn leafhopper populations is likely only minimal: the two host plants and leafhopper populations are separated by <10 km, while gene flow within the pestiferous population is uninterrupted over hundreds of kilometers, and the pestiferous population is arguably several orders of magnitude larger than the wild population and more prone to dispersal. Thus, it seems that at present, immigrant inviability does not represent a barrier to gene flow between the two corn leafhopper populations, as our results and those of others suggest, and does not contribute significantly to the leafhopper’s population genetic structuring (Dávila-Flores, 2012; Medina et al., 2012; Bellota et al., 2013; Dávila-Flores et al., 2013).

Overall, our results concerning immigrant inviability were surprising on two fronts. On one hand, they suggested an immigrant advantage on maize, and on the other no disadvantage or advantage for immigrants on perennial teosinte, contrary to the expected immigrant disadvantage on teosinte (Bellota et al., 2013; Dávila-Flores et al., 2013). Future studies should take a closer look at the advantages and disadvantages faced by immigrating pestiferous and wild leafhoppers on their nonassociated hosts, perennial teosinte and maize, respectively. Because immigrant inviability has been shown to play prominent roles in genetic structuring and host shifting in herbivorous insects, two features evident in corn leafhopper, it remains perplexing that our study did not uncover evidence of its occurrence.

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Disclosure

All authors are without conflicts of interest, including financial interests, relevant to the manuscript’s subject.

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