Effect of Host Plant Chemistry on Genetic Differentiation and Reduction of Gene Flow Among *Anastrepha fraterculus* (Diptera: Tephritidae) Populations Exploiting Sympatric, Synchronic Hosts

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ABSTRACT Herbivore host specialization includes changes in behavior, driven by locally induced adaptations to specific plants. These adaptations often result in sexual isolation that can be gauged through detection of reduced gene flow between host associated populations. Hypothetically, reduced gene flow can be mediated both by differential response to specific plant kairomones and by the influence of larval diet on some adult traits such as pheromone composition. These hypotheses could serve as a model to explain rapid radiation of phytophagous tephritid fruit flies, a group that includes several complexes of cryptic species. The South American Fruit Fly *Anastrepha fraterculus* (Wiedemann) is a complex of at least seven cryptic species among which pheromone mediated sexual isolation resulted in rapid differentiation. Cryptic species also exhibit differences in host affiliation. In search of a model explaining rapid radiation in this group, we studied host plant chemical composition and genetic structure of three host associated sympatric populations of *A. fraterculus.* Chemical composition among host plant fruit varied widely both for nutrient and potentially toxic secondary metabolite content. Adaptation to plant chemistry appears to have produced population differentiation. We found host mediated differentiation to be stronger between populations exploiting sympatric synchronic hosts differing in chemical composition, than between populations that exploit hosts that fruit in succession. Gene flow among such host associated populations was extremely low. We propose as a working hypothesis for future research, that for those differences to persist over time, isolating mechanisms such as male produced sex pheromones and female preferences resulting from adaptation to different larval diets should evolve.

KEY WORDS South American fruit fly, gene flow, host mediated differentiation, cryptic species complex

The abundance of continuous arrays from biotypes, to polymorphisms, to host races, to good species suggests that speciation may occur, perhaps frequently, in sympatry, and is more common than appreciated (Drés and Mallet 2002, Mallet 2008). Most studies on chemosensory speciation concern sexual isolation mediated by pheromone divergence, although adaptive divergence of chemosensory traits in response to factors such as hosts can also commonly drive the evolution of prezygotic barriers (Smajda and Butlin 2009). Among insects, diet chemical constituents are often precursors to the biosynthesis of pheromones. For example, *Drosophila serrata* Malloch flies bred on different diets can evolve both differences in male cuticular hydrocarbon profiles, and female preferences for such profiles (Rundle et al. 2005). If speciation is associated with host plant changes in phytophagous insects (Becerra 1997), then the evolution of pheromone blends might also be predicted to occur by association (Symonds and Elgar 2008).

Host plant switches in phytophagous insects can be favored by escape from natural enemies and reduced competition (Feder 1995, Feder et al. 1995, Zvereva et al. 2010), but often expose divergent populations to novel secondary plant metabolites that are potentially toxic. Herbivores can cope with such compounds in different ways (Glendinning 2002, Despres et al. 2007) and can adapt to novel larval diets in a few generations (Bernays and Graham 1988). When original and novel host plants are chemically different, colonization can lead to specialization, and larvae of host associated

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populations may perform better in their preferred host (Gross et al. 2004, Blair et al. 2010). However, larval adaptations to different diets can only persist if reproductive isolation evolves between adult populations exploiting different hosts.

Many herbivorous insects appear to have generalized diets over their full geographical range but result to be more specialized when diet breath is examined over several local communities (Fox and Morrow 1981). The *fraterculus* cryptic species complex in the genus *Anastrepha* Schiner is composed of a group of at least seven morphotypes that together range from Mexico to Argentina (Steck and Sheppard 1993). Such morphotypes differ from each other in karyotype, genetic composition, morphology, behavior, and host affiliation (Smith–Caldas et al. 2001, Aluja et al. 2003, Basso et al. 2003, Hernández–Ortiz et al. 2004, Selivon et al. 2005) and in some cases, exhibit some degree of reproductive incompatibility (Selivon et al. 2005, Vera et al. 2006, Cáceres et al. 2009).

The existence of cryptic species complexes, may be taken as evidence of rapid radiation (Clarke et al. 2004), and suggests that mechanisms causing reproductive isolation in sympatry may be at play. A famous example of rapid radiation of morphologically similar groups of tephritid species is the *pomonella* species group in the genus *Rhagoletis.* Host races in this group arise through shifts in host plants, and because all species of *Rhagoletis* Loew are monophagous and mate exclusively on the host plant (Bush 1969, Prokopy and Papaj 2000), this eventually produces reproductive isolation and interruption of gene flow (Berlocher and Feder 2002). Additionally, *Rhagoletis* adult eclosion matches the fruiting periods of host plants that differ in phenology by adjusting the length of diapause (Filchak et al. 2000). This results in additional temporal isolation among host races and cryptic species. Selection for host fidelity and occupancy of enemy free space also contributes in effectively reducing gene flow (Feder 1995, Feder et al. 1995) eventually leading to the formation of species (Berlocher 2000, Xie et al. 2008).

As opposed to *Rhagoletis*, flies in the genus *Anastrepha* do not necessarily mate on the host plant but rather exhibit a mating system where males release pheromones in leks often formed in nonhost trees to attract females (Aluja et al. 2000). Additionally, no known species of *Anastrepha* enters diapause and, in the particular case of *A. fraterculus* complex, they are highly polyphagous (Ovruski et al. 2003, Norrbom 2004, Segura et al. 2006). In consequence, the classic model of sympatric speciation through host race formation cannot be evoked to explain their radiation.

Results of recent experiments exploring reproductive isolation between two morphotypes in the *fraterculus* complex have shown that such morphotypes differ, among other things, in the composition of male pheromone (Cáceres et al. 2009). Additional experiments have shown that hybrids between the two morphotypes produce a novel mix of volatiles and that hybrid females preferentially respond to such novel volatile blends (Segura et al. 2011). It has been suggested that changes in hybrid female chemical perception could result in recognition of novel host plants. Such a mechanism could explain both the evolution of rapid reproductive isolation and differences in host affiliation. When dealing with novel plant chemistry, populations exploiting different plants could further diverge. This hypothesis is rooted on the fact that male pheromone some male *Anastrepha* pheromone components are also found in some fruits, and green leaf volatiles (Heath et al. 2000). If this mechanism of chemical speciation occurs in nature, then sympatric populations exploiting different sets of hosts should retain their genetic integrity and display reduced levels of gene flow among them.

As a component of a model to explain radiation of tropical phytophagous fruit flies and to test the hypothesis of chemical-mediated sympatric speciation, we examined the genetic composition and genetic structure of three populations of *A. fraterculus* associated to three sympatric hosts differing in chemical composition and measured interpopulation levels of gene flow.

Materials and Methods

Chemical Composition of Host Plant Fruit. The chemical composition of the three main host plants of *A. fraterculus* in North Western Argentina was examined: *Juglans australis* Griseb, an endemic species from the "Yungas" mountain forest, whose fruit ripen between December and January, *Prunus persica* L., an exotic host in Argentina whose fruit also ripen between December and January and *Psidium guajava* L., a neotropical host that ripens between March and June (Ovruski et al. 2003, Schliserman et al. 2004).

Chemical composition of fruit mesocarp of different host plants was compared by examining both primary metabolites (sucrose and protein) and secondary metabolites (total phenols, tannins, and UV-B absorbing compounds). Content was established by sampling for each host compound in six fruit from five trees at two time intervals (early and late season). For chemical determinations, collected fruits were kept in liquid nitrogen and then transported to the Plant Physiology Laboratory of the Faculty of Natural Sciences of the Universidad Nacional de Tucumán, Argentina.

Sucrose Content. Soluble sugars were extracted from 500 mg fresh weight (FW) of frozen fruit mesocarp by homogenization in 4 ml of 80% (vol:vol) ethanol with a mortar and pestle. The homogenate was heated in a water bath at 75°C for 10 min and the insoluble fraction removed by centrifugation at $5{,}000 \times g$ for 10 min. After a second extraction with 4 ml of 80% (vol:vol), ethanol supernatants were pooled and dried under a stream of hot air. The dry residue was resuspended in 1 ml of distilled water and desalted by filtration through an ion-exchange column (Amberlite MB3, BDH, Poole, Dorset, England, United Kingdom). Sucrose was determined according to methods by Cardini et al. (1955).

Soluble Phenolics and Tannins. Total soluble phenolic compounds and tannins were extracted from 100 mg FW of frozen fruit mesocarp with methanol 90% at 5°C for 12 h. After centrifugation at 5,000 rpm for 5 min

the supernatant was collected and used for soluble measurements. Total soluble phenolic compounds were determined by using the Folin–Ciocalteau reagent as described by Swain and Hillis (1959). Tannins were determined according to Hagerman and Butler (1978). Briefly, a methanolic extract was evaporated to dryness and resuspended in 0.2 ml of 96% ethanol. The ethanolic extract was added with 0.1% of bovine serum albumin in 0.2 M acetate buffer, pH 5.0 containing 0.17 M NaCl to a final volume of 1.5 ml, mixed thoroughly, left to stand for 15 min at room temperature, and then centrifuged at 13,000 × *g* for 10 min. Supernatant solution was decanted into a clean tube and 10 mM ferric chloride ($FeCl_3$; 0.5 ml) and SDS/TEA (sodium dodecyl sulfate/triethanolamine, 1.6 ml) reagents were added and left to stand for 30 min at room temperature. The absorbance of sample was measured at 510 nm after zeroing the spectrophotometer with a blank without fruit sample. Tannic acid was used as standard. Tannin content was expressed as milligrams of tannic acid per gram FW walnut fruit. SDS/TEA reagent contained 1 g SDS and 5 ml TEA in a final volume of 100 ml. Ferric chloride reagent was a 10 mM solution of anhydrous $FeCl_3$ in 0.1 M HCl.

UV-B Absorbing Compounds. UV-B absorbing compounds were extracted from 10 mg of FW mesocarp tissue in the dark with 2 ml of acidified methanol (methanol/water/HCl, 79:20:1) according to Mirecki and Teramura (1984). Absorbance was measured at 305 nm with a UV-VIS spectrophotometer (Hitachi U-2800A, Japan).

Protein Content. Soluble protein was extracted from 1 g FW of frozen walnut mesocarp tissue using 2.5 ml of 100 mM Tris-Cl buffer, pH 7.6 containing 1 mM β-mercaptoethanol, 10 mM $MgCl_2$ and 5 mM EDTA. Protein was determined according to Bradford (1976) using BSA as standard.

Collection and Processing of Biological Material for Genetic Analyses. Adults for genetic analyses stemmed from pupae recovered from field infested *P. persica*, *P. guajava*, and *J. australis* in perturbed native vegetation areas of Horco Molle (26° 45′S, 65° 20′ W, 500–600 m), Tucumán province, Argentina. Fruit collection was performed during December and January for both peaches and walnuts and during March for guava. All these fruit species are common *A. fraterculus* host plants in Tucumán (Ovruski et al. 2003). Infested fruit was recovered from 25 guava, 15 walnut, and 25 peach trees. Maximum distance between trees was ≈1,130 m., which is within the dispersal range of flies in the genus *Anastrepha* (Baker et al. 1986). Material selected for analysis consisted of 25 females from each of the three host species. Because some sympatric and morphologically similar species of *Anastrepha* coexist with *A. fraterculus* in the collected hosts, individuals were captured with an aspirator and cooled for 10 min in a refrigerator at 5°C to immobilize them before identification using a key by Zucchi (2000). Upon identification as *A. fraterculus*, individuals, still alive, were placed in 2 ml plastic vials and stored in an ultrafreeze at −80°C at PROMI awaiting DNA extraction. Frozen individuals were then transported to the laboratories of the Facultad de Ciencias Exactas y Naturales, UBA, in Buenos Aires in a thermos containing liquid nitrogen, where DNA was extracted.

DNA Extraction. DNA extraction was done with a lysis buffer composed of NaCl 0.1 M, Tris ClH 0.1 M pH 9.1, EDTA 0.05 M pH 8, SDS 0.5%, and $4.5.10^{-4}$ mg K Proteinase. Biological material was ground and homogenized in Eppendorf tubes with 200 μl of lysis buffer. Subsequently, 200 μl of the same buffer were added. The solution was incubated at 65°C for 30 min, and then cooled on ice for a few minutes; 8 M potassium acetate was added at a final concentration of one M (≈50 μl) and kept at 0°C for 30 min. The homogenized solution was centrifuged at 13,000 × *g* for 15 min. Supernatant was recovered and two volumes of 100% ethanol (900–1,000 μl) were added. After that material was kept at room temperature for 5 min to allow DNA precipitation. Then the solution was again centrifuged at 13,000 × *g* for 15 min. After centrifugation supernatant was discarded and the pellet was washed with 500 μl 70% ethanol.

Quantity and quality of extracted DNA was verified on 1% agarose gels stained with 10 μl ethidium bromide. Concentrations used in the gel were 5 μl of sample and two of loading buffer. Lambda phage (Universidad de Quilmes) digested with *Eco*RI and *Hind*III was used as molecular weight marker.

Inter Sample Sequence (ISSR) Analysis. ISSRs were carried out following a modification of the protocol used by Zietkiewicz et al. (1994). Nine different primers were initially screened to identify well amplified, polymorphic bands, selecting the primer [7(AC): CTT], which produced the highest level of bands per individual and the best readability, to be used for subsequent analyses.

The polymerase chain reaction (PCR) amplifications were carried out in a 25-μl reaction volume containing 15 ng of template DNA, 05 μl of 10 μM primer (Invitrogen), 0.25 μl 20 mM dNTPs (Biodynamics), 0.2 μl of *Taq* polymerase (5U/μl Invitrogen), 1.5 mM $MgCl_2$ 2 μl of 10× reaction buffer (Invitrogen), and 2 μl of 225 mM MgCl. The PCR program used for amplification in a MyCycler Thermal Cycler System (Bio-Rad) programmed as follows: an initial heating at 94°C for 3 min, followed by 35 cycles at 93°C for 30 s., 51.6°C for 60 s., 72°C for 90 s, and a final extension cycle at 72°C for 5 min.

PCR products were separated by electrophoresis in a horizontal gel box system through 1% 20 by 20 cm agarose gels stained with ethidium bromide. Twelve microliters of loading buffer was added to the total volume of each PCR product and then the sample was loaded in the gel. Two lanes were used to load a molecular weight marker (1Kb DNA Ladder, Invitrogen) so that band weight could be estimated. Electrophoretic conditions were 120 V (6V/cm) for 3 h in 1× TAE buffer. Bands were visualized using a Life Technologies UV transilluminator and photographed with an Olympus CAMEDIA C-3030ZOOM camera for later analysis. Only those bands that showed consistent amplification were scored (PCRs and gels were repeated twice). Smeared and weak bands were ex-

Table 1. Comparison among peach, guava, and walnut nutrient and secondary metabolite content (mean ± SD) for fruit collected from *Anastrepha fraterculus* infested host plants

Variables	Peach			Guava			Walnut		
	n	Metabolite content	P1[a]	n	Metabolite content	P2	N	Metabolite content	P3
UV-B Abs. Comp. (Abs. 305 nm)	12	0.21 ± 0.02	0.0435	10	0.12 ± 0.2	0.001	10	0.36 ± 0.03	0.004
Tot. sol. Phenols (μmol g^{-1}FW)	12	1.83 ± 0.26	0.0001	10	0.91 ± 0.9	0.001	10	5.14 ± 0.38	0.001
Tannins (mg g^{-1}FW)	12	0.17 ± 0.03	0.4343	10	0.19 ± 0.3	0.001	10	0.86 ± 0.04	0.001
Sucrose (μmol g^{-1}FW)	12	61.91 ± 5.24	0.0001	10	1.48 ± 5.4	0.001	10	59.93 ± 7.41	0.988
Proteins (μg g^{-1}FW)	12	183.40 ± 17.91	0.0001	10	66.28 ± 19.62	0.001	10	559.44 ± 25.3	0.001

[a] P1, peach-guava pairwise significance; P2, pairwise guava-walnut significance; P3, pairwise peach-walnut significance. Based upon Tukey (HSD) post hoc means comparisons ($P < 0.005$).

cluded. ISSR bands were scored as present (1) or absent (0) for each sample.

Data Analyses. To meet parametric analysis assumptions, we analyzed Log_{10} (X + 1) volume of tannins, total soluble phenolic, sucrose, protein, and UV-B absorbing compounds. Untransformed data are reported in figures to ease interpretation. Chemical content for different hosts were analyzed by MANOVAs followed by univariate ANOVAs and Tukey's honestly significant difference (HSD) test for multiple comparisons using Statistica (version 7) (Statsoft Inc., 2004) software.

For genetic analyses a binary matrix of band presence or absence was converted into allelic frequencies by a Bayesian method (Zhivotovsky 1999) with nonuniform prior distribution with a predetermined F_{IS} value of 0, using the software AFLP-SURV 1.0 (Vekemans et al. 2002).

Alelle frequencies, standard error values (*SE*), variance because of individual numbers (*Vind*) and variance because of number of loci (*Vloci*) were calculated. Genetic variability was quantified following the approach of Lynch and Milligan (1994) by the unbiased expected heterozygosity (*He*) (Nei 1978) and percentage of polymorphic loci (*P*). We analyzed population structure by means of nonhierarchical F_{ST}, and following the approach of Lynch and Milligan (1994), we estimated within population (host species) (*Hw*) and among population (*Hb*) variability components. Confidence intervals for the F_{ST} estimated were obtained by 1,000 random permutations of individuals among populations. F_{ST} statistics is equivalent to a nonhierarchical analysis of molecular variance (Excoffier 2003) that can be applied to dominant markers and is widely used in population structure analysis (Frankham et al. 2002). Indirect estimations of gene flow (*Nm*) were obtained from the differentiation among populations (F_{ST}) according to the relationship $Nm = (1\text{-}F_{ST})/4\,F_{ST}$ (Nei 1978). Using the same software, we also estimated pair-wise F_{ST} and unbiased Nei (1978) genetic distances.

Linear discriminant analysis (LDA) was conducted, trying to determine how best to separate known groups of individuals (associated to peach, walnut, or guava) and predict to which predefined class an individual belongs. This analysis was conducted using MASS software (Venables and Ripley 2002) of R ver. 2.13.0 software (R Development Core Team 2011).

Results

Chemical Analyses: Plant Chemistry. There were highly significant differences in chemistry among fruits of different plant species (Table 1). A MANOVA revealed highly significant (Wilks'λ = 0.0021; F = 86.155; $P < 0.001$) differences for tannins, UV-B absorbing compounds, total phenols, sucrose, and protein among fruits of the three host species.

A marked difference in content of chemical compounds of fruit of the three host species was observed. Primary metabolite content was the lowest for guava. In particular, sucrose content in peach was similar to that of walnut and significantly higher than guava's; while soluble protein content was higher in walnut fruit (Fig. 1). Secondary metabolite content (tannins,

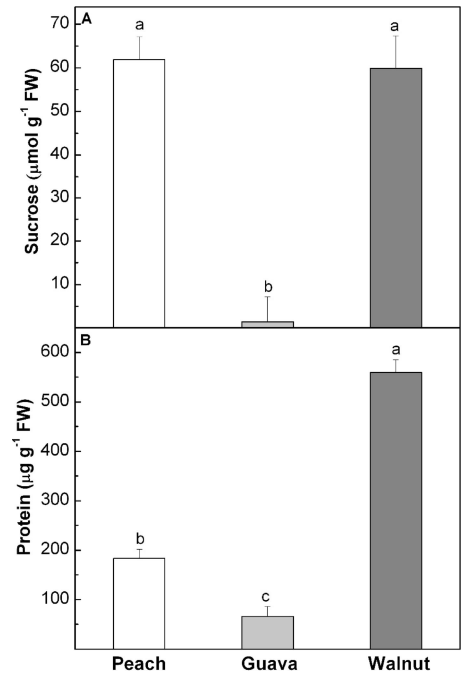

Fig. 1. Sucrose (A) and protein (B) content in fruits of *P. persica*, *P. guajava*, and *J. australis*. Data were tested by a MANOVA followed by Tukey's HSD test for multiple comparisons. Significant differences ($P < 0.05$) are identified by different letters over columns.

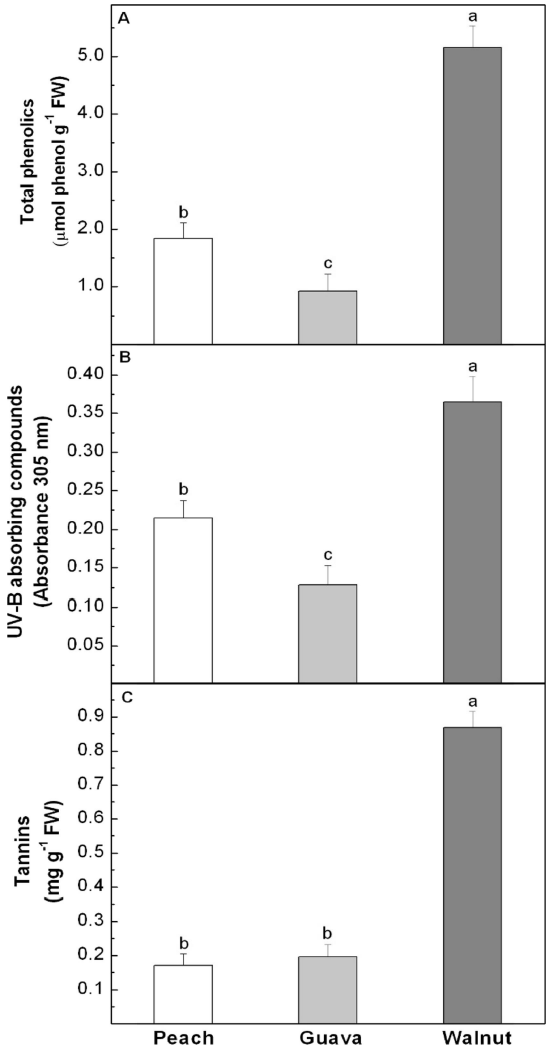

Fig. 2. Total phenolic compounds (A), UV-B absorbing compounds (B), and tannins (C), in fruits of *P. persica*, *P. guajava*, and *J. australis.* Data were tested by a MANOVA followed by Tukey's HSD test for multiple comparisons. Statistically significant differences ($P < 0.05$) are identified by different letters over columns.

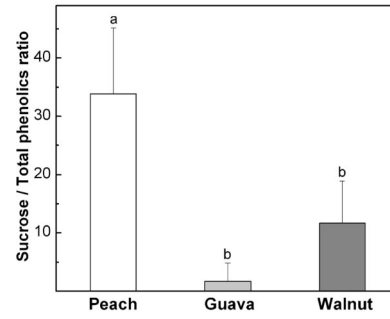

Fig. 3. Sucrose/total phenolics ratio in fruits of *P. persica*, *P. guajava*, and *J. australis.* Data were tested by a MANOVA followed by Tukey's HSD test for multiple comparisons. Statistically significant differences ($P < 0.05$) are identified by different letters over columns.

UV-B absorbing compounds, and total soluble phenols) was significant higher in walnut fruit than in the other two plants (Fig. 2).

Because phenolic compounds are important for plant chemical defense and potentially toxic to herbivores, while sucrose is a central carbohydrate for their nutrition, we evaluated the sucrose/total phenolics compound ratio for the three fruit species (Fig. 3). As shown in Fig. 3, the sucrose/total phenolics ratio was higher in peach, whereas that in guava and walnut the ratio was similar.

Genetic Analyses. ISSR amplifications yielded 14 distinct variable bands that were analyzed as different loci. All 14 loci were polymorphic for flies developing in peach and guava whereas only 10 (71.4%) were polymorphic for flies developing in walnuts (71.4%). Within population diversity (Hw) was 0.299 ($SD < 0.001$) and among population diversity (Hb) was 0.055 ($SD = 0.065$). Genetic differentiation among flies stemmed from different host species was highly significant. F_{ST} estimate was 0.154 ($P = 0$ based on 1,000 permutations), and the corresponding CI at 95% level was −0.017/0.200.

When comparing paired population genetic differentiation, the highest value was recorded between individuals stemming from peach and walnut ($F_{ST} = 0.179$), the lowest value was found between walnut and guava ($F_{ST} = 0.132$), while differentiation between peach and guava recorded an intermediate value ($F_{ST} = 0.155$). Assuming an equilibrium between drift and migration among populations using the overall F_{ST} value, a low number of migrants per generation was revealed $Nm = 1.3$.

Genetic Distances. The greatest Nei genetic distance was recorded between individuals recovered from peach and guava, the lowest value was recorded between individuals stemming from guava and walnut, while intermediate value was obtained from individuals stemming from peach and walnut (Table 2).

Discriminant Analysis. Discriminant analysis revealed that 100% variation among groups is explained by the two canonical axes. A tendency for individuals stemming from different host to group was evident in the corresponding plot (Fig. 4). Individuals stemming from walnut grouped closer to those of guava than to those recovered from peach. Based on 10,000 permutations the differences among individuals stemming from different hosts was highly significant ($P < 0.0001$). As observed in Table 3, the coincidence be-

Table 2. Nei's genetic distance values between groups of individuals stemming from three different host species

Population	Walnut	Peach	Guava
Walnut			
Peach	0.087		
Guava	0.062	0.090	

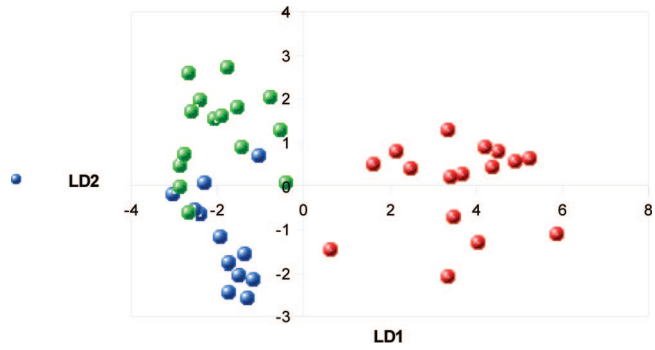

Fig. 4. Results of linear discriminant analysis on genetic composition of *A. fraterculus* individuals stemming from different host plants (blue: walnut; green: guava, red: peach).

tween a priori and a posteriori classification following the discriminant analysis was very high (93.5%).

Discussion

Our results revealed significant differences in chemical content among host plants and these differences may be driving high genetic variability in *A. fraterculus* populations exploiting different hosts, among which genetic differentiation was also significantly high. Specifically walnuts were found to be rich in protein and sugar but also in all secondary metabolites tested. Guavas had lower contents of both nutrients and defensive compounds, while peaches exhibited the highest sucrose/phenolics compound ratio. Of interest, despite the fact that host plants were all within the flight range of adult flies, gene flow among the studied populations was relatively low. Discriminant analysis confirmed grouping of populations according to host origin, illustrating greater differentiation in the population exploiting peaches.

Our results are in contrast to those of Malavasi and Morgante (1983) who studied the genetic structure of an *A. fraterculus* population using eight different hosts within a one ha orchard in Brazil. These authors failed to find allozyme differentiation according to host origin and found extremely low levels of heterozygosity ($H = 0.05$). They attributed the low level of differentiation and variability to the fact that *A. fraterculus* was polyphagous and multivoltine. However, subsequent studies in Argentina, revealed that *A. fraterculus* populations collected over a large area (Alberti et al. 2002, 2008) exhibited much higher allozyme variability (H ranging from 0.353 and 0.492). In concordance with the latter, we found that within a 4 ha sampling area, for sympatric hosts that fruit in synchrony (peaches and walnuts), *A. fraterculus* was genetically variable ($H = 0.299$) on the basis of ISSR.

Table 3. Percent coincidence between a priori and a posteriori classification after linear discriminant analysis

Host	Walnut	Peach	Guava	% error
Walnut	13	0	2	13,33
Peach	1	15	0	6,25
Guava	0	0	15	0
Coincidence		93,48%		

F_{ST} values for populations stemming from different hosts in our study are not very different from those reported by Malavasi and Morgante (1983) and are intermediate when compared with those obtained by Steck (1991) across the entire *fraterculus* range, where it is now known that distinct species exist (Hernández–Ortiz et al. 2004). These results and those reported above, again fall within boundaries of earlier studies on *A. fraterculus* population structure, which makes us believe that we obtained a reliable depiction of host populations at Horco–Molle. Across taxa, F_{ST} values for host populations recovered over a 4 ha plot, were greater than those reported for Medfly populations recovered over several continents (Gasperi et al. 2002).

A plausible explanation for genetic differentiation in our study is related to host plant chemistry. Specifically, the deme formation hypothesis for phytophagous insects proposes that insects adapt to defensive phenotypes of individual trees (Mopper et al. 1995). Here, differences in secondary metabolite content (e.g., juglanone, cyanide, and tannins) among different host plants could exert different selection pressures on larvae feeding on their pulp, where only individuals possessing certain alleles can survive in particular plants. Both walnuts and guavas are native, while peaches were introduced by Spanish colonists 500 yr ago. Greater genetic diversity of guava populations could be taken as evidence for an ancestral association between *A. fraterculus* and guava, which may not be apparent in walnuts and peaches because of the fact that perhaps in these hosts, larvae are exposed to more toxic environments and subjected to narrow genetic bottlenecks. In the case of sympatric synchronic hosts such as peach and walnut, escape from competition and natural enemies could select for increased reproductive isolation after host range expansion (Feder 1995, Feder et al. 1995, Mopper et al. 1995). Additionally, adaptations to cope with toxic secondary metabolites of walnut and peach may be costly but persist because of a tradeoff that allows individuals investing in detoxification access to higher nutrient content than in guava.

One of the most interesting results of our study was that we found greater differentiation between populations stemming from walnuts and peaches, which are sympatric and synchronic, than differentiation between walnut and guava, or peach and guava that produce fruit at different times. Additionally, assuming equilibrium between drift and migration, we found extremely low numbers of migrants per generation across hosts, with values falling below those reported across Argentina, and across several countries in South America (Steck 1991, Alberti et al. 2002). Because flies in the genus *Anastrepha*, exhibit a mating system where males emit pheromones to attract females from nonhost plants (Aluja et al. 2000), unless there are differences in male pheromone composition and on female preference for such pheromones, peach and walnut populations would completely homogenize after sexual maturity during mating. However, it has been recently demonstrated that different *A. fraterculus* morphotypes (Argentina and Perú), produce pheromones that differ in composition (Cáceres et al. 2009), and further, that laboratory hybrids between both morphotypes produce novel blends of pheromones, and hybrid females exhibit distinct preferences for such pheromone blends (Segura et al. 2011).

Another ecological requirement necessary to produce our results, barring differential survival of different genotypes developing in different hosts, is that mated females from reproductively isolated populations had to exhibit distinct host preferences. Otherwise, we would have been unable to detect the existence of both populations, because despite differences because of sexual isolation, some individuals of both differentiated populations would have been recovered from both hosts. Therefore, we have reason to believe that host odor recognition and response to male pheromone are linked in *A. fraterculus* females, as suggested earlier by Segura et al. (2011). Because pheromone constituents in many insect are plant derived volatiles (Landolt and Phillips 1997), we also have reason to believe that the use of different larval substrates can play a role in differentiation, as it has been found for several species of Diptera (Rundle et al. 2005). In this sense, it has been shown that diet has a profound influence on cuticular hydrocarbon composition in insects (Liang and Silverman 2000), and in flies larval host plant driven differences in cuticular hydrocarbons produce premating isolation (Stennett and Etges 1997). Moreover, the evolution of pheromonal communication can be explained in light of sexual selection, where female preference for cuticular hydrocarbons, detected as short distance volatiles, leads to rapid exaggeration of that character in the population, selecting for increased production and release in males (Ferveur 2005).

Ovruski et al. (2003) and Schliserman (2005) studied *A. fraterculus* host plant phenology in the Argentinean Yungas, and report guava as an alternate host after peach and walnut fruiting periods. Additionally, low temperatures during winter could significantly extend immature development and adult longevity, further contributing to bridge periods of host availability (Schliserman 2001, 2005). Such is the case for *A. ludens* in Northern Mexico, which in that subtropical area is bivoltine (Thomas 2003). It would be interesting to expand studies on genetic structure of *A. fraterculus* populations to see if differentiated populations stemming from walnut and peach recognize and use a different suite of hosts over the year.

In summary, our results appear to lend support to the notion that most herbivores represent specialized forms that arise as a result of natural selection and genetic drift to occupy distinct ecological niches. Frequently closer scrutiny (through molecular studies in particular) on "generalist species" reveals the existence of unsuspected niche partitioning and population differentiation favored perhaps by reduced competition and enemy free space. Because specialization fostered by local adaptations to specific host food plants can be reinforced by kairomones and sex pheromones (Loxdale et al. 2011), comparing male pheromone composition and female response of *A. fraterculus* host associated populations could provide further support for the differentiation hypothesis put forth in this study for the *fraterculus* cryptic species complex. Fitting the model to other tephritid groups undergoing rapid and recent radiation could verify its generality as a source of tropical fruit fly diversity.

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