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Host-associated genetic structure of Mexican populations of the cabbage aphid *Brevicoryne brassicae* L. (Homoptera: Aphididae)

L Ruiz-Montoya[1,2], J Núñez-Farfán[2] and J Vargas[2]
[1]*El Colegio de la Frontera Sur. Carretera Panamericana y Periférico Sur s/n, C.P. 29290 San Cristóbal de las Casas, Chiapas, México;* [2]*Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Ciudad Universitaria, C.P. 04510 Coyoacán, Distrito Federal, México*

Phytophagous insects can use different host plant species across their geographic distribution. Within a locality, however, their feeding can be restricted to one or two plant species. If host species constitute different selective regimes to herbivorous insects, genetic differentiation and host-associated local adaptation may occur. In this study, we describe the genetic structure of the aphid *Brevicoryne brassicae* L. associated to *Brassica campestris* L. and *B. oleraceae* var. *capitata* L., two plant species that occur sympatrically in four localities in the highlands of Chiapas, Mexico. The aim was to determine if the aphid populations are genetically structured in relation to the plant host species, and if such differentiation is consistent among localities. The genetic description of populations was made using 11 enzyme loci using cellulose acetate electrophoresis. Aphid genotypes were surveyed in two host plant species-associated populations within each of four localities at seven polymorphic loci (eight subpopulations in total). The genetic structure was assessed at the level of subpopulations, among localities, between hosts, and pairwise comparisons of hosts within locality, using Wright F-statistics. Genetic distance among localities and between host-associated populations within each locality was also estimated. We found that overall genetic differentiation was high ($F_{ST} = 0.22$), and that differentiation among localities ($F_{ST} = 0.13$) was higher than differentiation between hosts ($F_{ST} = 0.03$). All F_{ST} estimates were statistically significant. Pairwise comparisons of F_{ST} between hosts in each locality suggest high differentiation in two of them, and low but still significant differentiation in two other localities. Given that general environmental conditions are similar within localities, selection on each host species may produce genetic divergence within and among subpopulations of *B. brassicae*.
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Keywords: local adaptation; host races; genetic differentiation; *Brevicoryne brassicae*; Chiapas; Mexico; *Brassica* spp

Introduction

Phytophagous insects are often distributed in relation to the distribution of their hosts, and may feed upon a large number of host plant species throughout their geographic range (Futuyma, 1976). However, at the level of a local population, they may use a relatively restricted number of plant species. If the host plant species constitute different selective environments to herbivorous insects, adaptive genetic differentiation among populations could arise (eg Rausher, 1984; Futuyma and Peterson, 1985; Mopper and Strauss, 1998). Local genetic differentiation of insect populations in relation to different host plants has been suggested as being an incipient stage of sympatric speciation and an important phenomenon maintaining genetic diversity in insects (Bush, 1994; Berlocher and Feder, 2002; Drès and Mallet, 2002).

Correspondence: J Núñez-Farfán, Departamento de Ecología Evolutiva, Instituto de Ecología, UNAM, Circuito Exterior s/n, Ciudad Universitaria, C.P. 04510 Coyoacán, D.F. México. E-mail: farfan@servidor.unam.mx
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Aphids constitute excellent systems for assessing host-associated genetic structuring of insect populations. This is because many aphid species can often use more than one host plant species at the local scale, and thus experience different environment and selective regimes on different host plants (eg Via, 1991; Haack *et al*, 2000).

The host plants are considered to be an important factor that affects the genetic structuring of aphid populations (Steiner *et al*, 1985; Wöhrmann *et al*, 1986; Eggers-Schumacher and Sander, 1988; Tomiuk, 1990). Indeed, several studies have shown evidence that the genetic structure of aphids can be related to their host plants (eg De Barro *et al*, 1995a, b; Sunnucks *et al*, 1997; Via, 1999; Haack *et al*, 2000; Anstead *et al*, 2002; Lushai *et al*, 2002).

This study aimed to assess the genetic structure of the aphid *Brevicoryne brassicae* L. (Homoptera: Aphididae), both among and within localities from Chiapas, Mexico. *B. brassicae* uses, as host plants, *Brassica campestris* L. and *B. oleraceae* var. *capitata* L., which occur sympatrically in the Highlands of Chiapas, Mexico. Previous observations indicated differentiation in morphological and life history traits of this aphid in relation to these two hosts

(Ruiz-Montoya and Núñez-Farfán, unpublished data). Thus, besides the genetic differentiation among populations produced by spatial isolation, genetic differentiation between subpopulations (between hosts within localities) could be promoted by selection if the hosts impose differential selective pressure. This allows the assessment of genetic differentiation as a function of a biotic factor, host plant, independently of random subdivision by other means (eg predation or abiotic factors).

Materials and methods

Biology of *B. brassicae* L.

B. brassicae L. displays both sexual and asexual phases on an individual plant (with no alternation between host species) (Blackman and Eastop, 2000). In the study area, *B. brassicae* reproduces apparently only by parthenogenesis (L Ruiz-Montoya, personal observation). *B. brassicae* has been collected carefully by both yellow traps and on the host plants during the last 4 years in the Highlands of Chiapas. So far, there is no evidence of sexual reproduction in this aphid's populations (eg oviparous females, eggs or males; L Ruiz-Montoya, personal observation).

In the study area, *B. brassicae* feeds mainly upon *B. campestris* L. and *B. oleraceae* var. *capitata* L. *B. oleracea* is cultivated from the end of autumn (November), to the end of winter (February). *B. campestris* is an annual weed that grows near cultivated fields of *B. oleraceae*. Both hosts are available to aphids mainly in the winter. Small populations of *B. brassicae* can be found during summer on harvested plants of *B. oleraceae* left in the field, or on late-emerging plants of *B. campestris*.

Sampled localities

Four localities in the Highlands of Chiapas (Mexico), where the two host species occur sympatrically, were chosen for genetic analysis. Localities differ slightly in general environmental conditions (Figure 1), and are, on average, 12 km apart from one another. The altitude within the region varies from 1500 to 2600 m a.s.l. The natural vegetation is a *Pinus–Quercus* forest in different succesional stages (González-Espinosa *et al*, 1997). The mean annual temperature and rainfall are 16°C and 1500 mm, respectively. Rains occur from June to the end of September.

Sampling

Aphids were collected from plants of each host plant species selected at random in the localities of Balún, Chamula, Mitzitón and Teopisca (Figure 1). Within each locality, individuals of the two host plant species were separated from a few meters up to 200 m. In each locality, the nearest population of *B. campestris* to the cultivated field of *B. oleracea* was selected for aphid sampling. *B. campestris* grows in abandoned fields or in natural plant communities. Local farmers eliminate regularly the weeds from their cultivated fields, including *B. campestris*. Aphids were placed in vials, labelled and maintained in liquid nitrogen. In the laboratory, aphids were stored in a deep-freeze (−70°C) until electrophoretic analysis. At least 30 apterous adult aphids from different individual plants of each host species, in each locality, were screened to detect variation at the enzyme loci chosen. The total sample was: four localities (populations) and two subpopulations per locality (one for each host plant).

Electrophoresis

This was performed using horizontal cellulose acetate electrophoresis (Hebert and Beaton, 1993). Aphids were macerated in 15 µl of grinding bufffer (10 mg NADP and 100 µl β-mercaptoethanol in 100 ml water) (see Fisk *et al*, 1992). Five buffer systems were tested: CAAPM and TGE (Hebert and Beaton, 1993), BORATE (Richardson *et al*, 1986), and PHOSPHATE-1 and PHOSPHATE-2 (Fisk *et al*, 1992). In total, 18 enzymes/isozymes were assayed following the staining protocols of Hebert and Beaton (1993). Only the CAAPM and PHOSPHATE-1 buffer systems gave good resolution for 10 enzymes

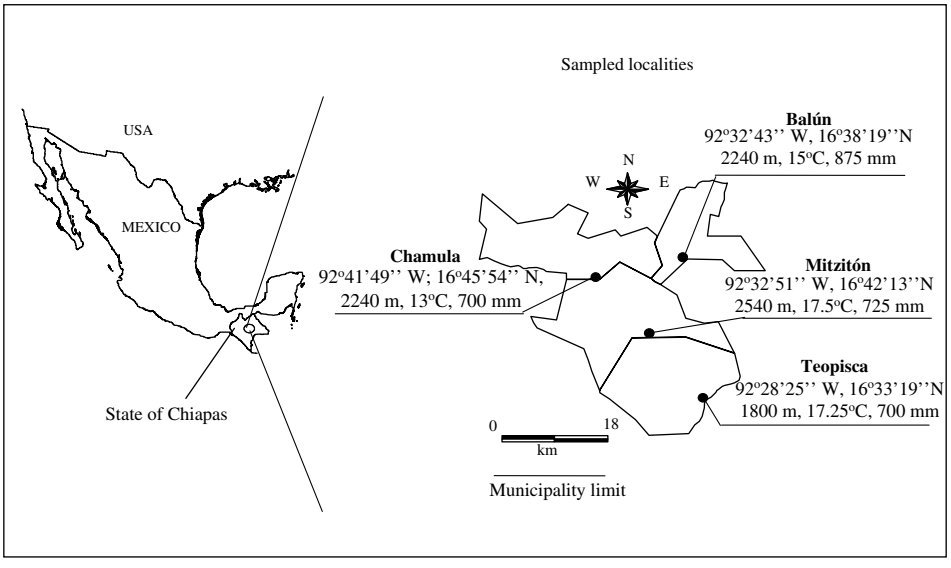

Figure 1 Geographic location and environmental characteristics of populations of *B. brassicae* in the Highlands of Chiapas, Mexico. (Geographic position, altitude above sea level, annual mean temperature and precipitation.)

Table 1 The enzymes/isoenzymes, buffers, number of loci and number of alleles resolved by electrophoresis of *B. brassicae* from Chiapas, Mexico

Enzyme (number of loci)	*International nomenclature*	*Abbreviated name*	*Number of alleles*	*Buffer system*
Malate dehydrogenase $NADP^+$ (1)	EC 1.1.1.40	ME	1	CAAPM[a]
Phosphoglucomutase (1)	EC 5.4.2.2	PGM	2	PHOSPHATE[b]
6-Phosphogluconate dehydrogenase (1)	EC 1.1.1.44	6PGDH	2	PHOSPHATE
Glucose-6-phosphate deydrogenase (1)	EC 1.1.1.49	G6PDH	2	PHOSPHATE
Alkaline phosphatase (1)	EC 3.1.3.1	ALP	2	PHOSPHATE
Malate dehydrogenase (1)	EC 1.1.1.37	MDH	2	CAAPM
Isocitrate dehydrogenase (1)	EC 1.1.1.42	IDH	2	PHOSPHATE
Carboxyl esterase (2)	EC 3.1.1.1	EST-1	1	CAAPM
		EST-2	2	CAAPM
Aspartate aminotransferase (1)	EC 2.6.1.1	AAT	1	PHOSPHATE
Fumarate hydratase (1)	EC 4.2.1.2	FUM	1	CAAPM

[a]CAAPM pH=7: 42.0 g citric acid (anhydrous), 50.0 ml 4-(3-aminopropyl) morpholine, make up to 1 l. Dilute 1: 4 CAAPM buffer:water for use.
[b]PHOSPHATE pH=7.0: 4.15 g $Na_2HPO_4 \cdot 12H_2O$, 1.31 g $NaH_2PO_4 \cdot 2HO$ in 1 l.

(Table 1). The electrophoresis was carried out using cellulose acetate plates of 76 mm × 76 mm (Helena Laboratories, Beaumont, TX, USA), run at ambient temperature, 95 V and 10 mA for 80 min.

Analysis

For each locality and host-associated population of *B. brassicae*, genotypic frequencies were scored and used to calculate the observed mean heterozygosity (H_o), and allelic frequencies. Allelic frequencies were used to estimate the mean number of alleles per locus (A), the average proportion of polymorphic loci using the 99% criterion (P), and expected mean heterozygosity (H_e) (Hartl and Clark, 1997). Heterogeneity of allelic frequencies among populations was evaluated by testing χ^2 (Workman and Niswander, 1970). Unbiased estimates of Nei's (1978) genetic distance (D) were obtained using the program TFPGA (Miller, 1997). Average genetic distances were estimated for all populations and host-associated populations.

In order to assess population genetic structure, estimates of f, F, and θ of Weir and Cockerham (1984), the equivalent of Wright's (1965) F-statistics, F_{IS}, F_{IT}, and F_{ST}, respectively, were calculated. F-statistics were estimated in four ways: (A) among all (eight) subpopulations of *B. brassicae*; (B) among the four localities (each including the two host-associated subpopulations); (C) between hosts (pooling localities for each host); (D) pairwise comparisons of F_{ST} between host species in each locality were obtained. The estimation of genetic differentiation was performed following the procedure of Weir and Cockerham (1984), using the FSTAT program (version 2.9.3; Goudet, 2001). Jack-knife and Bootstrap methods were used to obtain standard errors and confidence intervals of F-statistics. For each locus, F_{ST} departure from zero was tested by $\chi^2 = 2NF_{ST}\ (k-1)$, where k is the number of alleles, N the total number of analysed organisms, and $(k-1)(s-1)$ degrees freedom, where s is the number of subpopulations (Workman and Niswander, 1970). The statistical test for F_{IS} and F_{IT} was obtained by $\chi^2 = (F)^2N(k-1)$, with $k(k-1)$ degrees of freedom (Nei, 1977).

Indirect estimates of gene flow Nm, the mean number of individuals or migrants exchanged among groups per generation, was calculated as $Nm \approx 1/4(1/F_{ST}-1)$ since $F_{ST} \approx (1/(1+4Nm))$ (Wright, 1951).

Results

Seven out of 11 isozyme loci tested were polymorphic (Table 1), with an average of two alleles per locus (Table 1). High levels of polymorphism (45.5–63.6%) were found in all populations (Table 2). Heterogeneity in allele frequency between hosts within locality was found for five isozymes in Mitzitón, three in Balún, four in Chamula, and two in Teopisca (Figure 2). Likewise, differences in allele frequency in the same host were detected among localities (Figure 2).

High genetic diversity, expressed as expected heterozygosity (H_e), was found in all populations (range 0.262–0.42). At the locality level, the difference between the observed and expected heterozygosity (fixation index) for all loci indicates a deficiency of heterozygous individuals in three localities (ie inbreeding). However, at the level of host-associated populations within a locality, the fixation index indicated inbreeding in Mitzitón (in *B. campestris*) and Teopisca (in *B. oleraceae*). Contrary, there was an excess of heterozygous individuals in Mitzitón (*B. oleraceae*), Chamula (*B. campestris*), and Balún (*B. campestris*) (Table 2). These deviations from random mating expectations affect the magnitude of genetic differentiation (F-statistics) between hosts and among localities (see below).

Genotypic frequencies at G6PDH, EST-2, and 6PGDH loci deviated significantly from expected frequencies in all populations associated with *B. oleraceae*. In *B. campestris*, frequencies at the MDH, EST-2 and 6PGDH loci deviated from random mating expectations in three localities (Figure 3). A similar pattern was observed among populations.

All values of F_{IT} and F_{IS} obtained for each locus and average were significantly different from zero among subpopulations, localities, and between hosts. As expected for clonal populations, there is a deficiency of heterozygotes at the local (F_{IS}) and whole-population (F_{IT}) levels (Table 3a). Similarly, there is significant inbreeding among localities and between hosts (Table 3b, c).

Table 2 Average percentage of polymorphism (P), observed (H_o) and expected heterozygosity (H_e) and fixation index, (F=1−{H_o/H_e}) of host-associated populations of *B. brassicae* in Chiapas, Mexico

Locality	*Host species*	*Host species*					*Locality*				
		N	P (%)	H_o	H_e	F	N	P(%)	H_o	H_e	F
Mitzitón	*B. campestris*	30.1	63.63	0.268	0.422	+0.380					
	B. oleracea	37.8	45.45	0.373	0.338	−0.104					
							68.0	63.63	0.322	0.435	+0.258
Balún	*B. campestris*	42.0	63.63	0.311	0.262	−0.189					
	B. oleracea	36.0	63.63	0.335	0.337	+0.004					
							78.0	63.63	0.323	0.390	+0.171
Chamula	*B. campestris*	38.8	54.54	0.456	0.366	−0.247					
	B. oleracea	25.7	54.54	0.365	0.382	+0.044					
							64.5	54.54	0.418	0.388	−0.079
Teopisca	*B. campestris*	33.1	63.63	0.371	0.388	+0.042					
	B. oleracea	31.0	63.63	0.282	0.368	+0.232					
							64.1	63.63	0.329	0.389	+0.155
Total							274.7	63.63	0.352	0.444	+0.207

N, average number of individuals assayed.

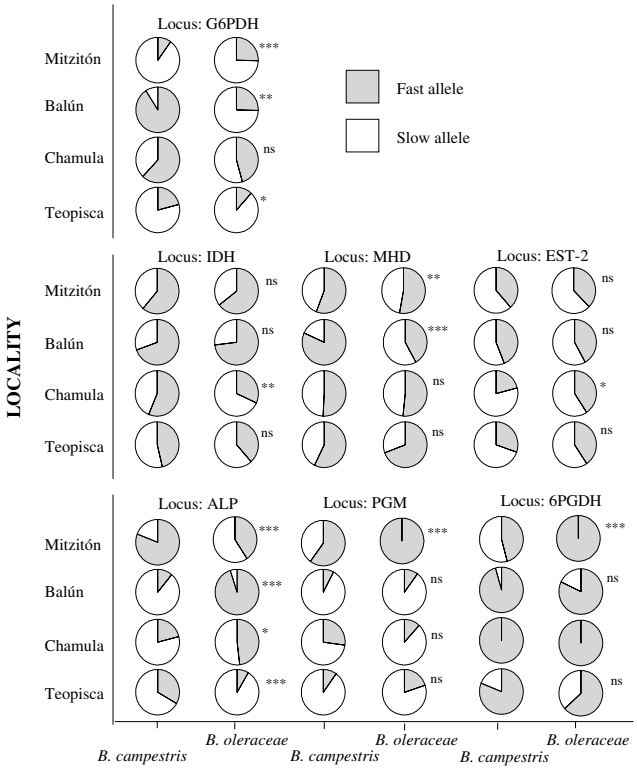

Figure 2 Heterogeneity tests for seven loci surveyed in *B. brassicae* associated to *B. campestris* and *B. oleraceae* in Chiapas, Mexico. *$P<0.05$; **$P<0.01$; ns, not significant.

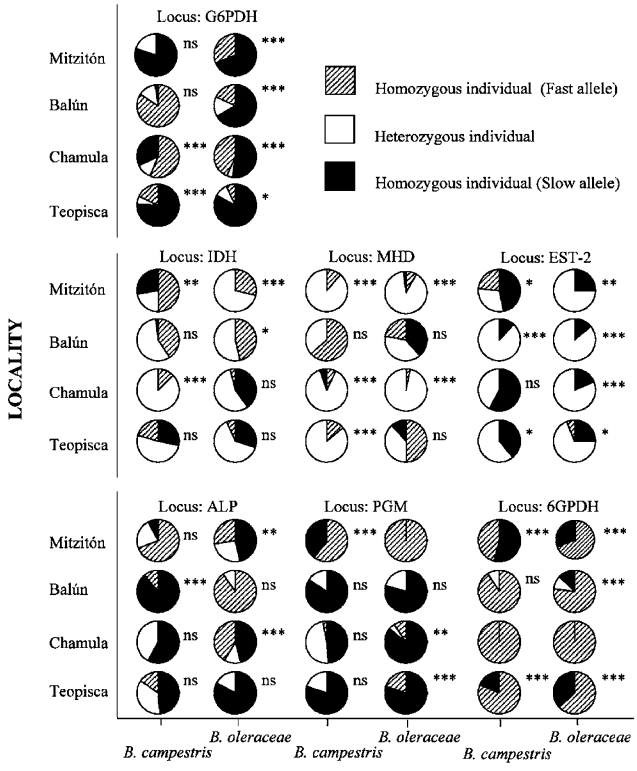

Figure 3 Observed genotypic frequencies at seven loci of *B. brassicae* on two host plants (*B. campestris* and *B. oleraceae*) in Chiapas, Mexico (Hardy–Weinberg departure test). *$P<0.05$; **$P<0.01$; ns, notsignificant.

The F_{ST} value estimated for the whole population was high and larger than the F_{ST} estimated among localities (Table 3a, b). Thus, the F_{ST} for the whole population includes a fraction explained by differentiation between hosts within locality. The value of F_{ST} between hosts (localities pooled within each host) indicates small but significant differentiation (Table 3c). Pairwise comparisons of F_{ST} within each locality detected high differentiation between hosts in two localities (Mitzitón and Balún, Table 4). The larger differentiation was observed in the locality of Balún (Table 4).

Genetic distance between the localities of Teopisca and Chamula was small (Figure 4). Furthermore, in each of these localities, the host-associated subpopulations showed small genetic distances between them (Figure 3). Contrary to this, Balún and Mitzitón showed

Table 3 Estimation of F-statistics: (a) Among eight subpopulations of *B. brassicaea*; (b) among four localities (which include the two host-associated subpopulations in each locality); (c) between hosts (pooling localities for each host)

Locus	F_{IT}	F_{ST}	F_{IS}	Nm
(a) Among subpopulations				
IDH	−0.194**	0.068**	−0.282**	3.42
MDH	−0.480**	0.046**	−0.552**	5.10
EST	−0.432**	0.019**	0.460**	12.90
ALP	0.623**	0.397**	0.374**	0.37
PGM	0.648**	0.431**	0.382**	0.33
G6PDH	0.798**	0.335**	0.696**	0.49
6PGHD	0.906**	0.256**	0.874**	0.72
Average	0.228**	0.219**	0.011**	0.89
Jack-knife	0.224	0.219	−0.010	
SD	0.229	0.070	0.216	
95% CI	−0.177	0.095	−0.316	
	0.612	0.343	0.449	
(b) Among localities				
IDH	−0.185**	0.065**	−0.267**	3.50
MDH	−0.486**	0.009*	−0.500**	27.50
EST	−0.431**	0.011*	−0.448**	22.90
ALP	0.611**	0.088**	0.574**	31.00
PGM	0.667**	0.421**	0.425**	0.34
G6PDH	0.799**	0.188**	0.752**	1.07
6PGHD	0.906**	0.128**	0.892**	1.70
Average	0.232**	0.129**	0.118**	1.68
Jack-knife	0.228	0.128	0.106	
SD	0.229	0.056	0.230	
95% CI	−0.175	0.045	−0.261	
	0.619	0.242	0.541	
(c) Between hosts				
IDH	−0.205**	0.0	−0.204**	
MDH	−0.487**	0.003ns	−0.492**	83.08
EST	−0.431**	0.005ns	−0.439**	49.75
ALP	0.615**	0.064**	0.589**	3.65
PGM	0.629**	0.004ns	0.627**	62.25
G6PDH	0.801**	0.118**	0.774**	1.86
6PGHD	0.903**	0.0	0.903**	
Average	0.218**	0.031**	0.193**	7.81
Jack-knife	0.214	0.031	0.186	
SD	0.231	0.019	0.228	
95% CI	−0.187	0.001	−0.198	
	0.622	0.067	0.601	

*$P<0.05$; **$P<0.01$; ns, not significant. Indirect estimates of gene flow by locus and average are provided.

high genetic distances in relation to Teopisca and Chamula. Also, subpopulations of *B. brassicae* in Balún and Mitzitón showed high genetic distances between hosts (Figure 3), agreeing with the pairwise estimates of F_{ST} (cf Table 4). A small amount of gene flow ($\ll 1$; indirect estimate, *Nm*) among all subpopulations was detected (Table 3a). Gene flow among localities and between hosts within localities resulted in higher estimates than those for all subpopulations (>1; Table 3b, c).

Discussion

A major goal of population genetics studies is to determine and quantify population substructure along with the level of gene flow among subdivided populations. In this sense, we identified genetic differentiation among all populations of *B. brassicae*, between localities and also, locally, we found further population subdivision associated with the host plant species used by this aphid. The host-associated genetic structure of insect populations is important in relation to host-race formation, a process that precedes speciation. The existence of host-mediated genetic differentiation is relevant for the analysis of the ecological causes that promote differentiation (Nason *et al*, 2002).

The genetic structure of aphid species is maintained by selection adjusting populations to the local environmental conditions (Tomiuk and Wöhrmann, 1984; Eggers-Schumacher and Sander, 1988; Tomiuk *et al*, 1991), by reduced gene flow (Loxdale and Brookes, 1990; Loxdale *et al*, 1998; Johnson *et al*, 2002; Massonnet *et al*, 2002), itself a function of species-specific flight behaviour dependent on their tendency and their ability to fly (see Loxdale *et al*, 1993, for a review), and by genetic drift (De Barro *et al*, 1995a). Perhaps, as in the case of the aphid *Methopolophium dirhodum*, differentiation between hosts in the localities of Mitzitón and Balún could be better explained if founder effects or genetic drift have occurred (see De Barro *et al*, 1995a).

Further subdivision in the genetic structure of populations may occur if the local environment is

Table 4 Pairwise comparisons of F_{ST} for populations of *B. brassicae* associated to the host plant species *B. campestris* and *B. oleracea*, within each locality

Locality	F_{ST}			
	Average	*Jack-knife over loci ± SD*	*95% CI*	
			Upper	*Lower*
Mitzitón	0.181***	0.1831 ± 0.084	0.337	0.038
Balún	0.381***	0.405 ± 0.171	0.624	0.046
Chamula	0.062**	0.062 ± 0.023	0.101	0.023
Teopisca	0.033*	0.033 ± 0.019	0.077	0.0046

*$P<0.05$; **$P<0.01$; ***$P<0.001$.

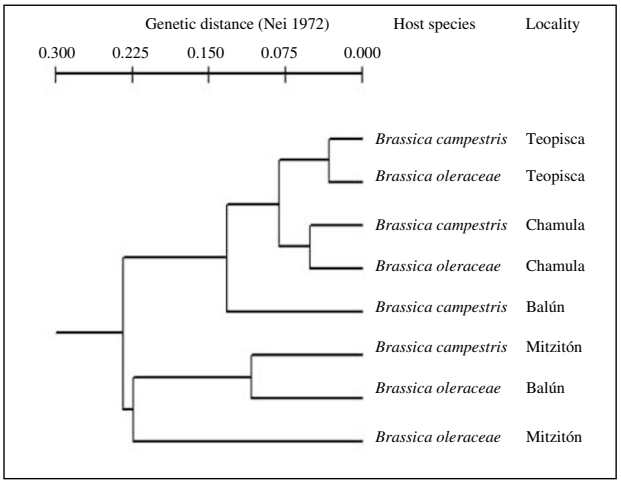

Figure 4 UPGMA phenogram of populations of *B. brassicae* from two host plants within each of the four localities in the Highlands of Chiapas.

heterogeneous. The presence of different host plants in a given habitat constitutes a heterogeneous environment to insect populations. Heterogeneity in gene frequencies and high differentiation between host-associated populations of *B. brassicae* in, at least, two localities (Table 4) suggests that either drift or selection may contribute to further genetic structuring of *B. brassicae* populations in the Highlands of Chiapas. The magnitude of genetic differentiation (F_{ST}) between hosts found in this study falls within the range of estimations obtained for other host-associated populations of aphids (range from 0.012 to 0.22; Via, 1999; Delmotte *et al*, 2002). In addition, Cole and Lynn (1996) found differences in allele frequency of *B. brassicae* at short distances (<1 km) using the 6PGDH locus, and such differentiation might be related to different host plant species. The geographic distances separating pairs of host-associated populations in the present study were even shorter than 1 km, and heterogeneity in allele frequencies was common (Figure 2).

Genetic differentiation between subpopulations of *B. brassicae* may be associated with the different environmental conditions represented by the two host plants (Via, 1999). These differences between hosts include nutritional, physiological, or micro-environmental conditions (Loxdale *et al*, 1985; Steiner *et al*, 1985; Berlocher *et al*, 1993; De Barro *et al*, 1995a, b; Eggers-Schumacher and Sander, 1988; Via, 1999). *B. campestris* and *B. oleraceae* are known to differ in nutritional quality (nitrogen content, humidity, minerals, crude protein; L Ruiz-Montoya, unpublished data), and secondary chemistry (Kjaer, 1976). Nutritional quality affects reproduction in aphids (Dixon, 1998). However, to what extent nutritional quality selects genotypes of *B. brassicae* that use one or the other host plant remains unknown.

Genetic differentiation and restricted gene flow between host-associated populations may promote local adaptation in phytophagous insects (Berlocher and Feder, 2002). Ample evidence of host plant differentiation has recently been found in other aphids (eg De Barro *et al*, 1995a, b; Sunnucks *et al*, 1997; Via, 1999; Lushai *et al*, 2002), supporting the view that this phenomenon is much more widespread than previously realized. Natural selection can contribute to host-related genetic structuring in *B. brassicae* if intense selection occurs during establishment in different hosts. A reciprocal transference experiment between hosts indicated that *B. brassicae* survived better on *B. campestris* than in *B. oleracea* (Ruiz-Montoya and Núñez-Farfán, unpublished data). Thus, further analysis of local adaptation and selection will be important in understanding the patterns of morphological, life history, and genetic differentiation found in this aphids species.

Several studies have revealed greater amounts of genetic variability among aphid populations (eg RAPD-PCR, DNA-microsatellite; Vanlerbergh-Masutti and Chavigny, 1998; Wilson *et al*, 2002; Llewellyn *et al*, 2003), and hence offer the potential to further assess host-associated genetic differentiation (Lushai *et al*, 2002).

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