Bulletin of Entomological Research (2004) **94**, 219–227 DOI: 10.1079/BER2004298

Diet breadth and its relationship with genetic diversity and differentiation: the case of southern beech aphids (Hemiptera: Aphididae)

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Abstract

Herbivorous insect species with narrow diet breadth are expected to be more prone to genetic differentiation than insect species with a wider diet breadth. However, a generalist can behave as a local specialist if a single host-plant species is locally available, while a specialist can eventually behave as a generalist if its preferred host is not available. These problems can be addressed by comparing closely related species differing in diet breadth with overlapping distributions of insect and host populations. In this work, diet breadth, genetic diversity and population differentiation of congeneric aphid species from southern beech forests in Chile were compared. While at the species level no major differences in genetic diversity were found, a general trend towards higher genetic diversity as diet breadth increased was apparent. The aphid species with wider diet breadth, *Neuquenaphis edwardsi* (Laing), showed the highest genetic diversity, while the specialist *Neuquenaphis staryi* Quednau & Remaudière showed the lowest. These differences were less distinct when the comparisons were made in the same locality and over the same host. Comparison of allopatric populations indicates that genetic differentiation was higher for the specialists, *Neuquenaphis similis* Hille Ris Lambers and *N. staryi*, than for the generalist *N. edwardsi*. Over the same host at different locations, genetic differentiation among populations of *N. edwardsi* was higher than among populations of *N. similis*. The results support the assumption that specialists should show more pronounced genetic structuring than generalists, although the geographical distribution of host plants may be playing an important role.

Introduction

The diversity of phytophagous insects is thought to be the result of the evolutionary process of host specialization (Jaenike, 1990; Bernays & Chapman, 1994). Host specialization takes place through the action of many factors that ultimately affect diet breadth (Fox & Morrow, 1981; Jermy, 1984; Bernays & Graham, 1988; Rausher, 1992; Bernays & Chapman, 1994). Given its importance in explaining the diversity of phytophagous insects, the conditions under which diet breadth is increased or reduced have been the matter of numerous studies. A long-standing hypothesis on this issue is that herbivorous insects with a

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restricted diet breadth (i.e. specialists) should be more prone to genetic differentiation than insects with a greater diet breadth (Price, 1980; Futuyma & Moreno, 1988; Peterson & Denno, 1998a,b). This higher genetic differentiation of specialists is based upon (i) the usually reduced population size and genetic diversity of specialists as compared to generalists, which can increase the effects of genetic drift, and also (ii) the patchier distribution of specialists' host-plants, which can reduce cohesion between populations, reducing gene flow, thus increasing isolation by distance (Kimura & Ohta, 1971; Peterson & Denno, 1998a). In contrast, generalists usually occur in larger, more diverse populations, and their host-plant distribution is rather continuous as a result of overlap of the distributions of all its host-plants. In other words, because suitable habitats (e.g. hosts) are patchier for specialists than generalists, gene flow should be relatively less among populations of specialists (Price, 1980; Futuyma & Moreno, 1988), which due to their reduced effective population size may also suffer genetic erosion at species level (Slatkin & Voelm, 1991; Ingvarsson & Olsson, 1997; Newman & Pilson, 1997). However, this hypothesis has recently found little support (Peterson & Denno, 1998a; Van Zandt & Mopper, 1998). Part of this lack of support seems to stem from the use of the terms generalist and specialist. For instance, a generalist can behave as a local specialist if a single host-plant is locally available, while a specialist can eventually behave as a generalist if its preferred host-plant is not available (Fox & Morrow, 1981; Bowers *et al*., 1992; Thompson, 1994).

Recently, these issues have been addressed and the indication made that, despite host spatial distribution, the specialist/generalist dichotomy *per se* is a poor predictor of the properties of insect populations, particularly concerning their genetic characteristics (Kelley & Farrell; 1998; Dobler & Farrell, 1999; Kelley *et al*., 2000). Kelley *et al*. (2000) suggested that this problem can be addressed by comparing population genetics of closely related species differing in diet breadth over the same geographical area with overlapping distributions of insect and host populations. Contrary to this expectation, studying differentiation in sister species of bark beetles, *Dendroctonus ponderosae* Hopkins and *D. jeffreyi* Hopkins (Coleoptera: Scolytidae), Kelley *et al*. (2000) found that the generalist species showed greater genetic differentiation between hosts than the specialist did on the same host over similar geographical distances, while the specialist showed the strongest levels of differentiation between geographical regions. A similar result was found for milkweed beetles *Chrysochus auratus* Fabricius and *C. cobaltinus* LeConte (Coleoptera: Chrysomelidae) by Dobler & Farrell (1999). Thus, geographical components seem to be relevant when determining the relationship between diet breadth and genetic structure of phytophagous insect populations.

Morphological differentiation among populations may also be a consequence of different diet breadths. Host-correlated morphological differentiation has also been described among insects (Sturgeon & Mitton, 1986; Langor & Spence, 1991, Bernays, 1991), albeit to a lesser extent than genetic differentiation. In sap-sucking insects, variations in the length of mouthparts and leg segments, and in the number of sensilla have been associated with different host-plant use (Kennedy, 1986; Moran, 1986; Heie, 1987; Carroll & Boyd, 1992; Bernays *et al*., 2000; Margaritopoulos *et al*., 2000). These findings support the adaptability of insect morphology to differential plant use, suggesting that wider diet breadth should be correlated with higher morphological variation in structures involved in host selection and feeding.

Aphids are widely distributed, frequently specialized, phloem feeding insects (Blackman & Eastop, 1994). Aphids can exhibit a variety of reproductive modes, from cyclic parthenogenesis to obligate parthenogenesis (Moran, 1992; Simon *et al*., 2002). The mode of reproduction of an aphid can be determined by its genetic characteristics and/or be the result of its interaction with the environment (Hales *et al*., 1997). The balance between different clonal lineages of aphids exhibiting different reproductive modes can lead to peculiar genetic structures of a species, a population, or an individual line within a species (Moran, 1992; Simon *et al*., 1999). Since aphids are highly host specific (Dixon, 1998), genetic differentiation according to the host and the existence of biotypes specifically adapted to some particular hosts may also occur (De Barro *et al*., 1995a,b; Via, 1991, 1999; Vanlerberghe-Masutti & Chavigny, 1998). However, the relationship between diet breadth and genetic structure of aphid populations has not been addressed.

In South American temperate forests, the endemic genus *Neuquenaphis* Blanchard (Hemiptera: Aphididae) is almost exclusively associated with *Nothofagus* trees (southern beech forests). Aphids of the genus *Neuquenaphis* reproduce by cyclic parthenogenesis alternating sexual reproduction with parthenogenesis, with sexual morphs having been described for several species, and are monoecious with non-host alternating generations completing their life cycle on the same host (Hille Ris Lambers, 1968; Blackman & Eastop, 1994; Quednau & Remaudière, 1994, R.L. Blackman, personal communication; C.C. Ramírez, unpublished data). *Neuquenaphis* species range from completely monophagous (i.e. *Neuquenaphis staryi* Quednau & Remaudière and *Neuquenaphis similis* Hille Ris Lambers) to polyphagous (i.e. *Neuquenaphis edwardsi* (Laing)) (Quednau & Remaudière, 1994; Fuentes-Contreras *et al*., 1997).

In this work, the relationship between diet breadth, genetic diversity and differentiation in *Neuquenaphis* populations from southern beech forests in Chile was assessed. The aim was to investigate whether or not in this aphid genus a restricted diet breadth is related to lower genetic diversity and higher genetic differentiation. Genetic diversity and differentiation among generalists and specialists coexisting in sympatry were also studied. To answer these questions, allopatric and sympatric populations of aphid species differing in diet breadth were studied with genetic markers, and their genetic diversity and degree of genetic and morphological differentiation were assessed. The main hypothesis tested was that a narrow diet breadth species (i.e. specialists) should be more prone to genetic differentiation and lower genetic diversity than one with a greater diet breadth.

Materials and methods

Insects

Four *Neuquenaphis* species known to differ in diet breadth on *Nothofagus* species (Fuentes-Contreras *et al*., 1997) were collected between November 1999 and January 2000 from four areas of central and southern Chile: Los Ruiles National Forest Reserve (35°52′S, 72°25′W), Alto Huelón (35°35′S,

Table 1. Location and size of collections of southern beech aphid (*Neuquenaphis* sp.) populations studied.

Neuquenaphis species	Location	*Nothofagus* species	Sample size for genetic studies	Sample size for morphometric studies
N. edwardsi	Nahuelbuta	*N. alpina*	10	22
		N. antarctica	7	22
		N. dombeyi	4	3
		N. obliqua	9	18
		N. pumilio	6	19
	Puyehue	*N. antarctica*	5	9
		N. betuloides	8	2
		N. pumilio	8	11
N. schlingeri	Los Ruiles	*N. glauca*	10	0
	Nahuelbuta	*N. obliqua*	10	0
N. similis	Nahuelbuta	*N. pumilio*	10	7
	Puyehue	*N. pumilio*	10	7
N. staryi	Los Ruiles	*N. alessandrii*	14	0
	Alto Huelón	*N. alessandrii*	22	0

72°25′W), Nahuelbuta National Park (37°48′S, 73°01′W), and Puyehue National Park (40°45′S, 72°19′W). The species collected were: *Neuquenaphis staryi* (a specialist found only on *Nothofagus alessandrii* Espin.), *Neuquenaphis similis* (a specialist found only on *Nothofagus pumilio* (Poeppl. & Endl.), *Neuquenaphis schlingeri* Hille Ris Lambers (an oligophagous species found on *Nothofagus glauca* (Phil.) and *Nothofagus obliqua* (Mirb.), and *Neuquenaphis edwardsi* (a generalist found on *Nothofagus dombeyi* (Mirb.), *Nothofagus obliqua*, *Nothofagus pumilio*, *Nothofagus antarctica* (Forst), *Nothofagus alpina* (Poeppl. & Endl.) and *Nothofagus betuloides* (Mirb.). Sexual morphs have been found in all these species during autumn in the last four years of recent studies (C.C. Ramírez, unpublished data). Aphids were collected using a standard beating tray from a minimum of five non-contiguous individual trees from each of the *Nothofagus* species present in each locality (table 1). The samples were preserved in 95% ethanol at –20°C, and split into two groups, one for genetic and the other for morphological analyses.

DNA isolation and RAPD–PCR analysis

DNA extracts were obtained using the 'salting out' method described for aphids by Sunnucks *et al*. (1996). In brief, aphids were individually homogenized in TNES extraction buffer (50 mM Tris-HCl pH 7.5, 400 mM NaCl, 20 mM EDTA, 0.5% SDS), and the homogenate was incubated at 37°C for 18 h in the presence of proteinase K (100 μ ml^{-1}). Proteins were precipitated by addition of 5M NaCl and subsequent centrifugation, and DNA was obtained by ethanol precipitation. DNA extracts were stored at –20 °C until their use.

Since random amplified polymorphic DNA – polymerase chain reaction (RAPD–PCR) markers have been successfully used to detect genetic polymorphism in several aphid species (Black *et al*., 1992; Al-Aboodi & Ffrench-Constant, 1995; Vanlerberghe-Masutti & Chavigny, 1998; Figueroa *et al*., 1999, 2002), a total of 24 decameric random primers (Keystone Labs, Biosource International USA) were tested. The use of these primers in studies on *Sitobion* aphids has been reported elsewhere (Figueroa *et al*., 1999, 2002).

Different DNA dilutions, $MgCl_2$ concentrations, and annealing temperatures were tested before defining the final conditions employed in the amplifications. Amplification reactions were performed in a final volume of 25 μl containing 2.5 μl 10 × PCR buffer (Invitrogen), 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 mM primer, 0.7 U of *Taq* polymerase (Invitrogen), 16.3 μl of ultrapure sterile water and 3 μl of a 1:50 dilution of re-suspended DNA. Amplifications were carried out in a Perkin Elmer 9700 thermal cycler, using a regime of 95°C for 5 min as initial denaturation step, followed by 40 amplification cycles (95°C for 1 min, 40°C for 30 s, 72°C for 1 min). A final extension step at 72°C for 10 min followed by cooling at 4°C completed the regime (Welsh & McClelland, 1990; Williams *et al*., 1990; Figueroa *et al*., 2002). The amplification products were separated by electrophoresis in 1.5% agarose gels in 1 × TAE buffer pH 8.0 at 100 V. The gels were stained with ethidium bromide (0.5 μg m^{-1}) and run against a 100-pb ladder (Invitrogen). A Polaroid photograph was taken as a permanent record. Each amplification was repeated twice to prove the reproducibility of the RAPD–PCR bands. Only well-amplified and reproducible bands were considered for the analysis of genetic polymorphism.

Estimation of genetic diversity and genetic differentiation

The RAPD–PCR phenotypes were deduced from the comparison between the banding patterns obtained for each individual analysed. The analysis was performed under the assumption that variation in banding patterns represents differences between independently amplified loci, and thus each locus was treated as a two-allele system, corresponding to presence/absence of well-amplified and polymorphic bands. Genetic diversity per primer was estimated using Shannon's diversity index (D′) for each locus, given as $-\sum p_i \ln p_i$, where p_i is the frequency of the presence or absence of one band (Lewontin, 1972), and by estimating Nei's gene diversity, h (Nei, 1973), both as implemented in the software POPGENE 3.2 (Yeh *et al*., 1999). The index was normalized to correct for differences in sample size: $D' = D/\ln N$, where N is the sample size. Index values were compared with one-way ANOVA followed by *a posteriori* comparisons.

In order to assess genetic differentiation of populations occurring in sympatry, RAPD–PCR data from the specialist *N. similis*, and the generalist *N. edwardsi* were subjected to hierarchical analysis of molecular variance (AMOVA) using the software ARLEQUIN (Schneider *et al.*, 2000). This analysis gives the components of the variance among host populations within localities (F_{st}). Population pairwise F_{st} values were calculated by a 1000 random permutations test. Estimates of number of migrants (Nm) between pairs of localities per generation were calculated using the Wright algorithm [$Nm = (1 - F_{st})/ 4F_{st}$] (Slatkin & Barton, 1989).

Estimation of morphological differentiation

In order to perform morphological comparisons between specialist and generalist species occurring in sympatry, the individuals collected were mounted on slides (Blackman & Eastop, 2000), and the following commonly used morphological measurements were performed: number of secondary rhinaria in antennal segment III, length of antennal segment III, distance from the base to the most distal secondary rhinaria in antennal segment III, distance from the top to the most proximal secondary rhinaria in antennal segment III, length of antennal segment IV, length of antennal segment V, length of base of antennal segment VI, length of processus terminalis, length of hind tibia, body length, length of ultimate rostral segment, width of siphunculi, length of siphunculi, width of cauda, length of cauda, length of segment II of hind tarsus, and ratio between length of processus terminalis and length of base of antennal segment VI. Specimens with missing values were not included in the analysis. Multivariate discriminant analysis was performed with the SPSS software (SPSS, 1996). Statistical differences between groups were determined by testing the significance of Mahalanobis distances from the discriminant function analysis. A stepwise analysis to determine those variables contributing the most to the discrimination between groups was also performed. Morphological analysis for the specialist *N. staryi* could not be performed because of the low abundance of specimens found in the field during this study.

Results

Genetic polymorphism

A ranking of primers according to the number of polymorphic and well-amplified bands was performed, and four primers giving rise to the highest number of polymorphisms were selected for further use: HN10 (5′-AGCCAGCGAA-3′), CFa9 (5′-GTCCCGACGA-3′), CFa8 (5′-GAACGGACTC-3′) and CFa5 (5′-TGCGGCTGAG-3′). Considering the whole sample, the percentages of polymorphic bands for each primer were 31.6, 24.1, 34.2 and 10.5%, respectively. The amplified bands ranged between 180 and 2060 bp.

Genetic diversity and diet breadth

At the species level (all populations pooled), the strict specialist *N. staryi* showed the lowest genetic diversity (h = 0.124 and D′ = 0.143, table 2). At the population level, when species with different diet breadths coexisted (i.e. *N. edwardsi*, *N. schlingeri* and *N. similis* in Nahuelbuta; *N. edwardsi* and *N. similis* in Puyehue), the genetic diversity was found to be higher in the species with wider diet breadth (h = 0.179 and D′ = 0.282 for *N. edwardsi* in Nahuelbuta; h = 0.130 and D′ = 0.204 for *N. edwardsi* in Puyehue, table 2). Interestingly, genetic diversity of *N. edwardsi* was lower in populations where diet breadth (number of available host-trees, table 1) was also lower (comparison between Nahuelbuta and Puyehue for *N. edwardsi* in table 2). Among local specialists, there were dissimilar genetic diversity values with some comparisons being significantly different (comparisons of genetic diversity between *N. schlingeri*, *N. similis* and *N. staryi* in table 2).

The genetic diversity differences were less distinct when the comparisons were made in the same locality and over the same host. For instance, *N. edwardsi* showed a higher genetic diversity than *N. schlingeri* on *N. obliqua* in Nahuelbuta ($F = 12.39$; df = 6; $P = 0.012$, and $F = 14.19$; df = 6; $P = 0.009$ for Nei and Shannon indexes, respectively). Nevertheless, genetic diversity did not differ between

Table 2. Genetic diversity and differentiation of *Neuquenaphis* species differing in diet breadth.

Neuquenaphis species	Location	Nei's gene diversity (h)	Shannon's diversity index (D′)	F_{st} (among locations)
N. edwardsi	Nahuelbuta	0.179	0.282	
	Puyehue	0.130	0.204	0.092 ***
	Overall	0.172 a	0.276 a	
N. schlingeri	Nahuelbuta	0.080	0.124	
	Los Ruiles	0.100	0.155	0.533 ***
	Overall	0.149 ab	0.227 ab	
N. similis	Nahuelbuta	0.152	0.230	
	Puyehue	0.122	0.188	0.235 **
	Overall	0.161 a	0.252 a	
N. staryi	Alto Huelón	0.045	0.071	
	Los Ruiles	0.012	0.020	0.548 **
	Overall	0.124 b	0.143 b	

Significant tests for F_{st}-values were conducted by 1000 random permutations of data within the AMOVA procedure. Different letters in columns indicate significant differences ($P < 0.05$, LSD *a posteriori* comparisons).
* $P < 0.01$; ** $P < 0.001$; *** $P < 0.0001$.

N. edwardsi and *N. similis* on the same host (*N. pumilio*) in Nahuelbuta ($F = 0.69$; df = 6; $P = 0.43$, and $F = 1.15$; df = 6; $P = 0.32$ for Nei and Shannon indexes, respectively). A similar result was found between *N. edwardsi* and *N. similis* in Puyehue ($F = 0.73$; df = 6; $P = 0.42$, and $F = 0.74$; df = 6; $P = 0.42$ for Nei and Shannon indexes, respectively).

Genetic differentiation of Neuquenaphis *species in relation to diet breadth*

Comparison of pairs of allopatric populations indicated that F_{st} values were higher for the strict specialists, *N. similis* and *N. staryi*, than for the generalist *N. edwardsi* ($F_{st} = 0.235$, $P < 0.001$, and $F_{st} = 0.548$, $P < 0.001$ for *N. similis* and *N. staryi*, respectively, and $F_{st} = 0.092$, $P < 0.0001$ for *N. edwardsi*; table 2). The oligophagous *N. schlingeri* also showed a high genetic differentiation when the comparison involved allopatric populations and different host plants (table 2). In contrast, over the same host at different locations (on *N. pumilio* in Nahuelbuta and Puyehue), the genetic differentiation among populations of *N. edwardsi* was higher (table 3, P-Np vs. N-Np value, $F_{st} = 0.303$, $P < 0.001$) than among populations of *N. similis* (table 2, $F_{st} = 0.235$, $P < 0.001$).

Hierarchical AMOVA including all *N. edwardsi* populations indicated that most of the differences occurred within hosts, with substantially smaller variance components for comparisons among localities, and among hosts within localities (table 4). The result of a random permutation test indicated that all these variance components were highly significant ($P < 0.001$). Pairwise comparisons showed that genetic differentiation was found mostly between allopatric populations (12 out of 15 allopatric comparisons, 2 out of 13 sympatric comparisons, proportions test: $Z = 3.03$, $P < 0.05$, table 3).

Morphological differentiation of Neuquenaphis *species in relation to diet breadth*

Multivariate morphological comparisons between individuals from the two southernmost locations, Nahuelbuta and Puyehue, did not show any significant differences for the specialist *N. similis* (Wilks' lambda: 0.110; $F = 1.49$; df = 11,2; $P = 0.47$) or for the generalist *N. edwardsi* (Wilks' lambda: 0.78; $F = 1.48$; df = 16,89; $P = 0.12$). However, within each of these localities, *N. edwardsi* showed significant morphological differences between *Nothofagus* hosts (fig. 1). Length of hind tibia, length of segment II of hind tarsus, length of siphunculi, length of the ultimate rostral segment, and length of cauda, were included by the stepwise discriminant analysis (with values of 1.30, 0.22, –0.51, –0.70 and –1.07 for standardized canonical coefficients, respectively).

Discussion

The question of whether or not a restricted diet breadth is related to lower genetic diversity and higher differentiation does not have a simple answer. For instance, while at the species level no major differences in genetic diversity were found among the aphid species differing in diet breadth, a general trend towards higher genetic diversity as diet breadth increased was apparent. Indeed, when local populations were considered, the aphid species with broadest diet breadth (*N. edwardsi*) showed the highest genetic diversity, while the strict specialist (*N. staryi*) showed the lowest. In relation to how patterns of genetic differentiation vary among generalists and specialists, it was found that, on the same host, the species with the narrowest diet breadth (*N. staryi*) showed the highest genetic differentiation. Only the oligophagous *N. schlingeri* showed a different

Table 3. Matrix of population pairwise F_{st}-values (below diagonal) and corresponding *P* values (above diagonal).

	N – *Nd*	N – *Np*	N – *No*	N – *Nan*	N – *Nal*	P – *Np*	P – *Nb*	P – *Nan*
N – *Nd*		0.227	0.000	0.061	0.000	0.000	0.000	0.000
N – *Np*	0.035		0.081	0.096	0.041	0.000	0.000	0.055
N – *No*	* 0.130	0.035		0.040	0.028	0.000	0.000	0.000
N – *Nan*	0.154	0.141	0.058		0.414	0.000	0.044	0.051
N – *Nal*	* 0.224	0.203	0.108	0.002		0.000	0.000	0.000
P – *Np*	* **0.325**	* **0.303**	* **0.169**	* **0.164**	* **0.078**		0.117	0.010
P – *Nb*	* **0.210**	* **0.222**	* **0.116**	**0.129**	* **0.145**	0.111		0.349
P – *Nan*	* **0.236**	**0.249**	* **0.143**	**0.126**	* **0.121**	0.086	0.029	

F_{st}-values for comparisons of allopatric host populations are shown in bold; comparisons which became significant after Bonferroni correction with $\alpha = 0.0018$ are marked with an asterisk. Significant tests for AMOVA and population pairwise F_{st}-values were conducted by 1000 random permutations of data. N, Nahuelbuta; P, Puyehue; *Nd*, *Nothofagus dombeyi*; *Np*, *Nothofagus pumilio*; *No*, *Nothofagus obliqua*; *Nan*, *Nothofagus antarctica*; *Nal*, *Nothofagus alpina*; *Nb*, *Nothofagus betuloides*; df, degrees of freedom.

Table 4. AMOVA results for RAPD–PCR data for the generalist aphid, *Neuquenaphis edwardsi*, from two localities, on different *Nothofagus* species.

	Df	Sum of squares	Variance component	Percentage of variation	*P*	
Among localities	1	59.424	1.240	7.32	0.033	$F_{ct} = 0.073$
Among hosts within localities	6	152.390	1.588	9.37	< 0.001	$F_{sc} = 0.101$
Within hosts	50	706.342	14.127	83.32	< 0.001	$F_{st} = 0.167$

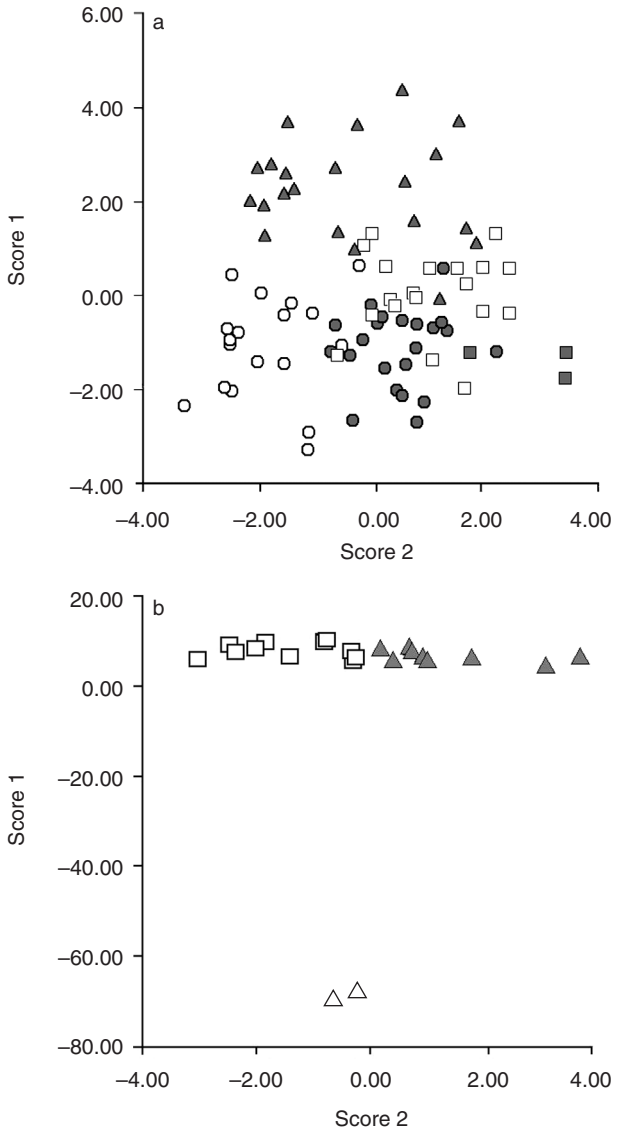

Fig. 1. (a) Plot of scores on the first (52.2% of total variance; Wilks' lambda: 0.093; F (64, 253)= 3.3; $P < 0.0001$) and second (88.8% of total variance; Wilks' lambda: 0.281; F (45, 194) = 2.3; $P < 0.001$) canonical variates for 84 samples of winged specimens of *Neuquenaphis edwardsi* from Nahuelbuta, on five *Nothofagus* hosts. Comparisons of Mahalanobis distance for groups were all significant ($P < 0.001$). (b) Plot of scores on the first (99.5% of total variance; Wilks' lambda: 0.0006; F (32, 8) = 10.2; $P < 0.001$) and second (100% of total variance; Wilks' lambda: 0275; F (15, 5) = 0.9; $P < 0.61$) canonical variates for 22 samples of winged specimens of *Neuquenaphis edwardsi* from Puyehue, on three *Nothofagus* hosts. Comparisons of Mahalanobis distance were significant ($P < 0.001$) for *N. pumilio* and *N. betuloides* groups, and for *N. antarctica* and *N. betuloides* groups. (●, *Nothofagus alpina*; ▲, *N. antarctica*; ■, *N. dombeyi*; ○, *N. obliqua*; □, *N. pumilio*; △, *N. betuloides*.)

trend, although in this case comparisons could not separate a host or location effect.

In the case of the aphid generalist *N. edwardsi*, genetic diversity was higher in locations where diet breadth was also higher (comparison of Nahuelbuta and Puyehue locations). This high local genetic diversity of *N. edwardsi*, relative to both strict specialist species and to the oligophagous species, *N. schlingeri*, may be the result of a greater out-crossing and gene flow between both allopatric and sympatric host-populations of *N. edwardsi*. The low F_{st} value for *N. edwardsi* relative to *N. similis* and *N. staryi* is also evidence for the rather high gene flow between the *N. edwardsi* populations studied (0.52 to 124.7 migrant individuals per generation between pairs of *N. edwardsi* populations, compared to 0.81 and 0.21 migrant individuals for *N. similis* and *N. staryi* populations, respectively). On the other hand, the small among-localities component of variance indicates a small effect of spatial distribution on degree of genetic variation, while the large within-host component of variance suggests that host-plant features are of great importance in determining the genetic structure of the *N. edwardsi* populations studied. Nevertheless, pairwise comparisons of F_{st} values showed that higher, significant values are found only between allopatric populations, suggesting that a geographical effect on genetic structure cannot be totally ruled out. This tendency to a greater genetic diversity and to a lower genetic differentiation of the generalist as compared with a specialist, is also observed when populations differing in diet breadth (a generalist vs. an oligophagous species) are compared in sympatry on the same host (*N. edwardsi* and *N. schlingeri* on *N. obliqua* in Nahuelbuta). Interestingly, a similar genetic diversity for *N. edwardsi* and *N. similis* (a specialist) on *N. pumilio* in Nahuelbuta and Puyehue was also found, with a higher genetic differentiation for the generalist between these two localities. This result can be explained in terms of host specialization of *N. edwardsi*. For instance, a higher morphological differentiation is observed for *N. edwardsi* when feeding on *N. pumilio* (fig. 1b). On the other hand, the performance (survival and reproduction rate) of this species has been found to be lower on *N. pumilio* than on other hosts and dependent on its provenance (previous host-plant) (C.C. Ramírez, unpublished data). Hence, it is apparent that only some pre-adapted genotypes of *N. edwardsi* can use *N. pumilio*, thus restricting the genetic variability on this host, and increasing its differentiation with other conspecific populations.

In the case of the specialists *N. staryi* and *N. similis*, a low but highly structured genetic diversity was found between their populations compared with populations of the generalist *N. edwardsi*. Although it was expected that the two specialists would show similar levels of genetic diversity and structuring, when these genetic features were compared between them, significant differences were found. *Neuquenaphis staryi* exhibited the lowest genetic diversity and the highest level of structuring between the two populations studied, as compared with *N. similis*. This was true despite the fact that in *N. similis* only fundatrices have been found, as compared with the other species studied in which all sexual morphs have been found (C.C. Ramírez, unpublished data), which would suggest a relatively more prominent asexual phase in *N. similis*, which would thus attain greater genetic structuring due to a balance between selection and gene flow. Hence, it is likely that ecological features of their host plants seem to be underlying this pattern. On the one hand, the populations of the tree *N. alessandrii*, the exclusive host of *N. staryi*, are quite patchy and scarce at present, the only extant forest areas over one hectare being the ones sampled in this study. Indeed, *N. alessandrii* has been pointed out as a species threatened with

extinction (Bustamante & Castor, 1998). This situation contrasts with that of the exclusive host tree of *N. similis*, *N. pumilio*, which is widely distributed all along the southern Andes (Ormazábal & Benoit, 1987; Donoso, 1993). Such host continuity and coverage enhances the chances of *N. similis* to outcross and exchange genes with other conspecifics from neighbouring populations, explaining its higher genetic diversity in relation to *N. staryi*. This hypothesis finds support in the much higher degree of genetic differentiation of *N. staryi* than *N. similis* populations, suggesting higher gene flow between *N. similis* populations. These results are in agreement with studies indicating that population genetic structure of phytophagous insects is affected by host distribution and availability (Thompson, 1994; Mopper, 1996), a factor that should be taken into account together with diet breadth when studying genetic structure of herbivores. Thus, the long-standing hypothesis that because suitable habitats (e.g. hosts) are patchier for specialists than generalists, predicting that gene flow should be relatively less among populations of specialists (Price, 1980; Futuyma & Moreno 1988), may also be plausible when specialists with different host plant availability are contrasted.

The morphological variation found in individuals of *N. edwardsi* collected from different *Nothofagus* species within a location, compared with the comparatively small genetic differentiation between these populations, suggests that such phenotypic variation could be the outcome of environmental factors and/or host features affecting aphid morphology. Few reports have shown host-based intraspecific morphological variation in sap-feeding insects (Kennedy, 1986; Moran, 1986; Heie, 1987; Carroll & Boyd, 1992). In this study, three traits bearing directly on the host–aphid interaction were among the highest canonical scores in the discriminant analysis: length of hind tibia and length of segment II of hind tarsus, two traits related to differential abilities in locomotion and adherence to plant surfaces (Moran, 1986), and length of the ultimate rostral segment. This last trait has also been found to be associated with host-plant use (Margaritopoulos *et al*., 2000), and its length may be an adaptation related to the ability to reach sieve elements of their host plants, similar to the pattern found in other Hemiptera showing adaptation in the length of the proboscis or 'beak' used to insert into the seeds of variably inflated fruits of particular host plants (Carroll & Boyd, 1992).

The results presented, similar to those of Kelley *et al*. (2000), do not support the study of Peterson & Denno (1998a), which found little evidence for the common assumption that specialists should show more pronounced genetic structuring than generalists. As suggested by Kelley *et al*. (2000), variations in genetic structuring may be the result of differences in scale of the comparisons made in each study. Indeed, Fox & Morrow (1981) indicated earlier that species with a wide host range can often be made up of more specialized populations, emphasizing the importance of local patterns. In conclusion, the trend towards higher genetic differentiation and lower degree of genetic diversity as diet breadth decreases supports the predictive value of diet breadth for genetic structure of phytophagous insects.

Acknowledgements

Financial support by FONDECYT (grant no. 1000775 to C.C.R.), the Presidential Chair in Sciences awarded to H.M.N., and the Millennium Science Initiative (P99–103-F ICM), are gratefully acknowledged.

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(Accepted 4 February, 2004)