Genetic and morphological variation in *Neuquenaphis* aphids on southern beeches (*Nothofagus* spp.)

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Abstract

Among phytophagous insects, the relationship between host range and genetic variation is not clear at all. Southern beeches (*Nothofagus* spp.) in Chile are colonised by aphids of the South American endemic genus *Neuquenaphis*. Species of *Neuquenaphis* spp. vary in degree of specialisation from those that can use only one *Nothofagus* host (e.g. *N. staryi* and *N. similis*), to others that are more generalists (e.g. *N. edwardsi*, *N. schlingeri*). The relationship between genetic variation (measured as genetic polymorphism of RAPD-PCR markers) and host range of naturally occurring *Neuquenaphis* populations was studied. Morphological comparisons were also performed. The relationship between genetic variation and host range of naturally occurring *Neuquenaphis* populations seems to follow a positive trend. The meaning of this result is discussed in the context of the biology of this group.

Introduction

Phytophagous insects are known to vary in their degree of host specialisation, with most species feeding on few plant genera or families (Bernays & Chapman, 1994). Depending on whether a species is specialist or generalist, exposure to heterogeneous environments (e.g. fragmented host distribution) will have different consequences in the genetic and/or morphological variation it exhibits. For instance, for a strict specialist, a patchy distribution of its host-plant may reduce gene flow between populations, leading to genetic isolation by distance that can be enhanced by genetic drift and/or selection (Peterson & Denno, 1998). Since host distribution is thought to be patchier for specialists than to generalists, inter-population genetic variation is expected to be higher for specialists (reviewed in Futuyma & Moreno, 1988; but see Peterson & Denno, 1998). In contrast, it has been argued that, even in sympatry, species using different host species (generalists) are likely to suffer host-based

selection pressures leading to genetic deme formation (Mopper, 1996). Thus, among phytophagous insects, the relationship between host range and genetic variability is not yet clear.

The South American endemic aphid genus *Neuquenaphis* is associated with *Nothofagus* trees (Hille Ris Lambers, 1968; Quednau & Remaudière, 1994), and shows high heterogeneity in its host range, with species closely associated to a single host (specialists) (e.g. *N. staryi*, *N. similis*), and others to several hosts (generalists) (e.g. *N. edwardsi*, *N. schlingeri*) (Fuentes-Contreras *et al.*, 1997). In order to explore the relationship between genetic variability and host range of naturally occurring aphid populations, the genetic polymorphism of RAPD (Random Amplified Polymorphic DNA) markers of four *Neuquenaphis* aphid species differing in host range was studied. On the other hand, to assess morphological variation, two *Neuquenaphis* species with contrasting host ranges were subjected to morphometric analysis.

Materials and methods

Four holocyclic *Neuquenaphis* aphid species found in three natural forest reserves in Central and Southern Chile were studied (Table 1). One thousand two hundred aphids were collected between November 1999 and January 2000 from Los Ruiles (35°52'S; 72°25'W), Nahuelbuta (37°48'S; 73°01'W) and Puyehue (40°45'S; 72°19'W). The samples were preserved in 95% ethanol at -20°C until their utilisation.

Table 1: Forest reserves and *Nothofagus* species where *Neuquenaphis* species were sampled.

Aphid Species	Localities		
	Los Ruiles	Nahuelbuta	Puyehue
N. staryi	*N. alessandrii*		
N. similis		*N. pumilio*	*N. pumilio*
N. schlingeri	*N. glauca*	*N. obliqua*	
N. edwardsi		*N. alpina*	*N. antarctica*
		N. antarctica	*N. betuloides*
		N. dombeyi	*N. pumilio*
		N. obliqua	
		N. pumilio	

DNA was extracted using the "salting out" method described by Sunnucks *et al.* (1996). Two decamer primers (HN4: 5'-CCG TAC TTG G-3'; HN8: 5'-AGT CAG CCA C-3') were used, which were selected from among 25 primers tested for the genus *Neuquenaphis*. The PCR reactions and amplification conditions were performed according to Figueroa *et al.* (1999). Under the assumption that variation in banding patterns represents differences in amplified loci, each locus was treated as a two-allele system, corresponding to presence/absence (1/0) of well-amplified bands. The RAPD phenotypes, allele frequencies and Nei's genetic distances modified for dominant markers (Lynch & Milligan, 1994) were obtained using the RAPDistance v1.04 package (Armstrong *et al.*, 1994).

Aphid species were determined following the key for the genus (Quednau & Remaudière, 1994). For the morphometric study, the samples were mounted according to Martin (1983), and the following parameters determined (Blackman & Eastop, 1994): number of secondary rhinaria in antennal segment III, length of antennal segment III, length of antennal segment III from the base to the most distal secondary rhinarium, length of antennal segment III from the top to the most proximal secondary rhinarium, 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, ratio between processus terminalis and base of antennal segment VI. Data were subjected to canonical analysis (Manly, 1994).

Results and discussion

Comparing the genetic variation (measured as the proportion of polymorphic loci) and host range (diet breadth) of the *Neuquenaphis* species studied, a trend of genetic variability to increase with host range was found (Table 2). This result was independent of sample size since the proportion of polymorphic loci of each species was standardised by the number of individuals sampled. It is interesting to note that specialist *N. staryi* showed the lowest level of genetic polymorphism. This must be due to the fact that this species is restricted to only the small remaining populations of its host *N. alessandrii*, whose degree of fragmentation and threatened conservation state (Bustamante & Castor, 1998) may generate a geographic isolation which in turn increases inbreeding and decreases local genetic variability. Despite the fact that *N. staryi* and *N. similis* have been reported as strict specialists (Fuentes-Contreras *et al.*, 1997), *N. similis* showed higher genetic variability than *N. staryi* (Table 2). This may be attributed to the broader distribution of the host plant of *N. similis* (*N. pumilio*, 35° to 55°S latitude than that of *N. stary* (*N. alessandrii*, around 35°S latitude), exposing separated populations to different selective and/or genetic drift forces, which may generate higher inter-population variation in *N. similis*.

Table 2: Number of host and RAPD-PCR polymorphisms from two primers (HN4 and HN8) for each *Neuquenaphis* species studied. The proportion of polymorphic loci for each primer (estimated as the number of polymorphic bands over the total number of amplified bands, following Vanlerberghe-Masutti & Chavigny, 1998), standardised by the number of individuals sampled (sample size in last column) is shown. Proportions were compared by one-tail χ^2-test for multiple proportions (Zar, 1994), hypothesising increasing proportion of polymorphic loci as the number of host species increases.

Species	Number of hosts	HN4 *	HN8 **	n
N. staryi	1	0.14	0.36	14
N. similis	1	0.55	0.55	20
N. schlingeri	2	0.80	0.60	20
N. edwardsi	6	0.75	0.72	65

*: P=0.0001 and **: P=0.11

Since *N. staryi* individuals exist at a single locality (Los Ruiles and other smaller patches in its surroundings), inter-population comparisons of genetic variation was possible only in the case of *N. similis*. Nevertheless, this species did not show variation in RAPD profiles between the localities where they were collected (Nahuelbuta and Puyehue), and

morphological comparisons also revealed lack of significant inter-population variation (Wilks' Lambda= 0.16; F [9,4] = 2.32, p=0.22). Thus, Nahuelbuta and Puyehue populations of *N. similis* were not different either genetically or morphologically, suggesting gene flow to be high enough to compensate local natural selection and/or genetic drift. It should be noted that winged morphs were not found in *N. similis*, while winged and wingless morphs were found in *N. staryi*. Therefore, it may be speculated that among strictly specialist aphids, distribution of their host plant seems to be more important than dispersal capacity as a factor affecting genetic variability.

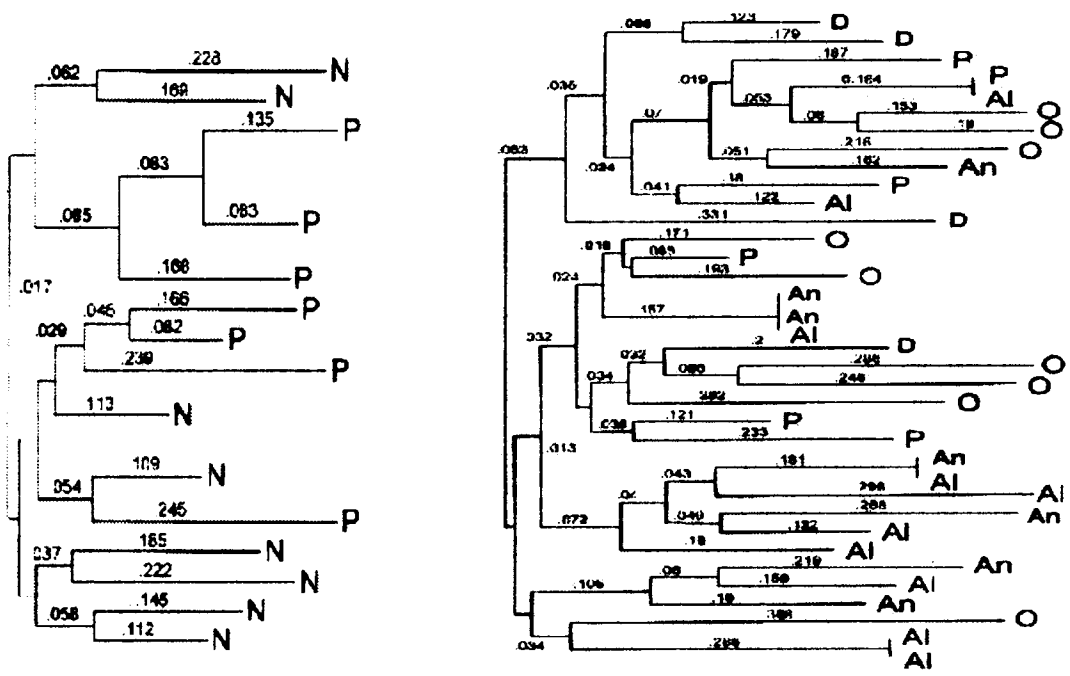

Figure 1: Neighbour-joining tree based on Nei's genetic distances for *N. edwardsi* RAPD profiles using primer HN4: A (right) on a single host (*N. antarctica*) and between two localities (N: Nahuelbuta and P: Puyehue); and B (left) between different hosts (Al: *N. alpina*, An: *N. antarctica*, O: *N. obliqua*, and P: *N. pumilio*) but within a single locality (Nahuelbuta).

The species with wider host range, *N. schlingeri* and *N. edwardsi*, showed higher degree of genetic polymorphism as compared with the specialists *N. staryi* and *N. similis* (Table 2). Given that *N. schlingeri* and *N. edwardsi* were found feeding on more host plants and in more localities than the specialist species, the higher genetic polymorphism found could be a consequence of their exposure to a wider range of biotic and abiotic factors, possibly expressed as higher genetic distance among aphid populations. To test this hypothesis, the genetic polymorphism of the most generalist species (*N. edwardsi*) was analysed at two levels: between localities considering the same host and between hosts within the same locality. Comparisons were performed by constructing Neighbour-Joining trees based on Nei's genetic distances corrected for dominant markers. The comparison of RAPD profiles of two geographically separated populations of *N. edwardsi* living on *N. antarctica* (Nahuelbuta and Puyehue) showed no significant divergence (very low bootstrap values, Fig. 1A). This suggests no restriction in gene flow, probably due to lack of isolation of populations despite the long distance (ca. 400km) between sites, although sample size could have been too

small as well. Nevertheless, these two populations were morphologically different (Wilks' Lambda=0.17; F [17,13]=3.61, P<0.01; with length of antennal segment III from the top to the most proximal secondary rhinarium, length of antennal segment IV, and body length significantly contributing to group discrimination). Similarly, within one locality (Nahuelbuta), *N. edwardsi* did not show differences in RAPD profiles between *Nothofagus* species (Fig. 1B), although significant morphological variation among hosts was found (Wilks' Lambda: 0.39; F[6,152]=15.440; P<0.0001; with the variables length of hind tibia and length of cauda contributing the most to the discrimination between groups). This result also suggests that the population of *N. edwardsi* in Nahuelbuta is only phenotypically affected by the host, possibly due to phenotypic plasticity. As in the case of *N. similis*, gene flow among localities and among host populations may be rather high and consequently populations are under continuous genetic homogenisation.

Although a trend towards higher genetic variability as host range increased was found, patterns of inter-population variation between localities and between host plants within a location as in the case of *N. edwardsi*, do not explain its higher genetic polymorphism. Genetic polymorphism among *Neuquenaphis* species studied herein may be due to intrinsically different genetic variation of each species, which are probably related to ecological factors affecting population abundance and dispersal capability.

Despite the inherent difficulties of the study of host-driven genetic patterns of natural populations, where balanced designs which can be set up in the laboratory may not be available, the examination of patterns of genetic variation among herbivore populations, as done herein, may still provide insight into factors that drive host association. Thus, although the relationship between genetic variation and host range of naturally occurring *Neuquenaphis* populations seems to follow a positive trend, the causes underlying this pattern deserve further studies.

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