

Genetic variability of the recently introduced aphid *Sitobion avenae* in Chile

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Abstract

The aphids *Sitobion avenae* and *S. fragariae* were first reported in Chile in 1974 and 1993, respectively. *Sitobion* aphids in Chile represent an interesting study case for population genetics because of their recent introduction and mode of reproduction. Samples were collected along a 950km latitudinal transect from different cultivated and wild host plants. *S. avenae* was distinguished from closely related *S. fragariae* using morphological and molecular criteria. Genetic diversity was assessed using five microsatellite loci, and genetic structure determined according to host plant and geographic zone. A lower genetic diversity and a higher heterozygosity were found in Chilean samples relative to European strains. These results are discussed in terms of founder effect, and anholocyclic reproduction by *Sitobion* aphids in Chile. A weak genetic structuring according to the host plant was observed, and is discussed considering host plant attributes. No differences were found between collection zones.

Introduction

Patterns of genetic variation within introduced populations have been reported. The importance of the introduction of generalist individuals compared with specialist individuals (genotypes) has been assessed (Lynch, 1984), and the level of plant preferences of the first migrants has been proposed as a key factor in the conquest of a new niche (Lynch, 1984). If the introduced genotypes are generalists, they will show an advantage in terms of flexibility to attack new crops in the new environment. In contrast, if the introduced genotypes are specialists, a high performance is expected only if the host species and environmental conditions are similar to those in the region of origin.

The potential modes of reproduction of an aphid are determined genetically and their expression is dependent on the environment (Hales *et al.*, 1997; Moran, 1992). Aphids evolve reproductive strategies specifically adapted to a geographic region or to a host-plant. As with most phytophagous insects, aphids are highly host specific (Dixon, 1998). The complex reproductive cycle of aphids can lead to peculiar population structures, including lineages of clonal colonies living on a host-plant and interacting with other lineages from different hosts (Shaposhnikov, 1987; Simon *et al.*, 1996a,b). In this work, we examine the genetic diversity and structure of populations of the introduced aphid species *Sitobion avenae* and *S. fragariae*, first reported in Chile in 1974 and 1993, respectively, on different cereals and wild graminaceous species (Apablaza, 1974; Remaudière *et al.*, 1993). The analyses include comparisons between most of the known host-plants comprising cultivated and wild Poaceae in a 950 Km latitudinal transect along Chile. Furthermore, we provide data showing that *S. avenae* and *S. fragariae* in Chile are largely anholocyclic.

Materials and methods

Sitobion aphids (n=1749) were collected between 1996 and 2000 around five places distant 0 to 200 (zone 1), 250 to 350 (zone 2), 500 to 600 (zone 3), 700 to 850 (zone 4) and 850 to 950 (zone 5) km south from Santiago, around the following latitudes: 33-34°, 35-36°, 37-38°, 39°, and 41°S. The host-plants collected included wheat (*Triticum durum* L.), oat (*Avena sativa* L.), cocksfoot (*Dactylis glomerata* L.), wild oat (*Avena fatua* L.), mouse barley (*Hordeum* spp.), and velvetgrass (*Holcus lanatus* L.). *Sitobion* individuals were determined according to morphological and molecular criterions (Appendix) (Figueroa *et al.*, 1999). Patterns of allelic diversity were examined at five microsatellite loci (*S3.R*, *Sm11*, *S5.L*, *Sm17* and *Sm10*) (Sunnucks *et al.*, 1996; Wilson *et al.*, 1997; Simon *et al.*, 1999; Wilson *et al.*, 1999).

Results and discussion

The genetic diversity was low in both *S. avenae* (52 multilocus genotypes) and *S. fragariae* (30 genotypes), and it was mainly constituted by a few common genotypes and by several infrequent or unique genotypes. In *S. avenae*, three genotypes (Sa1-Sa3) represented over 79% of the sample (Appendix), while in *S. fragariae* two genotypes (Sf1-Sf2) represented over 62% of the whole sample (Appendix). The lack of genetic diversity of *Sitobion* aphids found in Chile as compared with populations in the United Kingdom or France (Sunnucks *et al.*, 1997; Simon *et al.*, 1999), could result from a founder effect, or from the absence or rarity of sexual reproduction among Chilean populations.

With the exception of locus *Sm17* ($p=0.73$) in *S. avenae* and locus *S3.R* ($p=0.97$) in *S. fragariae*, all microsatellite loci widely departed from the HW equilibrium ($p<0.003$). In *S. avenae* populations, they were caused by significant homozygote excess (strong positive F_{IS} value, see Table 1) at locus *S3.R* and by significant heterozygote excess at the other four loci (strong negative F_{IS} value, see Table 1), while HW deviations were caused by heterozygote excess at all loci in *S. fragariae* populations (Table 1). As has been proposed by Delmotte *et al.* (2001) for the aphid *Rhopalosiphum padi*, the very high heterozygosity observed at almost all loci could be a consequence of a long-term asexuality, in which the appearance of new

alleles by mutations are expected to stay in the heterozygote state. On the other hand, the chance of production of asexual lineages by hybridization between *S. avenae* and *S. fragariae* (see below) could also be occurring (Delmotte *et al.*, 2001), but this hypothesis needs to be confirmed by using maternally inherited markers.

Table 1: Observed heterozygosities at different microsatellite loci in *Sitobion avenae* and *S. fragariae*.

Species	<i>S3.R</i>	<i>Sm11</i>	<i>S5.L</i>	<i>Sm17</i>	<i>Sm10</i>	Over all loci
<i>Sitobion avenae</i>	0.0235 (0.9016)	0.9765 (-0.9306)	0.9925 (-0.7203)	0.9981 (-0.9499)	0.8750 (-0.6208)	0.7731 ± 0.4220 (-0.6175)
<i>Sitobion fragariae</i>	0.9938 (-0.9843)	0.9969 (-0.4300)	0.9984 (-0.9646)	0.8125 (-0.6929)	0.9984 (-0.6634)	0.9600 ± 0.0825 (-0.7211)

In parenthesis, the F_{IS} value for each locus

Genetic differentiation of *Sitobion* aphids sampled from different host-plants was moderate. At a multilocus level in *S. avenae*, significant differences were observed comparing oat with cocksfoot grass and mouse barley (Fisher's method, $p < 0.03$) aphid populations, and between wheat and oat, cocksfoot grass and wildoat ($p < 0.0001$) aphid populations. In *S. fragariae* populations, highly significant differences were observed between wheat and oat, wildoat, and cocksfoot grass ($p < 0.0001$), between oat and wildoat, mouse barley, cocksfoot grass, and velvetgrass ($p < 0.0001$), and between wildoat and cocksfoot grass ($p < 0.0001$). Considering that some rare or unique genotypes of *Sitobion* aphids were restricted to a single plant, an additional comparison was performed. The frequencies of the commonest genotypes of *S. avenae* (3 genotypes) and of *S. fragariae* (2 genotypes) were compared considering all their host-plants, and highly significant differences still remained ($p < 0.0001$). The weak host-based differentiation found can be interpreted in terms of differential performances between aphid genotypes on host-plants with different chemical constituents (C.C. Figueroa, personal observation). Wheat and wild barley contain hydroxamic acids (Hx), a family of secondary metabolites with antibiotic and deterrent properties against aphids, while oat, wild oat and cocksfoot grass do not contain Hx (Copaja *et al.*, 1991; Barría *et al.*, 1992; Niemeyer & Pérez, 1995). Hence, different genotypes could differentially respond to chemical defences, preferring an alternative host-plant.

Considering the multilocus genotypes, only a weak geographic structure was detected among samples of *S. avenae* collected over the 950km range ($F_{ST} = 0.015$). This might represent an underestimation of structuring given the asexual reproduction of this aphid in Chile. Fisher's exact method for all loci showed highly significant differences between zones 1 and 2 in relation to the other zones ($p < 0.0001$). Although this might have been due to the absence of genotype Sa3 in zones 3 to 5, when genotype Sa3 was removed from the analysis, significant differences subsisted between geographic zones ($p < 0.001$). Hence, the differences observed were mainly caused by the presence of infrequent and unique genotypes. The analysis of the frequencies of the commonest multilocus genotypes of *S. avenae* showed they differed significantly between sites ($p < 0.0001$). As described for the host-plants, some infrequent and unique genotypes were restricted to a single site so that significant differences between sites were found when Fisher's exact tests were performed on the full array of *S. avenae* genotypes ($p < 0.001$).

No specialised genotypes were found. This contrasts with the reports of De Barro *et al.* (1995) and Sunnucks *et al.* (1997), who found specialised genotypes of *Sitobion* on cocksfoot. In contrast, we found a widespread generalist genotype (Sa1) with a comparable distribution

on most host-plants and not restricted to some geographic zone. These last results are in agreement with those obtained in France, where two *S. avenae* genotypes were widely found on many cereals (Haack *et al.*, 2000). In the present work, the patterns of genetic variation of introduced populations of *Sitobion* aphids in Chile showed the dominance of generalist genotypes over specialist ones. Hence, the generalist genotypes were able to colonise different Poaceae in the new environment, given the range of agroclimatic zones in Chile. In order to study the putative origin of Chilean *Sitobion* populations, we compared the genetic constitution of Chilean and French *S. avenae* populations. Our preliminary results show that the generalist *S. avenae* genotype Sa3 is the same at five microsatellite loci that the widespread genotype 2 found by Haack *et al.* (2000). This observation constitutes good evidence to hypothesise that Chilean populations of *S. avenae* were founded by at least one European generalist genotype. In addition, the alleles found among *S. avenae* populations at the five microsatellite loci screened are not restricted to Chile, since they also correspond to alleles commonly recorded in European and non-European populations (Sunnucks *et al.*, 1997; Simon *et al.*, 1999; Wilson *et al.*, 1999; Haack *et al.*, 2000). However, the analysis of a larger sample is needed to assess more precisely the putative origin(s) of introduction(s).

Most *S. avenae* and *S. fragariae* genotypes possessed alleles at the five microsatellite loci restricted to the species. However, 29 infrequent or unique genotypes included mostly *S. fragariae* or *S. avenae* alleles, but also alleles of the other species (Table 1). This apparent introgression of *S. fragariae*-like alleles in *S. avenae* is in agreement with Sunnucks *et al.* (1997), who pointed out the possibility that hybridisation between these species may occur. There are two possibilities for the appearance of hybrids. First, those putative hybrids could have originated locally (in Chile) through rare events of sexual reproduction. A second alternative is an introduction from countries where *S. avenae* and *S. fragariae* regularly undergo sexual reproduction and hence have a chance to hybridise, as they apparently do in the UK (Sunnucks *et al.*, 1997). To confirm the hybrid nature of these individuals, maternal markers, such as mtDNA, should be used (Sunnucks *et al.*, 1997).

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