Genetic variability of the recently introduced aphid
_Sitobion avenae_ in Chile

Figueroa Christian C., Simon Jean-Christophe, Dedryver Charles-Antoine, Niemeyer Hermann M.

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 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.), cockfoot (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 Delmote 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*.**

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

In parenthesis, the $F_{st}$ 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 wild oat ($p<0.0001$) aphid populations. In *S. fragariae* populations, highly significant differences were observed between wheat and oat, wild oat, and cocksfoot grass ($p<0.0001$), between oat and wild oats, mouse barley, cocksfoot grass, and velvetgrass ($p<0.0001$), and between wild oats 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; Barria 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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