Heredity (2005) 95, 24–33

www.nature.com/hdy

Genetic structure and clonal diversity of an introduced pest in Chile, the cereal aphid *Sitobion avenae*

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In Chile, the aphid *Sitobion avenae* is of recent introduction, lives on cultivated and wild Poaceae, and is thought to reproduce by permanent parthenogenesis. In order to study the genetic variability and population structure of this species, five microsatellite loci were typed from individual aphids collected from different cultivated and wild host plants, from different geographical zones, and years. Chilean populations showed a high degree of heterozygosity and a low genetic variability across regions and years, with four predominant genotypes representing nearly 90% of the sample. This pattern of low clonal diversity and high heterozygosity was interpreted as the result of recent founder events from a few asexually reproducing genotypes. Most geographical and temporal variation observed in the genetic composition resulted from fluctuations of a few predominant clones. In addition, comparisons of the genotypes found in Chile with those described in earlier surveys of *S. avenae* populations in Western Europe led us to identify 'superclones' with large geographical distribution and high ecological success, and to make a preliminary exploration of the putative origin(s) of *S. avenae* individuals introduced to Chile.
Heredity (2005) **95,** 24–33. doi:10.1038/sj.hdy.6800662
Published online 20 April 2005

Keywords: *Sitobion avenae*; microsatellites; genetic variability; introduced species; parthenogenesis

Introduction

Despite the severe ecological and economic damage caused by introduced species, the genetic and biological properties that allow invaders to become successful in a new niche have not been extensively addressed (Tsutsui *et al*, 2000, 2001; Sakai *et al*, 2001). Introduced species that quickly colonize extensive areas offer a good opportunity to decipher the mechanisms that can lead to short-term evolutionary changes, whether stochastic or deterministic, and might eventually lead to speciation. Population bottlenecks following introduction, and genetic drift in small founding populations, can strongly reduce genetic diversity. The mode of reproduction of the introduced organism plays also an important role in modulating these genetic effects (Lynch, 1984; Simon *et al*, 1999a). Surprisingly, patterns of genetic variation within introduced populations of parthenogenetic species have not been extensively studied (but see Nicol *et al*, 1997, 1998; Downie, 2002 for aphids). Genetic markers, particularly highly polymorphic ones, have proven useful to identify the genetic source of introduced populations and to document the temporal and spatial dynamics of recent invasions through characterization of their genetic structure (Davies *et al*, 1999). In addition, they provide the genetic background to design new strategies to control introduced pest species.

Aphids show a variety of reproductive modes, from cyclical to obligate (apomictic) parthenogenesis (Moran, 1992; Simon *et al*, 2002, 2003). In cyclical parthenogenetic (sexual) lineages, asexual multiplication alternates with the production of sexual forms, which mate and lay diapausing eggs resistant to frost. Conversely, obligately parthenogenetic (asexual) lineages continue to reproduce parthenogenetically all year round. However, because they do not produce resistant forms, they play a risky strategy that is only favoured in areas with mild winter (Rispe *et al*, 1998). In between, intermediate lineages are able simultaneously to overwinter as parthenogenetic individuals while also investing in the sexual production of frost-resistant eggs, that is, they adopt a 'bet-hedging' strategy that is favoured in regions with unpredictable winter climates (Dedryver *et al*, 1998; Halkett *et al*, 2004). This ecological link between sexual reproduction and cold resistance is thus expected to result in climatic clines: sexual lineages predominating in cold regions, which are replaced by asexual lineages where winters are mild and by intermediate ones where winter climate fluctuates. This picture is supported by empirical studies that show clines in aphid reproductive mode in spite of strong dispersal and gene flow (Simon *et al*, 1999a, Dedryver *et al*, 2001). Additionally, since host specialization is a common feature in aphids (Dixon, 1998), genetic differentiation according to the host plant and the existence of biotypes

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Received 29 June 2004; accepted 31 January 2005; published online 20 April 2005

specifically adapted to some host species have been reported in several aphid taxa (Vanlerberghe-Masutti and Chavigny, 1998; Via, 1991, 1999; Downie, 2000; Shufran *et al*, 2000; Via and Hawthorne, 2002).

In Chile, all cereal aphid species (ie *Sitobion avenae* (F.), *S. fragariae* (W.), *Rhopalosiphum padi* (L.), *Schizaphis graminum* (R.), *Diuraphis noxia* (K.), *Metopolophium dirhodum* (W.) and *M. festucae cerealium* (S.)) have been introduced and all apparently reproduce primarily through obligate parthenogenesis (Fuentes-Contreras *et al*, 1997). Since introduction, these aphids have caused severe damage to crops through invasion of both cultivated and wild Poaceae such as wheat, oat, barley, maize, cocksfoot grass and wild *Hordeum*, across a wide geographic zone covering Central-Southern Chile (Apablaza, 1974; Remaudière *et al*, 1993; Starý *et al*, 1994; Fuentes-Contreras *et al*, 1997).

S. avenae was introduced in Chile some 30 years ago (Apablaza, 1974), and has caused severe crop losses. This aphid is a nonhost-alternating species that lives on numerous species of Poaceae, including cereals and some pasture grasses of temperate climates. It is thought to originate from Europe and the Mediterranean zone but is now distributed worldwide (Blackman and Eastop, 1984). In Western Europe, *S. avenae* displays the full array of reproductive strategies described above, including sexual, intermediate and asexual lineages (Dedryver *et al*, 1998; Simon *et al*, 1999a; Dedryver *et al*, 2001).

Previous work on the genetic structure of a restricted sample of *S. avenae* in Chile using mainly RAPD-PCR markers showed a low genetic diversity and a lack of host-based genetic structure (Figueroa *et al*, 1999, 2002). In contrast, in European countries (eg France, UK and Romania), a high genetic variability and genetic differentiation according to the mode of reproduction and the host plant were found using microsatellite loci (De Barro *et al*, 1995; Sunnucks *et al*, 1997; Simon *et al*, 1999a; Llewellyn *et al*, 2003; Papura *et al*, 2003).

In this work, we examined the genetic diversity of *S. avenae* in Chile with microsatellites, considering the impact of reproductive mode, interaction with host plants and recent introduction on aphid population structure. The survey included individuals sampled mostly from wheat and oats along a 950 km latitudinal transect in Central-Southern Chile over 4 years. Additionally, we compared the identity of the genotypes found in Chile with that of clones that have been monitored in earlier surveys of *S. avenae* populations in Western Europe. This attempted (1) to detect clones with large geographical distribution and high ecological success and (2) to tentatively explore the putative origin(s) of *S. avenae* introduced to Chile.

Materials and methods

Aphid collection

Sitobion aphids ($N = 1749$) were collected over 4 years on available host plants where they were present at the time and site of collections (Figure 1). Aphids were sampled along a 950 km latitudinal transect across two agroclimatic zones. Valleys in central Chile with a predominant dry Mediterranean climate constitute zone 1 (Figure 1), while valleys in south-central Chile with a predominant rainy temperate climate constitute zone 2 (Novoa *et al*, 1989). Zone 1 was sampled in each of the 4 years while zone 2 was sampled only in 1997 and 2000. Six different host plants were sampled: wheat (*Triticum durum* L), oat (*Avena sativa* L), cocksfoot grass (*Dactylis glomerata* L), wild oat (*Avena fatua* L), mouse barley (*Hordeum murinum* L), and common velvetgrass (*Holcus lanatus* L). To limit the chance of sampling individuals from the same colony, each individual aphid was collected from a single host plant separated by at least 10 m from the next sample.

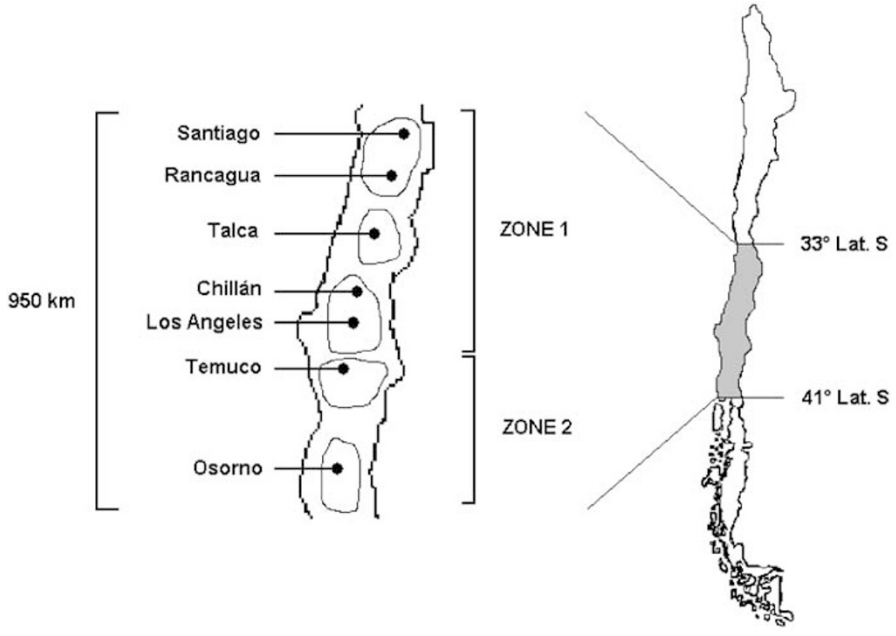

Figure 1 Schematic representation of the two main geographic zones in the latitudinal transect sampled: *Zone 1*: Central Chile (Santiago, Rancagua, Talca, Chillán and Los Angeles); *Zone 2*: South-central Chile (Temuco and Osorno).

All the samples were collected in 95% ethanol and preserved at −20°C prior to their utilization. Under these conditions, DNA was obtained, which remained of good quality after several months or years of preservation.

As *S. avenae* co-occurs in Chile with its closely related species *S. fragariae*, *Sitobion* individuals were determined as *S. avenae* or *S. fragariae* according to morphological and molecular criteria (Figueroa *et al*, 1999). As there is evidence for hybridization/introgression between these two *Sitobion* species, diagnostic alleles from loci *Sm10*, *Sm11* and *Sm17* were also used to discriminate between parental species and their putative hybrids (Sunnucks *et al*, 1997).

Microsatellite loci

Patterns of allelic diversity in Chilean populations of *S. avenae* were examined at five microsatellite loci. Loci *Sm10*, *Sm11* and *Sm17* were isolated from *S. miscanthi* (Sunnucks *et al*, 1996), while loci *S3.R* and *S5.L* were isolated from *S. avenae* (Simon *et al*, 1999a; Wilson *et al*, 2004). *Sm11* is an X-linked locus, while the other loci are autosomal (Wilson *et al*, 1997; A. Wilson, personal communication). Common genotypes were analysed at six additional microsatellite loci (*Sm12, R5.10, S16b, S17b, S3.43* and *S30* – Sunnucks *et al*, 1996; Simon *et al*, 2001; Wilson *et al*, 2004) in order to (i) assess whether individuals with identical genotypes at the initial five loci also matched at these additional loci, which would indicate that they were of clonal origin, and (ii) to compare the identity of common Chilean genotypes of *S. avenae* with that of their western Europe counterparts (see below).

Genomic DNA was extracted from wingless adult aphids according to Sunnucks and Hales (1996), and resuspended in 20–40 µl of sterile ultrapure water depending on the aphid size.

The PCR amplifications of microsatellite loci were prepared in a 15 µl reaction volume, including 0.5 U of *Taq* DNA polymerase (Invitrogen, USA), 10 × Mg^{2+} free reaction buffer, 2 mM $MgCl_2$, 200 µM dNTPs, 10 pmol of each primer (BiosChile-IGSA, Chile), and about 10 ng of aphid DNA. The PCR reactions were carried out in a Perkin-Elmer 9700 thermocycler using the following steps: an initial denaturation for 2 min at 94°C, and 40 cycles consisting of denaturation for 40 s at 94°C, annealing for 45 s with temperature depending on locus and elongation at 72°C for 45 s. For the last cycle, the elongation time was extended to 4 min.

Electrophoresis and silver staining

The PCR reaction was mixed with 4 × loading buffer (Sambrook *et al*, 1989), denatured for 3 min at 95°C, loaded on a 6% polyacrylamide–urea gel and subjected to electrophoresis in 0.5 × TBE at 1.0 kV. After electrophoresis, the gel was silver stained as follows. In brief, the gel was fixed in 10% ethanol, oxidized in 1% nitric acid, washed with ultrapure water and incubated for 20 min in a solution of silver nitrate (1 g/l). After incubation, the gel was washed and developed in a sodium carbonate/formaldehyde solution (30 g/l Na_2CO_3 and 540 µl/l of 37% formaldehyde). The reaction was stopped with 10% acetic acid and the gel washed with water and air-dried at room temperature overnight. The size of the alleles of each locus was estimated using a sequencing size ladder corresponding to the sequence of pGEM® – 3Zf(+) vector (Promega, USA).

Data analysis

Genetic diversity was evaluated using two indices as in Llewellyn *et al* (2003). First, the gross genotypic diversity (*GGD*) was calculated as *G*/*N*, where *G* is the number of different multilocus genotypes and *N* is the sample size. Second, the Shannon–Weaver diversity index (*H*) was calculated as $H = -\sum_i p_i \log_e p_i$, where p_i is the relative frequency of the *i*th genotype (Shannon and Weaver, 1949). This algorithm determines the genetic diversity in relation to the number of genotypes and their relative abundance in each population. This value can be expressed as e^H to obtain an index proportional to the actual number of genotypes found in each population.

Allelic frequencies were calculated using POPULATION software version 1.2.01 (available at http://www.cnrs-gif.fr/pge/bioinfo). Departures from Hardy–Weinberg equilibrium (HWE), linkage disequilibrium (LD) and genetic heterogeneity among the entire set or pairwise population samples were analysed using exact tests available in the Genepop package v. 3.2a (Raymond and Rousset, 1995). Significance of multiple pairwise comparisons was tested using Fisher's method (Genepop package). F-statistics were computed according to Weir and Cockerham (1984) using the same software, with bootstrapping according to Weir (1990). The effects of the host plant, zone and year of collection (as well as their interactions) on the distribution of the four most common genotypes (representing ca 90% of the total sample, see Results below) were analysed using generalized models assuming a binomial error and a logit link function (Mc Cullagh and Nelder, 1989) and performed with S-plus® statistical software (MathSoft, Cambridge, MA, USA) (Venables and Ripley, 1997). Due to plant availability and sampling constraints, sizes of aphid samples from the various host plants were extremely unbalanced. For that reason, only wheat and oat, on which *S. avenae* individuals were frequently sampled, were considered in the frequency distribution analysis. To investigate the genetic relatedness among Chilean multilocus genotypes, Goldstein's distances (a distance specifically developed for microsatellites, Goldstein *et al*, 1995) between individual genotypes were calculated with the POPULATION software and a Neighbour-joining tree was subsequently built.

The genotypic features of Chilean individuals were compared with those of French ones. This was achieved using data obtained on French *S. avenae* by Haack *et al* (2000), who had scored the same set of microsatellites, with the exception of locus *S3.R*. A subset (200 individuals) of the sample of Haack *et al* (2000) was therefore analysed for variation at *S3.R* for a full comparison with Chilean genotypes. After this additional genotyping, 56 five-locus genotypes were discriminated within this subset of French *S. avenae* and retained for comparison with Chilean genotypes.

Results

Occurrence of S. avenae *and* S. fragariae

Of the 1749 individuals analysed, 1052 were *S. avenae* (exhibiting diagnostic alleles exclusive to *S. avenae*), 640

were *S. fragariae* (exhibiting diagnostic alleles exclusive to *S. fragariae*), and 57 were considered as putative hybrids (sharing alleles from *S. avenae* and *S. fragariae*). Genetic data on *S. fragariae* and putative *Sa* × *Sf* hybrids are not presented in this paper, as they will be published elsewhere. *S. avenae* was mainly found on cultivated cereals (89.1%), but it was also present on wild grasses (with the exception of velvet grass) and particularly on cocksfoot grass (Table 1). On the other hand, *S. fragariae* preferentially occurred on wild pastures (57.3%), and it coexisted locally with *S. avenae* (data not shown).

Genetic diversity and within population structure revealed by microsatellite loci

Only 44 multilocus genotypes were distinguished within the whole sample of *S. avenae* individuals ($N = 1052$). Their allelic combination at each locus is indicated in Table 2. Genetic diversity, as measured by the GGD and Shannon–Weaver indices, was very low (Table 3). Overall, only 4% of the collections of Chilean *S. avenae* consisted of unique genotypes while the four most common ones (Sa1: 378 copies, Sa2: 340 copies, Sa3: 127 copies, and Sa4: 75 copies) represented over 87% of the sample (Table 3). Furthermore, the examination of several copies (from 4 to 10) for each of the eight most common genotypes at six additional microsatellite loci did not lead to the discrimination of further multilocus genotypes, suggesting that most genotypic diversity was resolved with the first five loci (data not shown).

An analysis of genotype proportions was carried out considering only a single copy of each of the 44 multilocus genotypes, so the results did not simply reflect the differential clonal amplification of the genotypes. Frequent deviations from the HW genetic equilibrium were detected at all loci (Table 4). The deviations were attributable to significant heterozygote excess at loci *Sm11*, *S5.L*, Sm17 and strong heterozygote deficit at locus *S3.R* (Table 4). Although failures in PCR reactions were not particularly observed at this locus, which would have been suggestive of homozygous null individuals, the possibility of null alleles cannot be completely excluded. No LD was detected among the microsatellite loci studied considering only the 44 genotypes.

Distribution of multilocus genotypes according to host plant, year and zone

The effect of year (1996, 1997, 2000), zone (zones 1 and 2) and plant (oat and wheat) of aphid collection was studied on the frequency distribution of each of the four most common genotypes (those representing ca 90% of the total sample).

Genotype Sa1 showed highly significant changes in frequency across years: while nearly absent before 1999, it was by far the most abundant genotype in 2000 (Table 3). In addition, Sa1 was significantly more frequent in zone 1 and on wheat, these two last factors being independent (Table 5). Genotype Sa2 also varied in frequency between years: being predominant in 1996, it showed a slight but significant decrease until 2000 (Table 3). Globally, Sa2 was more frequent on wheat than oat but the significant plant–year interaction indicated this was not true for all years (Table 5). Genotype Sa3 varied in frequency only between years (see Tables 3 and 5). Finally, year, plant and plant–year interaction influenced significantly the frequency distribution of Sa4. This genotype was frequent in 1997 but showed an abrupt decline in frequency the following years (Table 3). Globally, Sa4 was more common on oat than wheat, but this also depended on years as indicated by the significant plant–year interaction (Table 5).

Relatedness among Chilean multilocus genotypes of *S. avenae* and comparison with their French counterparts

The microsatellite tree (based on five loci) separated the 44 multilocus genotypes of *S. avenae* found in Chile into three main types (Figure 2). Group 1 gathered 28 genotypes (64%) that shared many alleles of closely related size. It is noteworthy that group 1 included three of the four most common clones, notably Sa1 and Sa2, which differed by a single allele at locus *S5.L* (Table 2). Similarly, group 2 included 10 highly related genotypes (23%) among them being Sa3, the third most common clone. The remaining six genotypes (13%) branched individually on the tree and were remote from each other and from groups 1 and 2 by long branches. The two main groups differed from each other by 10 allelic changes on average. For instance, Sa3 (group 2) differed from Sa1 (group 1) by 11 changes and from Sa2 and Sa4 (group 1) by 8–10 changes (Table 2).

The comparison of allelic frequencies at the five microsatellite loci between Chilean and French populations showed in Chilean genotypes a 34% reduction in allelic diversity as compared with French genotypes (19 and 28 alleles at the five loci for Chilean and French genotypes, respectively). Most alleles found in Chilean populations of *S. avenae* (ca 80%) were also found in French populations with only four alleles ($S3.R^{338}$, $Sm17^{181}$, $Sm10^{163}$ and $Sm10^{165}$) restricted to Chile. Apart from $S5.L^{223}$, the most frequent alleles in Chilean

Table 1 Distribution and abundance of *Sitobion avenae* in Chile according to the host plant, year of collection and agroclimatic zone

Host plant	*Zone 1*				*Zone 2*		*Total*
	1996	*1997*	*1999*	*2000*	*1997*	*2000*	
Wheat	28	65	62	440	42	125	762
Oat	35	21	ND	77	24	18	175
Cocksfoot grass	1	45	ND	4	13	7	70
Wild-oat	10	ND	ND	8	ND	9	27
Mouse barley	ND	ND	6	10	ND	2	18
Total	74	131	68	539	79	161	1052

ND: Not detected.

Table 2 Allelic combinations of the 44 multilocus genotypes of *Sitobion avenae* characterized in Chile

Genotype	*Microsatellite locus*				
	S3.R	*Sm11*	*S5.L*	*Sm17*	*Sm10*
Sa1	337/337	144/149	225/227	178/179	164/166
Sa2	337/337	144/149	223/227	178/179	164/166
Sa3	361/361	144/149	223/227	178/179	164/166
Sa4	337/337	144/149	223/227	178/179	164/164
Sa5	337/337	144/149	223/227	177/179	164/166
Sa6	337/361	144/149	225/227	178/179	166/166
Sa7	361/361	144/149	223/227	178/179	164/164
Sa8	337/337	144/149	223/227	177/178	164/166
Sa9	337/337	144/149	225/227	177/179	164/166
Sa10	337/337	144/144	223/227	178/179	164/166
Sa11	361/361	144/149	223/227	177/179	164/166
Sa12	337/337	144/149	225/227	178/179	166/166
Sa13	337/337	144/149	223/227	178/179	166/166
Sa14	337/337	149/149	223/227	178/179	164/164
Sa15	337/337	144/149	227/227	178/179	166/168
Sa16	337/337	149/149	223/227	178/179	164/166
Sa17	337/361	149/149	225/227	178/179	166/166
Sa18	361/361	144/149	223/227	177/178	164/166
Sa19	337/337	144/149	223/227	178/179	166/168
Sa20	337/361	144/149	223/227	178/179	164/166
Sa21	337/361	144/149	227/227	178/179	164/164
Sa22	337/337	149/149	223/227	178/179	166/168
Sa23	337/337	143/143	223/227	178/179	164/164
Sa24	337/337	143/148	223/227	178/179	164/164
Sa25	361/361	144/144	223/227	178/179	166/166
Sa26	337/337	144/149	223/227	177/177	164/164
Sa27	337/337	144/149	223/227	177/177	164/166
Sa28	361/361	144/149	223/227	177/179	166/168
Sa29	338/338	144/149	223/227	178/179	164/166
Sa30	338/338	144/149	223/227	178/179	166/166
Sa31	337/337	144/149	223/227	178/179	163/164
Sa32	337/361	144/149	223/227	178/179	164/164
Sa33	337/337	144/149	223/227	179/181	164/166
Sa34	337/337	144/149	225/227	178/179	163/165
Sa35	337/337	144/149	225/227	178/179	163/166
Sa36	337/337	144/149	225/227	178/179	164/164
Sa37	337/337	144/149	225/227	178/179	166/168
Sa38	361/361	144/149	225/227	178/179	164/166
Sa39	337/361	144/149	227/227	178/179	164/166
Sa40	337/337	148/149	223/227	178/179	164/164
Sa41	361/361	149/149	223/227	178/179	164/164
Sa42	361/361	149/149	223/227	178/179	164/166
Sa43	337/337	149/149	225/227	178/179	164/164
Sa44	361/361	149/149	227/227	177/179	164/166

Table 3 Multilocus genotypes of *Sitobion avenae* identified along all collection zones and years. The number of individuals collected per genotype is indicated for each sampling unit

Genotype	*Host plant*			*Zone 1*				*Zone 2*		*Total*	*Frequency*	*Cumulated frequency*
	Wheat	*Oat*	*Other*	*1996*	*1997*	*1999*	*2000*	*1997*	*2000*			
Sa1	346	21	11	0	0	48	309	0	21	378	0.359	0.359
Sa2	207	79	53	43	59	6	136	45	51	340	0.323	0.682
Sa3	89	23	15	8	2	11	79	1	26	127	0.121	0.803
Sa4	29	30	16	11	37	1	5	21	0	75	0.071	0.874
Sa5	21	1	0	0	0	0	0	0	22	22	0.021	0.895
Sa6	7	2	7	2	14	0	0	0	0	16	0.015	0.910
Sa7	4	8	2	1	9	1	0	3	0	14	0.013	0.923
Sa8	7	2	0	0	0	0	1	0	8	9	0.009	0.932
Sa9–Sa44	52	9	10	7	8	0	14	8	34	71	0.068	1.000
Total	762	175	115	72	129	67	544	78	162	1052		
Genotype number	34	15	16	12	12	6	10	10	19	44		
GGD	0.04	0.09	0.14	0.17	0.09	0.09	0.02	0.13	0.12	0.04		
e^H	5.42	5.38	6.08	4.37	4.66	2.56	2.99	3.66	8.59	6.21		

GGD = gross genotypic diversity; e^H = Shannon–Weaver genotypic diversity.

Table 4 Observed heterozygosities, exact test for the homogeneity of genotypic frequencies with respect to HW equilibrium, and F-statistics for the microsatellite loci studied in the 44 Chilean genotypes of *Sitobion avenae*

Locus	*Observed heterozygosity*	F_{IS}	*Heterozygote excess (P-value)*	*Heterozygote deficit (P-value)*
S3.R	0.136	0.719	1.000	<0.001
Sm11	0.750	−0.372	0.846	0.154
S5.L	0.909	−0.564	<0.001	1.000
Sm17	0.956	−0.556	<0.001	0.999
Sm10	0.591	0.009	0.443	0.601
Multilocus	0.668	−0.184	0.786	0.234

Table 5 Frequency distribution analysis of the most common multilocus genotypes of *Sitobion avenae* considering the interaction between year and zone of collection, and the host plant

Multilocus genotype	*Year*	*Zone*	*Plant*	*Interactions*
Sa1	**	**	**	NS
Sa2	**	NS	*	Plant–year (**)
Sa3	**	NS	NS	NS
Sa4	**	NS	**	Plant–year (**)

*$P<0.05$; **$P<0.005$; NS = nonsignificant.

genotypes were also the most common in France at each of the five microsatellite loci.

Comparing microsatellite profiles of the most common genotypes in the two countries at 11 loci showed that Sa1 shares exactly the same 11-locus genotype with G6, the third most common clone in the French sample studied by Haack *et al* (2000), indicating they derive from the same clone (Table 6). When the four loci in common with other genotypic surveys of *S. avenae* and the present study were considered (*Sm10*, *Sm11*, *Sm12* and *Sm17*), none of the four most common Chilean clones had a multilocus profile matching exactly that of either British (Sunnucks *et al*, 1997; Llewellyn *et al*, 2003, 2004) or other French (Simon *et al*, 1999a) genotypes.

Discussion

Chilean populations of *S. avenae* encompass a few heterozygous multilocus genotypes

In contrast to reports for European populations (Sunnucks *et al*, 1997; Simon *et al*, 1999a; Haack *et al*, 2000; Llewellyn *et al*, 2003, 2004; Papura *et al*, 2003), *S. avenae* populations in Chile showed a very low clonal diversity, independent of the host plant, geographic zone, or sampling season. As compared with the 4% of genotypic

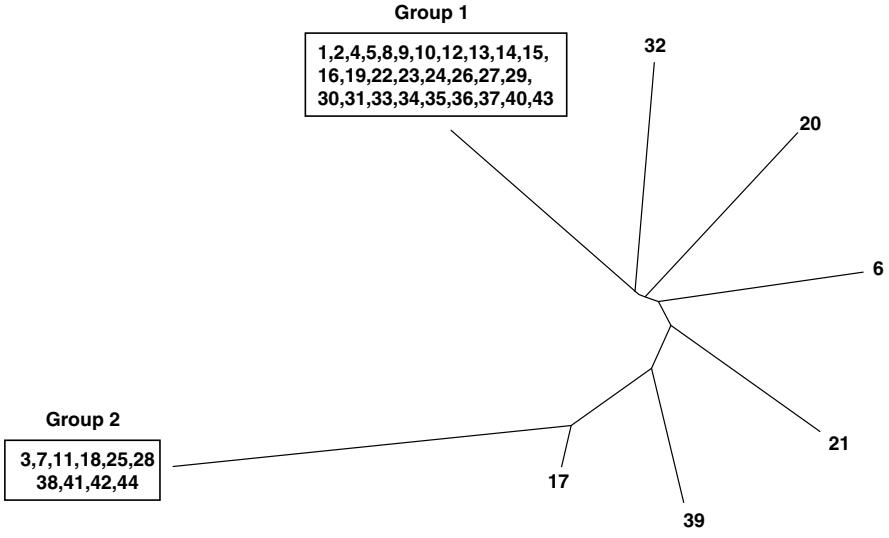

Figure 2 Neighbour-joining tree based on Goldstein's distance (Goldstein *et al*, 1995) calculated with five microsatellite loci for the 44 multilocus genotypes discriminated in Chilean populations of *Sitobion avenae*.

Table 6 Comparison between some of the most common genotypes of *Sitobion avenae* in Chile and France at 11 microsatellite loci

Genotypes	*Microsatellite locus*										
	Sm10	*Sm11*	*Sm17*	*Sm12*	*S5.L*	*S3.R*	*R5.10*	*S16b*	*S17b*	*S3.43*	*S30*
In Chile											
Sa1	164/166	144/149	178/179	169/169	225/227	337/337	272/272	162/176	199/199	185/185	164/164
Sa2	164/166	144/149	178/179	151/157	223/227	337/337	268/272	190/190	215/217	185/193	164/166
Sa3	164/166	144/149	178/179	139/151	223/227	361/361	272/272	210/214	205/209	185/193	164/166
Sa4	164/164	144/149	178/179	151/165	223/227	337/337	268/272	190/190	215/217	185/193	164/166
In France											
G2	164/166	144/149	178/179	165/165	225/227	345/361	268/272	150/206	199/205	185/185	164/166
G5	164/166	144/148	178/179	151/151	223/225	337/337	272/272	162/176	199/217	185/186	164/166
G6	164/166	144/149	178/179	169/169	225/227	337/337	272/272	162/176	199/199	185/185	164/164

diversity found in this work, Haack *et al* (2000) and Llewellyn *et al* (2003) have reported 26 and 46% of genotypic diversity (obtained with the same number of microsatellite markers), in French and British populations, respectively: about 10-fold more genotypic diversity than in this apparently asexual population (see below) of *S. avenae* in Chile. This low genetic diversity in Chilean populations could be the result both of their recent introduction (Apablaza, 1974) through founder effects, and/or of their absence or rarity of sexual reproduction among Chilean populations, as already proposed for other *Sitobion* species introduced to Australia (Sunnucks *et al*, 1996; Wilson *et al*, 1999).

The low genetic diversity of *S. avenae* in Chile was accompanied by an extremely high level of heterozygosity (>99% at some loci), which could have three complementary explanations. Firstly, the genotypes that became established may have been continuously parthenogenetic since their introduction because of the mild climate prevalent in areas where cereal crops are cultivated in Chile. After many rounds of parthenogenetic generations, *S. avenae* populations in Chile could have accumulated high levels of heterozygosity through allelic divergence at neutral nuclear loci, as previously proposed for other asexual lineages of aphids (for *Rhopalosiphum padi* see Simon *et al*, 1999b; Delmotte *et al*, 2001, 2002; for *Myzus persicae* see Vorburger *et al*, 2003; for *S. avenae* and other related species see Wilson *et al*, 1999, 2003; Papura *et al*, 2003). Alternatively, it has been found recently that asexual lineages of aphids can arise from hybridization: either between two distinct parental species or between differentiated lineages of the same species (Delmotte *et al*, 2002, 2003; Simon *et al*, 2003). *S. avenae* genotypes introduced to Chile could have such hybrid origin(s) that arose in their source populations that would readily explain their high heterozygosity. Finally, an array of genotypes with varying heterozygosity could have been introduced to Chile and selection then favoured heterozygous combinations due to heterosis or associative overdominance. The discrimination between these three hypotheses requires deeper information on Chilean genotypes obtained at allele sequence level along with heterozygosity-fitness correlation assessment.

Predominance of genetically related asexual lineages of *S. avenae* in Chile

Reproductive mode variation in aphids is strongly determined by climatic factors because of the existing link between sexual reproduction and the production of eggs resistant to frost (Simon *et al*, 2002). As a result, cyclically parthenogenetic genotypes tend to be more abundant in areas with cold winter while the opposite seems to apply to obligately parthenogenetic genotypes. Aphids were sampled mainly in valleys of central Chile, where climate is characterized by warm winters with no snowfall (Novoa *et al*, 1989). These environmental conditions would favour the prevalence of permanent parthenogenesis over cyclical parthenogenesis (Rispe *et al*, 1998; Dedryver *et al*, 2001). Since *S. avenae* populations were characterized by low clonal diversity with a few time-persistent genotypes, and a high heterozygosity for the markers studied, these genetic features provide evidence for a predominance of asexual reproduction in Chile. However, the possibility that some *S. avenae* genotypes have rare events of sexual reproduction in Chile cannot be excluded. Laboratory observations have revealed that some Chilean *S. avenae* genotypes still possess the ability to produce sexual individuals when they are placed at low temperatures and short day conditions (Dedryver *et al*, unpublished data). This was also the case for *Sitobion* aphids in Australia that reproduced asexually in the field but retained, at least for some clones, the capability of sexual reproduction in the laboratory (Wilson *et al*, 1999, 2003). Therefore, if genotypes still capable of sexual reproduction are present in colder regions of Chile (eg higher altitudes, austral territories) than those surveyed here, they may regularly generate recombined genotypes that could migrate and admix with the bulk of asexual clones. A phenotypic assessment of reproductive modes of Chilean genotypes of *S. avenae* is needed to explore in details the possibility of sexual events in Chile.

The analysis of genetic relationships among genotypes revealed two main groups clustering most genotypes (38 out of 44). Since genetic relatedness among genotypes belonging to the same group is very high, and assuming that obligate parthenogenesis is the primary mode of reproduction, this pattern suggests that groups 1 and 2 represent two different origins of introduction followed by genotypic diversification through mutation (Wilson *et al*, 2003). For example, in group 1, genotype Sa2 differed from Sa4 by only two alleles ($Sm10^{166}$ and $Sm12^{157}$) out of 22, strongly suggesting that they have diverged through mutations after being introduced to Chile. Clones that were at low frequency at the beginning of the survey (eg Sa5 and Sa8) could have acquired ecological traits (by mutation assuming the absence of recombination) that increased subsequently their fitness leading to an abrupt increase in frequency during the following years (Sunnucks *et al*, 1998). On the other hand, the remote position of the remaining six genotypes on the tree could be explained in several ways. For instance, they could originate from (i) supplementary introduction events that are continuously occurring from neighbouring or remote countries, (ii) scarce recombination episodes (see above) or (iii) gene flow from plants that have not been sampled in this survey. Alternatively, mutations could be producing new genotypes at a high rate, accounting for those unrelated genotypes, and for those genotypes that suddenly appeared in this 4-year study.

Broad ecological tolerance of common Chilean genotypes of *S. avenae* and the hypothesis of 'superclones'

Contrasting with previous works reporting host specialization in *Sitobion* (De Barro *et al*, 1995; Sunnucks *et al*, 1997, 1998; Lushai *et al*, 2002), none of the most common Chilean genotypes was strictly associated with any of the five host plants considered in the present study. This latter observation is in agreement with results obtained in France, where two genotypes of *S. avenae* were mainly found on maize but also occurred on other species of cereals (Haack *et al*, 2000). However, some Chilean genotypes showed significant differences in their frequency distribution between host plants. This could result from variation in performance and/or preferences among Chilean genotypes of *S. avenae*, some being more

specialists (eg Sa1, Sa2 and Sa4) and other being more generalist (Sa3) in character. Plant chemical defences such as hydroxamic acids (Niemeyer and Pérez, 1995) have been proposed as modulators of genetic variability in *S. avenae* populations (Figueroa *et al*, 2002). Recently, we have shown that the most common genotypes of Chilean *S. avenae* differ in their performance and survival when reared on oat (no defences) and wheat cultivars with different levels of hydroxamic acids (Figueroa *et al*, 2004). For example, Sa1 performed better on defended plants while Sa2 performed equally on plants with low and high hydroxamic acids. Thus, the extreme abundance of these two genotypes across Chile could be the result of clonal selection promoting the emergence of 'superclones' (Llewellyn *et al*, 2003; Vorburger *et al*, 2003), which would be characterized by a broad host range and a low variance in their performances on host plants with different defence chemicals levels. The 100% of identity at 11 microsatellite loci between the widespread Sa1 (Chilean) and G6 (French) genotypes gives additional support to the existence of 'superclones' in *S. avenae*. Further work is needed to check whether the Sa1/G6 genotype has an even broader distribution, as observed for a genotype of the aphid *Myzus antirrhinii* which is distributed across several continents and feeds on a wide range of host plants (Wilson *et al*, 2003).

Concluding remarks and future directions

In this paper, we presented the population genetic properties of the introduced aphid pest *S. avenae* in Chile, featured by a markedly low genetic variability, with a few genotypes having low plant preferences. This lack of genetic diversity in Chile is likely to result from the recent introduction of a few asexual genotypes. Some of these introduced genotypes seem to have achieved tremendous ecological success in a short period of time. However, clonal turnover may be very rapid as observed during this 4-year survey.

It is surprising that despite their lack of genetic variation, populations of *S. avenae* were able to successfully colonize different cultivated and wild host plants throughout different agroclimatic zones along hundreds of kilometres in Chile. This paradox of successful introductions of genetically poor invaders is not new. Although reductions in genetic diversity are generally considered detrimental, the Argentine ant, *Linepithema humile*, constitutes an example of how a genetic bottleneck can precede widespread ecological success when they invade a new niche (Tsutsui *et al*, 2000, 2001). However, aphids, by their asexual mode of reproduction (either partial or complete), their high dispersal capacities through direct flights or trade exchanges and the existence of 'superclones' seem to be particularly well suited to rapid conquest of new habitats.

Additional work is needed to get a clearer understanding on the origin and dynamics of this introduced pest species, which is also a requirement to design appropriate management strategies. Detailed studies should be conducted to identify the source(s) of populations of *S. avenae* introduced to Chile and to explain the biological and genetic reasons for the ecological success of introduced genotypes. It will also be intriguing to follow the fate of the clones that are currently have wide geographic and ecological ranges into the future, to establish whether there is evidence of an increased role for niche specialization and recombination as evolution proceeds in the novel Chilean environment.

Acknowledgements

This work was funded by FONDECYT Grants 2000060 and 3020051 to CCF, the Presidential Chair in Sciences awarded to HMN, and ECOS-CONICYT Grant C96B04. We thank JP Gauthier and Y Outreman for helping with statistical analyses, and two anonymous reviewers who provided valuable comments on the manuscript.

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