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Host range expansion of an introduced insect pest through multiple colonizations of specialized clones

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Abstract

Asexuality confers demographic advantages to invasive taxa, but generally limits adaptive potential for colonizing of new habitats. Therefore, pre-existing adaptations and habitat tolerance are essential in the success of asexual invaders. We investigated these key factors of invasiveness by assessing reproductive modes and host-plant adaptations in the pea aphid, *Acyrthosiphon pisum*, a pest recently introduced into Chile. The pea aphid encompasses lineages differing in their reproductive mode, ranging from obligatory cyclical parthenogenesis to fully asexual reproduction. This species also shows variation in host use, with distinct biotypes specialized on different species of legumes as well as more polyphagous populations. In central Chile, microsatellite genotyping of pea aphids sampled on five crops and wild legumes revealed three main clonal genotypes, which showed striking associations with particular host plants rather than sampling locations. Phenotypic analyses confirmed their strong host specialization and demonstrated parthenogenesis as their sole reproductive mode. The genetic relatedness of these clonal genotypes with corresponding host-specialized populations from the Old World indicated that each clone descended from a particular Eurasian biotype, which involved at least three successful introduction events followed by spread on different crops. This study illustrates that multiple introductions of highly specialized clones, rather than local evolution in resource use and/or selection of generalist genotypes, can explain the demographic success of a strictly asexual invader.

Keywords: aphids, asexuality, biological invasions, Buchnera, host specialization, legumes

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Introduction

Explaining differences in invasive success among species is a challenge for evolutionary and ecological theories (Sakai *et al*. 2001; Suarez & Tsutsui 2008), as well as for managing biological invasions (Kolar & Lodge 2001). Invaders may rely on genetic diversity, provided by large founding populations or multiple introductions, and permitting local adaptation (Kolbe *et al*. 2004; Dlugosch & Parker 2008; Suarez & Tsutsui 2008). In addition, invasive success depends on key traits that may already exist in source populations, such as niche breadth, phenotypic plasticity and reproductive mode (Facon *et al*. 2006). In particular, uniparental reproduction is often observed in areas of introductions or marginal environments (Glesener & Tilman 1978) for species showing variation in reproductive mode. Asexuality indeed circumvents the demographic cost of male production, the need to locate mates and inbreeding depression likely to affect initially small introduced populations (Frankham 2005). These demographic advantages however, contrast with the limited evolutionary potential usually associated with asexuality. It is thus expected that this reproductive mode benefits introduced populations that were already adapted to their new habitat (Facon *et al*. 2006) and that widespread clonal genotypes display broad ecological niche (Lynch 1984).

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Asexual taxa with clearly defined ecological ranges can provide empirical support for these predictions.

Aphids evolved intricate relationships with their host plants resulting in host specialization at both species and population levels (Dixon 1998) in combination to diverse reproductive strategies (Moran 1992; Simon *et al*. 2002). These range from lineages practicing cyclical parthenogenesis (hereafter named 'sexual' lineages) that yearly produce recombinant and frost resistant eggs, to fully asexual viviparous clones that do not thrive at freezing temperatures (Powell & Bale 2008). Strict parthenogenesis has accompanied the invasion of many introduced aphid species in warm regions throughout the world, leading to the spread of clonal lineages over wide areas and long timescales (Vorburger *et al*. 2003; Fuentes-Contreras *et al*. 2004; Figueroa *et al*. 2005). If these invasive clones feeding on many host plants seem to present broad ecological tolerance (Figueroa *et al*. 2005; Vorburger 2006), it is unclear whether invasive asexual aphids expanded their host ranges (Funk & Bernays 2001), or already displayed high plasticity in host use in their source populations (Figueroa *et al*. 2005).

The pea aphid, *Acyrthosiphon pisum* Harris (Homoptera: Aphididae), is a worldwide pest of palearctic origin (Eastop 1971) exhibiting host-specialized populations on different species of legumes (Fabacae) in its native range (Müller 1971; Ferrari *et al*. 2006). In particular, populations on pea and broad bean, red clover and alfalfa constitute three distinct biotypes, well characterized on a phenotypic and genetic basis (Sandström 1994; Simon *et al*. 2003; Bournoville *et al*. 2004; Frantz *et al*. 2006a). Specialized populations are also found on alfalfa and red clover in eastern USA (Via 1991, 1999) where this aphid was first reported on the continent 130 years ago (Thomas 1878). Despite their strong host specialization, pea aphid biotypes readily hybridize in the laboratory, producing genotypes of intermediate fitness (Müller 1971; Via *et al*. 2000). In addition to polyphagous populations found in California (Leonardo & Muiru 2003), this suggests that host specificity may evolve in *A. pisum*. This species also encompasses various reproductive modes, with 'sexual' lineages occurring in both continents (Eastop 1971; Via 1992; Mackay *et al*. 1993) and complete asexuality reported in warmer regions (Smith *et al*. 1999; Frantz *et al*. 2006b). The pea aphid thus presents variations in two key traits likely affecting its high invasive potential, that is, reproductive mode and ecological specialization. We investigated both traits in recently introduced Chilean populations, first reported only 40 years ago and infesting several legume crops (Rojas 2005), asking the following questions. Are introduced Chilean populations of the pea aphid genetically and ecologically similar to specialized populations found in its native range or do they display broader host specificity? Did multiple introductions and asexual reproduction contribute to the invasion process? To address these issues, we assessed the genetic diversity of Chilean populations of the pea aphid sampled on a range of host plants in various regions, using microsatellite markers. We also characterized the reproductive mode and the degree of host specialization of several representative genotypes. Finally, the assessment of genetic relatedness of Chilean populations with reference populations from Europe found on the same host plants allowed us to infer an evolutionary scenario underlying the invasive success of *A. pisum* in central Chile.

Materials and methods

Sampling of pea aphids

Chilean populations of *Acyrthosiphon pisum* were sampled on five crops: alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), pea (*Pisum sativum* L.), broad bean (*Vicia faba* L.) and lentil (*Lens culinaris* Medikus). As a rough estimation of the genotypic composition of *A. pisum* on uncultivated plants, we occasionally collected individuals on wild legumes in various locations: *Vicia sativa* L., *V. villosa* Roth, *V. benghalensis* L., *Lathyrus latifolius* L. and the endemic *Lathyrus subandinus* Phillipi and *Astragalus germainii* Phillipi. In order to distinguish between geographical and ecological (host plant) effects on genotypic variation, crop fields were sampled in three locations along a 570-km latitudinal transect consisting of three main localities: Talca (35°26′S, 71°40′W), Temuco (38°46′S, 72°38′W) and La Unión (40°19′S, 72°58′W) (see Table S1, Supporting information for details). Within each location, sampling points (crop fields) were separated by less than 10 km. Samplings took place between September and November, from years 2005–2007. At this season, all individuals were parthenogenetic females irrespective of the reproductive mode of their lineage (i.e. 'sexual' or asexual). One wingless adult aphid was selected per sampled plant (nymphs were taken in rare instances) and the distance between surveyed plants was at least 2 m, maximizing chances that all individuals originated from separate clonal colonies. Aphids were stored in 96% alcohol until DNA extraction. Thirty-five live individuals collected in Temuco in 2005 from pea, broad bean, alfalfa and red clover initiated parthenogenetic lines (hereafter referred to as 'laboratory clones') for laboratory tests (see below). Rearing took place in a climatic chamber at 18 °C and a photoperiod of 16 h on broad bean, a plant suitable for all known biotypes of *A. pisum* (Müller 1971).

DNA extraction and genotyping

Highly polymorphic loci allow to identify repeated multilocus genotypes (MLG) resulting from asexual reproduction (Sunnucks *et al*. 1996), and their possible associations with ecological or geographical factors. We

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genotyped individuals at seven microsatellite loci (Caillaud *et al*. 2004): *AlA09M*, *AlB07M*, *AlB08M*, *AlB12M*, *ApF08M*, *ApH08M* and *ApH10M*. They were among the most variable microsatellites available for the pea aphid, as estimated on French populations (Frantz *et al*. 2006a).

Aphid total genomic DNA was extracted in semideep well trays following the salting-out protocol (Miller *et al*. 1988) then suspended in 120 µL of ultrapure water. Loci were amplified simultaneously with fluorescent-labelled primers in single 7.5-µL polymerase chain reactions (PCR). The mix included approximately 100 ng of aphid DNA, 0.25 U of *Taq*, PCR buffer at 1× final concentration, 3 mM of $MgCl_2$ (all products from Promega), 0.1 mM of each dNTP, 60 nmol of primer for loci *AIA09M*, *AIB07M*, *ApF08M*, *ApH10M* and 96 nmol of primers for the other loci. Amplification steps were an initial denaturation at 95 °C for 2 min, followed by 35 cycles consisting of a denaturation step at 95 °C for 45 s, an annealing step at 56 °C for 45 s and an elongation step at 72 °C for 1 min. The last elongation was extended for 1 h. Diluted PCR product (0.5 µL of 7×) was added to 10 µL of high-die formamide containing 0.7% of 500 LIZ DNA ladder (Applied Biosystems). Electrophoresis of amplified fragments was carried out in a capillary sequencer ABI PRISM 3130xl (Applied Biosystems), following the instrument protocol GS POP7 DS33. Allele calls were automatically assigned by GeneMapper (version 3.5 and 3.7, Applera Corp.) and visually checked.

Assessment of genotypic diversity and reproductive mode

We followed the procedure recommended by Halkett *et al*. (2005) to infer the reproductive mode of Chilean populations of the pea aphid. Genotypic diversity was estimated by $(G - 1)/(N - 1)$ (Dorken & Eckert 2001), where G is the number of different multilocus genotypes and N the number of genotyped individuals. Linkage disequilibrium between loci (LD) was tested performing 1000 permutations, both on the whole genetic data set and considering only one individual per MLG, in Genetix version 4.05 (www.genetix.univ-montp2.fr/). Deviation from expected heterozygosity under Hardy–Weinberg expectations (F_{IS}) was tested using permutations in Genetix, considering only one individual per MLG. One thousand permutations were carried out. We also estimated the probability of genotypic identity, that is, the probability that two sampled individuals sharing the same multilocus genotype come from sexual reproduction events. We computed the lower bound of this probability: $P_{(ID)unbiased}$, under Hardy–Weinberg expectations, and its upper bound $P_{(ID)sib}$, under strict sibs reproduction, as recommended by Waits *et al*. (2001).

The sexual generation in aphids is triggered by photoperiodic changes occurring by the end of summer and beginning of autumn (i.e. increasing night length), which would occur from March to May in the sampled area. To assess the reproductive mode of Chilean populations on a phenotypic basis, three laboratory clones bearing the three commonest genotypes found in Chile (Ap1, Ap2 and Ap3, see 'results') were placed under conditions that mimic autumnal nigh length. Following Frantz *et al*. (2006b), three parthenogenetic females of each clone were isolated on broad bean plants and exposed to a 12-h photoperiod at 16 °C, simulating autumnal day length. Their progeny was surveyed for two generations, and individual morphs (i.e. parthenogenetic viviparous females, sexual oviparous females or males) were visually determined.

Assessment of host adaptation

To visually estimate the effect of host plants on the genetic structure of Chilean *A. pisum*, the relatedness between microsatellite genotypes was measured by their allele shared distance (Chakraborty & Jin 1993) computed with the software Population version 1.2.3 (http://bioinformatics.org/~tryphon/populations/) and plotted as a neighbour-joining tree (Saitou & Nei 1987) using TreeView version 1.6.6 (taxonomy.zoology.gla.ac.uk/rod/treeview.html). We performed 1000 bootstrap replicates to assess the robustness of the branches.

We evaluated the effects of the sampling plant, location and year on the proportions of the three most common MLGs (90% of the sample) collected in each field with a generalized linear model (GLM). The corresponding multinomial model is equivalent to a log-linear model where observations within categories (here the three genotypes) are treated as independent Poisson variables conditionally to the sample size (McCullagh & Nelder 1989). This is achieved by introducing an extra factor accounting for each sample (here, the fields). Categories of the multinomial response are then included in the model as a factor (here, genotype), and the tested effects (plant and location) appear as interactions with this factor. The model was fitted and its deviance components analysed under R version 2.5 (www.r-project.org/).

Laboratory clones harbouring seven different MLGs (Ap1, Ap2, Ap3, Ap4, Ap7, Ap11, Ap12, representing 98% of the sampled individuals, see Results), were retained for tests of host-plant specificity. These tests consisted of measurements of larval survival and fecundity on pea, alfalfa and red clover. Plants measured approximately 25 cm, and were 2 years old, except the fast-growing pea (1 month). Before the tests, clones were maintained separately at low density on broad beans for three generations: adults were removed once they had left a dozen of offspring on each plant, in order to avoid any crowding effect that might affect performance. On each test plant, 10 first instar nymphs of each clone, born during the night before the test, were settled (day 0). The plant was then wrapped in a cellophane sachet and kept under rearing conditions (18 °C and a 16-h

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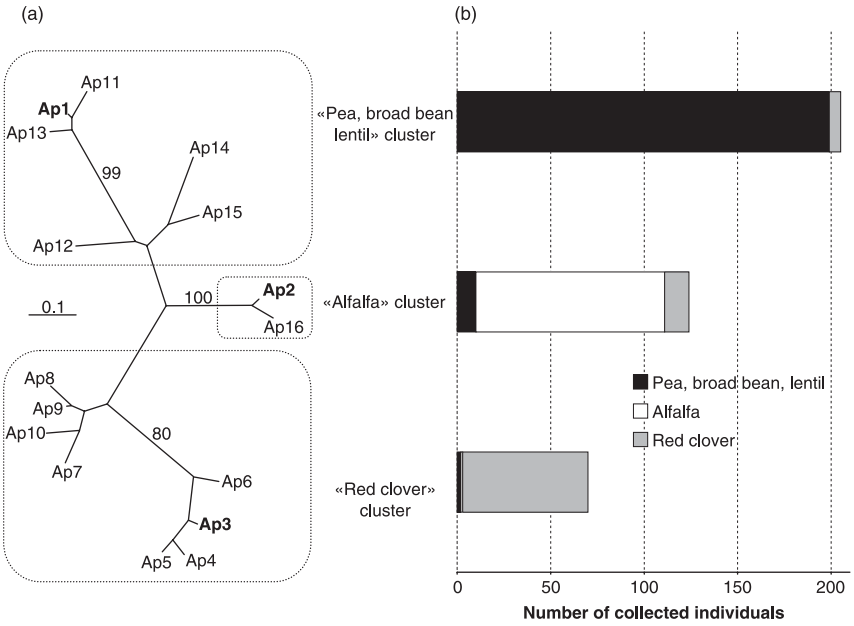

Fig. 1 (a) Genetic relatedness between 16 multilocus genotypes constituting Chilean populations of the pea aphid, unrooted NJ tree based on their allele shared distance at seven microsatellites. Numbers along main branches are bootstrap values above 70% (1000 samplings). The three most frequent genotypes (90% of the sample) are boldfaced. (b) Distribution of individuals belonging to the three groups of genotypes framed on the tree on the sampled crops (see also Table 1). For better readability, aphid distributions on pea, lentil and broad bean were considered together because they did not significantly differed (see Table 2b).

photoperiod). At day 8, surviving individuals were counted and five of them (or fewer, depending on survival rate) were kept on the plant. At day 19, all aphids on the plant were counted, as an estimate of fecundity. At this time, the offspring of focal parthenogenetic females were not mature, ensuring no confusion between the two generations. Three replications for each clone on each test plant were set in the same climate chamber, for a total of 63 tests. Effects of the plant, the clone and their interaction on larval survival at day 8 and colony size at day 19 were analysed using GLMs. To account for overdispersion, we used a quasibinomial distribution for survival data and a quasipoisson distribution for count data. Because tested clones appeared to belong to three genetic clusters having distinct host ranges (see results, Fig. 1b), the factor 'clone' was nested under a factor 'cluster' in the analysis. This allowed comparing variations in host specialization between and within clusters. In the analysis of colony size, the number of aphids left on the plant at day 8 was included as a first covariate. Once this variable fitted, the residual deviance in colony size was no longer conditioned by larval mortality, but mainly fecundity. GLMs were fitted, and their deviance analysed under R.

Test for multiple introductions in Chilean populations of the pea aphid

We tested the hypothesis of several introductions in Chilean pea aphids by assessing their genetic relatedness with European populations feeding on the same crops. We used a cytoplasmic marker, the primary endosymbiont of aphids, *Buchnera.* This symbiont shows strict maternal transmission (Clark *et al*. 2000; Funk *et al*. 2000) and greater polymorphism in some of its intergenic regions compared to mitochondrial genes conventionally used in intraspecific molecular phylogenetics (N. Moran, University of Arizona, personal communication). We analysed variation at the spacer between *groEL* and *efp* (corresponding to a pseudogene of *yjeK*), amplified in six of the Chilean laboratory clones and 21 French genotypes sampled on pea, alfalfa and red clover in 2006. We used primer 5′-GGCATGGGTGGAATG-ATGTA-3′ and primer 5′-TTCTATCAAACACGGCTCGTT-3′ to amplify a 760-bp fragment. The 30 μL PCR mix included approximately 200 ng of genomic DNA, 2 U of *Taq*, PCR buffer (1×), 75 nmol of $MgCl_2$, 3 nmol of each dNTP and 30 pmol of each primer. Amplification consisted in an initial denaturation at 94 °C for 4 min, followed by 30 cycles consisting of a denaturation step at 94 °C for 30 s, an annealing step at 60 °C for 30 s and an elongation step at 72 °C for 1 min. The last elongation was extended for 5 min. PCR products were purified using Sephadex G-50. Both strands were sequenced in an ABI PRISM 3730xl by Macrogen Inc. (Korea). Complete sequences were assembled from traces files with Staden package version 1.6 (http://staden.sourceforge.net/) and were aligned with BioEdit version 7.0.9 (Hall 1999). Sequences were deposited in GenBank under accession nos EU627161–EU627164. A statistical parsimony network of *Buchnera* haplotypes was built with TCS version 1.21 (Clement *et al*. 2000).

We also assessed the genetic relatedness between European and Chilean populations at the seven microsatellite loci, using a data set of 380 French and German genotypes sampled on pea, broad bean, alfalfa, red clover, and comprising sexual and asexual lineages (Frantz *et al*. 2006a, b; J. Peccoud *et al*. unpublished data). Combined with the 16 Chilean genotypes, this sample was submitted to a factorial

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Table 1 Distribution of the 16 microsatellite genotypes composing Chilean populations of *Acyrthosiphon pisum* on five crops sampled in three regions and on six wild plants

		Genotype															
Crop	Region	Ap1	Ap2	Ap3	Ap4	Ap5	Ap6	Ap7	Ap8	Ap9	Ap10	Ap11	Ap12	Ap13	Ap14	Ap15	Ap16
Pea	Talca	21										1					
	Temuco	26											2			1	
	La Unión	45	2									1					
Lentil	Talca	9											1				
	Temuco	8											1	1			
	La Unión	24	1			1											
Broad bean	Talca	25	2	1								4		1	1		
	Temuco	20	3										1				
	La Unión	3	2									3					
Alfalfa	Talca		22														
	Temuco		23														1
	La Unión		55	1													
Red clover	Talca	1	9	20		3	2										
	Temuco		4	32	1	1	2	2	2	1	1		5				
Wild plants																	
Astragalus germainii		1		3													
Lathyrus latifolius		3									1						
Lathyrus subandinus			3	2													
Vicia sativa		5	1									1					
Vicia villosa		5															
Vicia benghalensis		6		1								1					
Whole sample		202	127	59	2	5	4	2	2	1	1	10	12	2	1	1	1

correspondence analysis (FCA) implemented in Genetix, then plotted on the graph of the two main factors. This method was preferred over population clustering and assignment analyses, which assume sexual reproduction and were inappropriate because of the dominance of a few asexually reproducing genotypes in the Chilean data set (see Results).

Results

Field prevalence, genetic diversity and characterization of reproductive mode

In all sampled regions and most host plants, pea aphids were abundant, reaching densities > 100 individuals per plant. Although *Acyrthosiphon pisum* exhibits green and pink forms in other regions (Eastop 1971), all individuals from Chile were green. Among 432 individuals, 16 distinct multilocus genotypes, or MLGs, were identified (see Table S2, Supporting information for their allelic composition), resulting in a genotypic diversity ~0.035. Three MLGs (Ap1, Ap2 and Ap3) comprised 90% of the sample; other MLGs were shared by 12 or fewer individuals (Table 1). Significant LD was found for all 21 combinations of loci, considering all individuals and only one individual per MLG. Multilocus F_{IS} was significantly negative, indicating overall excess in heterozygosity. Three loci showed significant negative F_{IS} (AlA09M, AlB07M, and ApH10M), and none showed a significant heterozygote deficit. Assuming independent assortment at meiosis, the probability that two identical genotypes resulted from sexual reproduction was between $3.15*10^{-7}$ [$P_{(ID)unbiased}$] and $2.4*10^{-3}$ [$P_{(ID)sib}$].

When exposed to short photoperiods, the laboratory clone of genotype Ap1 produced 34% of males, the remaining offspring being parthenogenetic females. Clones of genotypes Ap2 and Ap3 produced no sexual forms at all.

Plant specialization in Chilean populations of the pea aphid

Dominant MLGs were found in the three locations, but clearly differed in their host ranges (Table 1). Genotype Ap1 represented 86% of the individuals from pea, broad bean and lentil but was almost never found on other hosts. Ap2 represented 98% of the pea aphids collected on alfalfa and was found at much lower frequency on the four other crops. Genotype Ap3 encompassed 60% of the pea aphids collected on red clover and was found just once on alfalfa and broad bean. Dominant genotypes were also prevalent on wild hosts, and Ap1 was the commonest on uncultivated *Vicia* sp. and *Lathyrus latifolius* (Table 1).

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Table 2 (a) Factors of variations in field abundance of three dominant genotypes of pea aphids sampled in central Chile. Environmental factors impacting the genotypic composition of aphid populations are represented by their interactions with the 'genotype' factor. The 'field' effect here just represents variations in sampling effort between fields. (b) Pairwise comparisons between plants. Above the diagonal: part of deviance explained by the genotype*plant interaction when only the two plants are considered in the model. Below the diagonal: associated *P* value. For both tables: *< 5%, **< 0.1%, NS > 5%.

(a)

Model parameters	d.f.	Deviance	*P* (> Chi)
Null	116		
field	38	20.6%	**
Genotype	2	8.5%	**
Genotype*plant	8	63.7%	**
Genotype*location	4	1.5%	*

(b)

Contrast	Pea	Broad bean	Lentil	Alfalfa	Clover
Pea	—	1.0%	0.0%	48.1%	51.0%
Broad bean	NS	—	1.0%	39.9%	53.4%
Lentil	NS	NS	—	44.1%	55.5%
Alfalfa	**	**	**	—	33.7%
Clover	**	**	**	**	—

The three commonest MLGs shared no or few alleles, but differed in only one or two alleles from less frequent MLGs, resulting in three clades with strong bootstrap support (Fig. 1a and Table S2). Other rare MLGs had weaker genetic relatedness to dominant clones, as more internal branches had low bootstrap values (< 50%, not shown). Less frequent MLGs tended to share the host ranges of their related dominant clone (Table 1), allowing the delineation of three clusters grouping genotypes by their plant origin (Fig. 1b). Accordingly, the 'genotype*plant' interaction explained nearly 64% of the variance in abundance of Ap1, Ap2 and Ap3 (Table 2a), indicating that the sampling plant had a major effect on the genotypic composition in the field. Sampling location also had significant effect, which may partly result from between-year differentiation because some locations were surveyed at different times. However, regardless of its basis, this regional differentiation was negligible in comparison to host-plant differentiation, explaining approximately 1.5% of the model deviance. Contrast analysis (Table 2b) revealed that populations sampled on pea, broad bean and lentil had similar genotypic composition, while genotypic composition differed significantly among aphid populations on other crops.

Consistent with field observations, variations of performance (larval survival and fecundity) mostly reflected host adaptations of aphid genetic clusters (delineated in Fig. 1) rather than differences between particular tested clones, which were insignificant or minor in comparison to the 'cluster*test plant interaction' (Table 3). Specifically, genotypes Ap1, Ap11 and Ap12 performed best on pea, while genotypes Ap3, Ap4 and Ap6 performed best on red clover and genotype Ap2 showed the highest performance on alfalfa (Fig. 2). The test plant alone also greatly affected aphid survival, which we attributed to the detrimental effect of alfalfa on nymphs (except for genotype Ap2). Pea and red clover were less selective at this early stage (results not shown). Larval survival on the most favourable plant was between 80 and 100%, suggesting that handling of nymphs had moderate impact on their survival.

Table 3 Factors of variations in the performance of Chilean pea aphid clones reared on three plant species. Upper half: sources of variations in survival after 8 days on a given plant. Lower half: sources of variations in fecundity up to day 19. The seven tested clones were nested within three genetic clusters, as delineated in Fig. 1(b)

Model parameters	d.f.	Deviance	*P* (> Chi)
Null GLM on larval survival	62		
Cluster	2	8.6%	***
Test plant	2	34.6%	***
Cluster*test plant	4	22.8%	***
Clone *within* cluster	4	1.9%	NS
(Clone *within* cluster)*test plant	8	3.6%	NS
Null GLM on colony size	62		
Number left on plant (day 8)	1	48.0%	***
Cluster	2	2.2%	***
Test plant	2	2.9%	***
Cluster*test plant	4	39.2%	***
Clone *within* cluster	4	0.6%	NS
(Clone *within* cluster)*test plant	8	2.0%	*

Genetic relatedness of Chilean pea aphids to European populations

Sequences of the pseudogene *yjeK* (726 aligned nucleotides) of *Buchnera* showed three polymorphic sites resulting in four haplotypes (Table 4). These haplotypes differed at five other sites from the published sequence of the *Buchnera* genome, obtained from a Japanese pea aphid clone (Shigenobu *et al*. 2000). Chilean and French individuals shared three haplotypes, grouping mostly aphids of the same host origin (Fig. 3). Haplotype 1 was typical of aphids specialized on pea, and haplotype 3 was characteristic of individuals specialized on red clover, in both cases irrespective of their geographical origin. The dominant Chilean MLG on alfalfa (Ap2) shared a haplotype with French individuals from the same plant, but also with one

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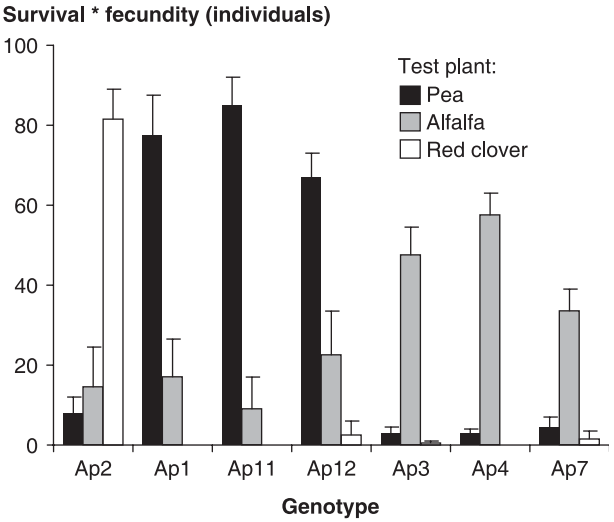

Fig. 2 Performance of seven Chilean laboratory clones of the pea aphid when reared on pea (black bars), red clover (grey bars) or alfalfa (white bars): product of mean larval survival by mean individual fecundity on each test plant, averaged on three replicates. Standard deviation across replicates is shown. Effect of the test plant on larval survival and individual fecundity (when applicable, see method) is significant for all clones.

Table 4 Polymorphism at the pseudogene of *yjeK* in *Buchnera* the primary endosymbiont of *Acyrthosiphon pisum*, among 27 individuals (21 France, 6 Chile). BuAPS refers to the haplotype of the *Buchnera* strain whose genome has been sequenced in *A. pisum*

Position:	146	294	398	462	469	583	619	638
BuAPS	G	—	A	C	T	G	C	T
Haplotype 1	C	A	A	T	T	A	T	C
Haplotype 2	G	A	A	T	G	A	T	C
Haplotype 3	G	A	A	T	T	A	T	C
Haplotype 4	C	A	G	T	T	A	T	C

specialized on pea. Haplotype 4 was found only in French pea aphids originating either from alfalfa or from red clover. Consistently, the FCA individuals' plot (Fig. 4) grouped European and Chilean microsatellite genotypes according to their hosts, although no MLG was shared between the two continents.

Discussion

Asexuality and host specialization in Chilean populations of Acyrthosiphon pisum

Genetic and biological data indicated that pea aphids in Central Chile reproduced mainly, if not exclusively, by strict parthenogenesis. In striking contrast to known 'sexual' populations (Simon *et al*. 2003; Frantz *et al*. 2006a), Chilean

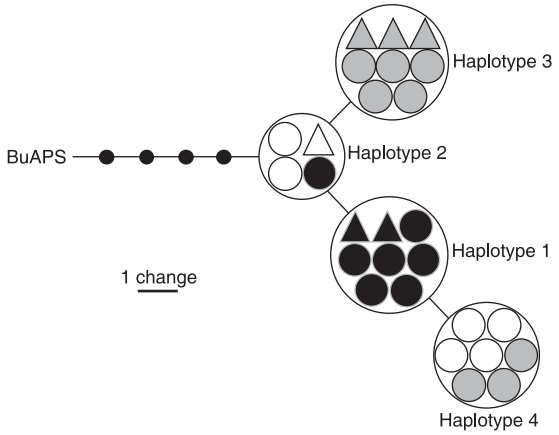

Fig. 3 Parsimony network of *Buchnera* haplotypes found in 21 pea aphids clones from France (circles) and six clones from Chile (triangles) specialized on either pea (black) alfalfa (white) or red clover (grey). Small circles represent undetected haplotypes. BuAPS represents the haplotype of the published *Buchnera* genome from a Japanese strain of *Acyrthosiphon pisum*.

pea aphids displayed a very low genetic diversity at the seven microsatellites and few highly abundant MLGs. This observation is unlikely to result from selection of particular MLGs throughout the year (e.g. Vorburger 2006), as samplings were carried out during spring. Individuals sharing the same MLG had less than 0.2% chance of arising from sexual reproduction. Consequently, we considered individuals of identical genotypes as belonging to the same asexually reproducing lineage, or clone. Accordingly, Chilean *Acyrthosiphon pisum* populations displayed linkage disequilibrium between all loci and negative F_{IS}, even considering one individual per MLG, which are typical signatures of clonality maintained for generations (Bengtsson 2003; Figueroa *et al*. 2005; Halkett *et al*. 2005). Sampling locations and years showed marginal effects on the genotypic diversity of Chilean populations, suggesting the stability of this reproductive mode throughout the surveyed range (570 km) and over time. Laboratory experiments showed that dominant Chilean genotypes were unable to produce sexual females when exposed to stimuli triggering sex induction in other pea aphids. Male production by the most frequent clone (Ap1) under laboratory induction was probably a vestigial trait as our results suggest a lack of females available for mating in the sampled area. In Europe, 'intermediate' pea aphid lineages can combine both reproductive modes by producing parthenogenetic individuals alongside sexual ones in autumn (Frantz *et al*. 2006b). This bet-hedging strategy selected under less predictable winter climates (Halkett *et al*. 2004) was, however, not evidenced in Chilean populations. Even if induction experiments did not fully mimic natural conditions of a gradually shortening photoperiod (Via 1992), the overall congruence of biological and

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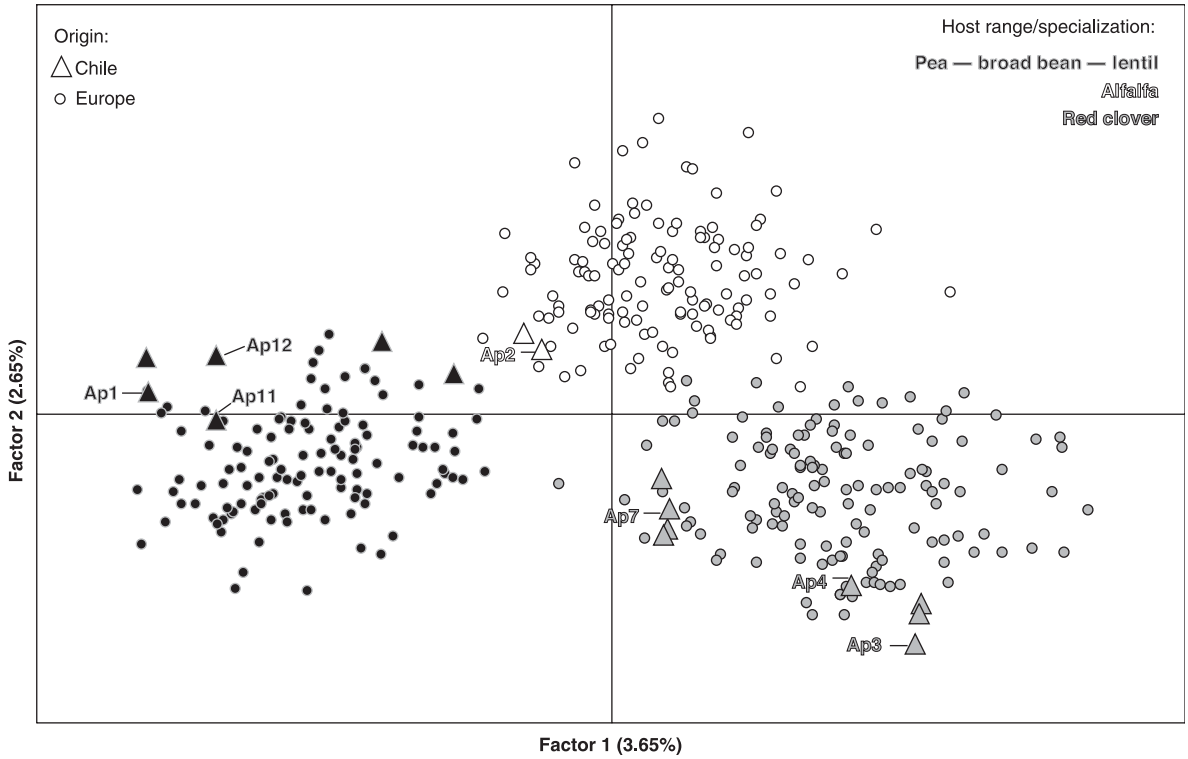

Fig. 4 Factorial correspondence analysis performed on 380 European and 16 Chilean pea aphid genotypes, individuals' plot of the two main factors. Genotypes were thus grouped according to their multidimensional genetic distance at seven microsatellites. For clearer visualization, only the Chilean genotypes whose plant specialization has been tested were named on the plot. Untested Chilean genotypes were assigned to the host range of their genetic group (see Fig. 1b).

genetic data strongly suggests the dominance of strict asexual reproduction in Central Chile for *A. pisum*.

Chilean pea aphids also appeared specialized onto different host-plants. Their genotypic composition and population structure were better explained by their collection plants than their geographical origins. Individuals from a given host-plant predominantly had one prevalent genotype or one of its close relatives. However, aphids from pea, broad bean and lentil did not significantly differ in their genotypic composition, suggesting that genotypes associated with legumes of the Vicieae tribe (pea, broad bean, lentil as well as introduced *Vicia* sp. and *Lathyrus latifolius*) belong to the same host specialized population. As a result, we identified three distinct genetic clusters; each specialized on alfalfa, red clover or the Vicieae. Consistency of this genetic clustering with host specialization of laboratory clones strongly suggests the existence of three host-specialized biotypes in Chilean populations of *A. pisum*.

Independent colonizations at the origins of Chilean populations of A. pisum

With the exception of lentil for which specialization by *A. pisum* has not been investigated in Europe, Chilean biotypes were very close to European ones in their host range (Frantz *et al*. 2006a) and degree of specialization (Bournoville *et al*. 2004). Furthermore, shared host plants best explained the genetic relatedness of sampled genotypes even between the two continents. European and Chilean biotypes on the same plants usually shared specific haplotypes of their primary endosymbiont *Buchnera* and their relatedness extended to nuclear markers. Common ancestry of European and Chilean biotypes, rather than parallel specialization on the same plants, is the more likely explanation for this pattern. Given the recent record of the pea aphid in Chile (Rojas 2005), and more broadly in America (Thomas 1878), common ancestors of Chilean and European biotypes would locate in Eurasia, covering the native range of *A. pisum* (Eastop 1971). In this scenario, Chilean populations of the pea aphid are likely to descend from three Eurasian ancestral biotypes that were already specialized on the sampled crops. At least three introductions of *A. pisum* to Chile were followed by the spread of asexual clones representing the three prevalent MLGs. The actual number of successful introductions of *A. pisum* into Chile that could account for observations cannot be firmly estimated. The clonal evolution of three specialized lineages, involving mitotic mutations at microsatellites, could

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explain the clusters of closely related genotypes within the three Chilean biotypes, as well as their strong LD and excess in heterozygosity (Figueroa *et al*. 2005; Halkett *et al*. 2005). This would imply a single successful introduction of an alfalfa-specialized parthenogenetic female, followed by diversification through mutations, leading to two genotypes differing at one allele. The two distinct subgroups of genotypes associated with red clover (Fig. 1a) could result either from two separate introductions followed by clonal evolution, the local diversification of a single clone and/or rare events of sexuality. The extent of genetic divergence between these two clover-associated lineages argues for the former hypothesis (Sunnucks *et al*. 1996), although separate introductions may not trace back to Eurasia. The divergence of the two clover-associated lineages may have occurred within America, before their introduction to Chile. The genotypic composition of the biotype specialized on pea, broad bean and lentil does not allow clear inference regarding the number of introductions of pea aphids on these crops.

Factors of successful introduction and invasiveness in A. pisum

The three independent invasions of Chilean crops by strictly clonal lineages of *A. pisum* evidenced here suggest higher invasiveness of asexual over sexual pea aphid lineages in this environment, even though the introduction and presence of 'sexual' lineages in Chile remains undetermined. Some of the general advantages conferred by asexuality likely apply to pea aphids, such as the avoidance of producing males and of inbreeding depression affecting genetically deprived populations (Frankham 2005). More importantly, because asexual aphid lineages do not produce diapausing winter eggs, they enjoy a much higher population growth rate than sexual lineages in climatic zones where their survival is allowed. Their prevalence in central Chile is in line with theoretical expectations of asexuality in aphids being selected under mild winter climate (Rispe & Pierre 1998) and with observations of other introduced aphid species in Chile and Australia, where aphid populations consist of a few clones (Vorburger *et al*. 2003; Fuentes-Contreras *et al*. 2004; Figueroa *et al*. 2005).

Advantages conferred by clonal reproduction are potentially balanced by a lower evolvability, which may particularly affect the surveyed populations. In contrast to other asexual invasives that can alternate with boots of sexuality, like many weeds (Baker 1974), freshwater snails (Facon *et al*. 2005) or water fleas (Mergeay *et al*. 2006), strictly asexual aphid lineages seem purely clonal, and would have lost all ability to genetically recombine (Wilson & Sunnucks 2006). Additionally, dominant Chilean clones of the pea aphid did not show greater tolerance in host use than known specialized populations, suggesting that their invasive potential may heavily depend on ecological constraints present in source populations, such as genetic trade-off preventing the evolution of polyphagous genotypes (Hawthorne & Via 2001), rather than adaptations during the invasion process. In this view, highly specialized parasites colonizing new regions while maintaining their hosts may not face new selective pressures, but on the opposite may enjoy reduced antagonistic biotic interactions (Glesener & Tilman 1978). One may thus expect specialized parasites to particularly benefit from asexuality during invasions.

Despite their expected low evolutionary potential, clonal lineages of *A. pisum* acquired endemic Chilean plants, *Lathyrus subandinus* and *Astragalus germainii*, as new hosts during the invasion. This apparent host range expansion may just result from pre-existing adaptations to using related plants, as other species of *Lathyrus* and *Astragalus* harbour the pea aphid in its native range (Eastop 1971). It still raises concerns about the invasive potential of *A. pisum* in Chile, as it is a pest of major importance (van Emden & Harrington 2007). Aphids may impact not only crops, but also wild ecosystems (e.g. Messing *et al*. 2007) by direct or indirect damages to plants, e.g. sap intake, transmission of viral diseases (van Emden & Harrington 2007). In this line, our results suggest that cultivated legumes and wild plants harbouring the same aphid clones could not be protected independently, as opposed to crops hosting different biotypes (Bourguet *et al*. 2000; Vialatte *et al*. 2005).

Under strict asexuality, Chilean uniclonal populations would more predictably respond to management strategies (Burdon & Marshall 1981) and may suffer from environmental changes, particularly from increasing biotic interactions such as predation and parasitism (Howard & Lively 1994). These populations are more likely to react to these selective pressure by clonal replacements (e.g. Facon *et al*. 2003; Figueroa *et al*. 2005) if new genotypes are introduced. Maintaining low genotypic diversity by controlling introduced crops against the presence of aphids, which have short autonomy off their host but high colonization abilities once established (Dixon 1998), is thus crucial for the management of this pest. Future evolutionary pathways of Chilean pea aphids do not completely exclude clonal evolution (Sunnucks *et al*. 1998) or rare events of recombination among genotypes, which may still be able to produce few sexual forms under the right conditions (Facon *et al*. 2005; Wilson & Sunnucks 2006). Testing these alternatives with further samplings across a wider spatial and temporal scale would provide new insights on the evolutionary potential of strictly asexual invaders.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Sampling points (fields) of pea aphids on five crops and in three locations of central Chile. Pea aphids from red clover were not collected in La Unión because this plant was not cultivated in this area

Table S2 The 16 different microsatellite genotypes found in 432 pea aphids sampled on several host plants and locations of central Chile. Numbers represent allele sizes (bp)

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