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Signatures of genetic bottleneck and differentiation after the introduction of an exotic parasitoid for classical biological control

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Abstract As biological invasions, intentional introductions often result in a loss of genetic diversity in the new founder populations. In classical biological control programs, natural enemies introduced into novel environments are likely to suffer from population bottlenecks. Unlike invasive populations, individuals for biological control are typically kept in quarantine during several generations before being released in the field. This procedure reduces further the effective population size of the introduced populations, which thus increases the effects of inbreeding and genetic drift, resulting in a greater loss of genetic diversity. This study addresses the genetic consequences of the introduction of the parasitoid wasp *Aphidius ervi*, a successful biocontrol agent of important aphid target-pests in Chile. This was assessed by examining the genetic diversity and differentiation at nuclear and mitochondrial genetic markers in terms of (1) the magnitude of the genetic diversity loss after 38 years of the introduction of *A. ervi*, (2) the current level of genetic differentiation between Chilean introduced populations and putative native populations from France, and (3) the genetic relationships and magnitude of the genetic diversity loss between introduced populations of *A. ervi* in Chile compared to those introduced in North America. The results provide evidence that parasitoid populations suffered the effects of a moderate genetic bottleneck during the introduction, showing further a strong geographical genetic differentiation between populations in the natal and novel environments. In addition mtDNA sequences analysis showed evidence of a single main event of introduction in Chile, unlike the North American situation, where there is evidence for multiple introductions. The significance of the loss of genetic diversity during introductions related to the success of parasitoids as biocontrol agents in classical biological control programs is discussed.

Keywords Genetic bottleneck · Classic biological control · Biological invasions · Aphid parasitoids · *Aphidius ervi*

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Introduction

Classical biological control is based on intentional introductions of exotic species to control certain target pest species (Eilenberg et al. 2001). This strategy frequently involves the introduction of propagules from the native range to a novel environment, which are expected to be affected by similar biogeographic (e.g., geographic isolation), demographic (e.g., population bottleneck and founder effect) and genetic (e.g., genetic drift and inbreeding) processes to those operating during natural or unintentional biological introductions (Guillemaud et al. 2011; Fauvergue et al. 2012). Hence, populations of introduced controllers usually carry a fraction of the genetic diversity from the source population (Roderick and Navajas 2003; Dlugosch and Parker 2008). Once introduced, these populations suffer from bottlenecks, resulting in elevated rates of inbreeding and inbreeding depression, losses of genetic diversity and fix deleterious alleles, thus reducing the adaptive potential of the populations (Nei et al. 1975; Frankham et al. 2002, 2011). However, unlike other biological introductions, individuals for biocontrol are usually kept in quarantine during several generations before being finally released into the targeted environments (Hufbauer et al. 2004). This procedure further reduces the effective population sizes of the released populations, increasing the effects of inbreeding and genetic drift on the loss of genetic diversity (Unruh et al. 1983). This loss of genetic diversity has been frequently related with a loss of the adaptive potential (i.e., lower fitness and poor performance) of introduced populations (Reed and Frankham 2003; Frakham 2005), which should limit their ability to respond to environmental changes (Hufbauer et al. 2004; Hufbauer 2002; Boer et al. 2012). Evolutionary processes occurring in the introduced populations are critical for successful establishment and effective biological control of target pests (Roderick and Navajas 2008; Roderick et al. 2012). For instance, adaptation of biological control agents to a novel environment could increase their fitness on target hosts. However introduced small populations could fail to adapt due to loss of genetic diversity through bottlenecks and inbreeding, causing decreased fitness and decline of biological control populations (Fauvergue et al. 2012). Moreover, phenotypic plasticity could minimize the risk of extinction arising from the maladaptation of populations to novel environments (Chevin and Lande 2010; Lande 2015). In addition, demographic processes could also play a role in the success of introduced biological control agents, such as Allee effects, where a decrease in fitness components occurs in response to a decreased population size (Fauvergue et al. 2012). Although the genetic consequences of biological introductions have been extensively studied in many species of plants and animals, little is known about the genetic consequences and their relationship to evolutionary processes occurring in populations of biocontrol agents in novel environments (Roderick et al. 2012; Fauvergue et al. 2012). Classic biological control could be considered as a natural manipulative experiment of biological invasions, with the advantage that introductions are planned, offering a better follow-up of the effects of biological introductions (Roderick and Navajas 2008). In this sense, post-release studies on biological control agents are important as they may further understanding of the key determinants to biological invasions and the success of the practice of classic biological control; a prerequisite for management strategies and the release of biocontrol agents of pests (Roderick et al. 2012).

Aphidius ervi (Hymenoptera: Aphidiidae) is a moderate generalist parasitoid of various aphid species (Hemiptera: Aphididae) in its native range (Eurasia) (Starý et al. 1993; Stilmant et al. 2008). The parasitoid *A. ervi* has been intentionally introduced into several countries from its native range with the aim of controlling introduced aphid pests in different agroecosystems (Starý et al. 1993). A well-studied case has been the introduction of *A. ervi* to North America, where records show that 1000 individuals from France and a small number of individuals (<20) from the Middle East were introduced by the USDA (United States Department of Agriculture) in 1959 (Hufbauer et al. 2004). According to Hufbauer et al. (2004), these introduced individuals were kept in quarantine during three months (3–6 generations) before being released into fields. Moreover, from 1976 to 1981, different Aphidiidae parasitoid species were introduced into Chile (South America) by the European USDA Laboratory established in France (Zuñiga et al. 1986a, b). A well-documented case reports the introduction of 271 live individuals of *A. ervi* in Chile, which were collected from France in 1976 (Zuñiga et al. 1986a, b). Those introduced individuals were also kept during a

non-specified quarantine period (1976–1981) for multiplication and for avoiding the introduction of parasitoid species other than *A. ervi*. During this period, a total of 560,784 *A. ervi* individuals, produced from a single founder population kept in quarantine, were released consecutively each year (Zuñiga et al. 1986b). Since then, *A. ervi* has shown to be highly efficient in controlling important aphid pest species on different agroecosystems, becoming one of the most common parasitoid wasps attacking the cereal aphid *Sitobion avenae* and the pea aphid *Acyrthosiphon pisum* in Chile (Gerding et al. 1989; Rojas 2005; Starý et al. 1993; Starý 1993; Zepeda-Paulo et al. 2013).

Hence, the present study addresses the genetic consequences of the intentional introduction of *A. ervi* in Chile, relating them to the success of this parasitoid wasp as biocontrol agent. We have analyzed the genetic diversity and differentiation at nuclear and mitochondrial genetic markers in terms of (1) the magnitude of the genetic diversity 38 years after the introduction of *A. ervi*, (2) the current level of genetic differentiation between Chilean introduced populations and putative native populations from Europe, and (3) the genetic relationships and magnitude of the genetic diversity between populations of *A. ervi* in Chile compared to those introduced in North America, based on previous reports by Hufbauer et al. (2004).

Materials and methods

Parasitoid sampling

Aphidius ervi individuals were obtained from pea aphids, *Acyrthosiphon pisum* (the most common host in Europe) collected in legumes in Europe and Chile. The pea aphid represents a complex of genetically differentiated host races adapted to different species of Fabaceae (e.g., alfalfa, pea, red clover) (Simon et al. 2003; Ferrari et al. 2008; Peccoud et al. 2008, 2009). Hence, parasitoid individuals in Europe were sampled from five populations on the alfalfa race of *A. pisum* (ALF-1, ALF-2, ALF-3, ALF-4, ALF-5) and from five populations on the red clover race of *A. pisum* (CL-2, CL-3, CL-4, CL-5 and CL-6), at six different locations in France and Switzerland during the spring of 2008 and 2009 (Table 1). These sampling sites were Le Rheu (R) and Domagné (D), located in the north-west of France, Mulhouse (M), Nancy (N) and Châlons-en-Champagne (C), located in the north-east of France, and Lausanne (L), located in the south-west of Switzerland close to the French border (Table 1). The single population from Switzerland was analyzed together with all French populations in the genetic population analysis. In Chile (the introduced range), two parasitoid populations of *A. pisum* were sampled on the alfalfa host race (ALF-6 and ALF-7) and the pea host race (P-1 and P-2) (Table 1). Similarly, two parasitoid populations were sampled on the grain aphid, *S. avenae* (S-1 and S-2) in wheat fields from Talca (Central Chile) and in oat fields from Valdivia (South of Chile) (Table 1). All parasitoid individuals were obtained by collecting live aphids from the field, which were subsequently transferred to the laboratory for rearing and parasitoid emergence under controlled conditions (20 ± 1 °C; 50–60 RH; L16:D8). This sampling method allows an unequivocal determination of the aphid host from which each individual parasitoid emerges. All newly emerged parasitoids were sexed, determined to the species level using a key to Aphidiinae parasitoids described by Starý (1995), and preserved in 95 % ethanol until DNA extraction. Finally, since the parasitoid *A. ervi* is a haplodiploid species (i.e., haploid males and diploid females) and only females provided genotypes for population genetic analyses, the analyses were performed using solely female parasitoids (Nyabuga et al. 2011).

Parasitoid genotyping

Parasitoid DNA was extracted using the "Salting-out" method (Sunnucks and Hales 1996). Genotyping was conducted at 12 microsatellite loci specific for *A. ervi* (Ae01, Ae03, Ae06, Ae08, Ae16, Ae20, Ae22, Ae27, Ae29, Ae32, Ae33 and Ae38) (Zepeda-Paulo et al. 2015). Each PCR reaction was set-up in 25 µL reaction volume containing 1 µL of genomic DNA, 1× PCR Buffer (20 mM Tris–HCl, 50 mM KCL), 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.25 mM of *reverse* primer, 0.25 mM of M13 primer, 0.25 mM of *forward* primer and 0.5 U of *Taq* polymerase (Hufbauer et al. 2004). Amplicons were analyzed in a capillary sequencer (ABI 3130xl, Applied Biosystems). After reading the sequences in the electropherograms using the GENEMARKER software (Softgenetics), the multilocus genotype for each parasitoid individual was obtained by combining the alleles from all microsatellite loci.

Table 1 Genetic diversity of parasitoid populations in the native (France) and introduced (Chile) ranges

Range	Pop code	Aphid host	Location	N		Na	Ne	A (SD)	Ho	He	uHe	Fis	Exact HWE test	M-ratio
Native	ALF-1	*A. pisum*-alfalfa race	Châlons en Champagne	39	Mean	8.3	3.5	5.8 (0.49)	0.569	0.679	0.688	0.165	<0.0001*	0.700
					SE	(0.8)	(0.4)		(0.04)	(0.04)	(0.04)	(0.02)		
	ALF-2	*A. pisum*-alfalfa race	Domagné	86	Mean	9.2	3.8	5.79 (0.53)	0.469	0.710	0.715	0.342	<0.0001*	0.743
					SE	(0.8)	(0.4)		(0.03)	(0.03)	(0.03)	(0.02)		
	ALF-3	*A. pisum*-alfalfa race	Mulhouse	13	Mean	5.8	3.8	5.67 (0.21)	0.605	0.716	0.753	0.150	0.0002*	0.656
					SE	(0.5)	(0.4)		(0.05)	(0.03)	(0.03)	(0.07)		
	ALF-4	*A. pisum*-alfalfa race	Nancy	11	Mean	5.6	3.4	6.33 (0.0)	0.507	0.683	0.722	0.262	<0.0001*	0.678
					SE	(0.5)	(0.4)		(0.05)	(0.03)	(0.03)	(0.05)		
	ALF-5	*A. pisum*-alfalfa race	Le Rheu	44	Mean	7.7	4.0	5.82 (0.46)	0.611	0.720	0.730	0.153	0.0004*	0.759
					SE	(0.8)	(0.5)		(0.03)	(0.04)	(0.04)	(0.02)		
	CL-2	*A. pisum*-clover race	Domagné	118	Mean	10.2	4.0	6.11 (0.33)	0.517	0.717	0.721	0.279	<0.0001*	0.811
					SE	(0.9)	(0.5)		(0.03)	(0.04)	(0.04)	(0.02)		
	CL-3	*A. pisum*-clover race	Mulhouse	39	Mean	8.3	3.9	5.7 (0.46)	0.607	0.712	0.721	0.141	<0.0001*	0.741
					SE	(0.6)	(0.4)		(0.03)	(0.03)	(0.03)	(0.04)		
	CL-4	*A. pisum*-clover race	Nancy	11	Mean	6.0	3.7	6.11 (0.0)	0.551	0.698	0.731	0.186	<0.0001*	0.704
					SE	(0.5)	(0.4)		(0.04)	(0.04)	(0.04)	(0.08)		
	CL-5	*A. pisum*-clover race	Le Rheu	26	Mean	8.2	3.7	6.71 (0.52)	0.639	0.709	0.725	0.097	0.0132*	0.731
					SE	(0.8)	(0.4)		(0.04)	(0.03)	(0.03)	(0.04)		
	CL-6	*A. pisum*-clover race	Lausanne	16	Mean	7.0	4.5	6.54 (0.35)	0.555	0.749	0.776	0.262	<0.0001*	0.643
					SE	(0.4)	(0.5)		(0.06)	(0.03)	(0.03)	(0.07)		
	Total populations			403	Mean	7.63	3.84	6.06 (0.33)	0.563	0.709	0.728	0.204	<0.0001*	0.805
					SE	(0.3)	(0.1)		0.014	(0.01)	(0.01)	(0.02)		
Introduced	ALF-6	*A. pisum*-alfalfa race	Valdivia	64	Mean	5.67	3.04	4.98 (0.19)	0.631	0.633	0.638	0.014	0.028*	0.613
					SE	(0.6)	(0.3)		(0.1)	(0.04)	(0.05)	(0.04)		
	ALF-7	*A. pisum*-alfalfa race	Talca	38	Mean	5.78	2.97	4.91 (0.2)	0.605	0.617	0.626	0.046	0.112	0.616
					SE	(0.7)	(0.3)		(0.07)	(0.05)	(0.05)	(0.05)		
	P-1	*A. pisum*-pea race	Valdivia	30	Mean	5.11	2.82	4.73 (0.24)	0.582	0.609	0.620	0.041	0.318	0.698
					SE	(0.6)	(0.3)		(0.05)	(0.05)	(0.05)	(0.04)		
	P-2	*A. pisum*-pea race	Talca	39	Mean	6.0	3.01	5.24 (0.29)	0.631	0.622	0.631	−0.028	0.518	0.639
					SE	(0.8)	(0.4)		(0.04)	(0.05)	(0.05)	(0.03)		
	S-1	*S. avenae*	Valdivia	55	Mean	6.44	3.07	5.12 (0.31)	0.620	0.639	0.646	0.029	0.084	0.634
					SE	(0.8)	(0.3)		(0.04)	(0.04)	(0.04)	(0.02)		
	S-2	*S. avenae*	Talca	61	Mean	6.56	2.87	5.25 (0.37)	0.627	0.631	0.639	0.008	0.125	0.633
					SE	(0.8)	(0.2)		(0.04)	(0.04)	(0.04)	(0.02)		

Table 1 continued

Range	Pop code	Aphid host	Location	N		Na	Ne	A (SD)	Ho	He	uHe	Fis	Exact HWE test	M-ratio
	Total populations			287	Mean	5.92	2.96	5.03 (0.26)	0.616	0.625	0.633	0.018	0.009*	0.684
					SE	(0.3)	(0.1)		(0.02)	(0.02)	(0.02)	(0.01)		

N sample size, *Na* no. of different alleles, *Ne* no. of effective alleles, *A* standardized allelic richness, *Ho* observed heterozygosity, *He* expected heterozygosity, *uHe* unbiased expected heterozygosity, *Fis* fixation index. *SE* standard error and *SD* standard deviation and M-ratio

* Indicates significant differences

A mtDNA sequence analysis using a subsample of 133 individuals collected from different aphid hosts in different zones (Central and South zone) of Chile was also performed by amplifying a segment of ~1300 bp, which includes portions of the COI and COII genes using the primers C1-J-1718 and C2-N-3661 described by Simon et al. (1994). These primers corresponded to the same previously reported by Hufbauer et al. (2004) to analyze the introduction of *A. ervi* in North America. The amplification of the mtDNA fragment was conducted at 25 µL volumes with 2.5 µL 10× PCR buffer, 2.5 mM $MgCl_2$, 0.24 mM dNTPs, 0.5 µM each primer, 0.5 U taq platinum (Invitrogen) and 2 µL DNA (~50 ng/µL concentration) using the following thermocycling conditions: 3 min at 94 °C, 37 cycles of 30 s at 94 °C, 60 s at 58.5 °C (annealing T°) and 90 s at 72 °C, with a final extension of 10 min at 72 °C. The amplicons were purified using PureLink® PCR Purification kit (Invitrogen) and sequenced on 3730xl DNA analyzer (Macrogen- Korea).

Microsatellite analyses

Genetic diversity within French and Chilean populations

To determine and compare the genetic diversity in the French and Chilean populations of *A. ervi*, a total of 403 individuals from 10 populations in France and 287 individuals from 6 populations in Chile were genotyped (Table 1). The term "parasitoid population" is used here operationally to describe a group of parasitoid individuals based on their ecological (different hosts) and spatial distributions (different localities) in both countries. Null alleles, scoring errors due to stuttering, and large allele dropouts were tested at the 12 microsatellite loci using Microchecker v.2.2.3 (van Oosterhout et al. 2004). The observed allele frequencies for the different loci in the French and Chilean populations were plotted using the package standArich v.1.0 in R (R Core Team 2012). Allelic richness of populations was computed and standardized by the multiple random reduction method (Leberg 2002) using the smallest sample size observed (N = 11) (Table 1), with standArich v.1.0 in R (R Core Team 2012). The genetic diversity for each population was calculated using Genalex v.6.5 (Peakall and Smouse 2012), examining the number

of alleles, the observed (Ho) and expected (He) heterozygosities, the allelic diversity and the inbreeding coefficient (F_{IS}). Deviations from the Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) between pairs of loci were tested for each population using GENEPOP v 3.4 (Raymond and Rousset 1995).

Signatures of genetic bottleneck

The occurrence of genetic bottlenecks during the introduction of *A. ervi* in Chile was studied using two methods. First, the mean observed Garza–Williamson index (*M-ratio*) was calculated across different loci. The *M-ratio* is based on the ratio between the number of microsatellites alleles (*K*) and the allele size range (*r*) (Garza and Williamson 2001). The number of alleles is expected to decline faster than the allele size range in populations that have crossed a bottleneck compared to large populations at genetic equilibrium. Thus, the *M-ratio* is expected to be smaller in populations that experienced a bottleneck (Peery et al. 2012). The *M-ratio* was calculated for each population in France and Chile, and over the pooled populations in both countries using the software M_P_Val (Garza and Williamson 2001). The critical *M-ratio* value (M_c) according to the sample size and number of loci, was estimated using the following parameters: the Watterson estimator of population mutation rate theta (θ) $= 5 = 4Ne\mu$; the average size of multi-repeat-unit mutations (δ_g) $= 3.1$; and the mean percentage of multi-repeat-unit mutations (p_g) $= 0.22$, as recommended by Peery et al. (2012) and implemented in the software Critical_M (Garza and Williamson 2001). As second method, a heterozygosity excess test was computed, which is highly sensitive to the loss of rare alleles during a bottleneck, inferring when the observed heterozygosity exceeds the expected heterozygosity under a mutation-drift balance model (Cornuet and Luikart 1996). The significances of a heterozygosity excess were computed using a one-tailed Wilcoxon signed-rank test (Luikart and Cornuet1998), using the software Bottleneck v1.2.02 (Piry et al. 1999) under three different models of microsatellite evolution: the infinite allele model (IAM), the stepwise mutation model (SMM) and the two-phase model (TPM), as recommended by Luikart and Cornuet (1998).

Genetic variation among parasitoid populations

To study the genetic partitioning among geographical ranges (France and Chile) and among populations within a geographical range and within populations, an AMOVA at three hierarchical levels was performed using the software Genalex v.6.5 (Peakall and Smouse 2012). The Fst (fixation index) was estimated to determine the degree of genetic differentiation by locus and by population using Genalex v.6.5 (Peakall and Smouse 2012). To study the genetic differentiation among parasitoid populations, the most probable number of clusters (k) containing different multilocus genotypes of *A. ervi* was estimated considering (1) all the multilocus genotypes identified in both geographical ranges (France and Chile) and (2) only genotypes from a same geographic range (Chile or France) using the software STRUCTURE v. 2.3.4 (Pritchard et al. 2000). Similarly, the distribution of genotypes was determined by computing the coefficient of ancestry for each multilocus genotype to the different inferred clusters, using a Bayesian assignment analysis implemented in the same software. An admixture model with correlated allele frequencies without a priori geographical information was used. The number of clusters (k) with the highest posterior probability after 10 replicates for each k (between 1 and 16 clusters) was determined. The model was tested by performing 100,000 MCMC runs with 10,000 burn-in periods before each run. The most probable number of clusters was determined using the log of the posterior probability and the average rate of change (delta k) for each value of k, using the method proposed by Evanno et al. (2005) and implemented in the software Structure Harvester v.6.93 (Earl 2012). The genetic similarity among all the multilocus genotypes and populations of *A. ervi* from France and Chile was examined using an individual assignment test with a factorial correspondence analysis (FCA) in Genetix v.4.05 (Belkhir et al. 1996).

Analysis of mtDNA sequence data

The mtDNA sequences were aligned using Geneious software v 5.6 (Drummond et al. 2011). To study the genetic relationships of different haplotypes among the *A. ervi* individuals collected from Chile and other regions, we compared a $\sim$600 bp sequence of the COI gene from *A. ervi* for samples collected in Chile

(n = 133) with North American (n = 45), Japan and Argentina (South America) (n = 2) and 23 sequences from different countries spanning their native range (Europe) previously reported by Hufbauer et al. (2004) (supplementary data 1). A median Joining network was constructed using the Network software v 4.6.1 (available at http://www.fluxus-engineering.com/sharenet.htm). The sequences were obtained from Genbank under the accession numbers: AY262762–AY262787 and AY427835–AY427886 (Hufbauer et al. 2004) (Supplementary data 1). Some ambiguous sequences reported by Hufbauer et al. (2004), may correspond to other parasitoid species (e.g., *Aphidius pisivorus*), these were not included in the present study.

Results

Genetic diversity in French and Chilean populations of *Aphidius ervi*

Null alleles were detected in three of the twelve microsatellite loci (Ae20, Ae22 and Ae38) considering the whole dataset. Therefore, the data set was subsequently analyzed only at nine microsatellite loci after removing those three loci. In four of the remaining nine loci (Ae06, Ae16, Ae27 and Ae29), the allele frequencies were similar across the French and Chilean parasitoid populations, with one or two alleles found at higher frequencies (>0.6) for populations from both geographical ranges (supplementary data 2). Indeed, these four loci showed a weak differentiation among populations compared to the other loci, with three loci showing no significant differentiation (see below). The other five loci (Ae01, Ae03, Ae08, Ae32 and Ae33) showed larger differences in their allele frequencies between the native and introduced ranges (supplementary data 2). However, this was not observed among parasitoid populations within the same range (i.e., in each country) (supplementary data 2). It should be noted that all the alleles amplified at these loci, which have been found at high frequencies in Chilean populations, were also present in French populations. In fact, from the 66 alleles (100 %) observed across all loci in the Chilean populations, a low percentage corresponded to private alleles (10.6 %), with only seven alleles restricted to Chile (supplementary data 2 and 3).

The genetic diversity was higher in all the ten parasitoid populations from France. The average number of different alleles, standardized allelic richness and the average number of effective alleles being all found to be higher in France (Na = 7.63, A = 6.06 and Ne = 3.84, respectively) than in the six populations from Chile (Na = 5.92, A = 5.03 and Ne = 2.96) (Table 1). Similarly, the overall expected heterozygosity was higher in the French populations (He = 0.709) compared to the introduced parasitoid populations in Chile (He = 0.625) (Table 1). However, all parasitoid populations from France deviated significantly from Hardy–Weinberg equilibrium due to heterozygote deficit (overall Ho = 0.563) (all Fis values >0.097; $p < 0.05$) (Table 1). Contrastingly, parasitoid populations in Chile were mostly at genetic equilibrium (overall Ho = 0.616), with the exception of a single population sampled from the *A. pisum*-alfalfa race, which showed a significant heterozygote deficit (Fis = 0.014; $p < 0.05$) (Table 1).

Signatures of genetic bottleneck in introduced populations of *A. ervi*

French populations showed a higher *M-ratio* (ranging between 0.643 and 0.811; overall *M-ratio* = 0.805) than Chilean populations (ranging between 0.613 and 0.698; overall *M-ratio* = 0.684) (Table 1). Since the *M-ratio* over the pooled French populations was above the expected critical $M_c = 0.744$ (estimated for the whole dataset), then no signature of bottleneck was detected in the native range. In contrast, the *M-ratio* for the pooled Chilean population and the *M-ratios* for each separate Chilean population were all below the critical *M-ratio* (Table 1), suggesting that the introduced populations have crossed a genetic bottleneck(s). However, the heterozygote excess analysis did not provide support for a recent genetic bottleneck (i.e., all loci fit the mutation-drift balance model) in the introduced Chilean populations of *A. ervi* for any of the three evolutionary models tested (IAM, *He* excess: $p = 0.102$), (TPM, *He* excess: $p = 0.787$), (SMM, *He* excess: $p = 0.995$). Similar results were observed for the French parasitoid populations at the three different evolutionary models used (IAM, *He* excess: $p = 0.326$), (TPM, *He* excess: $p = 0.993$), (SMM, *He* excess: $p = 0.999$).

Genetic variation between and within French and Chilean populations of *A. ervi*

The AMOVA analysis revealed a significant genetic differentiation between the French and Chilean populations (% variation = 6.8; *F*-statistic = 0.069; *p* value = 0.001), as well as within populations (Table 2). Populations within each country showed only 0.3 % of genetic variation (*F*-statistic = 0.003; *p* value = 0.002) (Table 2). The genetic differentiation between the pooled French and Chilean populations (global *F*st = 0.058*) was significant for most loci (*F*st values: Ae01 = 0.051**; Ae03 = 0.079**; Ae08 = 0.049**; Ae16 = 0.055*; Ae32 = 0.089** and Ae33 = 0.080**; **p* value = 0.05; ***p* value <0.01). However, some loci did not show significant differences (*F*st values: Ae06 = 0.033; Ae27 = 0.030; Ae29 = 0.038), observing similar allelic frequencies in both the French and Chilean populations (supplementary data 2 and 3).

All *F*st comparisons between pairs of French and Chilean populations showed significant genetic differentiation, the *F*st ranging from 0.025 to 0.110 (Table 3), while low genetic differentiation was observed between pairs of populations within the same geographical range (France or Chile) was observed (*F*st < 0.029) (Table 3). Among the French populations, four pairs of populations showed a significant genetic differentiation between each other, including the geographical and host-race level (ALF-1 with ALF-4, *F*st = 0.029, *p* = 0.018; ALF1 with CL-6, *F*st = 0.02, *p* = 0.018; ALF-4 with ALF-5, *F*st = 0.025, *p* = 0.018 and ALF-4 with CL-5, *F*st = 0.019, *p* = 0.036) (Table 3). The remaining French populations did not show any genetic differentiation between each other (Table 3). On the other hand, the Chilean populations did not show any significant genetic differentiation between all pairs of populations (Table 3).

Regarding the genetic structuring of the multilocus genotypes from France and Chile, they were assigned to different genetic clusters with the highest rate of change (delta *K*) for k = 2 (supplementary data 4), thus confirming the occurrence of two distinct genetic clusters, which correspond to the two countries of origin (Fig. 1a). Indeed, considering a coefficient of ancestry (q) greater than 80 %, the individuals assigned to the first cluster grouped 90 % of the French multilocus genotypes, while the individuals in the second cluster corresponded to 94 % of all the Chilean multilocus genotypes (Fig. 1a). In agreement with *Fst* comparisons, the Structure analyses including only multilocus genotypes from the same geographic range (Chile or France) showed a lack of genetic structure among genotypes within the same geographic range. The highest rate of change (delta *K*) occurred for genotypes within both geographic ranges (k = 2) (Fig. 1b, c and supplementary data 4). However, the genotypes within a same geographic range were not grouped into a k ≥ 1 cluster, because multilocus genotypes were assigned to each genetic cluster in a similar proportion (membership coefficient) (i.e., ~1/number of clusters) (Fig. 1b, c). Similarly, parasitoid populations were grouped according to their geographical origin based on the factorial correspondence analysis (FCA), which also detected two different genetic clusters (France and Chile) (Fig. 2a). However, the FCA on multilocus genotypes revealed that some genotypes from both France and Chile are not completely assigned according to their geographical origin (Fig. 2b), with some "overlapping" between French and Chilean populations, due to several alleles that are present in both countries at high frequencies (Fig. 2b).

Ancestry relationships among mtDNA haplotypes of *A. ervi* worldwide

A lower diversity was observed in Chile (0.388; SD of 0.04) than in North America (0.587; SD of 0.06), with a number of variable sites of 2 and 4, respectively. This haplotype diversity for North America was slightly

Table 2 Hierarchical analysis of the molecular variance (AMOVA) of parasitoid populations from France and Chile countries using microsatellite loci

Source of variation	*df*	Sum of squares	Variance components	F-statistic	Percentage of variation	*p* value
Among countries	1	154.8	0.225	0.069	6.8	0.001*
Among populations within countries	14	53.2	0.009	0.003	0.3	0.002*
Within populations	1364	4206.8	3.084	0.070	93	0.001*
Total	1379	4414.7	3.318			

* Indicates significant differences

Table 3 Pairwise comparisons of fixation index (Fst) between *A. ervi* populations from Chile (ALF-6, ALF-7, P-1, P-2, S-1 and S-2) and France (ALF-1, ALF-2, ALF-3, ALF-4, ALF-5, CL-2, CL-3, CL-4, CL-5 and CL-6) for all loci

	ALF1	ALF2	ALF3	ALF4	ALF5	CL2	CL3	CL4	CL5	CL6	ALF6	ALF7	P1	P2	S1	S2
ALF1		0.739	0.973	0.018	0.117	0.883	0.153	0.378	0.189	0.018	0.000	0.000	0.000	0.000	0.000	0.000
ALF2	0.000		0.919	0.054	0.991	0.721	0.991	0.964	0.270	0.387	0.000	0.000	0.000	0.000	0.000	0.000
ALF3	−0.013	−0.004		0.432	0.910	0.748	0.991	0.982	0.883	0.820	0.000	0.000	0.000	0.000	0.000	0.000
ALF4	*0.029*	0.024	0.009		0.018	0.054	0.378	0.667	0.036	0.694	0.000	0.000	0.000	0.000	0.000	0.000
ALF5	0.005	−0.007	−0.011	*0.025*		0.982	0.730	0.901	0.351	0.901	0.000	0.000	0.000	0.000	0.000	0.000
CL2	−0.002	0.000	−0.004	0.022	−0.006		0.991	0.964	0.883	0.315	0.000	0.000	0.000	0.000	0.000	0.000
CL3	0.006	−0.011	−0.025	0.007	−0.002	−0.018		0.739	0.225	0.649	0.000	0.000	0.000	0.000	0.000	0.000
CL4	0.007	−0.013	−0.029	0.005	−0.007	−0.021	−0.002		0.694	0.757	0.000	0.000	0.000	0.000	0.000	0.000
CL5	0.006	0.005	−0.010	*0.019*	0.003	−0.004	0.005	−0.002		0.207	0.000	0.000	0.000	0.000	0.000	0.000
CL6	*0.020*	0.007	−0.008	0.004	−0.006	0.006	0.000	0.000	0.009		0.000	0.000	0.000	0.000	0.000	0.000
ALF6	*0.072*	*0.060*	*0.055*	*0.106*	*0.076*	*0.064*	*0.067*	*0.077*	*0.097*	*0.076*		0.757	0.829	0.838	0.883	0.991
ALF7	*0.063*	*0.053*	*0.048*	*0.101*	*0.069*	*0.058*	*0.060*	*0.069*	*0.093*	*0.073*	−0.003		0.793	0.982	0.910	0.991
P1	*0.063*	*0.057*	*0.051*	*0.110*	*0.076*	*0.062*	*0.059*	*0.073*	*0.093*	*0.075*	−0.004	−0.003		0.568	0.892	0.991
P2	*0.062*	*0.052*	*0.051*	*0.102*	*0.071*	*0.059*	*0.058*	*0.062*	*0.089*	*0.071*	−0.005	−0.008	−0.001		0.919	0.991
S1	*0.053*	*0.047*	*0.037*	*0.093*	*0.065*	*0.048*	*0.052*	*0.061*	*0.084*	*0.064*	−0.004	−0.005	−0.005	−0.005		0.991
S2	*0.025*	*0.034*	*0.031*	*0.102*	*0.048*	*0.046*	*0.028*	*0.028*	*0.077*	*0.060*	−0.039	−0.041	−0.039	−0.031	−0.051	

Fst values are shown below diagonal and p values above the diagonal. The population codes are those described in Table 1

Italic sized cells contain Fst values having a p value <0.05 after 1000 permutations

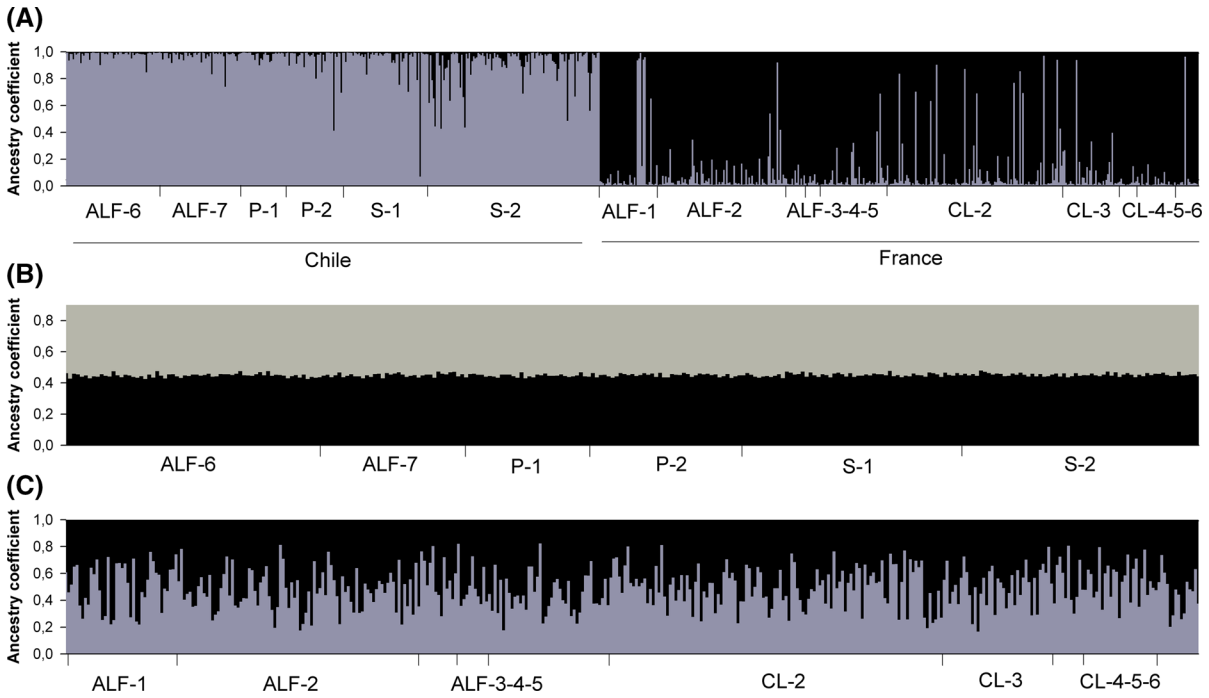

Fig. 1 Bayesian assignation analysis for the different multilocus genotypes of *A. ervi* sampled in Chile (ALF-6, ALF-7, P-1, P-2, S-1 and S-2) and France (ALF-1, ALF-2, ALF-3, ALF-4, ALF-5, CL-2, CL-3, CL-4, CL-5 and CL-6); ancestry coefficients for **A** each multilocus genotype from France and Chile, **B** only multilocus genotypes from Chile and **C** multilocus genotypes from France

lower than the previously reported (0.613) by Hufbauer et al. (2004), which can be explained due to a shorter portion of the COI gene sequence used in our study. Amongst the 133 *A. ervi* samples analyzed from Chile, four different haplotypes were identified, one corresponding to haplotype-A (HA, accession number KU194421) previously reported by Hufbauer et al. (2004), and three corresponded to new haplotypes (CH1, CH2 and CH3; accession numbers KU194423, KU194422 and KU194424 respectively) not previously reported (Supplementary data 1). These three latter haplotypes were found at low frequencies (14, 7.5 and 1.5 %, respectively), while haplotype-A was the most frequent (77 %) (Fig. 3). The Chilean haplotypes CH1, CH2 and CH3 derived from H-A, showing between 1 and 2 mutational steps (Fig. 3).

Among *A. ervi* sequences re-analysed from Hufbauer et al. (2004), a total of four haplotypes were found (HA, HB, HC and CAG2CA) in North America, corroborating the haplotypes previously reported (Hufbauer et al. 2004). Haplotype-A was the most common ($\sim$57 %) in North America, while the haplotype-B (HB) was observed in $\sim$15 % of samples. Both of them correspond to the most frequent haplotypes in samples from the native range (Fig. 3; Hufbauer et al. 2004). Indeed, haplotype-A was found in France, Hungary, Germany, Israel, Morocco and UK, while haplotype-B was found in France, Hungary, Italy and Turkey (Fig. 3; Hufbauer et al. 2004). The other two haplotypes present in North America (CAG2CA and HC) correspond to exclusive haplotypes found only in that country, and represented around 20 and 4 % of the sample, respectively. The haplotype CAG2CA was observed to derive from the most common haplotype (HA), while haplotype-C was not derived from this same haplotype, being closely related to a Japanese haplotype (Fig. 3; Hufbauer et al. 2004). This last observation has been explained by Hufbauer et al. (2004) as a possible and inadvertent introduction of *A. ervi* from Japan to North America.

Discussion

Genetic diversity in French and Chilean populations of *Aphidius ervi*: signatures of a genetic bottleneck after the introduction to Chile

The results of the present study suggest that introduced populations of *A. ervi* in Chile have indeed

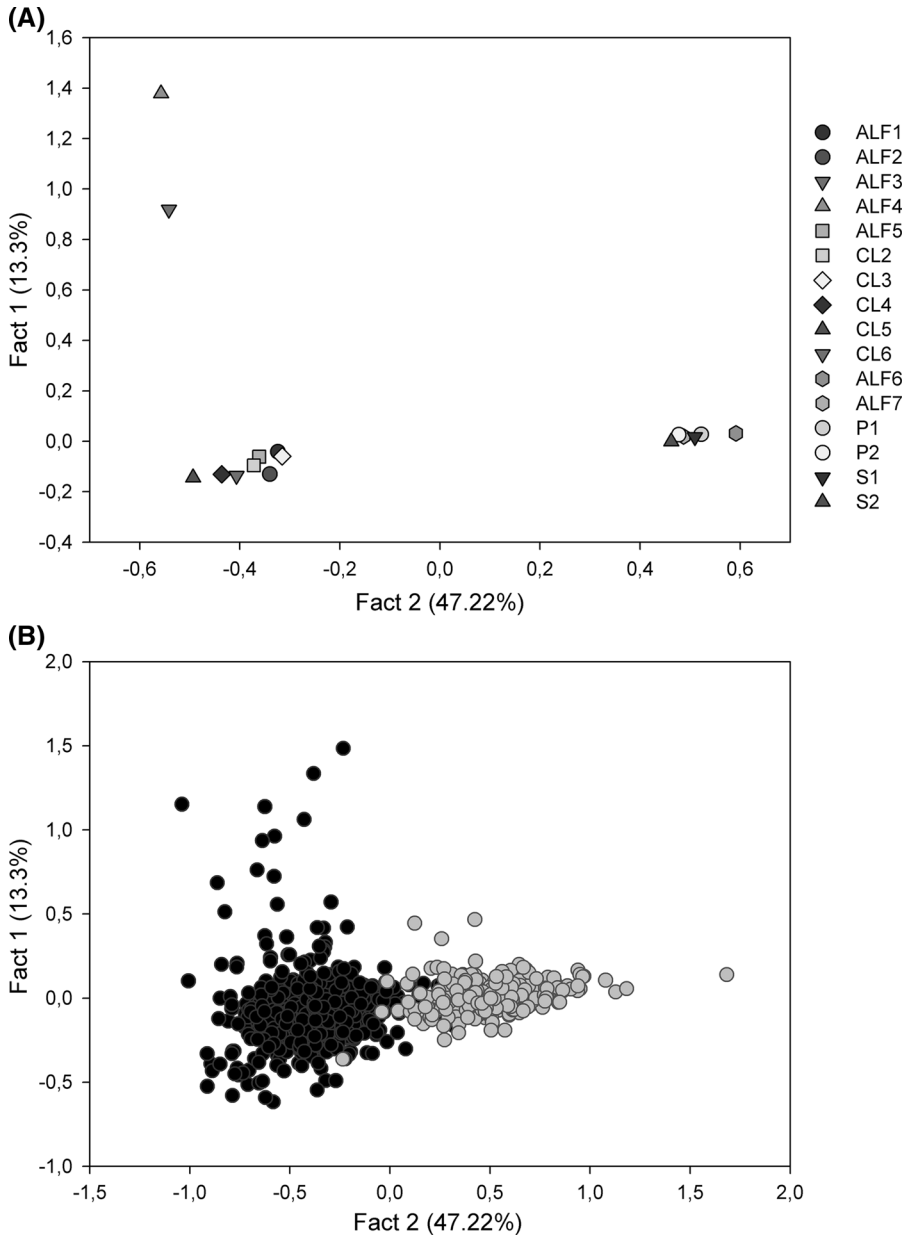

Fig. 2 Factorial correspondence analysis. Genetic similarity is plotted for **A** French (ALF-1, ALF-2, ALF-3, ALF-4, ALF-5, CL-2, CL-3, CL-4, CL-5 and CL-6) and Chilean (ALF-6, ALF-7, P-1, P-2, S-1 and S-2) populations, **B** different French (*black circles*) and Chilean (*gray circles*) multilocus genotypes of *A. ervi*

experienced a genetic bottleneck following their introduction from a putative French source, since we observed a significant reduction of allelic richness (*M-ratio*) in the introduced populations of Chile, when compared with the French populations of *A. ervi*. However, no heterozygote excess was observed in the Chilean populations (i.e., populations were at the mutation-drift equilibrium), showing no evident sign of a recent bottleneck. Since rare alleles are usually lost shortly after the introduction, populations under a recent bottleneck should show a significant heterozygote excess under a mutation-drift balance model (Garza and Williamson 2001). However, heterozygote excess persists only during a restricted number of

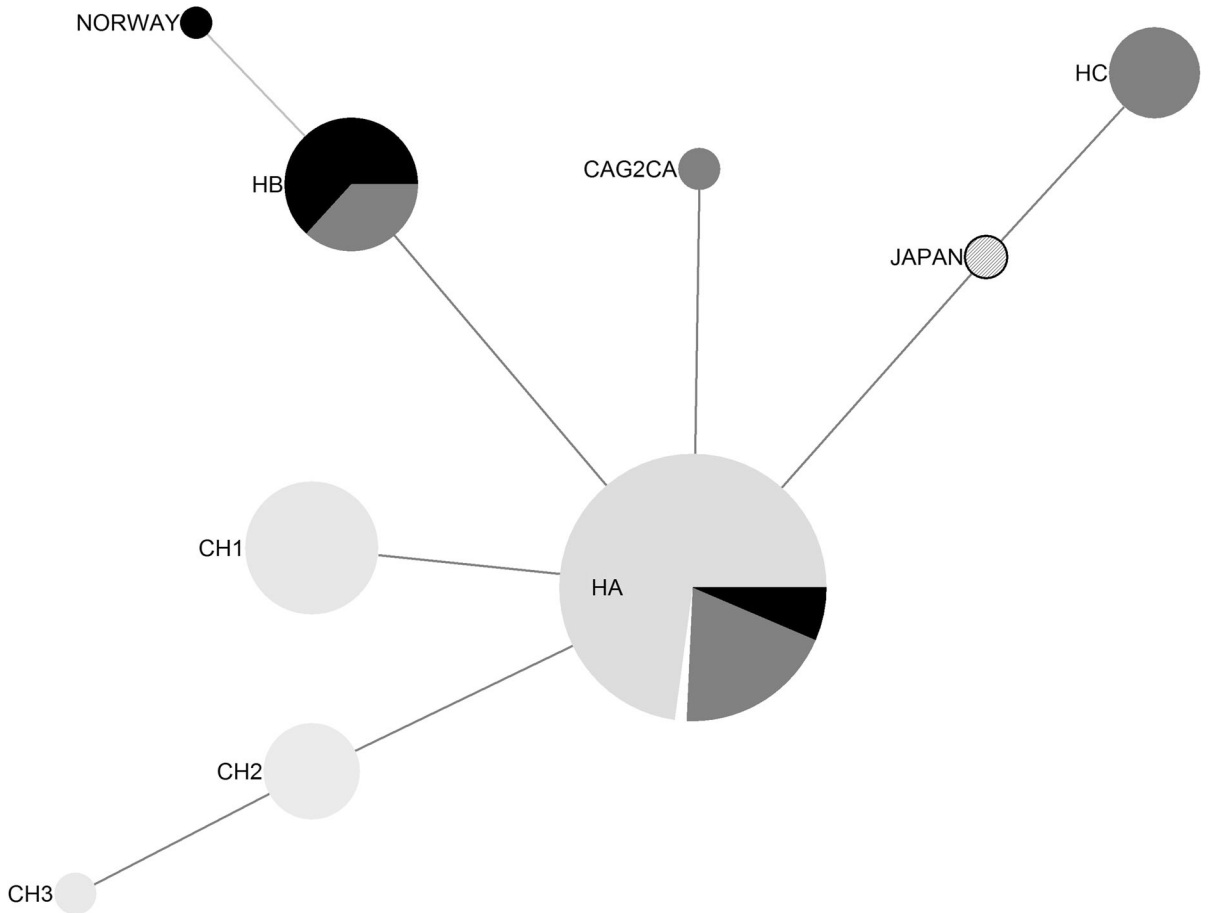

Fig. 3 Haplotype network of the *A. ervi* mtDNA sequences sampled from introduced populations in Chile (*light gray*), Argentina (*white*), Japan (*diagonal lines*) and North America (*dark gray*) and samples from the native range (*black*) belonging to different countries (France, Hungary, Germany, Israel, Morocco, UK, Italy, Turkey, Japan and Norway), described in supplementary data 1. *Circle size* represents the frequency of these haplotypes within the geographic ranges analyzed

generations (0.2–4Ne) until a new equilibrium is reached (Beebee and Rowe 2008). In contrast, the ratio between the number of alleles and the allele size range for a sample of microsatellite loci (*M-ratio*) changes more slowly, thus bottleneck signatures are expected to be retained for a longer time (Cornuet and Luikart 1996; Garza and Williamson 2001).

Our results are consistent with the few individuals of *A. ervi* initially introduced in Chile (only 271 individuals according to official reports) and the quarantine period (between 4 and 5 generations) before their release in the field (Zuñiga et al. 1986b). Moreover, microsatellites alleles reached a mutation-drift equilibrium in the introduced populations, which is consistent with the time elapsed from the introduction of *A. ervi* in Chile (over 30 years). Indeed, nearly 300 parasitoid generations have followed the introduction ($\sim$10 *A. ervi* generations per year; Hufbauer 2001), which may have restored the migration-drift balance as suggested by the heterozygote excess test (Hoban et al. 2013). However, genetic bottleneck tests should be interpreted with caution, because the error rate and power of tests to detect a bottleneck could be affected by other factors besides the recovery of the populations (Hoban et al. 2013). For instance, unlike heterozygote–excess test, that could be reasonably robust to incorrect assumptions about the mutation model, the *M- ratio* test could be more sensitive to the true and assumed proportion of multi-repeat-unit mutations (p_g) (Peery et al. 2012). The error rate type I for M-ratio test could increase when incrementing the difference between true and assumed proportions

of multi-repeat-unit mutations (p_g) (Peery et al. 2012). In this sense, the present analyses assumed a proportion of multi-repeat-unit mutations that might reduce the type I error rates for *M-ratio* test as recommended by Peery et al. (2012), overall being a conservative estimate of bottlenecks in the studied populations.

Other studies addressing the genetic consequences of insect introductions for weed biological control have rarely found a drastic loss of genetic diversity. Those insects include the sap-feeding mirid, *Eccritotarsus catarinensis* (Hemiptera: Miridae), the psyllid, *Boreioglycaspis melaleucae* (Hemiptera: Psyllidae) and the gall midge, *Spurgia capitigena* (Diptera: Cecidomyiidae), which demonstrate a limited effect of demographic bottlenecks on the genetic diversity of introduced populations (Lloyd et al. 2005; Franks et al. 2011; Taylor et al. 2011). On the other hand, studies carried out on aphid parasitoids have shown similar results to those reported in the present study. For instance, the aphid parasitoid, *Diaeretiella rapae* (Hymenoptera: Aphidiidae), shows a significant loss of genetic diversity in the introduced range, but without any evidence of a significant heterozygote excess (i.e., populations are under migration-drift balance) (Baker et al. 2003). In the case of the parasitoid *A. ervi* introduced to North America, the loss of genetic diversity was accompanied by a reduced fitness on the host races of the pea aphid (*A. pisum*) when compared to native parasitoid populations from France (Hufbauer et al. 2004). In that case, this pattern was only correlational and cause-effect could not necessarily be inferred (Hufbauer et al. 2004; Hufbauer and Roderick 2005). While it has been suggested that reduced neutral genetic variation is important for fitness (Reed and Frankham 2003), several studies in invasion biology show that this is not a universal trend (Nicol et al. 1998; Hollingsworth et al. 1998; Tsutsui et al. 2003). In contrast with what has been observed in North America (Hufbauer et al. 2004), Chilean introduced populations of *A. ervi* have proved to be highly efficient in controlling important target-pests in the field (Starý et al.1993; Zepeda-Paulo et al. 2013), without non-target effects (B. Lavandero, pers. comm.). In addition, reciprocal-transplant experiments have shown that these populations exhibit high phenotypic plasticity in host-related traits, enabling a high parasitism rate on alternative aphid hosts (*S. avenae* and *A. pisum*) (Zepeda-Paulo et al. 2013).

Our results suggest that a decrease of neutral genetic diversity in introduced populations is not necessarily related with negative impacts on fitness-related traits, which are relevant for host use in parasitoids. Certainly, the introduction of individuals in a novel environment typically exposes a founder population to novel selective pressures. However, introduced populations for biological control may be able to respond to selection when the allele diversity is not completely depleted after the introduction and can still confer a high fitness in the new environment (Fauvergue et al. 2012). On the other hand, an increase in plasticity favoring the adaptive evolution of new optimal phenotypes could be possible during the colonization of novel environments (Lande 2015).

For instance, positive effects of bottlenecks on fitness and dispersion during the introduction of biological control agents have been observed in the ladybird *Harmonia axyridis* released into eastern North America due to the purge of deleterious alleles followed by inbreeding depression (i.e., reduced fitness in offspring of related individuals) during the introduction (Facon et al. 2011; Lombaert et al. 2014). This particular invasive population (i.e., bridgehead population) of *H. axyridis* have been the source of multiple invasions into different novel environments on a world-wide scale (i.e., invasive bridgehead effect) (see Lombaert et al. 2010; Facon et al. 2011).

Indeed, Fauvergue et al. (2012) have shown how an increased homozygosity can have unexpected positive effects when recessive deleterious alleles are exposed to selection and purged (purging of genetic load) and/or when beneficial alleles are present in the population introduced into novel environments, thus increasing the mean fitness of the introduced populations.

In effect, bottleneck events in haplodiploid species (i.e., haploid-males and diploid-females) such as parasitic Hymenoptera could promote the expression of recessive deleterious alleles in haploid males due to increased homozygosity in populations experiencing inbreeding (Henter 2003). This implies that deleterious mutations should be purged at every generation, just as the inbreeding depression should be reduced in traits subjected to purifying selection in males (Charlesworth and Willis 2009; Tien et al. 2015). Hence, haplodiploid species should have a much lower genetic load than diploid ones, being able to recover genetic diversity more quickly or enough to compensate the negative effects of inbreeding (Henter 2003).

However, more studies are needed to elucidate the genetic consequences of bottlenecks on the fitness and ecological success of introduced biological control agents in novel environments, particularly in haplodiploid parasitoid species, considering that the consequences may be species-specific.

Genetic differentiation between French and Chilean parasitoid populations and relationships among mtDNA haplotypes of *A. ervi* worldwide

A significant genetic differentiation between the French and Chilean populations of *A. ervi* was observed. This was mainly determined by some microsatellite loci showing strong differentiation (*F*-statistics by locus and allele frequencies). The factorial correspondence analysis (FCA) revealed that several of the French and Chilean genotypes show a close relationship not congruent with the general geographic isolation (France and Chile are in different continents). In addition, a low genetic differentiation among parasitoid populations within the same country was observed, as well as the lack of host-associated genetic differentiation confirming previous studies on *A. ervi* (Bilodeau et al. 2013; Zepeda-Paulo et al. 2015). The lack of genetic differentiation among populations of *A. ervi* within a same geographic range was recently reported as a consequence of a high gene flow mediated largely by parasitoid males (Zepeda-Paulo et al. 2015).

The genetic differentiation between French and introduced Chilean populations could be the result of a founder effect followed by genetic drift during the pre and post-release stages of the biological control agents. Since a reduced effective population size could drastically increment the effects of genetic drift on allele frequencies (alleles can be randomly loss or fixed), the occurrence of genetic structuring is highly probable (Hufbauer and Roderick 2005). Founder events are expected to increase the levels of genetic differentiation between populations due to the strong action of genetic drift after introductions with such small founder populations (Estoup and Guillemaud 2010). Therefore, genetic differentiation between the introduced and native populations is expected when they have been isolated for a long time as reported for the parasitoid *Diachasmimorpha tryoni*, which show an increased genetic differentiation between the introduced (Hawaii) and native (Australia) ranges over time since its introduction (Vorsino et al. 2014).

Interestingly, in North America, where *A. ervi* was also introduced, a significant genetic differentiation at microsatellite loci was shown between the introduced and their source native range populations in France, but with a higher degree of admixture (Hufbauer et al. 2004) than the observed between France and Chile. The mtDNA sequence analysis showed lower haplotype diversity for the Chilean samples, compared to the North American samples. Of the four haplotypes found in Chile (H-A, CH1, CH2 and CH3), three corresponded to exclusive haplotypes, which are likely derived from the most common haplotype-A observed in Chile and in North America (Hufbauer et al. 2004). Haplotypes-A was found in several of the countries analyzed from the native range of *A. ervi*, including France a well-documented source for introductions to America (Chile and North America) (Zuñiga et al. 1986b; Hufbauer et al. 2004). The observation of a unique haplotype-A shared between the introduced populations from Chile and the native range, might suggest a single major event of introduction for Chile. This observation supports the historical records of *A. ervi* individuals introduced to Chile from France, where at least one of the main introduction campaigns to Chile was carried out (271 individuals) (Zuñiga et al. 1986b). Contrary with what occurred in Chile, Hufbauer et al. (2004) reported evidence of multiple introduction events for the introduced populations in North America. For instance, haplotypes-A and B found in the native range were also observed among the *A. ervi* sequences from North America, including the haplotype-C that would have derived from a reported Japanese haplotype (Hufbauer et al. 2004). Multiple introductions in North America from its native range have been proposed, since *A. ervi* is currently commercially available from a number of European companies for biological control in greenhouses (Hufbauer et al. 2004). In Contrast, the parasitoid wasp *A. ervi* in Chile is not used for augmentative control in greenhouses, so the single introduction event reported during the 70s would be the only likely source of parasitoids. Hence, in the absence of a genetic "buffering" through multiple introductions, longer divergence times and/or a strong bottleneck would result in high levels of differentiation of biological control agents in the natal and novel environments, as observed here between French and Chilean populations.

Implications for biological control agents in novel environments

We have studied the genetic consequence in a parasitoid wasp successfully introduced for controlling important aphid pests in a novel environment. Our study provides evidence of a moderate genetic bottleneck occurring immediately after the release of parasitoids *A. ervi*, which was followed by significant genetic differentiation between populations in the novel and natal environments, and with a high gene flow among populations within the same geographic range. Moreover, mtDNA sequences analysis suggests that *A. ervi* was established in Chile after a single main event of introduction. Despite the loss of neutral genetic diversity in the introduced populations and its genetic differentiation with native populations, *A. ervi* has proven to be highly effective in controlling several aphid pests in different Chilean agroecosystems. The latter is likely the result of multiple interacting factors. For instance, introduced populations of *A. ervi* in Chile have shown high plasticity for host-related traits, enabling them to succeed well on alternative aphid hosts (Zepeda-Paulo et al. 2013). The efficient use of alternative hosts could favor the biological control of the target-pest, since different hosts could act as sources and reservoirs of natural enemies in case of a low incidence of the target-pest and/or under environment disturbance (Starý et al. 1993), which could be particularly true for biological control agents introduced in novel environments. In addition, the high gene flow among parasitoid populations associated to different hosts (e.g., *A. pisum* and *S. avenae*) observed in Chile, reflects a high level of connectivity, which could buffer the negative effects of genetic drift when small populations are introduced. Another positive effect of the high level of connectivity among introduced populations concerns the minimization of release efforts, because a single release could be sufficient to cover a wide geographic area (Hufbauer and Roderick 2005). An additional factor affecting the success of biological control agents is related to propagule pressures in a novel environment. The combination between the number of releases (i.e., propagule number) and the number of individuals released (i.e., propagule size) could significantly improve the establishment of controlling agents due to demographic processes occurring in the introduced populations (e.g., environmental and demographic stochasticity and Allee effects) (Fauvergue et al. 2012; Grevstad et al. 2013). To our knowledge, a single founder population of *A. ervi* was introduced in Chile and kept in quarantine, from which several releases of thousands of individuals (>500,000 in total) were performed in the field. These releases could also have positively influenced the establishment chances and the evolutionary trajectory followed by the introduced population (as a potential bridgehead population). Therefore, it seems clear that pre-release studies regarding genetic and demographic effects on the performance and adaptability (e.g., adaptive phenotypic plasticity) of potential biocontrol agents on novel environments need to be addressed. Moreover, the actual pressures within a novel environment must also be considered especially for ecological interactions before the release of a potential biological control agent (see Vorsino et al. 2014). In addition, further post- release studies addressing the link between eco-evolutionary and demographic processes occurring on populations of biological control agents that are experiencing novel environments are needed to maximize the efficacy and safety of biological control programs and the implementation of optimal releases strategies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Baker D, Loxdale H, Edwards O (2003) Genetic variation and founder effects in the parasitoid wasp, *Diaeretiella rapae* (M'intosh) (Hymenoptera: Braconidae: Aphidiidae), affecting its potential as a biological control agent. Mol Ecol 12:3303–3311

Beebee TJC, Rowe G (2008) An introduction to molecular ecology. Oxford University Press, Oxford

Belkhir K, Borsa P, Chikhi L et al (1996) GENETIX 4.05, logiciel sous Windows TM pour la génétique des

populations. Laboratoire génome, populations, interactions, CNRS UMR 5000:1996–2004
Bilodeau E, Simon J-C, Guay J-F et al (2013) Does variation in host plant association and symbiont infection of pea aphid populations induce genetic and behaviour differentiation of its main parasitoid, *Aphidius ervi*? Evol Ecol 27:165–184
Boer JG, Kuijper B, Heimpel GE et al (2012) Sex determination meltdown upon biological control introduction of the parasitoid *Cotesia rubecula*? Evol Appl 5:444–454
Charlesworth D, Willis JH (2009) The genetics of inbreeding depression. Nat Rev Genet 10:783–796
Chevin LM, Lande R (2010) When do adaptive plasticity and genetic evolution prevent extinction of a density-regulated population? Evolution 64:1143–1150
Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014
Dlugosch K, Parker I (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449
Drummond A, Ashton B, Buxton S et al (2011) Geneious, version 5. 4. Geneious, Auckland
Earl DA (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361
Eilenberg J, Hajek A, Lomer C (2001) Suggestions for unifying the terminology in biological control. Biocontrol 46: 387–400
Estoup A, Guillemaud T (2010) Reconstructing routes of invasion using genetic data: why, how and so what? Mol Ecol 19:4113–4130
Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620
Facon B, Hufbauer RA, Tayeh A et al (2011) Inbreeding depression is purged in the invasive insect *Harmonia axyridis*. Curr Biol 21:424–427
Fauvergue X, Vercken E, Malausa T et al (2012) The biology of small, introduced populations, with special reference to biological control. Evol Appl 5:424–443
Ferrari J, Via S, Godfray HCJ (2008) Population differentiation and genetic variation in performance on eight hosts in the pea aphid complex. Evolution 62:2508–2524
Frakham R (2005) Genetics and extinction. Biol Conserv 126:131–140
Frankham R, Ballou JD, Briscoe AD (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge
Frankham R, Ballou JD, Eldridge MDB, Lacy RC, Ralls K, Dudash MR, Fenster CB (2011) Predicting the probability of outbreeding depression. Conserv Biol 25:465–475
Franks SJ, Pratt PD, Tsutsui ND (2011) The genetic consequences of a demographic bottleneck in an introduced biological control insect. Conserv Genet 12:201–211
Garza J, Williamson E (2001) Detection of reduction in population size using data from microsatellite loci. Mol Ecol 10:305–318
Gerding MP, Zuñiga ES, Quiroz CE, Norambuena HM, Vargas RM (1989) Abundancia relativa de los parasitoides de Sitobion avenae (F) y *Metopolophium dirhodum* (WLK) (Homoptera:Aphididae) en diferentes áreas geográficas de Chile. Agricultura técnica (Chile) 49:104–114
Grevstad F, Coombs E, McEvoy P et al (2013) Revisiting release strategies in biological control of weeds: are we using enough releases? 13th International symposium on the biological control of weeds, Hawaii, USA. US Forest Service Forest Health Technology Enterprise Team
Guillemaud T, Ciosi M, Lombaert E et al (2011) Biological invasions in agricultural settings: insights from evolutionary biology and population genetics. CR Biol 334:237–246
Henter HJ (2003) Inbreeding depression and haplodiploidy: experimental measures in a parasitoid and comparisons across diploid and haplodiploid insect taxa. Evolution 57:1793–1803
Hoban SM, Gaggiotti OE, Bertorelle G (2013) The number of markers and samples needed for detecting bottlenecks under realistic scenarios, with and without recovery: a simulation-based study. Mol Ecol 22:3444–3450
Hollingsworth M, Hollingsworth P, Jenkins G et al (1998) The use of molecular markers to study patterns of genotypic diversity in some invasive alien *Fallopia* spp. (Polygonaceae). Mol Ecol 7:1681–1691
Hufbauer RA (2001) Pea aphid-parasitoid interactions: have parasitoids adapted to differential resistance? Ecology 82:717–725
Hufbauer RA (2002) Evidence for nonadaptive evolution in parasitoid virulence following a biological control introduction. Ecol Appl 12:66–78
Hufbauer RA, Roderick GK (2005) Microevolution in biological control: mechanisms, patterns, and processes. Biol Control 35:227–239
Hufbauer R, Bogdanowicz S, Harrison R (2004) The population genetics of a biological control introduction: mitochondrial DNA and microsatellie variation in native and introduced populations of *Aphidius ervi*, a parasitoid wasp. Mol Ecol 13:337–348
Lande R (2015) Evolution of phenotypic plasticity in colonizing species. Mol Ecol 24:2038–2045
Leberg P (2002) Estimating allelic richness: effects of sample size and bottlenecks. Mol Ecol 11:2445–2449
Lloyd CJ, Hufbauer RA, Jackson A et al (2005) Pre-and post-introduction patterns in neutral genetic diversity in the leafy spurge gall midge *Spurgia capitigena* (Bremi) (Diptera: Cecidomyiidae). Biol Control 33:153–164
Lombaert E, Guillemaud T, Cornuet J-M et al (2010) Bridgehead effect in the worldwide invasion of the biocontrol harlequin ladybird. PLoS One 5:e9743. doi:10.1371/journal.pone.0009743
Lombaert E, Estoup A, Facon B et al (2014) Rapid increase in dispersal during range expansion in the invasive ladybird *Harmonia axyridis*. J Evol Biol 27:508–517
Luikart G, Cornuet JM (1998) Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conserv Biol 12:228–237
Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability of populations. Evolution 29:1–10
Nicol D, Armstrong K, Wratten S et al (1998) Genetic diversity of an introduced pest, the green spruce aphid *Elatobium abietinum* (Hemiptera: Aphididae) in New Zealand and the United Kingdom. Bull Entomol Res 88:537–543

Nyabuga FN, Loxdale HD, Heckel DG et al (2011) Temporal genetic structuring of a specialist parasitoid, *Lysiphlebus hirticornis* Mackauer (Hymenoptera: Braconidae) attacking a specialist aphid on tansy. Biol J Linn Soc 102: 737–749

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539

Peccoud J, Figueroa C, Silva A et al (2008) Host range expansion of an introduced insect pest through multiple colonizations of specialized clones. Mol Ecol 17:4608–4618

Peccoud J, Ollivier A, Plantegenest M et al (2009) A continuum of genetic divergence from sympatric host races to species in the pea aphid complex. Proc Natl Acad Sci 106: 7495–7500

Peery MZ, Kirby R, Reid BN et al (2012) Reliability of genetic bottleneck tests for detecting recent population declines. Mol Ecol 21:3403–3418

Piry S, Luikart G, Cornuet J-M (1999) BOTTLENECK: a program for detecting recent effective population size reductions from allele data frequencies. Montpellier, France

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

R Core Team (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49:1280–1283

Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. Conserv Biol 17:230–237

Roderick GK, Navajas M (2003) Genes in new environments: genetics and evolution in biological control. Nat Rev Genet 4:889–899

Roderick G, Navajas M (2008) The primacy of evolution in biological control. In: Proceedings of the XII international symposium on biological control of weeds: La Grande Motte, France, 22–27 April 2007. CABI, pp 403–409

Roderick GK, Hufbauer R, Navajas M (2012) Evolution and biological control. Evol Appl 5:419–423

Rojas S (2005) Control biológico de plagas en Chile. Historia y avances. Colección libros INIA N°12. 123p. Instituto de Investigaciones Agropecuarias (INIA), Centro Regional de Investigación La Cruz, La Cruz, Chile

Simon C, Frati F, Beckenbach A et al (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann Entomol Soc Am 87:651–701

Simon J-C, Carre S, Boutin M et al (2003) Host-based divergence in populations of the pea aphid: insights from nuclear markers and the prevalence of facultative symbionts. Proc R Soc Lond Ser B Biol Sci 270:1703–1712

Starý P (1993) The fate of released parasitoids (Hymenoptera: Braconidae, Aphidiinae) for biological control of aphids in Chile. Bull Entomol Res 83:633–639

Starý P (1995) The Aphidiidae of Chile (Hymenoptera, Ichneumonoidea, Aphidiidae). Deutsche Entomologische Zeitschrift 42:113–138

Starý P, Gerding I, Norambuena I et al (1993) Environmental research on aphid parasitoid biocontrol agents in Chile (Hym., Aphidiidae; Hom., Aphidoidea). J Appl Entomol 115:292–306

Stilmant D, Van Bellinghen C, Hance T, Boivin G (2008) Host specialization in habitat specialists and generalists. Oecologia 156:905–912

Sunnucks P, Hales DF (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I–II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). Mol Biol Evol 13:510–524

Taylor S, Downie D, Paterson I (2011) Genetic diversity of introduced populations of the water hyacinth biological control agent *Eccritotarsus catarinensis* (Hemiptera: Miridae). Biol Control 58:330–336

Tien N, Sabelis M, Egas M (2015) Inbreeding depression and purging in a haplodiploid: gender-related effects. Heredity 114:327–332

Tsutsui ND, Suarez AV, Grosberg RK (2003) Genetic diversity, asymmetrical aggression, and recognition in a widespread invasive species. Proc Natl Acad Sci 100:1078–1083

Unruh T, White W, Gonzalez D et al (1983) Heterozygosity and effective size in laboratory populations of *Aphidius ervi* [Hym.: Aphidiidae]. Entomophaga 28:245–258

Van Oosterhout C, Hutchinson WF, Wills DP et al (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Vorsino AE, Wieczorek AM, Wright MG et al (2014) Genetic analysis of an introduced biological control agent reveals temporal and geographic change, with little evidence of a host mediated shift. Biol Control 77:41–50

Zepeda-Paulo FA, Ortiz-Martínez SA, Figueroa CC et al (2013) Adaptive evolution of a generalist parasitoid: implications for the effectiveness of biological control agents. Evol Appl 6:983–999

Zepeda-Paulo FA, Lavandero B, Mahéo F, Dion E, Outreman Y, Simon J-C, Figueroa CC (2015) Does sex-biased dispersal account for the lack of geographic and host-associated differentiation in introduced populations of an aphid parasitoid? Ecol Evol 5:2149–2161

Zuñiga SE, Van den Bosch R, Drea J, Francis G (1986a) Control biológico de los áfidos (Homoptera: Aphididae) de los cereales de Chile. II. Obtención, introducción y cuarentena de depredadores y parasitoides. Agricultura Técnica (Chile) 46:479–487

Zuñiga SE, Van den Bosch R, Drea JJ et al (1986b) [The biological control project against the cereal aphids (Hom., Aphididae) in Chile. 2: Exploration, importation and quarantine of predator and parasitoid species]. Agricultura Tecnica (Chile)