

The population genetics of a biological control introduction: mitochondrial DNA and microsatellite variation in native and introduced populations of *Aphidius ervi*, a parasitoid wasp

R. A. HUFBAUER,*† S. M. BOGDANOWICZ* and R. G. HARRISON*

*Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853, USA, †Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 805 23-1177, USA

Abstract

Introductions of biological control agents may cause bottlenecks in population size despite efforts to avoid them. We examined the population genetics of *Aphidius ervi* (Hymenoptera: Braconidae), a parasitoid that was introduced to North America from Western Europe in 1959 to control pea aphids. To explore the phylogeographical relationships of *A. ervi* we sequenced 1249 bp of mitochondrial DNA (mtDNA) from 27 individuals from the native range and 51 individuals from the introduced range. Most individuals from Western Europe, the Middle East and North America shared one of two common haplotypes, consistent with the known history of the introduction. However, some *A. ervi* from the Pacific Northwest have a haplotype that is most similar to haplotypes found in Japan, raising the possibility of a second accidental introduction. To examine population structure and assess whether a bottleneck occurred upon introduction to North America, we assayed variation at 5 microsatellite loci in 62 individuals from 2 native populations and 230 individuals from 6 introduced populations. Introduced samples had fewer rare alleles than native samples ($F_{1,34} = 13.5$, $P = 0.0008$), but heterozygosity did not differ significantly. These results suggest that a mild bottleneck occurred in spite of the introduction of over 1000 individuals. Using a hierarchical Bayesian approach, the founding population size was estimated to be 245 individuals. AMOVA showed significant genetic differentiation between the European and North American samples, and a Bayesian assignment approach clustered individuals into four groups, with most European individuals in one group and most North American individuals in the other three. These results highlight that genetic changes are associated with founder events in rapidly growing natural populations, even when the founding population size is relatively large.

Keywords: biological control, introduced species, isolation-by-distance, microsatellite DNA, mtDNA, population bottle neck

Received 31 March 2003; revision received 18 September 2003; accepted 5 November 2003

Classical biological control is the suppression of exotic pests with predators, pathogens and parasites derived from the pests' area of origin. Most biological control introductions involve large numbers of individuals to avoid the loss of genetic variation that can be associated

with bottlenecks in population size. However, there are times in the course of an introduction during which the effective size of a founding population may be reduced. Initially, a finite number of individuals is sampled from a natural population. Those individuals typically must pass through quarantine to verify that no unwanted pathogens or other species are present. This may take several generations during which time population sizes may be relatively small and inbreeding may occur (Unruh *et al.* 1983). When released into the new range, only a small

Correspondence: R. A. Hufbauer. Present address: Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523-1177, USA. Fax: +1 970 491 3862; E-mail: hufbauer@lamar.colostate.edu

fraction of individuals may actually contribute to the next generation, shrinking the true founding population size even further. Thus, it is easy to imagine that even when an initial collection is large enough to avoid a significant bottleneck, a reduction in genetic variation may ultimately occur. Although hundreds of species have been released around the world for biological control, there are few data available on the genetic consequences of those introductions.

In general, bottlenecks in population size can reduce levels of genetic variation (Mayr 1954; Nei *et al.* 1975; Templeton 1980; Barton & Charlesworth 1984) and yet through epistasis can also increase additive genetic variation (e.g. Goodnight 1987, 1988; Carson 1990; Armbruster *et al.* 1998). Founder effects have been implicated in theories of population differentiation and speciation, but these theories remain controversial (e.g. Mayr 1954; Barton & Charlesworth 1984; Carson & Templeton 1984; Gavrilets & Boake 1998).

Most empirical studies on the effects of population bottlenecks have employed model organisms put through experimental bottlenecks (e.g. Bryant *et al.* 1986, 1990; Woodworth *et al.* 1994). However an increasing number of studies focus on accidental or intentional introductions of organisms. For example, Baker & Moeed (1987) found reduced genetic diversity and a reduction in the number of rare alleles in introduced populations of common mynas (*Acridotheres tristis*: Sturnidae, Passeriformes) relative to native populations, consistent with a very small effective size of the founding populations. Irvin *et al.* (1998) found a reduced number of alleles in New World populations of *Drosophila simulans*, but little change in heterozygosity suggesting that the New World populations have experienced a mild bottleneck. Merilä *et al.* (1996) reviewed several studies on the genetic consequences of introductions and found that reductions in levels of genetic variability were greater when the founding populations were smaller, and that less variation was lost if the rate of population growth following an introduction was high, as predicted by Nei *et al.* (1975).

Biological control agents have been introduced worldwide, and often their history is better known than the history of inadvertent introductions. However, to our knowledge the population genetic consequences of these introductions have been studied in only a few systems (Easteal 1985a,b; Krafusur & Obrycki 1996; Sanchez & Carde 1998; Slade & Mortiz 1998), although genetic diversity is generally thought to be important in their successful establishment and subsequent efficacy (reviewed by Hopper *et al.* 1993). The process of establishing populations of biological control agents closely parallels conservation efforts aimed at reintroducing species into their former ranges. Thus, closer examination of the genetic consequences of biological control introductions may give insights into how to best design reintroduction efforts.

Aphidius ervi (Hymenoptera: Braconidae) is a parasitoid wasp that was introduced into North America in 1959 to control the pea aphid, a pest species native to Eurasia that feeds on alfalfa, clover and other legumes (Angalet & Fuester 1977). *Aphidius ervi* is a solitary endoparasitoid: the female wasp lays one egg (typically) inside an aphid, the egg hatches and the larva feeds on the aphid's internal tissues, eventually killing the aphid. For the biological control introduction, about 1000 *A. ervi* individuals were shipped from France to the United States Department of Agriculture (USDA) in Maryland. Of these, 800 were collected as pupae directly from alfalfa fields in France and 200 were from a French laboratory culture established from collections in the same region (USDA Beneficial Insects Research Laboratory second quarter report 1959; Halfhill *et al.* 1972; Mackauer & Campbell 1972; Angalet & Fuester 1977). The insects were in culture at the USDA for less than three months before release (3–6 generations) and the populations grew quickly after release suggesting that the bottleneck imposed was relatively brief. In addition, a small number (< 20) of *A. ervi* from the Middle East were imported to California in 1965 and subsequently released. With an introduction of 1000 individuals, a bottleneck lasting much less than 100 generations, and a rapid rate of population increase following the bottleneck, the reduction in heterozygosity and loss of rare alleles should be minimal (Nei *et al.* 1975). However, due to the nature of the introduction process (outlined above) the effective size of the founding population may have been smaller than the census size.

Research on the ability of the parasitoids to overcome physiological defences of their pea aphid hosts documents that introduced *A. ervi* in North America (upstate New York region) are less able to develop successfully on pea aphids than are native wasps from France (Loire River Valley) (Hufbauer 2002). There also appears to be less variation among the introduced wasp populations than among native ones in the ability to overcome host resistance (Hufbauer 2002), suggesting that the introduction process may have led to nonadaptive evolutionary changes in the introduced wasps. Here, we examine patterns of presumably neutral genetic variation in native and introduced populations. Specifically, we (i) explore relationships among *A. ervi* from many localities using mitochondrial DNA (mtDNA) sequence data in an attempt to verify the source of North American *A. ervi* populations (Western Europe vs. the Middle East vs. other inadvertent introductions); and (ii) compare variability in microsatellite loci of native and introduced populations to assess the intensity of the population bottleneck (if any) that occurred during the introduction to North America. Ultimately, we hope to shed light on the cause of the reduced ability of introduced wasps to overcome host resistance.

Materials and methods

Sequencing protocols

The mtDNA sequence analysis included 27 individuals from 14 localities in the native range, and 51 individuals from 18 localities in the introduced range (Table 1). We used the polymerase chain reaction (PCR) to amplify an ~2000 bp segment of mtDNA which included portions of both COI and COII. We amplified and sequenced using the same primers (Table 2). We sequenced from both ends of the PCR product using a BigDye Terminator Cycle Sequencing Kit and an ABI 377 automated sequencer (PE Applied Biosystems). Sequences are available from GenBank

(COI Accession nos: AY262762–AY262787 and AY427835–AY427886; COII Accession nos: AY262788–AY262813 and AY427887–AY427938).

Analysis of mtDNA sequence data

Sequences were aligned using Megalign (DNASTAR, Inc.) and trimmed to the same length. There were no gaps in the two regions, making alignment straightforward. We constructed a haplotype network using tcs v. 1.1.3 (Clement *et al.* 2000) based on 1249 bp (639 from COI and 610 from COII) for all 78 *Aphidus ervi* that were sequenced (Table 1). Haplotype diversity, nucleotide diversity (π), theta (θ) per site (where $\theta = N_e\mu$ for mtDNA, and estimates the number

Table 1 Collection locations, sample codes, collectors, year collected. All individuals were sequenced from S1718 to A3991 unless noted

Collection location	Sample codes	Collector	Year*
<i>Native range</i>			
France, Lusignan 1	FR13, FR2,‡ FR3,‡ FR5,‡ FR6,‡ FR7,‡ FR8‡	R. Bournoville	1999
France, Lusignan 2	FRC3	R. Hufbauer	1997
Germany, Bayreuth	Germany†	W. Voelkl	2000
Hungary, Budapest	HU42, HU45, HU46, HU47, HU49	L. Polgar	1999
Israel, Kfar Yebespal	Israel	T. Unruh	1981
Italy (colony)	Italy†	F. Pennacchio	NA
Japan, Sapporo, Hokkaido (colony)	Japan H1†	H. Takada	1997
Japan, Nagano, Honshu	Japan N1, Japan N2	H. Takada	1999
Morocco, Zagora	Morocco Z1, Morocco Z10	T. Unruh	1980
Netherlands (colony)	Netherlands	J. van Schelt	1999
Norway	Norway†	T. Unruh	1982
Turkey (F1 colony)	Turkey 1, Turkey 10	T. Unruh	1984
United Kingdom, Rothamstead (colony)	UK12	D. Brooks, W. Powel	1998
United Kingdom, Rothamstead (colony)	UK5	D. Brooks, W. Powel	1999
<i>Introduced Range</i>			
Argentina, Castelar	Argentina 1	T. Unruh	1988
Argentina, Trebol	Argentina 2	T. Unruh	1987
Chile, Andes	Chile 1†	T. Unruh	1988
Chile, Andes (F1)	Chile 2	T. Unruh	1988
Ecuador	Ecuador	J. C. Monje	2000
USA, California, Grapevine	CAG1, CAG2, CAG3, CAG4, CAG5	R. & S. Hufbauer	1999
USA, California, Riverside	CAR1,‡ CAR2,‡ CAR4,‡ CAR5‡	R. & S. Hufbauer	1999
USA, California, San Pascual	CASP1†	T. Unruh	1984
USA, Colorado, Fort Collins	COM1,‡ COM2,‡ COM4,‡ COM5‡	R. Hufbauer	1999
USA, New York State, Geneva	NYG2,‡ NYG4,‡ NYG5‡	R. Hufbauer	1999
USA, New York State, Lansing	NYL1, NYL2, NYL3, NYL4, NYL5	R. Hufbauer	1999
USA, Oregon, Corvallis	ORCC21, ORCCA31	R. Hufbauer	1997
USA, Oregon, Madras	ORM1,†‡ ORM2,‡ ORM3,‡ ORM4,‡ ORM5‡	R. Hufbauer, M. McClure	1999
USA, Oregon, Wasco	ORW1‡	R. Hufbauer, M. McClure	1999
USA, Washington State, Othello	WAO1, WAO2, WAO3	R. Hufbauer	1999
USA, Washington State, Moses Lake	WAML1, WAML2, WAML3, WAML4	R. Hufbauer	1999
USA, Washington State, Pasco	WAP2, WAP3	R. Hufbauer	1999
USA, Washington State, Whidbey Island	WAWH1,†‡ WAWH2,†‡ WAWH3,‡ WAWH4, WAWH5,‡ WAWH6,‡ WAWH9‡	R. Hufbauer	1999

*Years of collection. For laboratory colonies, year of establishment provided when available.

†Individuals sequenced with S1751 rather than S1718.

‡Individuals also used in microsatellite analyses.

Gene	Location of 3'-end*	Sequence (5'-3')	Name†
COI	S1751	GGATCACCTGATATAGCATTC	C1-J-1751
	S1718	GGAGGATTTGGAAATTGATTAGTTCC	C1-J-1718
COII	A3661	CCACAAAATTTCTGAACATTGACCA	C2-N-3661
	A3587	CCAGGAGTAGCATCTACTTTAATACC	C2-N-3587

*A and S refer to antisense or sense strand, respectively, and the number refers to the position of the 3' nucleotide relative to published *Drosophila yakuba* sequence (Clary & Wolstenholme 1985).

†First three primers listed are from Simon *et al.* (1994), the last primer was designed based on *A. ervi* sequence and is named using the same naming scheme.

Table 2 Gene region, location of 3'-end, sequence, and name of PCR and sequencing primers

Collection location	Code	F/M	Sample size*	Mean no. alleles	Heterozygosity
<i>North America</i>					
Riverside, CA USA	CA	28/16	72	5.4	0.494
Fort Collins, CO USA	CO	12/6	30	4.8	0.545
Geneva, NY USA	NY	15/14	44	4.4	0.518
Wasco, OR USA	OR1	26/18	70	5.2	0.594
Madras, OR USA	OR2	24/22	70	5.2	0.520
Whidbey, WA USA	WAWH	25/24	74	3.2	0.486
<i>Europe</i>					
Lusignan, FR	FR	10/10	30	6.0	0.507
Budapest, Hungary	HU	24/18	66	9.4	0.527

Table 3 Collection locations, population codes, numbers of females and males (F/M), sample sizes (number of genomes), mean number of alleles per population across loci, and mean heterozygosity per population

*2F + M.

of differences per nucleotide expected between two randomly chosen alleles under mutation–drift equilibrium), and the average number of pairwise nucleotide differences (k) were calculated in DNASP v. 3.53 (Rozas & Rozas 1999). We performed these analyses on two groups: native individuals, and introduced individuals from North America (not including the five individuals from South America). Two individuals, one from the native and one from the introduced range, had very divergent mtDNA sequences (see below) and were excluded from the DNASP analyses to give a total of 26 native individuals and 45 introduced individuals.

Microsatellite protocols

A. ervi were collected as larvae (in parasitized aphids) and pupae (in aphid exoskeletons or 'mummies') for microsatellite analysis (Table 3). Collections were made from eight different locations, six North American and two European. Most populations had a sex ratio biased towards females. In all, we used 164 diploid females and 128 haploid males. Populations were defined operationally as *A. ervi* found in a single alfalfa field (~2–10 acres). Samples were taken from widely separated areas in each field in an effort to avoid sampling closely related individuals and to obtain an adequate sample of the genetic variation

present. Microsatellites were isolated from a mixed pool of European and North American *A. ervi* as described in Hufbauer *et al.* (2001). Microsatellite loci were amplified using PCR Express thermocyclers (Hybaid) in 10 μ L reactions containing 1 μ L genomic DNA, 1 \times PCR Buffer (20 mM Tris–HCl, pH 8.4, 50 mM KCl), 2 mM MgCl₂, 0.2 mM each dNTP, 2 pmol of each primer, 0.5 units *Taq* DNA polymerase (Life Technologies), and 0.1 μ L TaqStart antibody (Clontech). Amplification cycle conditions consisted of ~1 min at 90 °C, and then 35 cycles of 50 s at 95 °C, 1 min at annealing temperature (see Hufbauer *et al.* 2001 for details), 1 min 30 s at 72 °C, and then a final extension step for 45 min at 72 °C. Reactions were held at 0–4 °C before separation on an ABI 377 automated sequencer. Allele sizes were scored by comparison with internal size markers using GENOTYPER software (PE Applied Biosystems).

Microsatellite analyses

All microsatellite analyses were performed on the total number of chromosomes sampled: twice the number of diploid females plus the number of haploid males. The number of alleles per locus for each sample location was estimated using GENEPOP (GENEPOP ON THE WEB at <http://wbiomed.curtin.edu.au/genepop/index.html>; Raymond & Rousset 1995). Deviation from Hardy–Weinberg equilibrium

(HWE) was assessed using the exact probability tests implemented in GENEPOP. Heterozygosities were estimated using ARLEQUIN (v. 2.0) (Excoffier *et al.* 1992; Schneider *et al.* 2000).

We tested for evidence of a bottleneck in population size associated with the introduction of *A. ervi* to North America in three ways: with the program BOTTLENECK v. 1.2 (Cornuet & Luikart 1996; Luikart *et al.* 1998), with simple analyses of variance to compare the numbers of alleles and heterozygosities, and with the program MSVAR v.1.3 (Beaumont 1999; Storz & Beaumont 2002). BOTTLENECK utilizes the fact that allelic diversity is reduced faster than heterozygosity during a reduction in population size. This gives a higher than expected heterozygosity given the number of alleles per locus in a population that has recently undergone a bottleneck (see Cornuet & Luikart 1996), and a shift in mode of the frequency distribution of alleles from rarest alleles being the most frequent to more common alleles being more frequent (Luikart *et al.* 1998). We used the stepwise mutation model (SMM) and the two-phase model (TMP) because they may more realistically model microsatellite evolution than the infinite alleles model (Valdes *et al.* 1993; DiRienzo *et al.* 1994). BOTTLENECK relies on information from individual locations and does not compare between sample locations. We also wanted to contrast directly our introduced North American and native European samples. To do this, we compared the number of alleles per locus and the average heterozygosities between the native and introduced samples using analyses of variance that included the locus identifier as a covariate (JMP v. 5, SAS Institute, 2002). The number of alleles was natural log transformed for analysis to improve the normality of the residuals. The residuals for the heterozygosity analysis were not significantly different from normal. Finally, we employed MSVAR to estimate the current effective sizes, and the ratio of the current sizes (N_0) to the ancestral sizes (N_1) (where $r = \log_{10} N_0 / \log_{10} N_1$) of the populations represented by the North American and European samples (Beaumont 1999; Storz & Beaumont 2002). An r -value of 1 indicates no change in population size, whereas $r > 1$ suggests population expansion, and $r < 1$ suggests population decline. MSVAR assumes a stepwise mutation model (SSM) and estimates the posterior probability distribution of the population parameters using Markov chain Monte Carlo (MCMC) simulations based on the observed distribution of microsatellite alleles and their repeat number. We used the known history of the biological control introduction to set the distributions of the priors for North America. Priors for the ancestral population size (before introduction to North America) were set to range 10^4 – 10^9 , and priors for the current size were set to range 10^1 – 10^3 . We assumed a long-term population expansion of the European samples due to the expansion of agriculture. We ran five independent chains on subsamples of 100 chromosomes of the North American data and on all 96 chromo-

somes represented in the European data. Each chain was run for 10^9 MCMC iterations, recording parameter values every 10^5 MCMC iterations. The North American data set was subsampled to achieve convergence in a reasonable time (following Storz & Beaumont 2002). We combined the second half of each run for overall data sets comprised of 25 000 points for both the North American and European data sets. We report means and upper and lower quartiles (Storz & Beaumont 2002) of the current effective population sizes for each region (calculated in JMP v. 5, SAS Institute, 2002).

We implemented Mantel's test (Mantel 1967) to examine the relationship between genetic and geographical distances using the program IBID (ISOLATION-BY-DISTANCE; Bohonak 2002). Population pairwise θ_{ST} (an analogue of F_{ST} calculated in GENEPOP according to Weir & Cockerham (1984)) was used for genetic distance, and approximate geographical distances between sample locations were measured as linear distance in km between the towns closest to the sample locations. Analyses were performed for the North American location pairs. Geographic distance was natural log transformed for the analyses (Slatkin 1993). The results were qualitatively comparable with and without transformations of both genetic and geographical distance. One pair of sample locations (the Oregon samples) were quite close to each other. Close populations may not follow the same pattern of isolation-by-distance found in widely spread samples (Rousset 1997); therefore analyses also were done with and without that population pair. The results were qualitatively similar and the analyses excluding the close pair are not shown.

To examine the distribution of genetic variation among individuals, among sample locations, and between Europe and North America we performed an analysis of molecular variance (AMOVA) in ARLEQUIN (v. 2.0) (Excoffier *et al.* 1992; Schneider *et al.* 2000). We used the hierarchical model for genotypic data with several groups of populations and no within-individual level.

In addition to these analyses using sample locations as *de facto* populations, we used the program STRUCTURE (v. 2; Pritchard *et al.* 2000) to estimate the number of populations represented by the eight sample locations to give us a separate insight into how the genetic variation is organized. STRUCTURE uses a Bayesian, Markov chain Monte Carlo approach to cluster individuals into groups while minimizing Hardy–Weinberg disequilibrium and gametic phase disequilibrium between loci within groups. The number of populations (K) represented by the data can be calculated by comparing the estimated log probability of the data for different values of K (Pritchard *et al.* 2000). We performed a series of independent runs using K from 1 to 8 populations, a burn-in period of 40 000 MCMC iterations, and a data collection period of 10^6 MCMC iterations. We ran three independent simulations for each value of K . The

independent runs produced consistent results for the same value of K . We estimated $\Pr(X|K)$ for a uniform prior on $K = 1, 2, \dots, 8$ (Pritchard *et al.* 2000).

Results

Mitochondrial DNA sequence data

We identified 12 distinct haplotypes among the 78 mtDNA sequences (Fig. 1). The two most common haplotypes (A and B) were found in individuals from both the native and introduced range. These two haplotypes differed from each other by only a single nucleotide substitution. The third most common haplotype (C) included only western North American individuals (Oregon, Washington and one Colorado sample), and appeared (on the basis of a single synapomorphy) to be derived from a haplotype found in Nagano, Japan. With the exception of three individuals, all wasps had haplotypes only 1 or 2 steps removed from the most common haplotypes. The three divergent haplotypes (Ecuador 1, Japan H1 and NYG4) shared four nucleotide substitutions that distinguished them from haplotype A. In addition, these three haplotypes were characterized by 1, 12 and 16 unique nucleotide substitutions, respectively. Excluding the divergent Japanese individual, the 26 remaining samples from the native range had a total of 4 polymorphic sites. Haplotype diversity was 0.628 (SD of 0.057), nucleotide diversity (π) was 0.00066 (SD of 0.00001), θ per site calculated from the number of segregating sites was 0.00084, and the average number of pairwise nucleotide differences (k) was 1.048. The 45 introduced samples from North America, excluding the divergent NYG4 individual, had a total of 6 polymorphic sites. Haplotype diversity was 0.613 (SD of 0.064), π was 0.0009 (SD of 0.00014), θ per site was 0.0011, and the average number of pairwise nucleotide differences (k) was 1.129.

Microsatellite variation

All five microsatellite loci were polymorphic in each of the eight populations sampled. Five of the 40 locus/population combinations showed a significant deviation from HWE, controlling for multiple comparisons (France locus 4, $P < 0.004$; Hungary loci 51 and 74, $P < 0.004$ and $P < 0.012$, respectively; New York locus 4, $P < 0.028$; and Oregon 2 locus 4, $P < 0.04$; P -values are corrected for multiple comparisons using the Bonferroni approach). Significant deviations were caused by a heterozygote deficit in every case. The five deviations from HWE are unlikely to represent a Wahlund effect, as the sample locations were geographically limited, and it is reasonable to expect that the individuals within them mated randomly. Departure from HWE may be due to either null alleles or violations of other assumptions inherent to Hardy–Weinberg such as close linkage

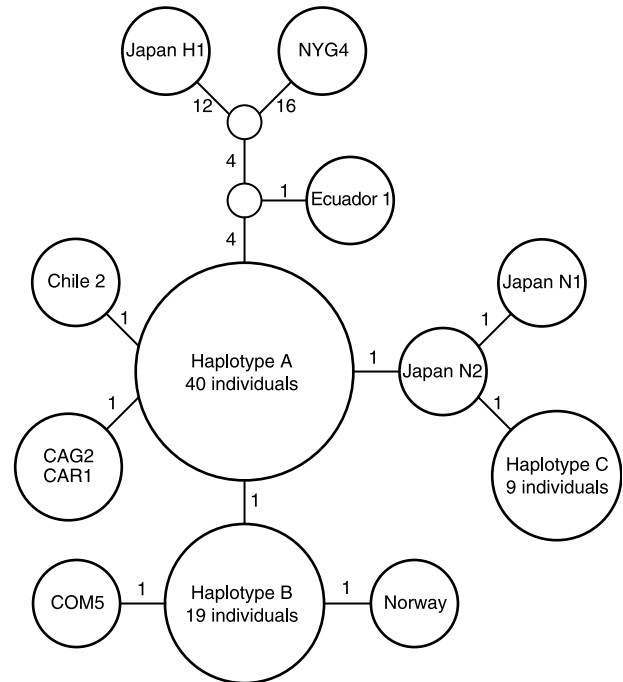


Fig. 1 Haplotype network of the mtDNA sequence data constructed using tcs v. 1.1.3. Numbers along branches indicate the number of nucleotide substitutions between haplotypes. Inferred haplotypes are shown with small circles. Sampled haplotypes have the name of the representative sample within, except for the three most common haplotypes. Membership of common haplotypes is as follows. *Haplotype A*: native individuals AerKop1, FR3, FR6, FR7, Germany, HU49, Israel, Morocco Z1, Morocco Z10, UK1, UK; introduced individuals Argentina 1, Argentina 2, CAG1, CAG3, CAG4, CAG5, CASP1, CAR2, CAR4, CAR5, Chile1, COM1, COM2, ORCC21, ORCCA31, ORM1, ORM3, ORM4, ORM5, WAML1, WAML2, WAML4, WAO1, WAO2, WAO3, WAP2, WAP3, WAWH3, WAWH6. *Haplotype B*: native individuals FR1, FR2, FR5, FR8, FRC3, HU42, HU45, HU46, HU47, Italy, Turkey1, Turkey10; introduced individuals NYG2, NYG5, NYL1, NYL2, NYL3, NYL4, NYL5. *Haplotype C*: no native individuals; introduced individuals COM4, ORM2, ORW1, WAML3, WAWH1, WAWH2, WAWH4, WAWH5, WAWH9. Sample names follow Table 1.

to areas under selection. Although the STRUCTURE analysis assumes HWE in order to group individuals into populations, with only 5 of 40 locus/sample combinations out of HWE, and with their distribution spread across 3 loci and 5 samples, we decided to perform the analyses despite the departures. Results should be interpreted conservatively.

BOTTLENECK detected only two populations with the consistent excess in heterozygosity expected with a strong bottleneck in population size: Riverside, CA and Wasco, OR. For both populations significance could only be demonstrated using the stepwise mutation model with the one-sided Standardized Differences Test, which requires more loci for reliable estimates (Table 4). Figure 2 shows

Table 4 Probability values for test of bottleneck effects calculated using the stepwise mutation model (SSM) and the two-phase model (TPM). Results for the two models were consistent except as indicated. The Sign Test compares the expected number of loci with heterozygote excess given that a bottleneck has occurred, with the observed number of loci with heterozygote excess. The Standardized Differences Test establishes if the average of standardized differences between observed and expected heterozygosities is significantly different from zero. For the Wilcoxon test, probabilities are for one-tailed tests of heterozygote excess. (See Cornuet & Luikart 1996 and Luikart et al. 1998 for details.) Correcting for multiple comparisons, only probabilities = 0.001 keep the risk of type 1 error at 0.05

Collection location	Sign test	Standardized Differences test	Wilcoxon test	Mode-shift
Riverside, CA USA	NS	< 0.0001 SMM NS TPM	NS	
Fort Collins, CO USA	NS	NS	NS	
Geneva, NY USA	NS	NS	NS	
Wasco, OR USA	NS	0.0001 NS TPM	NS	
Madras, OR USA	NS	NS	NS	
Whidbey, WA USA	NS	NS	NS	mode shift

Table 5 AMOVA testing population structure between the native and introduced populations. See text for details

Source of variation	d.f.	Sum of squares	Variance comp.	% of variation	P-value
Between EU and NA (native and introduced)	1	18.6	0.087	5.4	0.04
Between populations within EU and NA	6	31.7	0.068	4.2	< 0.0001
Within populations	448	659.8	1.47	90.45	< 0.0001

microsatellite allele frequency distributions in all populations. The graphs group alleles from all of the loci assayed into allele frequency bins following Luikart *et al.* (1998) and illustrate an overall trend of the introduced samples having fewer rare alleles than the native samples. Because sampling effort affects the number of alleles found, the graphs are grouped according to sample size, with one native sample location (Hungary or France) placed with the introduced populations of similar sample size for comparison.

The ANOVAS showed that the number of alleles per locus in the introduced samples (4.09) was significantly less than the number in the native samples (5.90, $F_{1,34} = 13.5$, $P = 0.0008$). Mean observed heterozygosity did not differ significantly between the native (0.53) and introduced (0.52) samples ($F_{1,30} = 0.45$, $P = 0.84$, Table 3).

The MSVAR analyses provided an estimate of the current effective population size in North America of 245 individuals, with lower and upper quartiles of 69 and 678, respectively. Given the very recent introduction, it is unlikely that the samples reflect new mutations, and thus this current size estimates the numbers of individuals that founded the North American populations. These estimates suggest that between 7 and 68% of the initial 1000 individuals founded the North American populations. The ratio of the population sizes, r , was 0.66. In contrast, the

current size of the European population was estimated at 7.8×10^4 , with quartiles ranging from 4018 to 2.8×10^5 , and showed modest population growth in the last 2000–3000 years ($r = 1.19$). The estimates of both ancestral (1.3×10^4) and current European population sizes were larger than estimates of the ancestral North American population size (4.2×10^3).

A significant pattern of isolation-by-distance was found among the North American samples ($r = 0.53$, $P = 0.012$, Fig. 3). Furthermore, although the AMOVA revealed that most of the variation was found within sample locations, there was significant variation between the native and introduced groups, and among sample locations within those groups (Table 5). Overall F_{ST} was 0.095 ($P < 0.0001$).

The STRUCTURE analysis produced results concordant with the AMOVA. There was a high (0.999) probability that the individuals from the eight sample locations represent four groups, with substantial differences among populations with respect to assignment of individuals to these four groups (Table 6). Wasps from the two European samples were assigned predominantly to cluster 4, whereas North American samples had only 8–24% of individuals assigned to that cluster. A majority of individuals from Whidbey Island and a substantial fraction of wasps from Oregon were assigned to cluster 1 (Table 6). Only 5–13% of individuals from other populations were included in cluster 1.

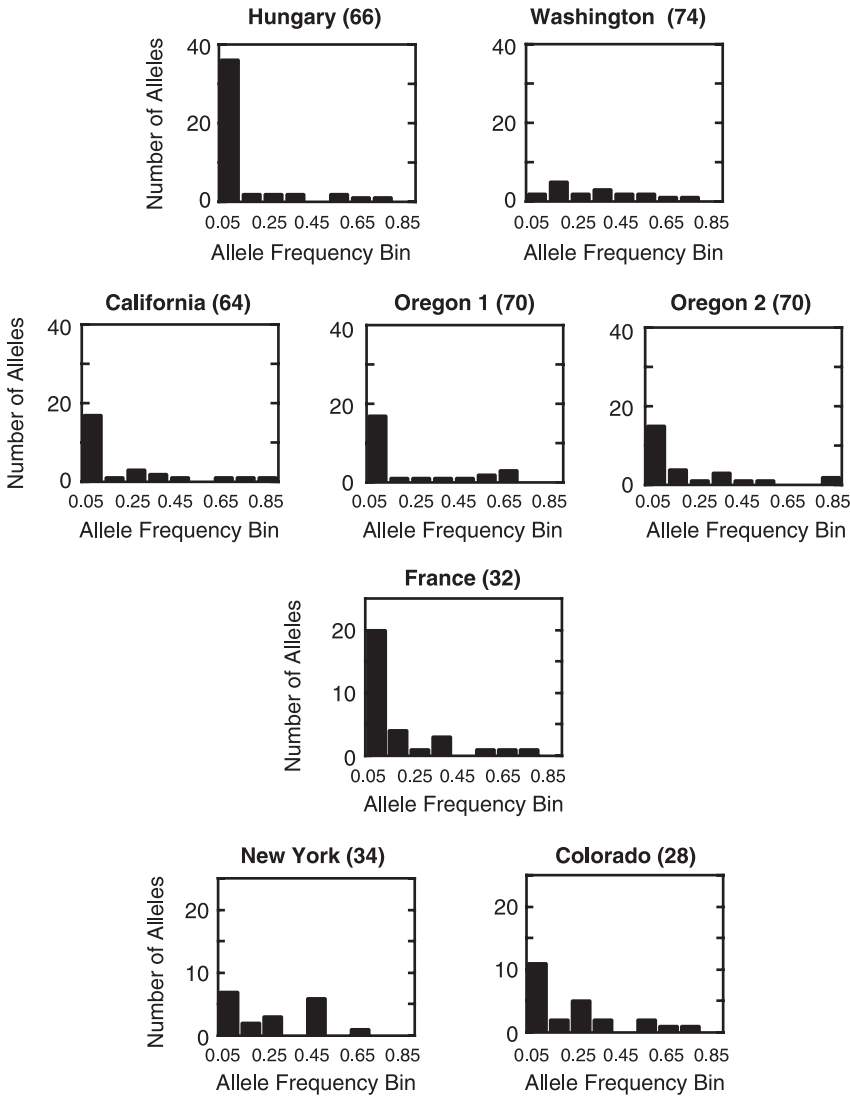


Fig. 2 The distribution of alleles present in different allele frequency classes in native (Hungary and France) and introduced samples. The number of chromosomes sampled is given in parentheses. The native samples are grouped with the comparably sized introduced samples that are appropriate for comparison.

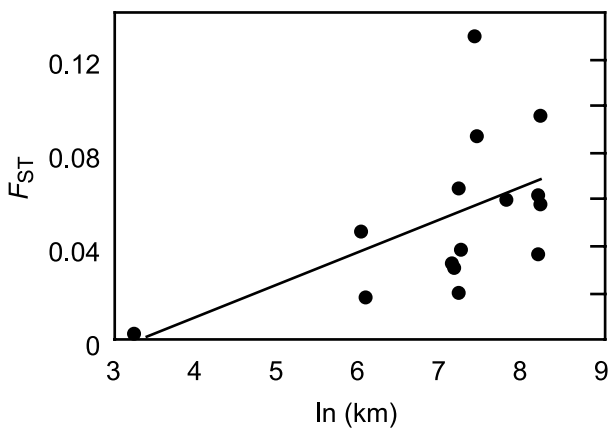


Fig. 3 Pairwise F_{ST} (θ_{ST}) plotted against the natural log of the distance (km) between populations for North American sample locations only.

Table 6 Proportion of membership of individuals from each sample location in each of the four population clusters inferred from the STRUCTURE analysis discussed in the text. Proportions greater than 0.5 are in bold.

Collection location	Inferred population clusters			
	1	2	3	4
North America				
Riverside, CA USA	0.132	0.456	0.267	0.146
Fort Collins, CO USA	0.049	0.550	0.165	0.237
Geneva, NY USA	0.088	0.281	0.493	0.139
Madras, OR USA	0.392	0.321	0.169	0.118
Wasco, OR USA	0.337	0.336	0.202	0.125
Whidbey, WA USA	0.620	0.173	0.119	0.088
Europe				
Lusignan, FR	0.121	0.119	0.151	0.609
Budapest, Hungary	0.066	0.086	0.246	0.602

Discussion

Mitochondrial DNA sequence data

Aphidius ervi from across the native and introduced ranges harbours haplotypes that with few exceptions differ by only one or a few nucleotide substitutions. One individual from Hokkaido, Japan was 1.6% different from the most common haplotypes, and a second individual from Geneva, New York differed by 1.9%. Takada & Tada (2000) found that *A. ervi* from the same the Hokkaido population sampled here differed in petiole colour from western European *A. ervi*, and also in their physiological ability to parasitize several different hosts. In addition, the Hokkaido population was partially reproductively isolated from the European population (Takada & Tada 2000). When other *Aphidius* species are included as outgroups, the Hokkaido individual is basal within the *A. ervi* clade. The sequence divergence between the closest outgroups and the most common *A. ervi* haplotype and the Japan 1 haplotype is 3–4% (Hufbauer & Bogdanowicz, unpublished data). Our data, in conjunction with the results of Takada & Tada (2000), indicate that the Hokkaido population is a distinct lineage, and may represent an incipient species.

The divergent individual from New York is more surprising. One explanation for this individual is that its mtDNA haplotype could represent *A. ervi pulcher* (Baker, syn. = *A. pisivorus* Smith) rather than *A. ervi ervi*. *A. ervi pulcher* is a subspecies that was found in the early 1900s in North America before the arrival of the pea aphid (Angalet & Fuester 1977). It is not known whether it is native to North America (Angalet & Fuester 1977), and Mackauer (1969) suggests it may have been inadvertently introduced. Mackauer & Finlayson (1967) report limited success crossing the two subspecies. The divergent mtDNA haplotype in our study may represent introgression of the *A. ervi pulcher* mtDNA into *A. ervi*. Alternatively, it could represent a separate inadvertent introduction of a divergent *A. ervi* group. Further sampling of the native range of *A. ervi* may reveal a closer match to this haplotype.

Most of the individuals from North America, Europe and the Middle East share one of two common haplotypes. This suggests that the individuals introduced from France did indeed survive to found a population in North America, but it does not rule out other introductions from Western Europe or other areas around the Mediterranean. This general lack of resolution can be common in phylogeographical analyses of recently introduced populations because they rely upon the previous existence of phylogenetically informative differences (reviewed in Roderick 2004). Despite the overall similarity of most of the haplotypes, an intriguing pattern emerged: haplotype C, which is shared by eight individuals from the Pacific Northwest of North America, and one from Colorado, seems to group

with the Japanese individuals from Nagano. Although no purposeful introductions from the main island of Japan (Honshu) are known to have occurred, trade and travel between cities such as Seattle, Tokyo and Kyoto is quite common. The haplotype C individuals may represent an inadvertent introduction from Honshu. A larger sample size, particularly from the native range, might reveal further patterns or relationships that we were not able to discern here. Caracristi & Schlötterer (2003) found an analogous pattern in microsatellite loci of *Drosophila melanogaster* populations. North American populations of *D. melanogaster* were thought to have originated from European populations, and European from African populations. However, Caracristi & Schlötterer (2003) found African alleles in North American populations that were not present in the European populations, suggesting an influx of individuals into North America directly from Africa.

Microsatellite loci

A population founded by 1000 individuals (which did not experience a subsequent bottleneck) would not be expected to exhibit measurable reductions in numbers of alleles and heterozygosity compared with the source population, particularly when the bottleneck is thought to have persisted for fewer than six generations and the subsequent growth of the population was rapid as is the case for *A. ervi* (Nei *et al.* 1975). Nevertheless, *A. ervi* in North America do exhibit some signs of a relatively mild population bottleneck: rare alleles were lost but overall genetic diversity (heterozygosity) was not significantly reduced. Given our limited sampling from the native range this comparison of the native and introduced samples is conservative. The unequal sampling effort biases the results towards finding fewer rare alleles in the native range, yet the native samples included more alleles than the introduced samples. Estimates of the size of the founding population from MSVAR ranged from 7 to 68% of the initial 1000 individuals that were imported.

Evidence for a bottleneck was more pronounced in the comparison of native and introduced samples and in the Bayesian MCMC simulations of population size than in the BOTTLENECK analysis. BOTTLENECK examines patterns of variation within single populations and is based on detecting a higher than expected heterozygosity given the number of alleles per locus within recently bottlenecked populations. It may take a more extreme reduction in population size than *A. ervi* experienced and a larger number of loci sampled for BOTTLENECK to detect excess heterozygosity.

The patterns observed here suggest that these approaches differ in their ability to detect mild bottlenecks. Pertoldi *et al.* (2001) found similar results: in their analysis of decline in the European otter, they observed reduced allelic diversity and dramatic declines in populations as

estimated using the approach of Beaumont (1999), but did not find changes in heterozygosity or significant results using BOTTLENECK.

Within North America there was significant genetic structuring among the sample locations. The AMOVA indicated that the European samples were significantly different from the North American samples, and that further differentiation of individual population pairs also occurred within those groups. The STRUCTURE analysis supported these patterns, grouping the samples into four populations, one of which contained 60% of the individuals from the two European sample locations, but < 25% of the individuals from each of the six North America sample locations. Furthermore, the northwestern North American populations (those that contain individuals with mtDNA haplotype C) have a high proportion of membership of individuals in cluster 1, to which only a small fraction of other North American and European populations are assigned. If assignment to cluster 1 reflects derivation from an inadvertent introduction from Japan, then allele frequency data for microsatellites from the Honshu population should assign many or most individuals from that population to cluster 1.

The assignment of individuals from a given sample location to several of the inferred populations is likely largely to be due to the relatively recent introduction and expansion of *A. ervi* populations in North America. Another factor in the high degree of mixing shown by STRUCTURE may be ongoing gene flow between western Europe and North America: *A. ervi* is commercially available for biological control in greenhouses from several companies, some of them European. Together the AMOVA and STRUCTURE analyses suggest that over the course of the last 45 years some of the populations in North America have become differentiated through restricted gene flow or founder effects.

The isolation-by-distance analysis suggests significant geographical restriction of gene flow among the introduced samples. However, the pattern of variation may simply result from founder effects associated with the initial introduction, the introduction of individuals from the Middle East into California in 1965, and a possible inadvertent introduction from Japan to the Pacific Northwest. Thus the question remains whether the patterns reflect conventional isolation-by-distance through restricted gene flow, or founder events combined with limited time for subsequent gene exchange. Our findings contrast with those of Tsutsui & Case (2001) who tested for isolation-by-distance in native and introduced North American populations of Argentine ants (*Linepithema humile*). They found significant isolation-by-distance in the native range, but not in the introduced range. They hypothesize that the introduced populations have higher gene flow across comparable distances because of lower rates of intercolony aggression associated with reduced genetic variation in the intro-

duced populations. It is also possible that insufficient time has passed for isolation-by-distance to evolve; however, the introduction of the Argentine ant into North America occurred almost 200 years ago (~600–800 generations). Similar to our findings, Goodisman *et al.* (2001) found significant isolation-by-distance of a vespid wasp that was inadvertently introduced into Australia sometime after 1959. Thus, in some situations, apparent isolation-by-distance can appear quite rapidly in a new geographical range.

Founder effects subsequent to introduction may also contribute to differentiation. The habitats used by *A. ervi* are ephemeral and disturbed: a single alfalfa field lasts only 4–7 years. When a new field is planted it is colonized with a subset of individuals from the surrounding population. Such a cycle of local extinction and recolonization can lead to differentiation (Nürnberg & Harrison 1995).

A. ervi from the native and introduced range differ in their ability to overcome pea aphid resistance to parasitism, with introduced wasps from New York State being less effective at parasitizing pea aphids specialized on alfalfa than native French wasps (Hufbauer 2002). This apparently maladaptive change is associated with the introduction of *A. ervi* for biological control. However, the relatively mild imprint of the introduction on presumably neutral population genetic patterns makes it unlikely that the decreased ability of the parasitoids to attack certain populations of aphids is linked to a severe bottleneck in population size.

Although the data presented here do not help explain the low virulence of introduced *A. ervi* populations (Hufbauer 2002), they do suggest that the process of introduction for biological control can have unintended genetic consequences. This may be more pronounced during introductions of species that are rare in their native range, and therefore difficult to collect in large numbers. During future biological control introductions, analysis of samples of individuals from different stages during establishment could help identify at what point reductions in effective population sizes occur. In addition, it may be advisable to form isofemale or family lines of multivoltine insects prior to release to increase the chance that all the individuals that are imported contribute to the gene pool. These and other captive breeding approaches can be effective tools for maintaining both fitness and genetic diversity (e.g. Delpuech *et al.* 1993; Roush & Hopper 1995; Bryant *et al.* 1999; see also Roderick & Navajas 2003). Such methods would be more difficult to implement with univoltine insects due to the challenge of reproducing annual temperature and moisture regimes timed with the appropriate stages of their hosts in a laboratory or greenhouse setting. However, it may be feasible to increase numbers in a single generation to minimize the probability of losing alleles represented in the individuals brought through quarantine. While implementing such programmes, it is important to avoid the declines in fitness that can occur under relaxed selection (Bryant

& Reed 1997) and problems associated with adaptation to laboratory conditions (e.g. Edwards & Hoy 1995).

Through experimental efforts comparing different approaches for establishing populations of classical biological control agents it may also be possible to evaluate the true relevance of neutral variation for the success of biological control. Often there is little relationship between quantitative traits and neutral loci (Reed & Frankham 2001). Most of the experimental data on these patterns come from model laboratory systems. Biological control introductions may provide experimental systems in which to examine such patterns outside the laboratory, in organisms less sensitive than species that are actively being conserved, and such an examination should in the long run improve the efficacy and even safety of biological control.

Acknowledgements

We thank M. McClure, S. Hufbauer, R. Bournoville and L. Polgar for assistance in sampling for the microsatellite work, L. Peres for assistance collecting data, A.P. Norton for support and intellectual input, and S. Gillies for building STRUCTURE to run on a Macintosh. Many thanks to colleagues in Table 1 for providing samples for the mtDNA analyses. S. Rauth provided useful comments on earlier versions of the manuscript. This work was substantially improved by the reviews of C. Schlötterer and two anonymous referees. This research was supported in part by NSF grant DEB-9815380 to RGH, Colorado Agricultural Experiment Station grant 156381 to RAH, and the Evolutionary Genetics Core Facility of Cornell University.

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Ruth Hufbauer works on the ecology and evolution of invasions and of biological control. Steve Bogdanowicz is interested in levels and patterns of molecular variation, primarily within and among insect taxa. Rick Harrison has for many years used molecular markers to answer questions about evolutionary genetics, population structure, and systematics. The current focus of the Harrison laboratory is the study of speciation and the evolution of reproductive isolation.
