The genetic consequences of a demographic bottleneck in an introduced biological control insect

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Abstract Population bottlenecks may result in the loss of genetic diversity, with potentially negative consequences for species of interest in conservation biology, including rare species, invasive species and biological control agents. We examined mtDNA sequence data and four variable microsatellite loci (SSRs) in the melaleuca psyllid *Boreioglycaspis melaleucae*, which was introduced from Australia to Florida as a biological control agent of the invasive plant *Melaleuca quinquenervia*. We sampled psyllids in the native and introduced ranges as well as individuals stored from the original founding population. There was a clear loss of mtDNA haplotype diversity, as well as a loss of rare microsatellite alleles, in the introduced range. However, there was little genetic differentiation between the home and introduced ranges, and no evidence for a genetic bottleneck based on an analysis of heterozygosity with the microsatellite markers. Overall, the data showed that the demographic bottleneck had a limited effect on the genetics of populations in the new range.

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Introduction

When species colonize a new location, a relatively small number of individuals may found the new populations (Elton 1958; Debach and Rosen 1991). This bottleneck may result in a loss of genetic diversity (Nei et al. 1975; Templeton 1980; Barton and Charlesworth 1984; Hartl and Clark 2007), which can limit the evolutionary potential of the populations (Avisé and Hamrick 1996; Frankham and Ballou 2003). This evolutionary potential can be particularly critical to success in a novel environment (Cox 2004).

Genetic bottlenecks can occur in species intentionally released as biological control agents against invaders, since they are often collected in limited quantities from a small number of sites (Debach and Rosen 1991). Genetic diversity may be further reduced due to mortality in transit and inbreeding in quarantine. While many biological control agents have established, others have not been as successful (Debach and Rosen 1991; Pemberton 2000; Louda et al. 2003; Culliney 2005). Loss of genetic diversity could be one factor limiting successful establishment of biological control agents.

Most studies of genetic bottlenecks attempt to infer demographic factors such as the minimum population size and duration of the reduction based on genetic data (Chen et al. 2006; Barker et al. 2009). Some approaches assume that following a demographic bottleneck, allelic diversity is reduced faster than heterozygosity, so that a bottleneck can

be inferred if observed heterozygosity is larger than expected at mutation-drift equilibrium given a particular mutation model (Cornuet and Luikart 1996). Other approaches assume that the largest effect of a bottleneck is a loss of rare alleles (Slatkin 1985). However, it is difficult to test assumptions and relative merits of these approaches unless the actual demographic information is known.

Work on intentionally introduced species such as biological control agents can often take advantage of a large amount of information on the timing, duration and intensity of a demographic bottleneck because of records of the introduction (Debach and Rosen 1991; Hufbauer et al. 2004; Goolsby et al. 2006). We took advantage of a unique opportunity to investigate the population genetics of an introduced species with a very well characterized introduction history and with genetic material available from the original founding population. The melaleuca psyllid *Boreioglycaspis melaleucae* (Moore) (Hemiptera: Psyllidae) is a sap-feeding insect that was introduced into Florida in 2002 to aid in the control of the tree *Melaleuca quinquenervia* (Cav.) Blake (melaleuca), which is originally from Australia and is one of the most invasive species in the Florida Everglades (Purcell et al. 1997; Serbesoff-King 2003; Wineriter et al. 2003). Not only are the precise collection locations in Australia and introduction locations in Florida known, there were preserved insects from the population that had been transferred through a quarantine facility, the sole point of entry, prior to release.

We sampled adult psyllids from natural populations throughout the native range of Australia and the introduced range in Florida, as well as from individuals stored in quarantine that were originally used as the founding populations (Table 1). We extracted nuclear and mitochondrial DNA (mtDNA) from individual adult psyllids and sequenced the nuclear DNA for highly variable microsatellite (SSR) markers and mtDNA for sequence variation in a known variable region. We analyzed spatial genetic variation and population genetic structure within and across populations and continents to examine the genetic consequences of the introduction of this biological control agent.

Materials and methods

Study species

We investigated population genetics of the insect *B. melaleucae*, the melaleuca psyllid. Host range testing showed that *B. melaleucae* feed only on *M. quinquenervia* and three other closely related species in the *M. leucadendra* complex (Purcell et al. 1997; Wineriter et al. 2003). The psyllids feed on the sap of leaves of melaleuca seedlings and mature trees, and excrete a distinctive white flocculence.

Table 1 Sampling locations

Pop	Name	Latitude	Longitude	Country
1	Estero	26.42537	−81.80884	USA
2	Praire Pines	26.72713	−81.86823	USA
3	Punta Gorda	26.90676	−82.06856	USA
4	Clewiston	26.75412	−80.93422	USA
5	Homestead	25.60856	−80.54893	USA
6	USDA Lab	26.08447	−80.23788	USA
7	Fort Pierce	27.42649	−80.40645	USA
8	Lake Conway	28.45487	−81.34009	USA
9	Sarasota	27.12132	−82.45361	USA
10	Picayune	26.10429	−81.63579	USA
11	Lox Slough	26.90783	−80.17204	USA
12	Colony 97-2/4/5	−27.51167	152.99683	QUA
13	Colony 97-6A	−27.31067	153.02367	QUA
14	Colony 98-1	−27.51167	152.99683	QUA
15	99-1	−27.51167	152.99683	QUA
16	EMD	−26.79933	152.98650	QUA
17	SWD	−26.37900	152.98667	QUA
18	Keirle Park, Sydney	−33.78500	151.28083	AUS
19	Toukley Lions Park	−33.27167	151.56267	AUS
20	Boomerand Drive	−32.34083	152.52083	AUS
21	Great Lakes	−32.18317	152.52100	AUS
22	Perch Hole	−31.53400	152.86200	AUS
23	Woolgoolga	−30.14000	153.19350	AUS
24	Arrawarra	−30.05750	153.18400	AUS
25	Brisbane	−27.51167	152.99683	AUS
26	Ewan Maddock Dam	−26.79933	152.98650	AUS
27	Hervey Bay Rd	−25.44650	152.73800	AUS
28	Dundowran Rd	−25.34750	152.77933	AUS

Shown are the latitude and longitude of the collections sites for populations of the melaleuca psyllid *B. melaleucae* in the introduced range (Florida—USA), native range (Australia—AUS), and the original founding populations (Quarantine—QUA). The *population numbers* correspond to the numbers on the maps on Fig. 2. Some of the populations were collected from the same locations (same latitude and longitude) but at different times so are listed separately

This feeding reduces plant growth and survival (Franks et al. 2006; Morath et al. 2006; Center et al. 2006; Rayamajhi et al. 2008; Tipping et al. 2008). The psyllids pass through distinctive life stages, developing from eggs, generally laid on the margins of leaves, to first through fifth instar nymphs. In the final stage of development, they become alate adults. The time for one generation is about 1 month under ideal conditions. The adults mate sexually, and there is no known parthenogenesis in this species.

For the introduction of this species, colonies of *B. melaleucae* were collected from six sites that were each less than 100 km from Brisbane, Australia between May 1997 and December 2001 (Center et al. 2006). The number

of individuals collected per site ranged from ten to several hundred and included all life stages. From these collections, six separate shipments of the psyllid were made to the quarantine facility in Gainesville, Florida, where the insects were maintained for host range testing (Wineriter et al. 2003; Center et al. 2006). From this quarantine population, a total of 45,965 individuals were released to south Florida during the period of February 12 through April 18, 2002. Adult psyllids were originally released in 8 *Melaleuca* stands that were widely distributed across the range of the invasive tree in south Florida (Center et al. 2006). From these initial release sites, *B. melaleucæ* dispersed at a rate of 4.7 km/year (Center et al. 2006) but additional redistribution efforts resulted in the movement of over two million *B. melaleucæ* individuals from these original release sites to new *Melaleuca* stands that occurred outside the natural dispersal abilities of the insect.

Sampling design

In 2004 (2 years post-introduction), we collected *B. melaleucæ* individuals from the native range of Australia and the introduced range in Florida, as well as from the original release population that had been stored in quarantine. The collection sites (populations) are shown in Table 1. There were 11 collection sites in Australia, 11 in Florida and six colonies from quarantine that represented unique collections from the source locations. The sampling locations were throughout the current range of the psyllid in both Australia and Florida. From each population, two individuals were used for analysis of mtDNA and up to 20 were used for analysis of microsatellites. The psyllids collected were all mature adults, and both males and females were included in the sampling.

Genetics

For microsatellite development and population genetic analysis, DNA was extracted from whole wild-collected adult psyllids using the ZR genomic DNA kit (Zymo Research, Orange, California) following the solid tissue protocol. In the mtDNA sequence analysis, two individuals from each of 28 populations were amplified using PCR. An approximately 650 bp fragment spanning the COI-tRNA-COII region was generated using the primers UEA9 (Lunt et al. 1996) and C2-N-3389 (Simon et al. 1994). The PCR conditions were denaturation at 94°C for 5 min, 41 cycles of denaturation at 94°C for 30 s, annealing at 48°C for 30 s, and extension at 70°C for 30 s, with a final extension of 70°C for 10 min. Some amplifications required reducing the annealing temperature to 45°C and four additional cycles for a total of 45. Amplified products were purified prior to sequencing by incubation with 3 µl Exonuclease and

2 µl Shrimp Alkaline Phosphatase for 30 min at 37°C, followed by 15 min at 80°C for enzyme deactivation. Products were sequenced from both ends using the same primers and BigDye Terminator Cycle Sequencing Kit v3.1, and were run on an ABI3730xl automated sequencer (Applied Biosystems, Foster City, CA). Sequences were edited and aligned using Sequencher (GeneCodes, Ann Arbor, MI) and trimmed to the same length.

Microsatellite markers were developed by the genetics facility at the Savannah River Ecology Laboratory following techniques described in Glenn and Schable (2005). Twenty-two microsatellite loci were identified (Suppl. 1). Of these, only four were shown to have clean amplification and variability in sample populations and were used in the analyses (Table 2).

We amplified the microsatellite loci in 10 µl PCR reactions, using either 0.5 or 1.0 µl of template DNA, following a program of 1–2-min cycle at 94°C; 30 cycles of 92°C for 30 s, 58°C for 1 min, and 72°C for 45 s; one cycle at 72°C for 3 min. The amplicons were separated and visualized on an ABI 3100 Genetic Analyzer (Applied Biosystems) using ABI ROX500 as a reference, and genotype scoring was performed using STR and (Hughes 1998). Because microsatellite genotyping was performed on DNA extracts from whole insects, it is possible that DNA from stored male sperm may have also been present in extractions from adult females and that samples included not only mtDNA from the target individual but also from mtDNA in the stored sperm. However, we did not observe low-intensity allele bands as sperm genotyping would produce. Further, we did not find any samples that possessed multiple different mtDNA haplotypes, which would be expected if sperm were sampled and the sperm was derived from fertilizations from different males.

Statistical analyses

We analyzed population genetic parameters and the genetic consequences of the demographic bottleneck in several ways. With the microsatellite data, allele frequencies and genetic diversity statistics were calculated with Arlequin 3.1 (Excoffier et al. 2005). These parameters were compared among the Australia, Florida and quarantine populations using analysis of variance (ANOVA) in SPSS (version 12), with pairwise post-hoc comparisons made using the Least Significant Difference (LSD) test. The Garza and Williamson (G–W) statistic (Garza and Williamson 2001; Excoffier et al. 2005), also calculated with Arlequin 3.1, is the ratio between allele size and range. This statistic is expected to reflect a recent bottleneck because the number of alleles should be more reduced than allelic range, such that G–W should be near zero in

Table 2 Sequences of microsatellite loci used in the population genetic analyses of the psyllid *B. melaleuca*

Locus	Primer	Sequence (5′–3′)	Product
16G	Forward	CAGTCGGGCGTCATCA GGA AGA GTG GGA AGA GT	(AAGT) _n
	Reverse	GTG GTT CAA CAT GAT ACA AT	
12D	Forward	GGAAACAGCTATGACCATG ACC TTT ACT TTA ACA CAT ATT TC	(AG) _n
	Reverse	AAA TTG TTT GCG ATA GAA	
B6F	Forward	TTT GTT GTA TTG ATT TTG TAG TTA	(AG) _n
	Reverse	CAGTCGGGCGTCATCA CGG GTA AAA GGA AAT CAT	
B9F	Forward	GGAAACAGCTATGACCATG AAG TTA CAT TGA TGA AAT ACA TA	(ACAT) _n
	Reverse	CTC CCT AAT CTT TGA CAA C	

recently bottlenecked populations and near one in stable populations (Garza and Williamson 2001). Population structure statistics were computed using Fstat 2.9.3.2 (Goudet 1995). This program estimates *F*-statistics following Weir and Cockerham (1984). We performed a hierarchical AMOVA, which partitions genetic variance into among source continents, among populations, among and within individual components. We also looked for evidence of a genetic bottleneck by comparing observed heterozygosities with heterozygosities expected under mutation-drift equilibrium with Bottleneck 1.2. If a bottleneck has occurred, there should be a loss of rare alleles, leading to an excess of heterozygotes compared with expectations under mutation-drift equilibrium (Cornuet and Luikart 1996). Because we used microsatellites, alleles that are similar in size are likely to be more closely related than alleles that are more different in size. We thus used the stepwise mutation models (SMM) and two-phase models (TMP) in Bottleneck (Cornuet and Luikart 1996) to generate the predicted heterozygosities. We performed an analysis of population genetic structuring and assignment tests using the model-based clustering analysis implemented in STRUCTURE (Falush et al. 2003; Pritchard et al. 2000). STRUCTURE uses genetic data to assign individuals to a particular number (*K*) of predefined populations. We performed the STRUCTURE analysis at *K* = 1 to *K* = 8 and determined the most likely level of *K* by determining the modal value of delta *K*, as described by Evanno et al. (2005). We conducted 100,000 iterations of “burn-in” followed by an additional 10,000 iterations of data collection, using a model of correlated allele frequencies (Falush et al. 2003). In all of the methods using the microsatellite data, the low number of markers (four) we had available could potentially limit our statistical power to detect a bottleneck, and the results should thus be interpreted with caution. However, the markers we used were highly variable, providing a substantial amount of population genetic information, and help us to determine if a bottleneck can be detected using a small number of genetic markers.

Results

The native and introduced ranges differed in mtDNA haplotype composition, based on 56 individuals sampled for eight polymorphic loci (Fig. 1). Seven haplotypes were found in the native range of Australia. Of these, only one (the most common haplotype) also was found in individuals from both quarantine and the introduced range of Florida. The second most common haplotype was found in Florida and quarantine but was absent in Australia. While

Fig. 1 Haplotype network map based on psyllid mtDNA haplotypes. The size of each box corresponds to the number of individuals represented by that haplotype. The individuals are from Australia (A, 22 sampled), Florida (F, 22 sampled) and quarantine (Q, 11 sampled). One line between two haplotypes represents one mutational step between the haplotypes. The *small circle* is an implied haplotype that was not sampled

five of the eight haplotypes were found only in Australia, only two haplotypes were found in Florida, and neither of these was unique to Florida (Fig. 1).

Australia shows clear spatial separation of the haplotype groups (Fig. 2). The most common haplotype (A) is found only in the north of the range near Brisbane, which is the area where insects were collected for introduction to Florida. The other haplotypes found in the north of Australia, C and D, differ from haplotype A by only one and two nucleotides, respectively. The only haplotypes found in Florida are A and B; haplotype B differs from haplotype A at a single nucleotide. The A and B haplotypes are intermixed in Florida. The haplotypes found in the central coast of Australia are found only in this region, while the southern range near Sydney contains a different unique haplotype (Fig. 2).

The microsatellite data showed 95 total alleles for the four polymorphic loci used in the study. These four loci were highly polymorphic (16–30 alleles per locus). Gene diversity, allelic diversity, observed and expected heterozygosity, and allelic size range were significantly smaller in the native range of Australia than in the introduced range of Florida (Table 3). The quarantine populations showed the lowest levels of allelic diversity, observed and expected heterozygosity and allelic size range. However, the quarantine populations had higher levels of gene diversity than the native range populations (Table 3). The G–W statistic was 0.277 (± 0.009) in the introduced range, which was low, as expected following a bottleneck. However, the

G–W statistic in the native range was 0.303 (± 0.057) which was not significantly different from the introduced range. Analysis of variance revealed significant effects of source location (Australia, Florida, quarantine) on gene diversity, allelic diversity, observed and expected heterozygosity, and allelic size range, but not the G–W statistic (Table 4).

There was little genetic structure within source locations and low genetic differentiation between the native and introduced ranges for the microsatellite markers. A hierarchical AMOVA showed that only 0.7% of the genetic variation was partitioned among continents, compared with 8.1% among populations and 10.1% among individuals (Table 5). The continents appeared fairly genetically homogenous, with $F_{st} = 0.088$ in Australia and $F_{st} = 0.009$ in Florida (Table 6). The quarantine population showed greater structure, with $F_{st} = 0.217$. The total number of alleles ($t_{1,20} = 2.34$, $P = 0.03$) and allele size range ($t_{1,20} = 2.09$, $P = 0.05$) are larger in Australia than in Florida (Table 6). Observed and expected heterozygosity did not differ between the continents. Genetic diversity (expected heterozygosity) tended to increase from south to north in Australia (Fig. 3).

Comparisons of observed with expected heterozygosity gave no evidence for a genetic bottleneck in the Florida populations. Under the stepwise mutation model (SMM), three loci showed significant heterozygosity deficiency rather than excess (Table 7), which is the opposite of what is expected following a bottleneck. None of the loci showed significant heterozygosity deficiency or excess under the two-phase model (TPM; Table 7). A Wilcoxon test showed that all loci fit mutation-drift equilibrium under the SMM ($P = 0.19$) and TPM ($P = 0.06$) models.

Generally more rare and unique (private) alleles appeared in the native range of Australia than in the introduced range of Florida. For example, at loci B6F and B9F, all of the unique alleles were in Australia and none were in Florida. At the 16G locus, nine alleles (two rare and seven private) were found only in Australia, while only four alleles (one rare and three private) were found only in Florida. None of the unique alleles were found in the quarantine populations.

In the STRUCTURE analysis, $K = 1$ emerged as the most likely number of clusters in the dataset, indicating that both the quarantine populations and the introduced populations were likely drawn from the sampled native (Australian) populations (Fig. 4). Modest population genetic structuring was also evident at $K = 2$ and $K = 3$. In particular, some of the quarantine populations (e.g. populations 12, 14 and 15) stood out as relatively uniform, although population 12 (Colony 97-2/4/5) appeared to be distinct from populations 14 and 15 (Colony 98-1 and 99-1, respectively).

Fig. 2 Map of sampling locations and mtDNA haplotypes. Grey dots are the sampling locations. The psyllid population numbers are from Table 1. The letters indicate unique mtDNA haplotypes. The '*' symbol indicates locations where collections were made for the original introductions into Florida

Table 3 Genetic diversity by population

Source	Pop	n	Genes	Alleles	H _o	H _e	Range	G–W
USA	1	20	42.0	9.25	0.7381	0.7999	32.5	0.27957
USA	2	20	37.5	9.00	0.7566	0.8058	36.0	0.27473
USA	3	20	39.5	10.75	0.7303	0.7694	40.0	0.29679
USA	4	20	39.5	10.75	0.7592	0.8175	39.5	0.28113
USA	5	20	37.0	9.25	0.6548	0.7919	34.5	0.27500
USA	6	20	39.0	10.50	0.6289	0.8048	35.5	0.28470
USA	7	20	39.5	11.00	0.7954	0.8184	50.5	0.28335
USA	8	20	37.0	9.50	0.7034	0.7941	34.0	0.26893
USA	9	20	39.0	8.50	0.6355	0.7427	28.5	0.28571
USA	10	20	37.5	8.75	0.5599	0.6790	44.0	0.24441
USA	11	20	36.0	9.00	0.6549	0.7914	32.5	0.27280
			38.5	9.66	0.6925	0.7832	37.0	0.27701
QUA	12	20	39.5	3.25	0.4665	0.4901	11.5	0.35943
QUA	13	20	38.0	5.75	0.5531	0.7350	17.5	0.30882
QUA	14	20	39.0	4.75	0.4612	0.5881	14.5	0.33380
QUA	15	20	39.0	3.00	0.4875	0.4836	9.5	0.30967
QUA	16	19	35.0	6.25	0.6565	0.7285	23.5	0.28439
QUA	17	20	38.5	8.50	0.5760	0.7346	29.0	0.27410
			38.2	5.25	0.5335	0.6266	17.6	0.31170
AUS	18	8	16.0	4.25	0.4375	0.4875	21.0	0.50504
AUS	19	20	37.0	7.00	0.5402	0.6369	22.5	0.32088
AUS	20	4	6.5	2.75	0.5625	0.6161	16.0	0.34293
AUS	21	11	18.5	6.00	0.5917	0.6710	20.5	0.27673
AUS	22	20	40.0	7.50	0.5500	0.6750	21.5	0.34052
AUS	23	5	10.0	6.25	0.7500	0.8889	39.0	0.20015
AUS	24	19	36.5	11.5	0.7383	0.8578	46.0	0.28175
AUS	25	20	39.0	6.25	0.4750	0.6941	26.5	0.24088
AUS	26	20	38.5	9.50	0.6960	0.8149	33.5	0.29089
AUS	27	20	29.5	10.00	0.6586	0.7973	33.5	0.28626
AUS	28	20	39.5	11.75	0.6961	0.8517	43.5	0.30443
			28.3	7.52	0.6087	0.7265	29.4	0.30822
Total	28	506						
Avg.			34.4	7.88	0.62550	0.72735	29.88	0.29670

Shown are source (United States—USA; Australia—AUS; and quarantine—QUA), population number, sample size, average number of gene copies, average number of alleles, observed heterozygosity (H_o), expected heterozygosity (H_e), allelic size range, and the Garza and Williamson (G–W) statistic, which is the ratio between allele size and range. Averages for each source location are shown in *bold* below each group, and the overall average is shown in the last row

Discussion

The populations of psyllids that we studied went through a known transfer of individuals across continents at a known recent time with a known number of individuals transported to and from specific, identified locations. The populations in Florida have thus passed through a demographic bottleneck of known parameters, giving us a unique opportunity to study the effects of this founder event on the genetics of these populations. We found some evidence of a genetic effect of this demographic bottleneck

as a loss of genetic diversity, since there were several microsatellite alleles and mtDNA haplotypes found in the native range but absent in the introduced range. This finding validates theoretical expectations of the genetic consequences of founder events (Nei et al. 1975; Templeton 1980; Barton and Charlesworth 1984). Because a loss of genetic diversity can critically influence population dynamics and evolution, our findings have important implications for the practice of biological control.

The genetic consequences of the demographic bottleneck in the introduced range could also be seen through the

Table 4 Summary analysis of variance (ANOVA) table of the effects of source location (United States, Australia, quarantine) on gene diversity (genes) allelic diversity (alleles), observed heterozygosity (H_o), expected heterozygosity (H_e), allelic size range (range), and the Garza and Williamson (G–W) statistic for microsatellite data

Variable	MSE	<i>F</i>	<i>P</i>
Genes	341.5	4.95	0.0150
Alleles	38.9	8.64	0.0010
H_o	0.052	6.73	0.0050
H_e	0.048	4.81	0.0170
Range	737.2	10.82	<0.0001
GW	0.004	1.33	0.2830

MSE is mean square error. Degrees of freedom are 2 (numerator) and 25 (denominator) for all tests. Significant effects ($P < 0.05$) are shown with *bold*

Table 5 Hierarchical analysis of genetic structure, using AMOVA, for microsatellite data

Source	D.f.	S.S.	Var comp	Percent	<i>P</i>
Among continents	2	18.8	0.01	0.67	<0.0001
Among populations	25	145.5	0.12	8.12	<0.0001
Among individuals	474	716.4	0.15	10.13	<0.0001
Within individuals	502	607.0	1.21	81.08	<0.05

The continents include Australia (native range), the US (introduced range), and the quarantine groups. Shown are degrees of freedom (d.f.), sums of squares (S.S.), variance components (Var Comp), percentage of variation (Percent) and significance tests (*P*). Results were obtained using the software program Arlequin 3.1

genotype and haplotype composition and diversity. In the native range of Australia, there was clear geographic separation of mtDNA haplotypes, with seven total haplotypes found and different sets of haplotypes present in the northern, central, and southern portions of the range. This pattern is consistent with other studies that showed similar genetic differentiation in this same region of the eastern coast of Australia (Arthur et al. 2008). In the introduced range of Florida, there were only two mtDNA haplotypes. One of these haplotypes was found in both the northern range in Australia and in the quarantine population. The other haplotype was found in quarantine and was not in the Australia samples, but was only a single base substitution different from a haplotype that was found in the northern range in Australia. This indicates that the second haplotype was likely present in Australia but at a low enough frequency that it was not present in our sample. Psyllids introduced into Florida through quarantine were originally collected from the northern part of the range in Australia, near Brisbane (Center et al. 2006). Thus only this subset of northern range genotypes were introduced into Florida,

Table 6 Population genetic statistics for microsatellite data

Statistic	USA	AUS	QUA	All
F_{it}	0.126	2.47	0.312	0.208
F_{st}	0.009	0.088	0.217	0.086
F_{is}	0.117	0.174	0.122	0.134
Alleles	17	20.25	11.25	23.75
H_o	0.69	0.61	0.53	0.65
H_e	0.79	0.81	0.77	0.80
Range	59	64.5	34	67
G–W	0.34	0.35	0.33	0.34

Genetic structure statistics F_{it} , F_{st} and F_{is} were determined using Fstat 2.9.3. Allelic richness, observed and expected heterozygosities (H_o and H_e), allele size range and the G–W statistic were determined using Arlequin 3.1. Parameter estimates are for the introduced range in Florida (USA), the native range in Australia (AUS), quarantine (QUA), and for all individuals grouped together (All)

Fig. 3 Genetic diversity cline in Australia. Shown is psyllid genetic diversity (expected heterozygosity) from microsatellite data plotted against population location in Australia. The populations are in order from south to north, corresponding to numbers in Table 1**Table 7** Analysis of bottleneck effects in the introduced range (Florida) populations for microsatellite data

Locus	H_e Obs	H_e TPM	<i>P</i>	H_e SMM	<i>P</i>
16G	0.836	0.882	0.0680	0.917	<0.0001
12D	0.884	0.889	0.3640	0.922	0.0190
B6F	0.904	0.874	0.1360	0.911	0.2760
B9F	0.547	0.692	0.0790	0.782	0.0020

Shown are loci, observed heterozygosity (H_e Obs), heterozygosity expected at mutation drift equilibrium under the two-phase model (H_e TPM), heterozygosity expected at mutation drift equilibrium under the step-wise mutation model (SMM) and the probabilities of heterozygosity deficiency or excess (*P*) under each model. *Bold* indicates $P < 0.05$

indicating a genetic diversity reduction in the introduced range.

Consistent with the results from the mtDNA data, many microsatellite alleles that were found in Australia were absent in Florida, and this was especially true for the unique and less common alleles. This shows that rare alleles are particularly informative and useful in detecting genetic bottlenecks (Slatkin 1985). Only a few alleles were found in Florida and not in Australia. This most likely reflects limited sampling, with these alleles probably occurring at low frequencies in Australia but not in the sample for this study. Alternatively, there could have been mutations at one or more loci in Florida following introduction. Although, mutation rates at microsatellite loci are particularly high (estimated at around 5×10^{-6} for *Drosophila* in inbred mutation accumulation lines (Vázquez et al. 2000)), it would be unlikely that mutations occurring between the time of the introduction of the psyllids (2002) and the time of our sampling (2004) would have affected our inferences. Thus differences in the genetic composition of the Australia and Florida populations is more likely due to the consequences of the demographic bottleneck than to the occurrence of new mutations.

Interestingly, genetic diversity, averaged across populations, was higher in the introduced range but total genetic

diversity within sources was higher in the native range. This finding is consistent with the higher degree of genetic structure and differentiation in Australia than in Florida, which is also as expected following a founder event. The STRUCTURE analysis produced groupings of some individuals consistent with their history and geographic distribution. Overall, the level of clustering with the highest likelihood was observed at $K = 1$. This result likely reflects the ancestry of the introduced and quarantine populations from ancestral populations in the native range (Australia). Some genetic structure was also observed at higher levels of K . For example, some of the quarantine populations appeared to be relatively homogenous, but distinct from other populations. Individuals in introduced populations were characterized by patterns consistent with a history of admixture, as evidenced by scattered assignment to different populations.

While there was a signature of a bottleneck from the mtDNA data and the population assignment tests using the microsatellite data, other methods using the microsatellite information did not show a bottleneck. These methods included an analysis of genetic structure, comparisons of genetic diversity, the $G-W$ statistic and comparisons of observed with expected heterozygosity. One of the most widely used methods for examining genetic bottlenecks,

Fig. 4 **a** Results of genetic population assignment analysis of microsatellite data using STRUCTURE, at $K = 2$ and $K = 3$ and **b** ΔK values for each level of K . Each individual is represented by a *thin vertical bar*, and the probability of assignment to each hypothetical populations is indicated by the *shaded portion* of the bar. The population numbers correspond to those given in Table 1. For convenience, the individuals are shown grouped together by population, and the ordering of individuals within each population is arbitrary

comparisons of observed with expected heterozygosities under mutation-drift equilibrium (Cornuet and Luikart 1996), showed no evidence for a genetic bottleneck in our study. A two-phase mutation model showed no significant differences between observed and expected heterozygosities, and a stepwise mutation model showed a larger than expected observed heterozygosity at 3 of the 4 loci, in direct contrast with expectations following a genetic bottleneck. The reason these methods did not detect a genetic bottleneck may be that these statistics are strongly influenced by the more common alleles (Hartl and Clark 2007). Genetic bottlenecks may eliminate rare alleles, with little effect on these genetic structure parameters. Thus methods based on examining rare alleles, mtDNA haplotypes, and assignment tests can be more powerful for detecting bottlenecks than those based on genetic structure. It is also important to note that the relatively low number of microsatellite markers used in this study could have limited the power of these methods to detect bottlenecks. However, the results indicate that rare alleles and geographic patterns of mtDNA haplotypes are more informative for detecting bottlenecks than genetic markers with alleles at intermediate frequencies.

The microsatellite data showed low genetic structure, with little differentiation between the native and introduced ranges and a very low amount of the total genetic variation explained by location. The low genetic structure was shown both within populations and among populations based on hierarchical AMOVA. This would indicate that the psyllids are relatively homogeneous genetically both within and across the native and introduced ranges. However, a low value of F_{ST} does not necessarily indicate low differentiation among populations, especially for highly variable loci such as microsatellites (Jost 2008). The lack of spatial genetic structure in the introduced range is not surprising. Because the introduction to Florida occurred recently (in 2002), there likely has been insufficient time for genetic differentiation among populations in the introduced range.

Like our study, several others also showed that recent founder events produced a loss of rare alleles but did not reduce heterozygosity. Hufbauer et al. (2004) examined the population genetics of a parasitoid wasp (*Aphidius ervi*) introduced as a biological control agent using microsatellite markers and mitochondrial DNA. They found no significant difference in heterozygosity between native and introduced range insects, but they did find substantial genetic structuring and differentiation, with several distinct haplotypes separating the different source locations and a reduction in allelic diversity, especially rare alleles, in the introduced range. Similarly, Baker et al. (2003) examined the genetics of another parasitoid wasp introduced for biological control and also found no loss of heterozygosity

but a loss of genetic diversity and rare alleles in the introduced range.

While the founder effect has been well described theoretically (Barton and Charlesworth 1984), the degree to which founding events result in a bottleneck in natural populations as well as the population genetic consequences of species introductions in general is less well understood. Recently, it has also become clear that the genetic consequences of species introductions can be complex. In some cases, introduced populations have lost large amounts of genetic diversity; in other cases, genetic diversity is higher in introduced populations than in native source populations. For example, genetic studies found no evidence of a bottleneck in introduced populations of the Western corn rootworm, *Diabrotica virgifera* (Kim and Sappington 2005), in the oriental fruit fly, *Bactrocera dorsalis* (Aketarawong et al. 2007), or in the walnut husk fly, *Rhagoletis completa* (Chen et al. 2006). Genetic bottlenecks may be uncommon in invasive species because of multiple introductions, or because species that undergo bottlenecks may not establish or become invasive. Furthermore, there is often a lag phase during which the population levels of an introduced species remain low before eventually growing at a rapid rate (Cox 2004). During this latency period, multiple introductions can occur and may offset the loss of genetic diversity in the original introduction. Finally, different introduced species may hybridize with each other or with native species in their new range, resulting in higher levels of genetic diversity relative to source populations in the invader's native range (Ellstrand and Schierenbeck 2000; Suarez and Tsutsui 2008).

Despite the loss of genetic diversity that has occurred from founder events in introduced species, many of those species have been highly abundant and successful, at least over the short term following introduction. Ross and Shoemaker (2008) found a vast population expansion of the invasive fire ant (*Solenopsis invicta*) despite a substantial loss of genetic diversity that appeared to be caused by the introduction of a very small number of individuals. Similarly, Argentine ants attain enormous population sizes in their introduced range despite losing substantial genetic diversity during introduction and establishment (Tsutsui et al. 2000; Suarez et al. 2008).

Loss of genetic diversity associated with bottlenecks should be considered in biological control programs. The loss of population fitness associated with a loss of genetic diversity is expected and well known from many studies of rare species (Avisé and Hamrick 1996; Frankham and Ballou 2003). Furthermore, a loss of genetic diversity may not be a problem over the short term but may leave the population more susceptible to disease and reduce the ability of the population to evolve following future changes in environmental conditions or in the target species. Thus

future biological control programs should consider widespread sampling in the source location, maintaining large populations in quarantine facilities and introducing large numbers of these individuals into the new range, and repeated sampling from the source location and introductions into the new range to limit the loss of genetic diversity in the biological control agent (Madeira et al. 2001).

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