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Invasion success and genetic diversity of introduced populations of guppies *Poecilia reticulata* in Australia

ANNA K. LINDHOLM,* FELIX BREDEN,† HEATHER J. ALEXANDER,† WOON-KHIONG CHAN,‡ SUMITA G. THAKURTA‡ and ROBERT BROOKS*

**School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney 2052 Australia, †Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6 Canada, ‡Department of Biological Sciences, National University of Singapore, 14 Science Drive, Singapore 117543, Republic of Singapore*

Abstract

High genetic diversity is thought to characterize successful invasive species, as the potential to adapt to new environments is enhanced and inbreeding is reduced. In the last century, guppies, *Poecilia reticulata*, repeatedly invaded streams in Australia and elsewhere. Quantitative genetic studies of one Australian guppy population have demonstrated high additive genetic variation for autosomal and Y-linked morphological traits. The combination of colonization success, high heritability of morphological traits, and the possibility of multiple introductions to Australia raised the prediction that neutral genetic diversity is high in introduced populations of guppies. In this study we examine genetic diversity at nine microsatellite and one mitochondrial locus for seven Australian populations. We used mtDNA haplotypes from the natural range of guppies and from domesticated varieties to identify source populations. There were a minimum of two introductions, but there was no haplotype diversity within Australian populations, suggesting a founder effect. This was supported by microsatellite markers, as allelic diversity and heterozygosity were severely reduced compared to one wild source population, and evidence of recent bottlenecks was found. Between Australian populations little differentiation of microsatellite allele frequencies was detected, suggesting that population admixture has occurred historically, perhaps due to male-biased gene flow followed by bottlenecks. Thus success of invasion of Australia and high additive genetic variance in Australian guppies are not associated with high levels of diversity at molecular loci. This finding is consistent with the release of additive genetic variation by dominance and epistasis following inbreeding, and with disruptive and negative frequency-dependent selection on fitness traits.

Keywords: additive genetic variation, bottleneck, introduced species, invasion success, neutral genetic diversity, *Poecilia reticulata*

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Introduction

Species that invade or that are introduced into new environments provide opportunities to study evolutionary change and diversification, particularly the consequences of random genetic drift and natural selection (Endler 1986; Lee 2002). If populations in new environments are founded by a small number of individuals, or if they go through bottlenecks after reintroduction, allelic diversity will be reduced relative to the source population (Nei *et al*. 1975). Both drift and mating between related individuals will result in an increased probability that two alleles in a given individual at a given locus are identical by descent. This probability is the coefficient of inbreeding, *f*. A rise in the mean coefficient of inbreeding within a population is expected to lead to an erosion of additive genetic variance at the rate of $(1 - f)\ V_A$ (Falconer & Mackay 1989). Such a loss of additive genetic variance will slow evolutionary responses to selection (Falconer & Mackay 1989), potentially

Correspondence: Anna K. Lindholm, Present address: Anthropological Institute and Museum, University of Zürich, Winterthurerstrasse 190, CH-8057, Zürich, Switzerland. Fax: +41 44 635 4780; E-mail: anna.lindholm@unsw.edu.au

constraining introduced populations from adapting to the new environment and thus invading it successfully.

Three alternative processes may free invading populations from the constraints of reduced additive genetic variation. First, in small populations, dominance and epistasis may result in an increase in additive genetic variance, particularly under inbreeding (Goodnight 1988; Willis & Orr 1993). Second, negative frequency dependence may maintain high levels of additive genetic variation (Barton & Turelli 1989; Falconer & Mackay 1989). Last, populations in new environments may have been established via multiple introductions or via admixture or hybridization of different source populations, resulting in enhanced allelic diversity, additive genetic variance and heritability relative to source populations that will increase evolutionary response to selection (Ellstrand & Schierenbeck 2000; Grant & Grant 2000; Kolbe *et al*. 2004).

Guppies, which are small live-bearing fish, offer a particularly interesting opportunity to study invasion success as there is a wealth of information on their biology (Houde 1997). Moreover, through accidental or deliberate release, guppies have successfully colonized at least 32 countries in the Americas, Europe, Asia, Australasia, and Africa (Froese & Pauly 2000; FAO 2004). They are native to South America from Venezuela to Brazil and nearby islands (Rosen & Bailey 1963; Welcomme 1988).

In Australia, multiple introductions of guppies are likely to have occurred. They were probably first brought to Australia around 1910. At this time guppies were sent through the Colonial Office to various tropical colonies for mosquito control (Vipan 1910). By 1912 guppies were already present in Australia, as the efficacy of the guppy in mosquito larvae control was tested in Brisbane in Queensland (Cooling 1912) and later in Adelaide in South Australia (Borthwick 1923). Several city councils, including that of Brisbane (Abell 1913; Borthwick 1923), likely played a role in the dissemination of guppies by encouraging the addition of mosquito-eating fish to stagnant water bodies. In 1929 the guppy was listed among mosquito-eating fish introduced to Australia (Hamlyn-Harris 1929). During World War II, it is believed that soldiers spread guppies and *Gambusia* in northern Queensland for biocontrol (J. Johnson, personal communication), a period in which there was an epidemic of malaria in Cairns (Patrick 1987). Today, guppies primarily occur in Queensland near sugarcane fields, where they may also have been used for mosquito control (McKay 1987), and in coastal drainages near urban areas (Allen *et al*. 2002).

Guppies are also popular ornamental fish in Australia, and introductions may have resulted from escapes or releases from aquaria or outdoor breeding ponds (McKay 1984). The most important source of ornamental guppies in the Australian pet trade until very recently has been Singapore (Glenn Briggs, personal communication). Guppies were introduced to Singapore in the 1900s (FAO 2004) and were found in feral populations in streams before 1940 (Herre 1940). From 1972–1977, Singapore exported more than 43 million tropical fish to Australia (UNCTAD/GATT 1979). From 1992 to 1994, Singapore was the top exporter of tropical fish in the world, and its main export was the guppy (Cheong 1996). If introductions resulted from releases of ornamental fish, then Singapore would be the most likely immediate source. Ornamental or 'fancy' guppies would have undergone artificial selection and inbreeding during domestication, and would be expected to have a different genetic architecture than either wild populations or populations resulting from introductions for biocontrol.

High levels of additive genetic variance have been found in laboratory studies of two introduced populations of guppies. In an introduced Australian population (Alligator in this study), male body size and colour traits showed high heritabilities (h^2) and coefficients of additive genetic variation (CV_A). Of 13 traits measured, overall h^2 estimates of seven traits were above 0.50, while CV_A estimates from six were above 20% (e.g. for body area $h^2 = 0.87$ and $CV_A = 11.8$ and for orange area $h^2 = 0.96$ and $CV_A = 67.3$; Brooks & Endler 2001a). In the same population, female mate preference functions were negligibly heritable (Brooks & Endler 2001b). An introduced South African population showed responses to artificial selection consistent with high heritability for both male colour and female preferences (Brooks & Couldridge 1999), as has been found in native populations (Houde 1994). For introduced guppies, high heritability of male colour traits is remarkable as much of the variation for these traits is Y-linked (Brooks & Endler 2001). Like mitochondrial loci, Y-linked loci have only one quarter of the effective population size of autosomal loci (Halliburton 2004). In guppies, variance in reproductive success of males (Becher & Magurran 2004) will further reduce the effective population size of Y chromosomes. Genetic bottlenecks should therefore have a particularly strong impact on variation in Y-linked traits in guppies.

Introduced populations, such as those in Australia and South Africa, may have high levels of additive genetic variance as a result of population admixture through multiple introductions, through the release of additive genetic variance by dominance and epistatic interactions among alleles, or through disruptive and negative frequency-dependent selection. If the first scenario pertains, neutral genetic markers will show similarly high levels of variation, whereas the latter two scenarios are consistent with small amounts of genetic variation typical of single introductions. In this study we examine genetic diversity in seven introduced guppy populations in northern Queensland, Australia, using nine autosomal microsatellite loci and one mtDNA locus, and use these to test the prediction of high

genetic diversity due to admixture of populations during multiple introductions. As mtDNA is especially suited to tracing founder events (Moritz *et al*. 1987), we use mtDNA haplotype diversity to estimate how many source populations there were, and their geographical origin. We also explore the hypothesis that Australian feral guppies descend from ornamental fish bred in Singapore.

Materials and methods

Sampling

Adult male and female guppies were captured from 5–12 April 2002 at seven sites in north Queensland, Australia (Fig. 1; Table 1). Guppies were air transported to Sydney and populations were separately housed in large tanks in a greenhouse at the University of New South Wales for use in several studies. Tissue samples were obtained from tailfin clips of live animals, or from muscle tissue of animals that were euthanized or died naturally. Thirty-nine guppies and one *Poecilia parae* [a sister taxon (Breden *et al*. 1999)] were also sampled from Patientia near Georgetown, Guyana, South America, in February 2002. Samples were stored in 20% DMSO salt solution (Amos & Hoelzel 1991) before DNA extraction. DNA was isolated from all samples by salt precipitation using Puregene Tissue Kit (Gentra) according to manufacturer's instructions.

Singapore ornamental guppies of the inbred varieties Tuxedo (see Khoo *et al*. 1999), Greensnake (Phang *et al*. 1989) and Red Tail (see Fernando & Phang 1990) were obtained from Swee Hing & Brothers Aquarium Company, Singapore, in 1995 and kept as stocks in the Genetics Lab, Department of Biological Sciences, National University of Singapore, until 2002. One individual from each of these three stocks were sampled in 1996 with an additional individual from a laboratory stock of feral guppies collected from Nee Soon, Singapore. These four individuals were analysed in 1996. After 1995–1996, a disease outbreak in Singapore led to wide-scale replacement of local guppy stocks with those from other countries that farm guppies. In 2001 laboratory stocks were eliminated and genetic samples destroyed. We could therefore not sample additional individuals nor evaluate the effect of selective breeding on genetic diversity.

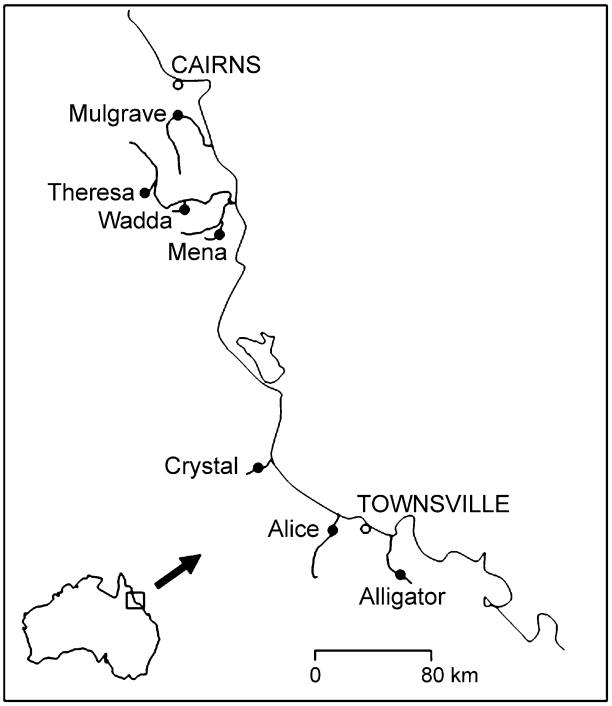

Fig. 1 Sampling locations in northern Queensland.

Measuring genetic diversity: mitochondrial DNA sequencing

Four hundred ninety-eight base pairs of the mitochondrial control region were sequenced for Queensland guppies (Table 1), two Guyanese guppies, and for *Poecilia parae*, the outgroup. Primers Lpro and 13R were used for initial amplification and sequencing (Ptacek & Breden 1998). Polymerase chain reaction (PCR) products were cleaned using the QIAquick PCR purification kit (QIAGEN). Sequencing products were ethanol precipitated and sequenced on an ABI 377 or 3730 DNA sequencer. Haplotypes were deposited in GenBank under Accession nos DQ097186–90.

Table 1 Sampling locations from south to north, relative population sizes, capture effort in person-hours, and sample sizes for mtDNA sequencing and microsatellite genotyping

Sample site (creek or river)	Location	Number captured	Capture effort	Number sequenced	Number genotyped
Alligator	19.45°S, 146.97°E	514	4.5	6	56
Alice	19.32°S, 146.60°E	6	3	6	6
Crystal	18.38°S, 146.33°E	163	8	6	52
Mena	17.65°S, 145.97°E	160	9	6	49
Wadda	17.60°S, 145.83°E	138	1	6	58
Theresa	17.50°S, 145.62°E	94	8	6	55
Mulgrave	17.12°S, 145.45°E	100	8	5	38

Identification of source populations

To determine the likely population origin of Queensland feral guppies, we compared their haplotypes to those of 128 wild guppies from the native range (see Table 2 for details) from Taylor & Breden (2000), Alexander & Breden (2004, unpublished), and this study, and three domesticated and one wild variety of Singapore guppies. Sequences from Singapore guppies were amplified using primers L15926 (Kocher *et al*. 1989) and H16498 (Shields & Kocher 1991), giving 490 bp fragments (GenBank Accession nos DQ097191-94). Sequences from the control region of native guppies and the outgroup were aligned to those of Australian guppies using CLUSTAL_X and trimmed to a uniform length, giving a 510-bp segment including gaps. The Singapore sequences overlapped this trimmed segment by 386 bp.

Phylogenetic analysis was performed using maximum likelihood, which performs well with missing data (Dunn *et al*. 2003). We first reduced the data set to a total of 66 haplotypes by excluding duplicate haplotypes from the same sampling location, and then used the likelihood-ratio test of MODELTEST version 3.5 (Posada & Crandall 1998) to select the best-fit model of nucleotide substitution for use in tree construction. We then estimated the maximum-likelihood tree using Felsenstein & Churchill's (1996) algorithm implemented in PHYLIP and calculated bootstrap support based on a majority-rule consensus of maximum-likelihood trees from 1000 bootstraps. As this algorithm treats indels as unknown nucleotides, thereby losing information, we also calculated bootstrap support using 5000 maximum-parsimony replicates in PAUP 4.10b treating gaps as a fifth state.

Table 2 mtDNA sequences used in phylogeographic analyses and the frequency of Queensland haplotypes 1 and 2 and Singapore haplotype S out of *N* animals sequenced at each location

				Freq. haplotype			
Region	Sampling area	Tributary of	*N*	1	2	S	GenBank Accession nos
Suriname	Lelydorp	Surinam River	1				AF228605
North Guyana	Patentia	Demerera River	2	1.0			DQ097187–88
North Guyana	Bartica	Essequibo River	1				AF170257
East Guyana	New Amsterdam	Berbice River	5				AF228609, DQ102578, DQ102581–83
East Guyana	Springlands	Corentyne River	3				AF228608, DQ102560, DQ102563
West Venezuela	Guanare River		6				AF170255, AF228615, AY135451, AY135457, AY135466, AY135473
East Venezuela	Isla de Margarita		1				AF228610
East Venezuela	Rio San Miguel		4				AF538280, AY135454, AY135465, AY135468
East Venezuela	Cumaná	Rio Manzanares	19				AY373767–68, AY373770–73, AY373779–80, AY373787–88, AY373791, AY373796, AY373798, AY373805–07, AY373812–14
East Venezuela	Yaguaracual		3				AY373776–78
East Venezuela	Paria Peninsula		11				AY373765–66, AY373782–6, AY373795, AY373803–04, AY373808
East Venezuela	Mira Flores/ Arenas/ Cumanocoa	Rio Manzanares	10				AY373764, AY373792–93, AY373799–802, AY373809–11
West Trinidad	Arima River	Caroni	9		0.33		AF228623, AF170265–66, AY135450, AY135452, AY135460, AY135475–77
West Trinidad	Aripo River	Caroni	4		0.25		AF170268, AY135470, DQ102585–86
West Trinidad	Guanapo River	Caroni	4		0.75		AF170267, AY135449, AY135472, AY373762
East Trinidad	Turure River	Oropuche	2		0.50		AF327266, AY135469
East Trinidad	Quare River	Oropuche	7				AF170261, AF193897–98, AF529246, AF52951–53
East Trinidad	Oropuche River		12				AF170259–60, AF193899, AF529244–45, AF529247, AF529249–50, AF529255–57, AF538279
East Trinidad	Aqui River	Madamas	3				AF170262, AF529248, AF529254
East Trinidad	Rio Grande		3				AF170258, AF170269–70
North Trinidad	Paria River		6				AF193902, AF228624, AY135448, AY135453, AY135459, AY135474
North Trinidad	Marianne River		5				AF19301, AY135456, AY135462–63, AY135467
North Trinidad	Yarra River		7				AF228625, AF170263–64, AY135455, AY135461, AY135464, AY135471
Total			128			0	

Measuring genetic diversity: microsatellites

Nine polymorphic microsatellite loci were amplified from individuals of the seven Queensland populations (Table 1): TCTG and sat 4 (Taylor 1999), TTA (Taylor 1999; but primers were redesigned), D6, D15 and D21 from Seckinger *et al*. (2002), Pr39 and Pr80 from Becher *et al*. (2002) and Pr67 (Becher & Magurran 2004). Primers were labelled with fluorescent markers and PCR products run on an ABI 377 DNA Sequencer or an ABI 3730 DNA Analyser. The mean number of loci scored per individual was 8.97. There was no evidence that these loci deviated from linkage equilibrium, or from Hardy–Weinberg equilibrium within each population, tested using FSTAT version 2.9.3 (Goudet 2001) and Bonferroni adjustments to significance tests, thereby meeting assumptions of the following tests.

Testing for population admixture

We first examined population structure to determine whether the number of genetic clusters estimated from mtDNA haplotypes correspond to that of nuclear markers. STRUCTURE 2.1 uses a Bayesian model implemented by a Markov chain Monte Carlo method to estimate how many different populations (genetic clusters in Hardy–Weinberg equilibrium) are in a data set, irrespective of sampling location (Pritchard *et al*. 2000; Falush *et al*. 2003). We estimated the most likely value of K (number of populations) using the admixture model with correlated allele frequencies, as populations might be closely related to each other.

If there were no admixture of populations with different mtDNA haplotypes, then populations sharing mtDNA haplotypes should be more similar to each other in microsatellite allele frequencies than populations of a different haplotype. Using a nested AMOVA design, we partitioned the estimated variance in microsatellite allele frequencies into the proportion attributable to differences in mtDNA haplotypes, the proportion attributable to populations within haplotypes, and that due to individual variation within populations.

We then examined genetic relationships between populations on a finer scale. As the Australian populations have been established over a period of less than 100 years (see Introduction), which equates to less than 200–300 generations, changes in microsatellite allele frequencies through mutation [at rates of 10^{-3} to 10^{-4} per locus per generation (Ellegren 2000)] are likely to have a minor influence on allele frequencies compared to the effects of genetic drift upon introduction. We therefore favoured methods that are based on evolutionary models of genetic drift rather than microsatellite mutation. First, we looked for evidence of gene flow between populations by identifying individuals of migrant ancestry within the data set using STRUCTURE 2.1 (Pritchard *et al*. 2000; Falush *et al*. 2003). We subsequently tested for population ancestry using the F model from STRUCTURE 2.1 (Falush *et al*. 2003). In this model a single parameter, F, analogous to F_{ST}, is calculated for each population. F estimates the degree of divergence from a common ancestral population by drift, with populations assumed to have diverged at the same time. Differences in rate of drift correspond to differences in effective population size after divergence (Nicholson *et al*. 2002; Falush *et al*. 2003). If one population has not diverged at the same time from the hypothetical ancestral population, but is rather descended from another population in the data set, its F value decreases when its ancestor is excluded (Falush *et al*. 2003). We therefore compared F values (using 40 estimates) omitting and including a likely source population for some of the Queensland populations, assuming that each sampling location represented an independent population.

As a complementary approach to investigating population relatedness, we used microsatellite allele frequencies to estimate phylogenetic trees using two methods. First, we constructed a restricted maximum-likelihood phylogenetic tree using Felsenstein's (1981) method implemented in PHYLIP. It assumes a Brownian motion model, and therefore, that loci evolve only by drift. The second method estimated a neighbour-joining tree from Nei's genetic distance D (Nei 1972), also using PHYLIP (Felsenstein 2004). Nei's genetic distance measure is based on the infinite alleles model and is the most widely used measure for codominant data (Lowe *et al*. 2004). For both trees, allele frequencies were bootstrapped 1000 times using MICROSAT (http://hpgl.stanford.edu/projects/microsat).

Testing for bottlenecks

Introduced populations are often subject to founder effects and bottlenecks that result in low genetic diversity. We looked for evidence of recent bottlenecks using BOTTLENECK version 1.2.02 (Piry *et al*. 1999). Populations undergoing reductions of effective population size often lose rare alleles at polymorphic loci. Loss of rare alleles affects heterozygosity expected at mutation–drift equilibrium more than observed heterozygosity, leading to an excess of observed heterozygosity (Luikart & Cornuet 1998). Bottleneck tests for heterozygosity excess relative to expectations under mutation–drift equilibrium using different mutation models: the infinite allele model (IAM), one-step stepwise-mutation model (SMM), and the two-phase model (TPM). For the TPM we used a model of 90% single-step mutations and 10% multistep mutations (Garza & Williamson 2001), and a variance of 10. The Wilcoxon's signed rank test, which is more powerful than the alternatives when using a small number of loci (Piry *et al*. 1999), is the reported statistic.

Results

mtDNA haplotype diversity and sources of Queensland guppies

Two haplotypes, differing by 17 nucleotides (3.4%), were found among Queensland populations, but no nucleotide diversity was found within populations. Haplotype 1 was found exclusively in guppies from Alligator and Mena, which are nonadjacent sites (Fig. 1); while haplotype 2 was found in all guppies sequenced from Alice, Crystal, Wadda, Theresa and Mulgrave. These results are consistent with founder effects, and suggest that there were a minimum of two introductions of female guppies in Queensland, with no admixture of source populations.

Sources of Queensland guppies were identified (Table 2). Guyana is very likely to be the source of haplotype 1 guppies, as the two sequences obtained from Patentia, Guyana, are identical to haplotype 1. Haplotype 2 occurred in 7 of 18 guppies from the Caroni River drainage system, in western Trinidad. This haplotype was also found in one of two guppies from the Turure River, an admixed population, the result of a translocation of male and female guppies in 1957 from the Arima River in the Caroni drainage to the Turure River in the Oropuche River drainage (Shaw *et al*. 1992; Becher & Magurran 2000). Thus, the Caroni drainage is very likely the source of haplotype 2 guppy populations. A single haplotype (named S) was found among the four Singaporean samples that differed by 2/386 nucleotides from haplotype 2, and this haplotype was not observed in any of the Australian populations.

Phylogenetic analyses (Fig. 2) showed strong bootstrap support [from maximum likelihood (94%) and from parsimony (76%)] for a clade containing haplotype 1 and Patentia. Support for the clade of guppies sampled from the Caroni River drainage, Trinidad, and haplotypes 2 and S was low (55%), indicating that more sequence data are required to fully resolve this node.

Incorporating microsatellite data to investigate population admixture

If guppies from sample sites sharing the same mtDNA haplotype were actually interbreeding populations, then most genetic variation should be captured by two genetic clusters. Analyses using STRUCTURE 2.1 (Table 3) showed that log-likelihood ratios steadily decreased as K (the number of genetic clusters in Hardy–Weinberg equilibrium) increased from one to six, and then began to plateau at K = 6. The plateau indicates that most of the genetic variation was captured in six genetic clusters, rather than two. The six clusters correspond to the sample sites Alligator, Mena, Wadda, Theresa and Mulgrave, with Alice and Crystal together as the final cluster. Seven clusters, corresponding to the seven sample sites, were slightly more likely, and eight clusters, which split Mulgrave into two populations, plus the other six sample sites, minimized the likelihood ratio. Similar results were obtained when data were analysed using the independent allele frequencies model. We then examined how individuals were assigned to genetic clusters, using K = 6, 7 and 8. With seven clusters, the estimates of membership coefficients within sample sites appeared to be maximized, and population membership was assigned consistent with population of origin except for one female identified as of migrant descent (95% probability interval: 0.798–1.0 for K = 7), regardless of the value of K. Eight clusters split Mulgrave guppies into two groups, which did not correspond to their pool of origin. These results suggest that considering each sampling location as a separate population is reasonable, that Crystal and Alice are closely related, and that the one Theresa female consistently assigned to the Wadda genetic cluster is evidence of recent gene flow between these sites.

Table 3 Log-likelihood ratios for different K

K	Ln $P(X \mid K)$
1	−7647.4
2	−6758.1
3	−6106.6
4	−5583.0
5	−5107.2
6	−4897.1
7	−4887.4
8	−4871.0
9	−4872.0

Analysis of molecular variance (Table 4) attributed 2% of variation in microsatellite allele frequencies in Australian

Table 4 Analysis of molecular variance, nesting population variance in allele frequencies at nine microsatellite loci within the two mtDNA haplotypes

Source of variation	d.f.	SS	MS	Est. var.	% variation	P value
Among haplotypes	1	229.1	229.1	0.16	1.9	0.001
Within haplotypes among populations	5	840.1	168.0	3.89	47.5	0.001
Within haplotypes within populations	307	1317.4	4.3	4.29	48.5	0.001
Total	313	2386.6	401.4	8.34		

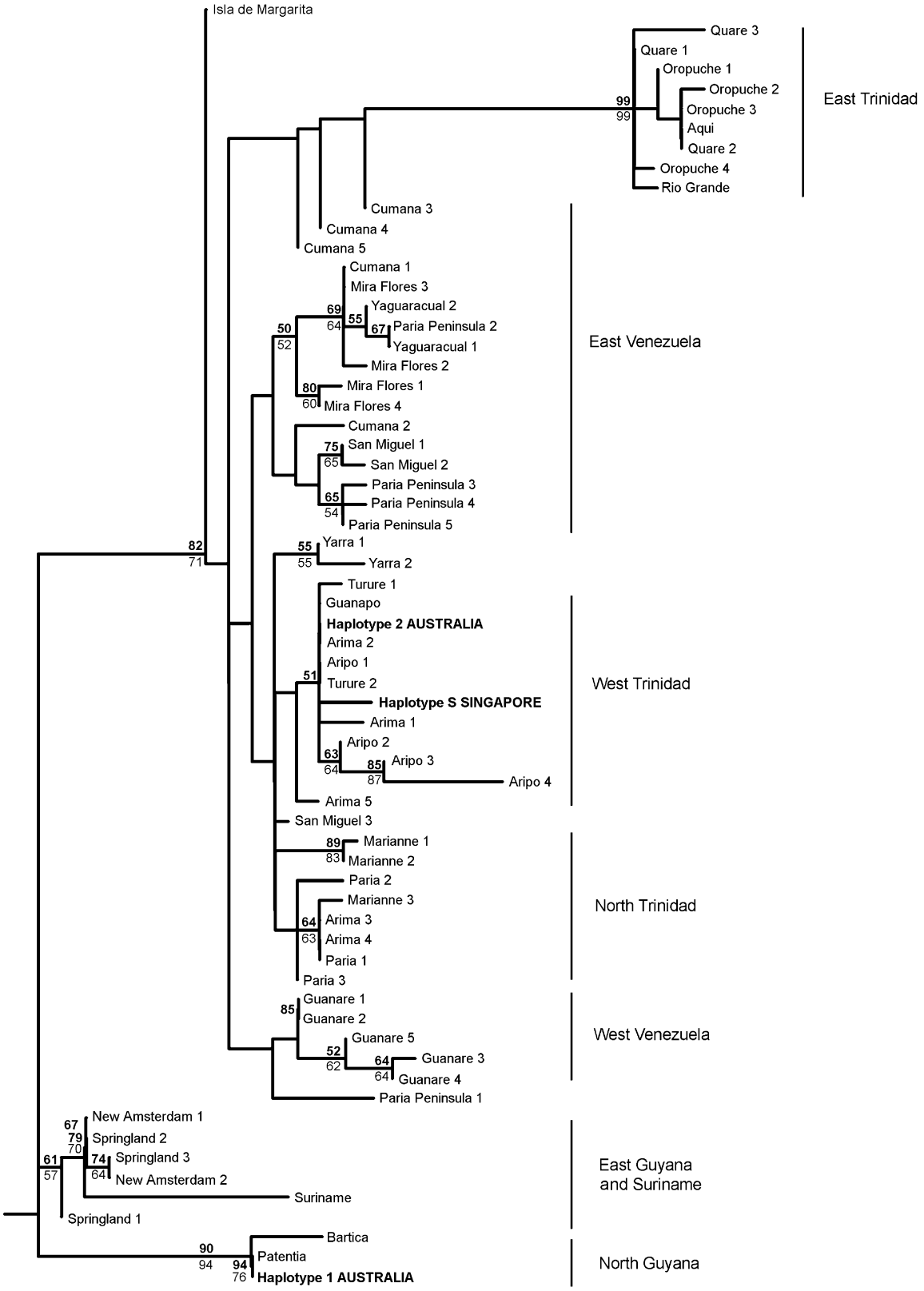

Fig. 2 Maximum-likelihood phylogenetic tree based on mtDNA control region sequence. Bootstrap values above 50% for 1000 bootstraps are indicated by the upper numbers in bold. In plain type below are bootstrap values above 50% for 5000 parsimony bootstraps. The tree was rooted using *Poecilia parae* (Breden *et al*. 1999).

populations to differences in mtDNA haplotypes, 48% of variation to population differences within haplotypes, and the remaining 49% to individual variation within populations. The small amount of microsatellite structure accounted for by mtDNA haplotype suggests that populations of the same haplotype have diverged, not only by drift, but also as a result of gene flow from populations of different haplotype.

Historical gene flow between populations of different mtDNA haplotype is further suggested by the observation that not all alleles from the haplotype 1 populations of Mena and Alligator were detected in their mtDNA source population in Guyana. All 13 of such alleles from Mena and 8 of 12 from Alligator were detected in Australian haplotype 2 populations. As an alternative explanation to gene flow, we explored whether the presence of these alleles may be due to mutations in the Mena and Alligator populations. As about 90% of microsatellite mutations appear to be single-step, and 10% multistep (Garza & Williamson 2001), we expected a similar distribution within our samples. Allele sizes did not conform to these expectations, as only 31% of the 13 alleles from Mena, and 50% of the 12 alleles from Alligator could have arisen within these populations as single-step mutations giving a change of one repeat unit. These alleles differ from nearest size classes by a mean (± 1 SD) of 4.8 ± 4.4 repeats in Mena, and 2.2 ± 1.6 repeats at Alligator. Moreover, these populations are of recent origin (see Introduction), rendering mutation an unlikely source of the majority of these alleles.

To explore population divergence by drift, we used STRUCTURE 2.1 (Falush *et al*. 2003) to estimate *F* values, which estimate the relative amount of drift that different populations have undergone from a common ancestral population (Nicholson *et al*. 2002; Falush *et al*. 2003). Estimates of *F* (Table 5) identified Wadda as the most highly differentiated of the Australian populations, while Mulgrave and Alice are the least differentiated, compared to a common ancestor. When any one Australian population was removed from the data set, only small changes in *F* were seen, suggesting that none was the ancestor of another. Adding the Guyana population to the model allowed a test of the hypothesis that it is the source of the Mena and Alligator populations. Mena showed a significant increase in *F* (along with Mulgrave and Alice), as indicated by its negative pairwise *t* statistic, while Alligator showed a nonsignificant increase (Table 5). Wadda showed the opposite trend.

Phylogenetic relationships were also inferred by tree construction using restricted maximum likelihood and neighbour joining of Nei's (1972) genetic distance measure. The trees are similar (Fig. 3a, b). In both, Alligator is the closest branch to Guyana, Alice and Crystal cluster together, while Wadda has a relatively long branch length. Bootstrap support was low overall, indicating that populations were not highly divergent in microsatellite allele frequencies.

Genetic diversity and evidence for bottlenecks

Allelic diversity and heterozygosity was lower in all Queensland populations compared to the Guyana population (Table 6), suggesting founder effects and/or bottlenecks. We found strong evidence for a recent bottleneck at both Theresa and Mulgrave (Table 7), as tests using three different models for microsatellite mutations produced similar results. At the remaining Queensland sites, with the exception of Alice, there is weak evidence for recent

Table 5 Mean values of *F*, paired sample *t*-test values and their probabilities (in bold, significant after sequential Bonferroni correction) for each population in Queensland, before and after adding Guyana to the data set

		Alligator	Alice	Crystal	Mena	Wadda	Theresa	Mulgrave	Guyana
All Queensland	Mean *F*	0.371	0.208	0.397	0.296	0.519	0.403	0.179	
	SD	0.023	0.042	0.004	0.017	0.003	0.013	0.006	
Include Guyana	Mean *F*	0.378	0.287	0.395	0.347	0.457	0.401	0.269	0.130
	SD	0.003	0.052	0.003	0.004	0.002	0.002	0.002	0.003
	Paired *t*	−2.09	−10.20	2.42	−17.99	106.52	1.17	−96.53	
	P	0.043	**0.001**	0.020	**0.001**	**0.001**	0.251	**0.001**	

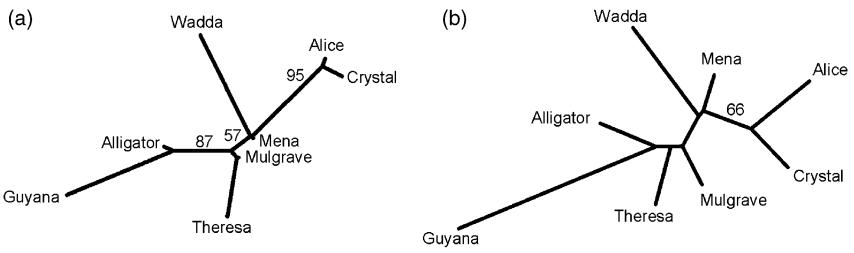

Fig. 3 Phylogenetic trees based on microsatellite allele frequencies (a) maximum-likelihood tree (b) neighbour-joining tree of Nei's genetic distance (Nei 1972). Bootstrap values above 50% for 1000 replicates are indicated.

Table 6 Number of alleles over all microsatellite loci, allelic diversity (mean number of alleles) and mean observed and expected heterozygosity

Site	Number of alleles	A	H_O	H_E
Alligator	34	3.8	0.41	0.42
Alice	30	3.3	0.63	0.53
Crystal	31	3.4	0.49	0.48
Mena	34	3.8	0.54	0.53
Wadda	21	2.3	0.36	0.34
Theresa	30	3.3	0.51	0.48
Mulgrave	42	4.7	0.61	0.64
Guyana	85	9.4	0.71	0.68

Table 7 One-tailed P values for a Wilcoxon test of heterozygote excess under three mutation models (BOTTLENECK version 1.2.02)

Site	IAM	SMM	TPM
Alligator	0.027	0.809	0.680
Alice	0.285	0.633	0.633
Crystal	0.014	0.590	0.455
Mena	0.010	0.500	0.410
Wadda	0.024	0.125	0.102
Theresa	0.004*	0.008	0.004*
Mulgrave	0.001*	0.064	0.007*
Guyana	0.150	0.787	0.752

*Significant after sequential Bonferroni correction.

bottlenecks, with significant results only before Bonferroni correction and for the least conservative model, the infinite alleles model. If the populations of Mena and Alligator are assumed to descend from the Guyanese population, and the effects of gene flow and any other processes that affect Hardy–Weinberg equilibria are ignored, then the inbreeding coefficient (f) can be calculated using the equation $H_f = H_r(1 - f)$, where H_f is heterozygosity in an inbred population and H_r is heterozygosity in a reference population (Halliburton 2004). Under these assumptions, $f = 0.38$ for Alligator, and $f = 0.22$ for Mena, indicating substantial population-level increases in the inbreeding coefficient relative to the Guyanese population.

Discussion

Genetic diversity was low in all Queensland populations: a single mtDNA haplotype was found in each population, while genetic diversity (heterozygosity and allelic diversity) at microsatellite loci was low in all Queensland populations relative to a wild population. These patterns accord with expectations of neutral genetic diversity in populations founded by a small number of individuals from the same source and/or which have passed through small bottlenecks (Nei *et al*. 1975). Furthermore, there was strong evidence that two Queensland populations have recently gone through a bottleneck.

Despite low genetic diversity, we found evidence for population admixture. Variation in microsatellite allele frequencies among populations segregated to only a very small extent within mtDNA haplotypes. This is most likely the result of male-biased gene flow between populations of different haplotypes. Male-biased gene flow will not affect the distribution of mtDNA haplotypes, as they are inherited matrilinearly. In guppies, males migrate at a higher rate than females (Croft *et al*. 2003), supporting this interpretation. One female of migrant ancestry was detected among Queensland populations, providing evidence for recent gene flow between populations in the same river system. Low bootstrap support for nodes within phylogenetic trees based on microsatellites indicated that populations were not highly differentiated, except for the cluster of Crystal and Alice. F statistics were also similar across most populations. The low level of population differentiation in microsatellite allele frequencies is consistent with historical gene flow between populations, or of multiple introductions of males. Given the popularity of guppies as aquarium fish, and their widespread distribution in northern Queensland waterways, human-assisted migration or multiple introductions of males cannot be discounted as a source of gene flow. However, as gene flow has not led to a high level of neutral genetic diversity within populations, a likely scenario is that migration events or multiple introductions alternating with bottlenecks have reduced genetic variation. Strong skew in reproductive success (Becher & Magurran 2004) could also have contributed to loss of variation.

Sources for Queensland populations

There are a minimum of two source populations for the seven introduced Australian populations studied, with one in Guyana near the capital, Georgetown, and one in the Caroni drainage, western Trinidad. Both identified sources are near capital cities in countries that were part of the British Empire, which is consistent with a scenario in which the British Colonial Office sent guppies between colonies for mosquito control (Boulenger 1912). Singapore strains also appear to originate from within the Caroni River system, but differences in their mtDNA haplotype to that of Queensland guppies suggests that they are not directly related.

The Guyanese population was genotyped to test the hypothesis that it is an ancestral population to the Queensland populations with which it shares a common mtDNA haplotype, Alligator and Mena. Phylogenetic trees supported relatedness between the Guyana population and Alligator (haplotype 1), while F-tests supported relatedness to Mena (haplotype 1) and Mulgrave (haplotype 2). Although these two approaches differed in which populations were

identified as related to the Guyanese population, each identified one population known to share an mtDNA haplotype with Guyana, providing support for the hypothesis that it is an ancestral population.

Low neutral but high additive genetic diversity

Successful invasive species are generally thought to have high genetic diversity, which allows them to escape the harmful effects of inbreeding (Keller & Waller 2003; Spielman *et al*. 2004) and adapt to their new environment (Sakai *et al*. 2001; Allendorf & Lundquist 2003). Genetic sampling of contemporary populations of guppies in Queensland provide no evidence for high neutral genetic diversity, despite invasion success in Queensland and elsewhere in Australia: Northern Territory (Letts 2004), Western Australia (collections of the Western Australian Museum and the American Museum of Natural History), Norfolk Island, New South Wales (Australian Museum collections) and the territories of Christmas Island and Cocos (Keeling) Island (Western Australian Museum) and many other countries. All seven populations in Queensland had relatively low neutral genetic diversity and only one mtDNA haplotype was detected within each population. Compared to a wild Guyanese population, heterozygosity at nuclear loci averaged 72%, and allelic diversity averaged 37% (Table 6).

The discrepancy between high levels of additive genetic variance in autosomal and Y-linked traits seen in the Alligator population (Brooks & Endler 2001a) and the reduction of neutral genetic diversity and evidence of founder effects and/or bottlenecks reported here may be due to two processes. The first is disruptive selection in combination with negative frequency-dependent selection, which is expected to increase additive genetic variance (Falconer & Mackay 1989). Guppies from Alligator are subject to strong disruptive sexual selection (Blows *et al*. 2003). Male guppies in this population, as in others, are highly variable phenotypically (Brooks & Endler 2001a). Some of this phenotypic variation is maintained by disruptive selection on male ornaments, as there is no single combination of male ornaments that females find attractive (Brooks & Endler 2001a; Blows *et al*. 2003), in combination with negative frequency-dependent selection. Such negative frequency dependence is supported by the presence of female preferences for rare male phenotypes (Farr 1977; Hughes *et al*. 1999).

The second process is the release of additive genetic variation following bottlenecks (Goodnight 1988; Willis & Orr 1993), which has been observed experimentally (e.g. Cheverud *et al*. 1999). With inbreeding coefficients up to $f = 0.5$, some low-frequency recessive alleles become more common through drift, thereby increasing additive variance (Robertson 1952; Willis & Orr 1993). Additive variance can also be increased through conversion of epistatic or dominance genetic variance to additive genetic variance (Meffert 2000). As some loci become fixed, epistatic interactions between loci, and dominance interactions between alleles, may be transmitted as additive effects (Walsh & Lynch 1998; Meffert 2000). However, it is very difficult to predict empirically whether a bottleneck will lead to an increase or a decrease in additive genetic variance (Walsh & Lynch 1998; Barton & Turelli 2004).

Our results reaffirm the difficulty of predicting the response of species to new environments (Allendorf & Lundquist 2003) and to population bottlenecks (Cheverud *et al*. 1999). In the case of guppies, diversity at 10 genetic markers was low and indicated founder effects and/or bottlenecks and was therefore not consistent with the documented high levels of additive genetic variance nor the replicated pattern of successful invasion. Similarly, in a meta-analysis molecular genetic variation did not correlate with heritability (Reed & Frankham 2001). In other taxa, high (e.g. Novak & Mack 1993; Kolbe *et al*. 2004) as well as low levels of neutral genetic diversity (e.g. Tsutsui *et al*. 2000; Colautti *et al*. 2005) characterize invasive populations. Neutral genetic diversity is therefore unlikely to predict the potential for invasion success and adaptation. Guppies, however, meet some of the life history characteristics of introduced fishes that have established successfully in the Great Lakes (Kolar & Lodge 2002), as they grow relatively rapidly and are tolerant of a wide range of salinity and temperatures (Meffe & Snelson 1989). Genetic variation underlying life history traits such as these and the processes generating this variation within populations founded by a small number of individuals are likely to be more important in capturing the potential for invasion success than simple measures of neutral genetic diversity.

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This project forms part of a larger study on the evolution of sexually selected traits in introduced Australian guppies by Anna Lindholm, Rob Brooks and John Endler. Felix Breden and Heather Alexander (PhD student at Simon Fraser University) use phylogeography as a tool to investigate speciation and trait evolution in poeciliid fishes. Woon-Chan Khiong and Sumita Thakurta are interested in the genetic background of introduced guppies in Singapore.
