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Genetic and ecological dynamics of species replacement in an arid-land river system

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Abstract

Museum records indicate that *Hybognathus placitus* was introduced into the Pecos River, New Mexico during the early 1960s. Approximately 10 years later, a congener, *Hybognathus amarus*, was extirpated from the system. We used microsatellite and mtDNA data, ecological data and modelling, and a computer simulation approach to reconstruct the history of invasion and species replacement. To identify the potential role of hybridization and introgression, we genetically screened *H. amarus* (*n* = 389) from the Rio Grande, New Mexico, and *H. placitus* (*n* = 424) from the Pecos River, New Mexico using four nuclear microsatellites and a partial fragment of the mtDNA *ND4* gene. Assignment tests excluded hybridization as a primary factor in species replacement and suggested a role for interspecific competition. Genetic analyses showed that *H. placitus* were introduced into the Pecos River from at least two genetically distinct source populations in the Canadian and Red rivers, Oklahoma. Lotka–Volterra models of interspecific competition indicated that the number of founding individuals could have been as few as 20 for *H. placitus* to have competitively displaced *H. amarus* in the Pecos River in 10 to 15 generations. Observed differences of allele frequencies between source and founder populations indicated that between 32 and 115 *H. placitus* individuals founded the Pecos River. Genetic and ecological data suggest that interspecific competition could have led to species replacement in this arid-land river system.

Keywords: assignment test, competitive exclusion, *Hybognathus amarus*, *Hybognathus placitus*, hybridization, Lotka–Volterra model

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Introduction

North America has the richest fauna of temperate freshwater fishes in the world, *c.* 800 native species in the waters of Canada and the United States (Page & Burr 1991). The spectacular ichthyofauna, however, has been heavily influenced by anthropogenic events over the last century that caused the decline, imperilment, and extinction of many freshwater fishes (Deacon *et al*. 1979; Williams *et al*. 1989; Burkhead & Jenkins 1991; Etnier & Starnes 1991; Moyle & Leidy 1992; Warren & Burr 1994). Aquatic ecosystems in southwestern North America are particularly at threat because they have a large proportion of endemic taxa. For example, 30% of the fish species inhabiting the Rio Grande are endemic (Carlson & Muth 1989). The southwest has also one of the most altered fish faunas in the United States. Boydstun *et al*. (1995) and Rahel (2000) documented that *c.* 25–50% of the fish species in the southwest (i.e. Arizona, New Mexico, Nevada, Utah, and Texas) are nonindigenous. Loss of endemics from biological invasions is homogenizing southwestern aquatic ecosystems at an alarming rate, and is one of the most prominent forms of biodiversity loss worldwide (McKinney & Lockwood 2001). Therefore, documenting and understanding ecological and genetic mechanisms attributing to the loss of biodiversity is a high priority. Using genetic and ecological data, this study addresses the extirpation of *Hybognathus amarus* (Rio Grande silvery minnow) in the Pecos River, New Mexico after the successful establishment of a non-native congener, *Hybognathus placitus* (plains minnow).

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Hybognathus is a widespread genus of North American minnows containing seven species. They are characterized by a long coiled intestine that facilitates digestion of benthic microflora (Page & Burr 1991). As herbivores, these species presumably play a vital role in the transfer of nutrients and energy in aquatic ecosystem where they occur. Yet, over the last decade some fishes in this guild (i.e. freshwater, herbivourous, pelagic spawners, Cross & Moss 1987) have dramatically decreased in abundance presumably because of habitat alteration and river fragmentation and intermittency (Taylor & Miller 1990; Bestgen & Platania 1991; Cook *et al.* 1992; Scheurer *et al*. 2003; Dodds *et al*. 2004).

H. amarus (Girard, 1856) is currently listed as endangered (Federal Register 1994), but historically, was among the most abundant fishes throughout its range (occurring in the Rio Grande and Pecos river systems from northern New Mexico to the Gulf of Mexico) (Trevino-Robison 1959; Bestgen & Platania 1991). This species is now confined to a *c*. 275 km stretch of the Rio Grande between Cochiti Dam and Elephant Butte Reservoir, NM. *H. placitus* Girard, 1856 is widely distributed in rivers and streams of the midwest (Page & Burr 1991), but did not overlap with *H. amarus* historically. In the early 1960s, *H. placitus* expanded into the Pecos River system, presumably by bait bucket introduction(s) (Bestgen & Platania 1991). By the mid-1970s, *H. placitus* completely replaced *H. amarus* in the Pecos River (Bestgen & Platania 1991). The disappearance of Pecos River *H. amarus* in *c.* 10 years likely resulted from hybridization or competition with *H. placitus*.

There is evidence that hybridization between species occurred at low frequency. As part of a larger systematic study of *Hybognathus*, Cook *et al*. (1992) found molecular (allozyme) and morphological (basioccipital width) evidence for hybridization between *H. amarus* and *H. placitus* (Pecos River). In a survey of morphological variation, Bestgen & Propst (1996) also detected two possible hybrids from a 1964 Pecos River collection of fishes (ASU 1308). Based on their findings and those of Cook *et al*. (1992), Bestgen & Propst propose hybridization and introgression as partially responsible for the elimination of *H. amarus* from the Pecos River. However, sustained hybridization and introgression would be necessary to convincingly invoke hybridization as a primary factor in *H. amarus*'s extirpation from the Pecos River. Thus, we tested for the presence of hybridization and introgression by examining mitochondrial DNA (mtDNA) and microsatellite loci in large, geographically representative samples of both species. We asked three additional questions with molecular data and an ecological modelling approach. Could displacement of *H. amarus* result from interspecific competition? What is the source population(s) of *H. placitus*? How many *H. placitus* individuals were introduced into the Pecos River?

Materials and methods

Sampling and DNA extraction

Genetic samples for *Hybognathus amarus* ($n = 389$) were collected from localities that spanned the species' current geographical range (Alò & Turner in press). We sampled *Hybognathus placitus* from four of eight major river drainages representing 50% of its current geographical range: Pecos River, NM ($n = 424$); Canadian River, NM ($n = 25$); Red River, OK ($n = 20$); and Moreau River, SD ($n = 20$). Canadian and Pecos river (1987, $n = 16$) samples, which are fishes used by Cook *et al*. (1992), allow us to directly compare hybridization results of Cook *et al*. (1992) with findings from our study. In the Pecos River, *H. placitus* samples ($n = 408$) were collected prior to spawning in April 1998, 2002, and 2003 from 13 localities (Table 1). To compare relatedness among mtDNA haplotypes, we included two other *Hybognathus* species (*Hybognathus hankinsoni*, $n = 5$; *Hybognathus nuchalis*, $n = 5$) (Table 1), and rooted the topology with *Notropis atherinoides* ($n = 1$, GenBank Accession no. AY116196). All specimens (excluding *N. atherinoides*) were deposited in the Museum of Southwestern Biology, Division of Fishes, University of New Mexico (Table 1). Total genomic DNA was isolated from ethanol-preserved tissue using standard organic extraction procedures (Sambrook *et al*. 1989).

mtDNA analysis

Double-stranded polymerase chain reaction (PCR) amplifications targeting a 328-bp segment of the mtDNA *ND4* gene were performed following the protocol of Alò & Turner (in press). Variation among individual fragments was visualized using single-stranded conformational polymorphism (SSCP) procedures of Moyer *et al*. (in press), except that a PCR annealing temperature of 50 °C was used. Nucleotide sequences of representative haplotypes from each gel (*c.* 20%) were verified by direct sequencing with an ABI BigDye Terminator (Applied Biosystems) cycle sequencing kit and an ABI 377 automated sequencing apparatus. Nucleotide sequences were aligned by eye.

Microsatellite analysis

Comparisons of genetic diversity between *H. amarus* and *H. placitus* involved four loci, *Ca6*, *Lco3*, *Lco6*, and *Lco7*. Primer information for *Lco3*, *Lco6*, and *Lco7* is found in Turner *et al*. (2004) and for *CA6* in Dimsoski *et al*. (2000). We multiplexed all four loci in 10-μL reactions using the following conditions: 1 × *Taq* reaction buffer (Promega), 2.25 mM $MgCl_2$, 0.2 mm each of dNTP, 1.0 μm each primer, and 0.375 U *Taq* polymerase (Promega). PCR conditions were an initial denaturation at 94 °C, followed by 25 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s. Prior to

Table 1 Locality and museum catalogue information of *Hybognathus placitus* specimens examined for this study

Year	Species	*n*	Drainage	Pecos River mile	MSB cat. no.
1987	*H. placitus*	25	Canadian River, NM		MSB 4634
1987	*H. placitus*	16	Pecos River, NM	522.2	MSB 4646
1998	*H. placitus*	100	Pecos River, NM	604.9	MSB 49944
2002	*H. placitus*	4	Pecos River, NM	585.3	MSB 49951
2002	*H. placitus*	51	Pecos River, NM	588.1	MSB 49952
2002	*H. placitus*	23	Pecos River, NM	604.9	MSB 49953
2002	*H. placitus*	24	Pecos River, NM	607.5	MSB 49954
2002	*H. placitus*	40	Pecos River, NM	621.0	MSB 49955
2003	*H. placitus*	11	Pecos River, NM	522.2	MSB 49945
2003	*H. placitus*	37	Pecos River, NM	575.8	MSB 49946
2003	*H. placitus*	37	Pecos River, NM	588.1	MSB 49947
2003	*H. placitus*	10	Pecos River, NM	594.7	MSB 49948
2003	*H. placitus*	32	Pecos River, NM	604.9	MSB 49949
2003	*H. placitus*	39	Pecos River, NM	611.0	MSB 49950
2003	*H. placitus*	20	Moreau River, SD		MSB 49907
2003	*H. placitus*	20	Red River, OK		MSB 49870
1987	*H. hankinsoni*	3	South Platte, CO		MSB 4806
2001	*H. hankinsoni*	1	Wolf River, WI		MSB 49957
2002	*H. hankinsoni*	1	Black River, WI		Fin clip only
1987	*H. nuchalis*	5	Buffalo River, MS		MSB 4807

The year indicates when each sample was collected, *n* is the sample size used in this study, and the MSB catalogue no. indicates that voucher specimens are deposited in the Museum of Southwestern Biology, University of New Mexico. Surveyed Pecos River localities are denoted by river miles.

electrophoresis, 1.2 μL of PCR products were mixed with a 1.2 μL solution containing 62.5% formamide, 25% bromophenol blue, and 12.5% Genescan ROX350 size standard (ABI Prism, Applied Biosystems). Microsatellite reactions were visualized on an ABI 377 Prism (Applied Biosystems) using fluorescently labelled forward primers and analysed using GENESCAN version 3.1.2 (Applied Biosystems).

Phylogenetic analysis

We estimated haplotype relationships using the maximum-likelihood (ML) method (Felsenstein 1981) implemented by PAUP* version 4.0b10 (Swofford 1998). Model selection and parameter estimation were conducted with hierarchical likelihood ratio testing (LRT) implemented using MODELTEST version 3.06 (Posada & Crandall 1998). Once a model and associated parameter values were identified, we employed the following ML heuristic search strategy: tree-bisection–reconstruction (TBR) branch swapping on 100 random additional replicates with the MulTrees option in effect. We assessed support by bootstrap resampling (Felsenstein 1985) (no. of pseudoreplicates = 500); heuristic tree searches: TBR branch swapping on an initial NJ tree with MulTrees option in effect).

Population dynamics and computer simulations

Microsatellite genotypic data were analysed for departures from Hardy–Weinberg equilibrium (HWE) expectations with Fisher exact test (Louis & Dempster 1987) as implemented in GENEPOP 3.1b (Raymond & Rousset 1995). We also tested for departures from HWE among localities in each sample year for Pecos River-2002 and -2003 datasets To test for population structure among Pecos River-2002 and Pecos River-2003 localities, we used analysis of molecular variance (AMOVA) with ARLEQUIN version 2.000 (Schneider *et al*. 2000). Linkage disequilibrium (LD) between all pairs of loci was tested with GENEPOP. For population comparisons (i.e. Pecos vs. Canadian + Red rivers), one minus the proportion of shared alleles (1 – PSA) (Bowcock *et al*. 1994) was used as an indicator of the genetic divergence among populations using the program PSACALC (Noor *et al*. 2000).

To evaluate various colonization scenarios, we used the simulation program MULTSIM (Noor *et al*. 2000). MULTSIM simulates founder events from allele frequency distributions observed in source populations and allele frequency differences between source and sink populations. The program randomly selects alleles from the source population based on their relative frequencies and begins a new population with a specified number of founders. The program then calculates 95% confidence intervals for the estimated number of founders by creating a frequency distribution of genetic divergences (1 – PSA) via bootstrapping the source population allele frequencies for each locus.

MULTSIM assesses the number of founders based on differing colonization scenarios. First, we simulated a founding event of $n = 1 – 100$ individuals followed by an expansion in population size ($n = 100\,000$) increasing by a

factor of five for four generations (Slow 1). Second, the same simulation was performed but the population size was expanded by a factor of 10 for each generation (Slow 2). Lastly, we bottlenecked the initial founding population for 10 generations and then allowed the population to grow instantaneously to a very large size (Slow 3). MULTSIM uses a haploid model; therefore, each locus was analysed separately. The 1 – PSA values were generated from allele frequencies for Pecos River (2003 individuals) vs. Red + Canadian river samples. MULTSIM sampled from the source population allele frequencies 2000 times to create the frequency distribution of possible genetic divergences, measured as 1 – PSA.

Hybridization analysis

We used the program STRUCTURE version 2.1 (Pritchard *et al*. 2000) to assess the degree of historical hybridization between *H. amarus* and *H. placitus* (Pecos River). STRUCTURE probabilistically assigns individuals to a given population or jointly assigns them to two or more populations if the genotype is admixed (Pritchard *et al*. 2000). We were interested in assessing the degree of introgression for *H. amarus* alleles in *H. placitus* from the Pecos River. Thus, using the characterized microsatellite data for known *H. amarus* from the Rio Grande, and known *H. placitus* from the Red and Canadian rivers, STRUCTURE assigned *H. placitus* (Pecos River) to one or both of these populations. We assume that *H. amarus* Pecos River and Rio Grande populations were not genetically different — an assumption corroborated by Bestgen & Propst (1996) who found only minor morphological divergence among these populations.

Three independent STRUCTURE runs were performed to assess the use of prior population information and differing allele frequency models. Initial parameters for the first run (run1) were (i) no prior population information; (ii) an admixture model with a correlated allele frequency model (see Pritchard *et al*. 2000); and (iii) all other parameters set to default. The second run was identical to the first except we chose the independent allele frequency model. The last run explored the effects of prior population information for *H. amarus* (Rio Grande), *H. placitus* (Red River), and *H. placitus* (Canadian River) populations — all parameter settings were the same as run1 except that the ancestry model was set to prior population information. All run lengths were 1×10^6 steps with the initial 1×10^5 steps discarded as burn-in.

Ecological modelling

Theoretical dynamics of interspecific competition between *H. placitus* and *H. amarus* were explored using Lotka–Volterra models. Our goal was to determine the set(s) of parameters for which *H. placitus* could outcompete *H. amarus* in 10–15 years (the presumed time frame for the disappearance of *H. amarus* in the Pecos River; Bestgen & Propst 1996). The logistic growth equations, which model the change in population growth through time, are:

$$\frac{dN_1}{dt} = r_1 N_1 \frac{[K_1 - (N_1 + \alpha N_2)]}{K_1}$$

$$\frac{dN_2}{dt} = r_2 N_2 \frac{[K_2 - (N_2 + \beta N_1)]}{K_2}$$

where parameters r_1 and r_2 are the intrinsic rates of growth for *H. amarus* and *H. placitus*, respectively; N_1 and N_2 are the starting populations sizes of *H. amarus* and *H. placitus*, respectively; K_1 and K_2 are the equilibrium population sizes for each species in absence of the other; α is a measure of the inhibitory effect of *H. placitus* on the population growth of *H. amarus*; and β is the inhibitory effect of *H. amarus* on the population growth of *H. placitus*. Survivorship to maturity of young of year *H. amarus* and *H. placitus* is unknown; therefore, we estimated reproductive output ($R_0 = 40$) using a conservative estimate of survivorship to maturity (5%) and an average fecundity of 800 (fecundity estimate from Taylor & Miller 1990). Based on R_0 and a discrete generation time (t) of one (Taylor & Miller 1990; Platania & Altenbach 1998), the intrinsic rate of growth is 3.69 ($r = \ln R_0/t$). Our estimate of K_1 and K_2 are based on Pecos River *H. placitus* census estimates obtained from population monitoring data by the New Mexico Department of Game and Fish (years 2000–2002). The census size ($N = 2.5 \times 10^5$) of *H. placitus* is the average of two estimates of N, where N_1 is the product of mean density, reach length (1.3×10^7 m), and average channel width, and N_2 is the product of average number of individuals per meter and total reach length (see Appendix for calculations of N_1 and N_2). Assuming $r_1 = r_2$, $K_1 = K_2$, and an initial *H. amarus* population at carrying capacity (2.5×10^5), we addressed the effects of changing the number of introduced *H. placitus* ($N_2 = 20$, 100, and 500) and the parameters α and β (the values of N_2 were chosen to resemble a range of values thought to be appropriate for a bait bucket introduction). Once we found a range of values for α and β, we held these parameters constant and relaxed the assumptions of $r_1 = r_2$ and $K_1 = K_2$. We set $r_1 = 1$, 2, or 4, holding $r_2 = 3.69$ (performing the reciprocal comparisons) and set $K_1 = 1 \times 10^2$, 10^3, and 10^4, holding $K_2 = 2.5 \times 10^5$. All combinations of parameters were assessed using $N_2 = 20$, 100, and 500 introduced *H. placitus*.

Results

Phylogenetic analysis

Likelihood ratio tests chose the TrNef + Γ [$\alpha = 0.5274$; R(a) = 1.00, R(b) = 18.36, R(c) = 1.00, R(d) = 1.00, R(e) = 8.76, R(f) = 1.00] model of sequence evolution. Phylogenetic

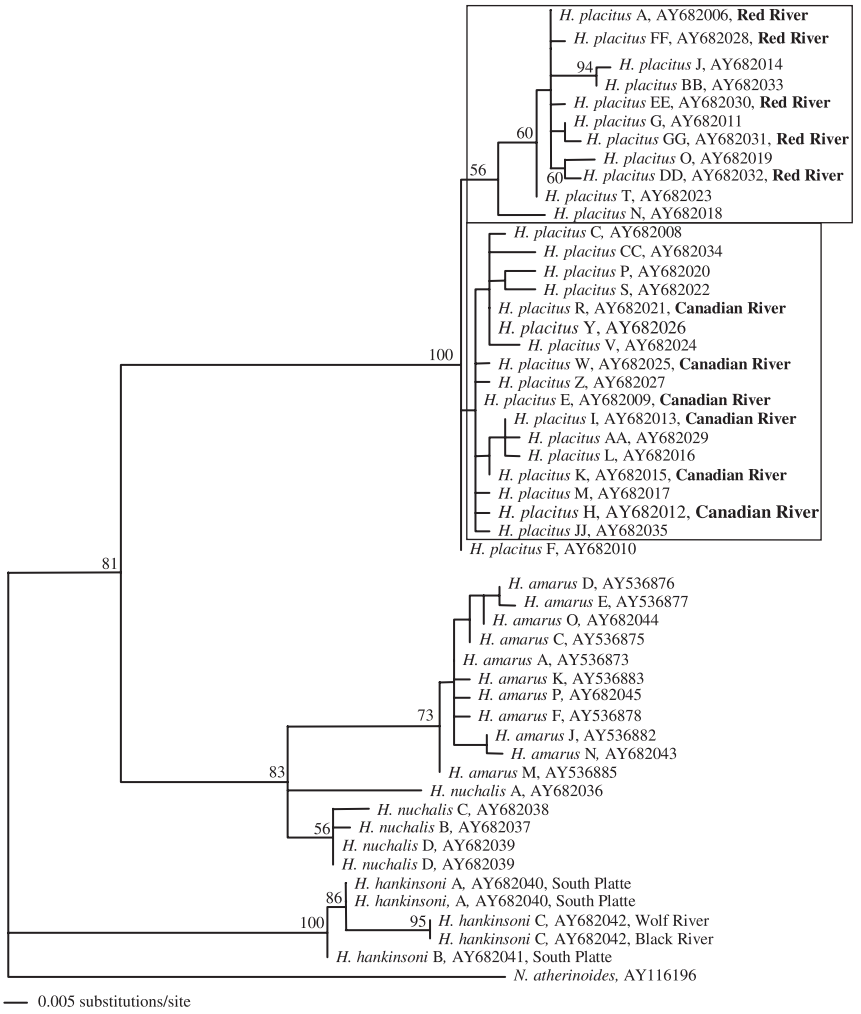

Fig. 1 Pecos River *Hybognathus placitus* halpotype relations inferred with partial mtDNA *ND4* sequences. Nodal values are bootstrap percentages out of 500 replicates; values < 50% are not reported. Letters after operational taxonomic units (OTUs) represent individual haplotypes. Haplotypes common between Pecos and Canadian or Pecos and Red rivers are designated by Canadian and Red rivers. All other numbers after OTUs represent GenBank Accession nos. Boxes indicate two major clades in Pecos River *Hybognathus placitus* samples.

analysis of *Hybognathus* mtDNA haplotypes reveals well resolved and monophyletic *Hybognathus amarus* and *Hybognathus placitus* clades (Fig. 1). There are 29 haplotypes present from surveyed *H. placitus* (Pecos River) samples forming a polytomy among three major clades with one clade comprising haplotypes found in the Pecos and the Canadian rivers, a second clade comprising haplotypes found in the Pecos and the Red rivers, and a third clade consisting of haplotype F from the Pecos River (Fig. 1). Seventeen haplotypes comprise the first *H. placitus* clade, of which, six are shared between Canadian River samples. Haplotypes E and I are in relatively high frequency in Canadian River (38% and 24%, respectively), but in lower frequencies in the Pecos River (7% and 1%, respectively). The frequency of the remaining shared haplotypes is ≤ 4%. The second clade consists of 11 haplotypes with five shared between Red River samples. Haplotype A is in relatively high frequency for both Pecos and Red river samples (48% and 80%, respectively), and the frequency of the remaining shared haplotypes is ≤ 0.5%. No haplotypes are shared between Red and Canadian river samples. Moreau River, SD samples of *H. placitus* share haplotypes with *H. argyritis* (Moyer, unpublished); therefore, these samples are excluded from subsequent analyses.

Population dynamics and computer simulations. All *H. placitus* populations conform to HWE expectations (all $P > 0.05$), and no spatial structuring among populations exists in either the Pecos River 2002 or 2003 samples ($F_{ST} = -0.001$, $P = 0.69$ and $F_{ST} = 0.005$, $P = 0.08$, respectively). No evidence of genotypic LD was uncovered (all $P > 0.05$). Average gene diversity ($\hat{H}$), calculated from all nuclear loci, is similar among Pecos River-1998 ($\hat{H} = 0.79 \pm 0.032$), Pecos River-2002 ($\hat{H} = 0.79 \pm 0.037$), Pecos River-2003 ($\hat{H} = 0.79 \pm 0.034$), Red River ($\hat{H} = 0.76 \pm 0.034$), and Canadian River ($\hat{H} = 0.57 \pm 0.16$) samples. mtDNA gene diversity is comparable to nuclear estimates and similar among Pecos-1998 ($\hat{H} = 0.80$), Pecos River-2002 ($\hat{H} = 0.71$), Pecos River-2003 ($\hat{H} = 0.75$), and Canadian River ($\hat{H} = 0.81$) samples. The Red River estimate ($\hat{H} = 0.37$) is less than nuclear and mtDNA estimates.

Table 2 Minimum and maximum number of founding *H. placitus* using three colonization scenarios

			Slow 1		Slow 2		Slow 3	
Locus	No. of haplotypes	1 – PSA	min.	max.	min.	max.	min.	max.
mtDNA	13	0.4450	2	15	2	16	2	115
CA6	6	0.1880	1	15	1	14	17	114
Lco7	12	0.2837	3	12	3	10	20	88
Lco3	6	0.3655	1	4	1	4	3	32
Lco6	6	0.2458	1	7	1	6	2	51
Autosomal average			2	10	2	9	11	71

One minus the proportion of shared alleles (1 – PSA) is the genetic divergence between Pecos and Canadian + Red river samples. Slow 1, Slow 2, and Slow 3 are three differing colonization scenarios (see text for details).

Qualitatively, simulations using MULTSIM indicate no difference in numbers of founding individuals between Slow 1 and Slow 2 models (Table 2). There is a considerable difference between Slow 3 and Slow 1 or Slow 2 models. The Slow 3 model predicts roughly a 10-fold increase in the maximum number of founding individuals. There is considerable variation in the maximum number of founders for each locus, but the average estimate based on autosomal loci for the Slow 1 and Slow 3 models is 10 and 71, respectively. The autosomal estimate is similar to mtDNA estimates (Table 2).

Hybridization analysis. Little evidence of hybridization is revealed among STRUCTURE runs, assuming different ancestry or allele frequency models. Known *H. amarus* samples (i.e. Rio Grande population) are assigned correctly to the *H. amarus* population 99% of the time (385 of 389 individuals). Similarly, known *H. placitus* (i.e. Red and Canadian river populations) group with the *H. placitus* population 93% of the time (42 of 45 individuals). Pecos River *H. placitus* are assigned 98% of the time (409 of 419 individuals to known *H. placitus* populations (i.e. Red and Canadian river populations). Analyses indicate that 10 individuals (2%) maybe of hybrid origin. STRUCTURE misassigned five individuals (1%) as a result of the possession of shared ancestral alleles (*Lco7* alleles 141, 148, 151, 153) between known *H. amarus* and *H. placitus*. The remaining five putative *H. placitus* share one allele (either *Lco3* allele 151 or *Lco6* allele 176) with *H. amarus* samples. This indicates that these individuals are of hybrid origin. Based on our interpretation of microsatellite and mtDNA data, fishes that Cook *et al.* (1992) postulated to be hybrids instead retain pleisomorphic alleles at allozyme loci they surveyed.

Ecological modelling

Exploration of various values for Lotka–Volterra parameter α and β indicate that α = 1.2 and β = 0.8–0.3 (Table 3) are optimal estimates (i.e. the smallest difference between parameters), for which the displacement of 99% of 2.5×10^5 *H. amarus* could occur in a 10–15-year period. Increasing or decreasing values of α and β result in faster or slower displacement times of *H. amarus*. A difference of α and β of 0.5 (α = 1.2 and β = 0.7) is the smallest for which *H. placitus* could displace *H. amarus* in the presumed time frame of 10–15 years. Relaxing the assumptions that $r_1 = r_2$ and $K_1 = K_2$ have little consequence on the number of generations until the displacement of *H. amarus* (Table 4). Decreasing values of r_1, in comparison to r_2, result in an increase in generation time to displacement. Decreasing values of K_1, in comparison to K_2, result in a dramatic decrease in generation time to displacement (*c.* 1–2 generations; data not shown). Numbers (N_2 = 20, 100, and 500) of introduced *H. placitus* have little effect on the number of generations until the displacement of *H. amarus* (Table 3). In all cases, increasing values of N_2 decrease estimates of generation time to the displacement for *H. amarus*.

Table 3 Optimal values of Lotka–Volterra parameters α and β and the number of generations needed for the displacement of *H. amarus* in the model system

			No. of generations		
α	β	α – β	20	100	500
1.2	0.9	0.3	32	27	23
1.2	0.8	0.4	20	18	15
1.2	0.7	0.5	15	14	12
1.2	0.6	0.6	13	12	10
1.2	0.5	0.7	11	10	9
1.2	0.4	0.8	10	9	8

Parameter α measures the inhibitory effect of *Hybognathus placitus* on the population growth of *Hybognathus amarus*; β is the inhibitory effect of *H. amarus* on the population growth of *H. placitus*. The number of generations for the displacement of *H. amarus* (initial census size = 2.5×10^5) was modelled using N_2 = 20, 100, and 500 founding *H. placitus*. Carrying capacities (2.5×10^5) were held constant between the species.

Discussion

Genetics of introduction

There are four outcomes for recently created admixtures of two or more congeners. First, the introduced taxon is an unsuccessful invader and is limited by factors such as low propagule pressure or narrow physiological tolerance to the new system (Lodge 1993; Duncan *et al*. 2001; Marchetti *et al*. 2004). Second, invading and native species coexist by altering relative abundance of resources in a patchy landscape (Schluter 1995; Amarasekare & Nisbet 2001; Abrams & Chen 2002). Next, hybridized and introgressed lineages can persist with one, both, or neither of the parental taxa (Huxel 1999; Epifanio & Philipp 2000; Scribner *et al*. 2001). Last, if competing taxa have identical ecological requirements, then the invading taxon is capable of displacing the native taxon (i.e. competitive exclusion; Hardin 1960).

We can eliminate the first and second scenarios because *Hybognathus placitus* is abundant in the Pecos River, whereas *Hybognathus amarus* no longer occurs in this system. Yet, can the displacement of *H. amarus* by *H. placitus* be ascribed to a hybridization event or, alternatively, to competitive interactions? Previous studies (Cook *et al*. 1992; Bestgen & Propst 1996) provide only anecdotal evidence that *H. placitus* × *H. amarus* hybrids existed, albeit at low frequencies. Cook *et al*. (1992) found five of 20 *H. placitus* (Pecos River) samples had alleles in common with *H. amarus*, but they could not eliminate the possibility that these alleles were pleisiomorphic characters from an ancestral gene pool. Furthermore, Bestgen & Propst (1996) surveyed museum collections of *H. amarus* ($n = 90$) and *H. placitus* ($n = 60$) from the Pecos River and described only two potential hybrids based on morphometric measurements. Predicting the outcome of a hybridization event is often difficult (Scribner *et al*. 2001), yet the low number of putative *H. placitus* × *H. amarus* hybrids found in previous studies contradicts theoretical (Epifanio & Philipp 2000) and empirical (Echelle & Connor 1989) findings. Epifanio & Philipp (2000) simulated hybridization events between two taxa varying three parameters: relative fitness of parental and F_x taxa, initial frequency of parental lineages, and mating preference coefficients (i.e. positive vs. assortative mating). Over a wide range of initial parameter values, Epifanio & Philipp (2000) documented that the frequency of parent and F_1 taxa decrease to < 1% in a relatively short time period (< 5 years). These findings indicate that recently hybridized populations should comprise F_x hybrids at frequencies > 99%. The results of Epifanio & Philipp (2000) are corroborated by Echelle & Connor (1989) who documented extensive genetic introgression of *Cyprinodon* (pupfishes) species pairs (in < 5 years) following the introduction of *Cyprinodon variegatus*. Based on the findings of Epifanio & Philipp (2000) and Echelle & Connor (1989), the expected frequency of Pecos River *H. placitus* × *H. amarus* F_x hybrids should be > 99%. Therefore, genetic screening of *Hybognathus* samples from the Pecos River should reveal a substantial proportion of admixed alleles and haplotypes if hybridisation and introgression occurred.

Assignment tests and phylogenetic analysis of *H. placitus* (Pecos River) samples (including samples analysed by Cook *et al*. 1992) do not indicate that recent hybridization or introgression has occurred in high frequencies between *H. amarus* and *H. placitus*. No shared mtDNA haplotypes exist between *H. amarus* and *H. placitus* samples in this study. The assignment tests, based on four microsatellite loci, clustered 98% of *H. placitus* Pecos River samples with known *H. placitus* from the Canadian and Red rivers. Although our data indicate five putative *H. amarus* × *H. placitus* hybrids, additional screening of more known *H. placitus* may reveal that alleles used to classify these individuals as hybrids are in fact shared ancestral polymorphisms. Because of the apparent lack of hybridization and introgression between these species, we believe that the extirpation of *H. amarus* is a result of fitness differences between it and *H. placitus*.

Hardin (1960) predicted that two species with identical ecological requirements could not occupy the same environment. Although the more streamlined body shape of *H. placitus* may suggest adaptation to higher velocity habitats (Bestgen & Propst 1996; Scheurer *et al*. 2003), *H. amarus* and *H. placitus* appear to inhabit similar river environments (Sublette *et al*. 1990) and share similar life histories (*H. placitus*, Taylor & Miller 1990; *H. amarus*, Platania 1995). To our knowledge, documented cases of competitive exclusion in fishes are rare, but the apparent lack of hybridization between these species in the Pecos River suggests that competitive interactions between *H. amarus* and *H. placitus* or among these species and other Pecos River fishes (i.e. diffuse competition, MacArthur 1972) may have caused the displacement of *H. amarus* from the Pecos River. Our data, however, do not allow us to ascertain the direct cause of the competitive interaction.

Location and number of source populations

If multiple divergent source populations founded the Pecos population, we would predict a haplotype topology consisting of multiple divergent haplotype clades — assuming the source populations are divergent from each other. Furthermore, source and sink population haplotype frequencies should be similar. mtDNA data produce two major groups corresponding to *H. placitus* from the Canadian and Red river drainages. Haplotype A is in relatively high frequency in both the Red and Pecos river samples, and similarly, haplotype E is in high frequency in the Canadian and Pecos rivers. All other shared haplotypes

are in relatively low frequencies (< 4%) in the Red, Canadian, and Pecos drainages. The diversity and phylogenetic pattern of Pecos River *H. placitus* mtDNA haplotypes suggests that invasions occurred from multiple, genetically divergent source populations (putatively from the Canadian and Red river drainages), although more than two source populations could have invaded the Pecos River (see succeeding discussions).

Bestgen *et al*. (1989) speculated that *Notropis girardi*, which commonly co-occurs with *H. placitus* in its native range, was accidentally included in a bait shipment of *H. placitus* and introduced into the Pecos River in the late 1970s. The introduction of *N. girardi*, however, appears much later than that of *H. placitus* (late 1970s vs. early 1960s; Bestgen *et al*. 1989; Bestgen & Platania 1991). The discrepancy in dates could be explained by more than one *H. placitus* introduction — one in the early 1960s and another in the late 1970s. This explanation is consistent with our molecular findings.

Ecological modelling

Our molecular data exclude hybridization as a primary factor in the displacement of *H. amarus* by *H. placitus*, perhaps indicating that competitive interactions are responsible. Yet, is competitive displacement ecologically plausible in a 10–15-year time period based on our understanding of the life history and demography of these species? Modelling competitive interactions using a Lotka–Volterra framework indicates that *H. placitus* can displace *H. amarus* in this short time. Furthermore, the degree of competition is rather small: 1.2 individuals of *H. placitus* have collectively the same inhibitory effect on *H. amarus* as one individual of *H. amarus*, and 0.4–0.9 *H. amarus* individual have the same effect on *H. placitus* as one *H. placitus* individual.

There are caveats to the Lotka–Volterra model, which is based on the logistic model of population growth. The model is deterministic with four underlying assumptions: a homogenous and stable environment; the effects of migration are limited; coexistence is reached at equilibrium; and competition (constant over time) is the only biologically relevant interaction (Begon *et al*. 1996). Assumptions are necessary simplifications and may be unrealistic, and there are a variety of factors not included in the model that can influence the outcome of competitive interactions (e.g. environmental change, disease, and chance). Therefore, our findings based on the Lotka–Volterra model are a first approximation.

Number of founding individuals

We used ecological and genetic approaches to estimate the maximum and minimum number of *H. placitus*. The ecological method indicates a minimum of 20 *H. placitus* may have founded the Pecos River, and is concordant with the genetic simulations using the Slow 1 and Slow 2 models. Although ecological and genetic data indicate approximately 20 effective founders, this number is an underestimate of the actual number of colonizers. Genetic data reveal 30 mtDNA haplotypes, and assuming the genetic diversity was bottlenecked after each introduction, the number of founding individuals is predicted to be > 30. The Slow 3 model, which bottlenecks the genetic diversity for 10 generations, simulates the lag phase (Kowarik 1995; Crooks & Soulé 1999) of introduced populations, and is a more realistic model for the introduction of *H. placitus*. For autosomal and mtDNA data, the Slow 3 model predicts a maximum of 32–115 individuals colonized the Pecos River, which agrees with ecological estimates.

The number of colonizers must be treated with caution for several reasons. Our genetic simulations assume a haploid model; therefore, various population and life history dynamics (e.g. skewed sex ratios or high variance in male or female mating success) may influence the results (Noor *et al*. 2000). The haploid model seems appropriate, the sex ratio of *H. placitus* appears equal and there is no evidence (e.g. sexual dimorphism) for large variances in male mating success (Taylor & Miller 1990). We also assume that founding sources originated in the Canadian and Red rivers and that no introduced haplotypes have gone extinct. Genetic data can not exclude the possibility that more than two source populations contributed to the introduction because numerous rare haplotypes exist in the Pecos River population that are not present in samples from the Canadian and Red rivers. These haplotypes may represent haplotypes from other source populations; alternatively, they are unsampled, rare haplotypes persisting in the Canadian and Red rivers. Based on the pattern of haplotypes and the small sample sizes used in genetic screening of putative source populations, we predict that more intensive surveys of these populations would reveal rare haplotypes surveyed from the Pecos River. Lastly, we assume that allele frequencies between propagule and source populations are similar (i.e. the effect of genetic drift between the propagule and source populations is minimal). Although *H. placitus* is declining throughout much of its range (Pigg 1987; Federal Register 1994), populations in the Canadian and Red rivers appear stable (Sublette *et al*. 1990); therefore, the effects of genetic drift between source and founder populations should be negligible. Based on these assumptions and the presence of rare Pecos River haplotypes unaccounted for in the source populations, the number of introduced individuals is a conservative estimate.

Conclusions

Understanding causal mechanisms underlying the extirpation of endemic species as a result of biological invasion is often difficult and requires detailed and accurate records

from long-term ecological studies or from museum collections. This study demonstrates the importance of combining knowledge gleaned from genetic and ecological data in an effort to understand the mechanisms for each phase of invasion and species replacement. Using genetic data, we were able to (i) exclude the role of hybridization with regard to the extirpation of *H. amarus;* (ii) document multiple *H. placitus* invasions and the invasion sources; and (iii) statistically determine the number of *H. placitus* that invaded the Pecos River. Lastly, we corroborate our genetic data using life history and demography data for *H. placitus* and *H. amarus*, and show that competition between these species is plausible in the given time frame ascertained from museum collections.

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Gregory Moyer's primary research interests focus on using molecular markers in conjunction with ecological and demographic data to explore the evolutionary processes underlying the diversification, maintenance, and exctinction/extirpation of fish species. Megan Osborne is a post-doctoral research associate. Her research involves using molecular data to understand the causes and genetic consequences of population decline, and to monitor the genetic impacts of supportive breeding in endangered desert fishes. Thomas Turner's research is broadly focused on ecology and evolution of aquatic organisms and systems. He is especially interested in uncovering relationships of life history, ecology and genetic diversity.

Appendix

Population monitoring and estimation of adult census size (N)

Estimates of adult numbers (N) for this study were generated from Pecos River *Hybognathus placitus* population monitoring data acquired during February and April 2000–2002 (R.D. Larson, unpublished). Sample months were selected because they represented the period when reproductively capable age one individuals comprised the vast majority of the population and because spawning for the year had not yet occurred. Fish abundance data were obtained from 17 monitoring sites established in the middle Pecos River from Fort Sumner to Brantley Reservoir.

The geomorphology of the Pecos River permits effective sampling by wading and seine netting. The braided river channel with sand and gravel substrate provides diverse aquatic habitats including main channel, shoreline runs and plunge pools with nonzero current velocity, and backwater habitats where current velocity is zero. Fishes were obtained using a $3 \times 1.5 \times 0.01$ m (mesh) seine net, and the total distance seined in an individual seine haul was measured in meters. Approximately 17 seine hauls were conducted at each monitoring site, with individual seine hauls chosen to sample evenly across habitat types, but selected haphazardly within habitat category.

Catch-per-unit-effort data for Pecos River *H. placitus* were used to estimate density (number of fish per meter squared) at each monitoring site by dividing the number of fish captured by the total area sampled (see Table below). Two estimates of N are provided; N_1 is the product of mean density, reach length (1.3×10^7 m), and average channel width (40 m), and N_2 is the product of average number of individuals per meter and total reach length.

	2000	2001	2002
No. sampled	187	389	176
Total area sampled (m^2)	1.3×10^7	1.3×10^7	1.3×10^7
Ave. no./m^2	0.024	0.073	0.039
Ave. no./m	0.055	0.114	0.052
N_1	3.1×10^5	9.6×10^5	5.1×10^5
N_2	1.8×10^4	3.8×10^4	1.7×10^4
$N_{1\ \text{harmonic mean}}$	4.8×10^5		
$N_{2\ \text{harmonic mean}}$	2.1×10^4		
N^a	2.5×10^5		

[a]is average of N_1 and N_2.