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Populations of Pecos pupfish (*Cyprinodon pecosensis*) differ in their susceptibility to hybridization with sheepshead minnow (*C. variegatus*)

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Abstract The importance of sexual selection in hybridization between species that evolved in allopatry is poorly understood. Even less is known about differences between populations in their resistance or susceptibility to genetic introgression with a congener. Here we examine behavioral differences between populations of the native Pecos pupfish (*Cyprinodon pecosensis*) and their potential effects in facilitating or retarding hybridization with an introduced congener, the sheepshead minnow (*C. variegatus*). We quantified competitive interactions, establishment of territories, and mating success of several naturally occurring *C. pecosensis* populations and an artificially created laboratory population (control) against *C. variegatus*. Experiments were conducted in large outdoor tanks during the summer months when high temperatures favor breeding activity in pupfish. *C. pecosensis* populations differed in agonistic behavior, territoriality, and in mating success when competing against *C. variegatus*. Differences in the competitive ability of males from different *C. pecosensis* populations suggest that the rate of introgression with *C. variegatus* may vary, depending on the population of *C. pecosensis* it comes in contact with. Our results suggest that large, stable *C. pecosensis* populations may be relatively resistant to hybridization with *C. variegatus*. Interpopulation variation in male behavior, shaped by sexual selection, may be important in determining the incidence and rate of hybridization between the two congeners. Experimental studies are a

powerful tool in elucidating behavioral processes, such as the action of intrasexual selection, that facilitate or retard the coalescence of lineages through initial hybridization and subsequent genetic introgression after secondary contact between allopatric species.

Keywords Hybridization · Sexual selection · Male–male competition · Geographic variation · Cyprinodontidae

Introduction

Hybridization, or interbreeding between species that have come into secondary contact after evolving in allopatry, is widespread in both plants (Arnold 1997) and animals (Grant and Grant 1992, 1997; Dowling and Secor 1997). Genetic studies have revealed the prevalence of hybridization and genetic introgression between closely related lineages in vertebrates (e.g. Lamb and Avise 1986; Pierotti and Annett 1993; Szymura 1993; Good et al. 2000; Roques et al. 2001; Randler 2002). Molecular studies suggest that introgression is an important and pervasive process both in the evolution of lineages, by enhancing genetic diversity through lineage coalescence, and the extinction of lineages, through the complete or partial replacement of one species' genome by that of another (Hubbs 1955, 1961; Miller et al. 1989; Levin et al. 1996; Rhymer and Simberloff 1996). Hybridization and introgression are important processes in the evolution of fish. Over 40% of the threatened, endangered, and recently extinct fishes in North America have been in some way impacted by introgression (Williams et al. 1989). It is thus surprising that the behavioral mechanisms underlying this process are poorly understood.

Most studies of natural hybridization emphasize the importance of ecological factors in the establishment and maintenance of hybrid zones. The role of sexual selection is less well studied (but see Pierotti and Annett 1993; McDonald et al. 2001; Collins and Luedem 2002; Randler 2002). Even less is known about differences in reproductive behavior between populations and how these may

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affect susceptibility to hybridization and introgression. Differences between populations, such as the physical size of the habitat, productivity, population stability, density, availability of breeding habitat, and presence of competitors and predators, have marked effects on the breeding system, and on the intensity of sexual selection. Populations may differ in male agonistic behavior, shaped by the intensity of intrasexual competition for access to females or resources required by females, such as breeding substrate (Kodric-Brown and Mazzolini 1992). Populations may also differ in the intensity of intersexual selection, and the types of male traits females use to discriminate among potential mates (e.g. Houde 1997). Thus competitive interactions among males and female preferences for particular behavioral and/or morphological traits of males may facilitate or retard hybridization between species. For example, strong female preferences for traits displayed by heterospecific males would facilitate hybridization, while a strong preference for attributes shown by conspecific males would retard this process. Similarly, if competitive ability is highly correlated with access to females or breeding substrates, then the species with aggressive males has an advantage and will obtain a disproportionate number of matings, thus affecting the rate of hybridization.

Differences between populations in the strength of sexual selection and their effect on the hybridization process have been largely ignored because, with the exception of populations on islands, most terrestrial vertebrates are rarely found in small, disjunct populations. By contrast, freshwater fishes often occur as multiple small, isolated, somewhat differentiated populations with different breeding systems and life-history traits that reflect both genetic and environmental differences (e.g. examples in Foster and Endler 1999; Downhower et al. 2000). Therefore, behavioral differences may have an important impact on susceptibility or resistance to introgression and/or replacement by alien genes.

We studied the role of intrasexual selection in hybridization between two congeners, the Pecos pupfish (*Cyprinodon pecosensis*) a native of the Pecos River, N.M., USA, and the sheepshead minnow (*C. variegatus*), an invasive exotic. Hybridization between *C. pecosensis* and *C. variegatus* is of interest because, unlike many examples of hybridization in the wild, hybrids are not only viable, but are superior to *C. pecosensis* (Echelle and Connor 1989; Rosenfield and Kodric-Brown 2003).

Currently, the hybrid swarm occupies most of the geographic range of *C. pecosensis* and is extending southward along the Pecos River well beyond the historic range of the invaded species (Echelle et al. 1997). Previous work has demonstrated an important role of sexual selection, and particularly male–male competition, in promoting hybridization and genetic introgression between these two species. Specifically, *C. pecosensis* were less aggressive than male *C. variegatus* or their F1 hybrids and obtained fewer matings as a result (Rosenfield and Kodric-Brown 2003).

There are several advantages to using experiments to study the role of sexual selection in hybridization between these two congeners:

1. The role of sexual selection can be examined separately from ecological factors, such as habitat preferences and resource exploitation.
2. The small size of pupfish and their territorial breeding system make them ideally suited for experimental manipulations to examine the importance of behavioral interactions in contributing to male reproductive success under realistic seminatural conditions.
3. The importance of geographic variation in behavioral traits of *C. pecosensis* populations on the rate of hybridization and introgression can be determined by comparing male competitive interactions and reproductive success between different populations of *C. pecosensis* and the introduced *C. variegatus* in identical, controlled settings.
4. The breeding system of both species has been studied both in the field and the laboratory (Itzkowitz 1977, 1981; Kodric-Brown 1977, 1983, 1988).
5. Geographic variation in the behavior of subpopulations and the structure of the breeding system has been documented (Kodric-Brown and Mazzolini 1992).

Here we address the question of whether *C. pecosensis* males from different populations differ in competition for mates and how this might affect the rate of hybridization and introgression with *C. variegatus*.

Natural history and historical background

The hybridization between *C. pecosensis* and *C. variegatus* provides an opportunity to study an ongoing dynamic process, as well as the underlying mechanisms. Echelle and Connor (1989) report that in the early 1980s a small number of *C. variegatus* were introduced into the Pecos River where they opportunistically hybridized with the native *C. pecosensis*. Molecular studies of the hybrid swarm suggest that introgression was extremely rapid; in less than a decade hybrids replaced the Pecos pupfish in more than half of its historic range (500 linear km of the Pecos River; Echelle and Connor 1989; Childs et al. 1996; Echelle et al. 1997).

The Pecos pupfish originally occurred in the lower Pecos River of New Mexico and Texas. It has a small geographic range compared to its congener. Currently it is restricted to small, geographically isolated populations inhabiting a few sinkholes and small springs in the flood plain of the Pecos River in New Mexico and western Texas. *C. variegatus* has an extensive geographic range and is distributed along the east coast of North America from Massachusetts to the Yucatan Peninsula in the Gulf of Mexico. It inhabits estuaries and coastal lagoons. Both species tolerate a wide range of temperatures and salinities.

Phylogenetically, the two species are closely related; both are part of the *C. variegatus* species complex (Echelle and Echelle 1978, Echelle et al. 2004). Like most pupfish, the two species are behaviorally and morphologically similar. Males establish and defend breeding territories over substrates favored by females as oviposition sites (Itzkowitz 1977, 1981; Kodric-Brown 1977). Females visit male territories, oviposit, then leave. There is no parental care of the demersal eggs, except for the incidental benefit of the presence of an aggressive territorial male who keeps out potential egg predators, including conspecific males and females. In *C. pecosensis*, male reproductive success is positively correlated with the quality of oviposition substrate in his territory as well as the expression of his breeding coloration (Kodric-Brown 1977, 1983; Kodric-Brown and Mazzolini 1992). Territorial males develop breeding coloration, which intensifies with spawning and/or agonistic interactions, particularly fights and displays (Kodric-Brown 1983, 1996). The two species differ primarily in adult size and shape of body; *C. pecosensis* are smaller and not as deep-bodied as *C. variegatus* (Echelle and Echelle 1978). Males also differ in breeding coloration. *C. pecosensis* males develop a dark blue coloration, which is uniformly distributed over their entire body; pectoral and pelvic fins vary in color from yellow to colorless. *C. variegatus* males are a pale greenish-blue dorsally with an intense blue stripe on the nape, have a red belly and red fins that are outlined with an outer black stripe, and a distinctive black caudal tail stripe.

Methods

All experiments were conducted outdoors on the campus of the University of New Mexico during the summers (17 May–2 August) of 1998–2001, in circular fiberglass tanks filled with water of 10‰ salinity. Observations of male behavior were made between 1000 and 1600 hours. Source populations of *C. pecosensis* stocks were from Bitter Lake National Wildlife Refuge, Chavez County, N.M., USA (BLNWR), Mirror Lake and Figure Eight Lake in Bottomless Lakes State Park, Chavez County, N.M., USA. The University of New Mexico (UNM) population consisted of offspring of reciprocal crosses between BLNWR and Figure Eight Lake fish and was produced by placing males from one *C. pecosensis* population in a stock tank with females of another population. Thus offspring of these crosses were outcrossed *C. pecosensis*. Two stock tanks of these fish were produced each year. Females were from the same source populations. Source populations of *C. variegatus* males used in these experiments were from Lake Balmorhea, Reeves County, Tex., USA (the presumed source population of colonists introduced into the Pecos River), the laboratory of Dr. P. Klerks (Louisiana State University, LaFayette, La., USA), and the first generation offspring of fish from these two populations raised in our laboratory. Both populations of *C. variegatus* represent the Gulf form of this species, which is genetically distinct from the form inhabiting the Atlantic coast of North America (B. Turner, Virginia Polytechnic, unpublished data). F1 hybrids were produced in the laboratory from reciprocal crosses of *C. variegatus* and *C. pecosensis*. A pair was kept in a 37.85-l aquarium for 24–48 h, allowed to breed, then removed to a stock tank. The resulting young were reared to maturity and used in subsequent experiments.

All fish were housed in indoor aquaria in same-sex groups at similar densities for at least 2 weeks prior to being tested.

Individual males and females were used only once. Males were taken from different aquaria to eliminate the effects of familiarity. In each trial we measured the standard length (SL: tip of snout to base of caudal peduncle) of both males and females. Males were matched for body mass, which was estimated from regressions of standard length on mass (weight) for each species and each sex (Rosenfield, unpublished data). During trials, males were identified by body shape and breeding coloration.

Reproductive success of each male was determined by recording the number of spawning sequences with females. Each spawning sequence includes a single or multiple spawning act with a single female. Pairs engage in a distinctive spawning sequence, which consists of the male wrapping his anal fin around the female's venter and the pair performing a synchronized jerking motion, which signals the release of sperm and a single egg (Barlow 1961). The pair may either separate, or move a short distance over the substrate, still in tandem, while the female continues to oviposit.

The following male agonistic behaviors were recorded: chases of opponents and fights. Fights are interactions between two males, which consist of swimming in tight circles with the head of one male less than 1 cm from the tail of the other one, and occasionally, biting.

We conducted two types of experiments. The two experiments differed in duration, number of competitors, population density, and operational sex ratio, to reflect differences in the level of competitiveness. In the dominance experiment we determined the short-term relative competitiveness and mating success of *C. pecosensis* males from different populations in dyadic contests with *C. variegatus* or F1 hybrids under conditions of lower density (6.30/m²) and a sex ratio with a stronger female bias (1 male: 3 females). In the territoriality experiment we compared long-term competitiveness, measured as territory establishment, and mating success of *C. pecosensis* males from different populations when competing with *C. variegatus* males for 1 week. Here males competed for territories and matings with three other males (see below), at a higher density (8.85/m²), and a less female-biased sex ratio (1 male:1.5 females).

Dominance

Dyadic aggressive interactions between two males, one *C. pecosensis* and the other either *C. variegatus* or F1 hybrid, and spawning activities with six females (three of each species) were recorded during three 15-min observation periods throughout a day. In the morning, competing males were simultaneously placed in the tank with the females, allowed to acclimate for 15 min, then observed for 15 min between 1000 and 1700 hours. All trials were conducted in large outdoor tanks (1.8 m in diameter) divided in half with an opaque fiberglass partition. A piece of travertine rock, which served as a spawning substrate for females and a focal point of a male territory, was placed in the center of each compartment. Independent trials were conducted simultaneously in each compartment of the tank. Source populations for *C. pecosensis* males were from BLNWR ($n=6$ trials), Figure Eight Lake ($n=8$ trials), Mirror Lake ($n=20$ trials), and the first generation offspring of fish from these populations raised in our laboratory (UNM stock, $n=29$). For each male we recorded agonistic behavior (chases and fights), as well as spawning activity.

Territoriality

Aggressive interactions, establishment of territories, and mating success were recorded for four males, two *C. pecosensis* and two *C. variegatus*, and six females, either *C. pecosensis* ($n=19$ trials) or *C. variegatus* ($n=18$ trials). Trials with F1 hybrid males were not included because few such trials were conducted with males from the Mirror Lake population. Each trial was run for 1 week to evaluate the stability of territories. Trials were conducted in tanks (1.2 m in diameter) containing four clay flower pot trays (17.5 cm

in diameter) filled with travertine chips of approximately 1 cm². These trays were placed equidistantly to each other and served both as focal points of territories and as spawning substrates for females. Source populations for *C. pecosensis* males were Figure Eight Lake ($n=14$ trials), Mirror Lake ($n=11$ trials), and UNM stock ($n=12$ trials). BLNWR stock was not used in this experiment because limited stocks would have permitted only very small sample sizes. Male behavior and spawning activity was recorded during two 30-min observation periods, the first usually 24–48 h after the fish were introduced into the tank. By this time males had established well defined territories. The second observation period was taken 4 days after the beginning of the trial to note any changes in the arrangement of territories and their ownership. We recorded agonistic behavior among males (fights and chases), as well as the location and size of each territory. Territory boundaries were determined from visual observations. Besides the four trays that were used as focal sites of territories, other landmarks, such as markings on the sides of the tanks, were used to accurately record the position and the boundaries of each territory. Territory sizes were calculated by measuring the longest and shortest dimension of the area a male patrolled during the two 30-min observation periods. If a portion of a male's territory overlapped with that of another male, the area of overlap was divided equally between the two males. The proportion of substrate defended by *C. pecosensis* males in each trial included both the territory size of each male and the number of males (one or two) defending territories. The intensity of breeding coloration was recorded for each male at the end of each observation period using the classification scheme developed by Kodric-Brown and Mazzolini (1992).

Statistics

We performed two types of analyses. We compared the aggressive behavior (fights and chases), spawning success, and the size of territories across *C. pecosensis* populations to determine if populations differed in these behaviors. We also compared the relative rates of spawning and the relative area of substrate defended by *C. pecosensis* males across populations, to determine if these intraspecific behavioral differences affected the competitive outcome between species. Analyses of aggressive behavior and spawning success were performed for individual males in the dominance experiments, but were based on the mean values for the two observation periods for each species in the territoriality experiments, since we could not readily distinguish among individuals. In the dominance experiments we did not find significant differences between *C. pecosensis* populations when males competed against *C. variegatus* or F1 hybrids, so we combined these trials (two-way ANOVA, effect of competitor $F=1.643$, $df=1,55$, $P=0.205$). Data on male aggressive behavior (chases and fights), male spawning success, and territory size were square-root transformed to meet the assumptions of parametric statistics, and analyzed with parametric tests (one-way ANOVAs with Fisher's post hoc comparison LSD test). Two-tailed, paired t -tests were used to determine if male types differed in aggressiveness and spawning success in each population. An arcsin square root transformation was used on proportion of spawnings and proportion of area defended by *C. pecosensis* males of each population. The color scores for male nuptial coloration of the three *C. pecosensis* populations in the territoriality experiment were analyzed with nonparametric tests (Kruskal-Wallis one-way ANOVA based on ranks).

Results

Dominance

C. pecosensis males from different populations differed in their aggressive behavior (Fig. 1A; $F=4.023$, $df=3,55$, $P=0.012$), with Mirror Lake males engaging in more

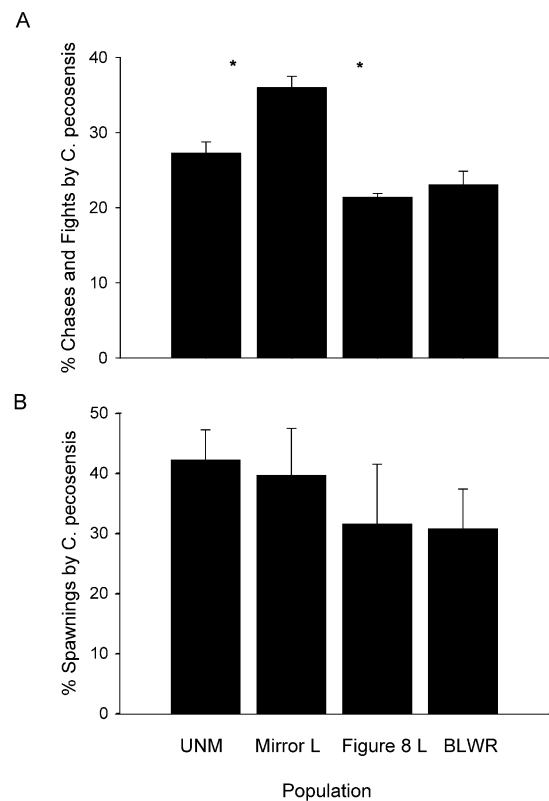


Fig. 1 **A** Percent chases and fights (mean±SE) of *Cyprinodon pecosensis* males from different populations competing against *C. variegatus* and F1 hybrid males in dominance trials. Males from the Mirror Lake population performed more aggressive behaviors than males from other populations ($P<0.05$). **B** Percent spawnings (mean±SE) obtained by *C. pecosensis* males from different populations competing against *C. variegatus* and F1 hybrid males in dominance trials. Percent spawnings by *C. pecosensis* males did not differ among populations

chases and fights than males from the other three populations (Fisher's LSD test: Mirror vs UNM: $P=0.004$; Mirror vs Figure Eight: $P=0.007$; Mirror vs BLNWR: $P=0.055$). No other significant interpopulation differences were detected (Fisher's LSD test: $P>0.47$ in all remaining comparisons). There were no differences in the proportion of spawnings by *C. pecosensis* males among populations (Fig. 1B; $F=0.497$, $df=3,55$, $P=0.686$).

Territoriality

Male spawning success was not affected by the species of females used in the territoriality experiments. *C. pecosensis* males obtained a similar proportion of spawnings in trials with *C. pecosensis* females (43.28 ± 5.38 SE) as they did in trials with *C. variegatus* females (41.49 ± 5.55 SE; $t=1.445$, $df=36$, $P=0.161$), therefore we combined the data.

Populations differed in their agonistic behavior (Fig. 2; $F=4.547$, $df=2,43$, $P=0.016$). Males from the Mirror Lake population engaged in more chases and fights with

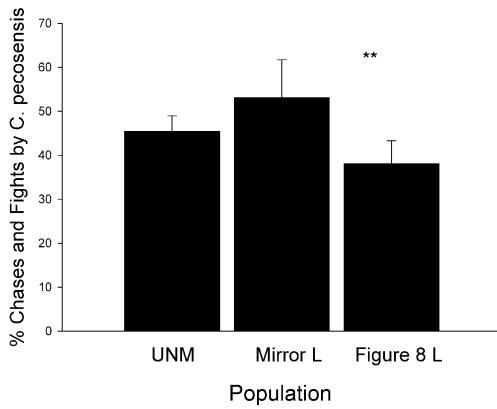


Fig. 2 Percent chases and fights (mean \pm SE) of *C. pecosensis* males from different populations competing against *C. variegatus* males in territoriality trials. Males from Mirror Lake were more aggressive than those from Figure Eight Lake ($P<0.001$). Other comparisons between populations were not statistically different

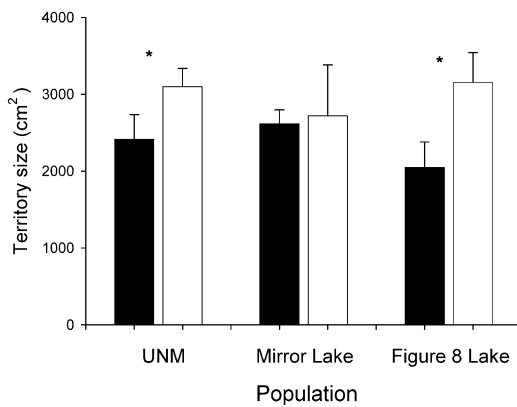


Fig. 3 Sizes of territories of *C. pecosensis* males (■) from Mirror Lake were similar to those of *C. variegatus* males (□); in the other two populations territories were much smaller than those of *C. variegatus* males ($P<0.05$)

C. variegatus males than those from Figure Eight Lake (Fisher's LSD test: $P=0.0004$). Other comparisons of competitive abilities between populations were not significantly different (Mirror Lake vs UNM: $P=0.163$, UNM vs Figure Eight Lake: $P=0.142$). A comparison of territory sizes of *C. pecosensis* males from the three populations with those of their competitors showed that males from Mirror Lake defended territories of similar size to those of *C. variegatus* males (Fig. 3; $t=0.134$, $df=8$, $P=0.896$). Territories of males from Figure Eight Lake and UNM were smaller than those of their competitors (Figure Eight Lake: $t=-2.441$, $df=10$, $P=0.034$, UNM: $t=-1.812$, $df=11$, $P=0.05$). A comparison of the proportion of area defended by *C. pecosensis* males from the three populations showed differences among populations ($F=4.722$, $df=2,29$, $P=0.039$). Males from Mirror Lake defended proportionally more area than those from Figure Eight Lake (Fisher's LSD test: $P=0.048$). The other two comparisons (Mirror vs UNM: $P=0.233$, UNM vs Figure

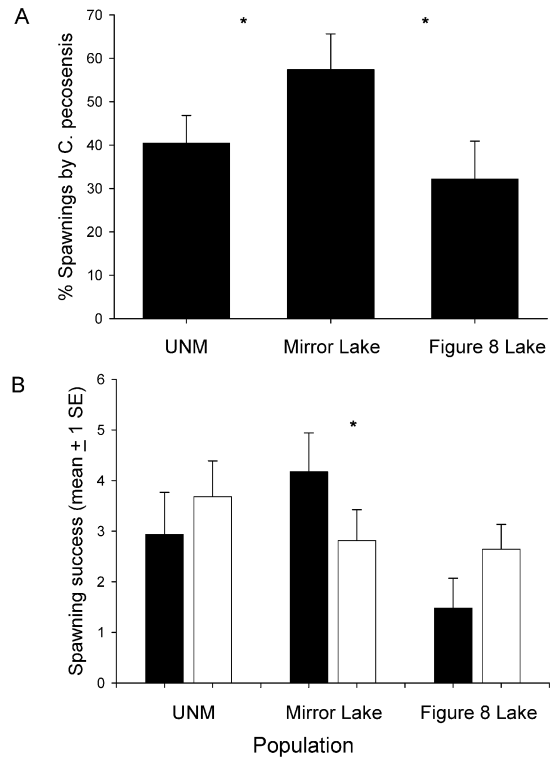


Fig. 4 **A** Percent spawnings obtained by *C. pecosensis* males from different populations when competing against *C. variegatus* males in territoriality trials. Males from Mirror Lake obtained proportionally more spawnings than those from other populations ($P=0.045$). Other comparisons between populations were not statistically different. **B** Spawning success (mean \pm 1SE) of *C. pecosensis* males (■) from different populations when competing against *C. variegatus* males (□) in territoriality trials. Mirror Lake males spawned more often than *C. variegatus* males ($P=0.05$). Spawning success of *C. pecosensis* males from Figure Eight Lake and the UNM population did not differ from that of *C. variegatus* males

Eight Lake: $P=0.369$) were not significantly different. Proportion of available area defended incorporates both territory size and the number of males defending territories.

Males from Mirror Lake obtained a significantly higher proportion of spawnings than males from Figure Eight Lake or those from the UNM stock when competing for spawnings with *C. variegatus* males (Fig. 4A; $F=3.34$, $df=2,40$; $P=0.045$, Fisher's LSD test: Mirror vs UNM: $P=0.013$, Mirror vs Figure Eight: $P=0.011$), but there were no differences between UNM and Figure Eight Lake males ($P=0.967$). Populations also differed in the mean number of spawnings compared with those of *C. variegatus* males (Fig. 4B). Males from Mirror Lake obtained a greater number of spawnings than *C. variegatus* males ($t=0.156$, $df=10$, $P=0.051$). Spawning success of males from Figure Eight Lake and the UNM stock did not differ from that of their competitors (Figure Eight Lake: $t=-1.188$, $df=13$, $P=0.262$; UNM: $t=-1.097$, $df=11$, $P=0.296$).

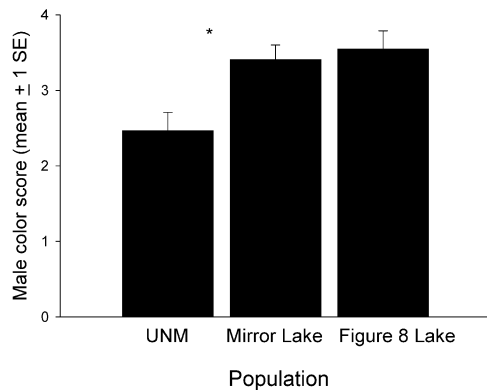


Fig. 5 Color scores (mean±SE) of *C. pecosensis* males (■) from different populations competing with *C. variegatus* males (□) in territoriality trials. The breeding coloration varies from score 1 (least) to score 4 (most). Males from Mirror Lake and Figure Eight Lake had similar color scores. Color scores of the UNM population were lower than those of males from Mirror Lake and Figure Eight Lake ($P<0.05$)

Males from the three populations differed in the expression of their breeding coloration (Fig. 5; Kruskal-Wallis ANOVA, $H=7.240$, $df=2,27$, $P=0.027$). Males from Mirror Lake and Figure Eight Lake had similar color scores (Fisher's LSD test: $P=0.536$), but they were higher than those of the UNM population (Fisher's LSD test: UNM vs Mirror Lake: $P=0.045$; UNM vs Figure Eight Lake: $P=0.012$). Color scores of *C. variegatus* males did not differ with respect to the origin of the Pecos pupfish populations with which they competed (mean= $2.47\pm 0.26SE$; Kruskal-Wallis ANOVA by ranks: $H=0.309$, $df=2,27$, $P=0.857$).

Discussion

The competitive ability of *C. pecosensis* males differed between populations. The results show that differences among *C. pecosensis* populations were similar in both short-term dyadic interactions (dominance) and longer-term interactions involving several males during the establishment of territories (territoriality). In the territoriality experiments, males from the Mirror Lake population were more aggressive, and defended larger territories when competing against *C. variegatus* males than males from Figure Eight Lake. Males from the UNM stock, a genetically mixed and outbred population, were more similar in their agonistic behavior to males from Figure Eight Lake than to those from Mirror Lake.

Mirror Lake males had a higher spawning success than males of the other two populations, but only in the longer-term experiments where the males established territories and interacted with several competitors (Fig. 4). Interestingly, the greater competitive ability of Mirror Lake males in the dyadic dominance experiments did not translate into higher reproductive success, since the proportion of spawnings did not differ between males

from the four populations (Fig. 1B). Experimental studies by Kodric-Brown (1988) and Rosenfield and Kodric-Brown (2003) have shown that population density, rather than sex-ratio variation, affected male reproductive success.

The two experimental protocols combined, controlled for factors such as differences in population density and operational sex ratio that affect the intensity of male–male competition for territories and the quality of oviposition substrates that in turn affect female choice of males (Kodric-Brown 1983). The experiments were designed to incorporate many factors shown to be important in the pupfish mating system under natural conditions. For example, in the territoriality experiment, adults could breed for 1 week, since at the peak of the breeding season territorial tenure for males of both species is on the order of 5–10 days (Kodric-Brown 1977; Itzkowitz 1981).

Although the design of our experiments did not allow us to investigate the relative importance of intra- and intersexual selection in the observed mating patterns, both processes contribute to the observed interpopulation differences in competitive ability and reproductive success of *C. pecosensis* males. Since females of both pupfish species mate with males defending territories, male–male competitive interactions to a large extent determine male reproductive success. In this study territory quality was associated with territory size, namely the number of trays a male was defending. Spawning success is positively correlated with territory size in both *C. pecosensis* and *C. variegatus* (Rosenfield and Kodric-Brown 2003), so similar territory sizes of Mirror Lake males competing against *C. variegatus* males might be thought to imply similar spawning success. This was not the case, however, because Mirror Lake males had significantly higher spawning success than *C. variegatus* males (Fig. 4). Territory size alone cannot explain this pattern.

Female choice may also have contributed to the observed pattern of generally higher mating success of males from the Mirror Lake population. In previous field studies and controlled experiments females discriminated among territory owners and preferentially spawned with males with specific attributes, such as large body size (Itzkowitz 1977), intense breeding color, and high display rates that indicate physical vigor (Kodric-Brown 1983, 1996; Kodric-Brown and Nicoletto 1993). In the present study, female choice of males was not based solely on such male attributes as the expression of breeding coloration. *C. pecosensis* males from both Figure Eight Lake and Mirror Lake showed a similar expression in breeding coloration (Fig. 5) but Mirror Lake males secured a greater proportion of matings than Figure Eight Lake males. Territories of Figure Eight Lake males were much smaller than those of *C. variegatus* males, indicating that more intense breeding color by itself was not sufficient to compensate for smaller territories. Thus under these experimental conditions, the reproductive success of males depended primarily on the ability to acquire and defend a territory. Competitive interactions

among males for territories rather than female choice of particular male attributes accounted for the observed differences between *C. pecosensis* populations.

The spawning success of *C. pecosensis* and *C. variegatus* males was not affected by the species of females used in the territoriality experiments. Females mated with territorial males irrespective of whether these males were *C. pecosensis* or *C. variegatus*. The visual association preference of *C. pecosensis* females for heterospecific males observed in other experiments with somewhat different designs (Rosenfield and Kodric-Brown 2003) was not evident in these territoriality trials. Our results imply that species-specific mate recognition and assortative mating are not sufficiently well developed to prevent extensive hybridization between *C. variegatus* and any populations of *C. pecosensis*.

The rate at which the two species hybridize should depend on the initial number of exotics and the opportunities for reproduction, which, to a large extent are determined by the ability to monopolize suitable breeding substrates. In competition with Mirror Lake males, where fewer *C. variegatus* are able to obtain territories, the majority of *C. pecosensis* females would likely mate with conspecific territorial males (as would some *C. variegatus* females). In competition with Figure Eight Lake males, where *C. variegatus* are competitively superior and able to defend large territories with several breeding sites, a much greater proportion of both *C. pecosensis* and *C. variegatus* females would likely mate with *C. variegatus* males. Thus the initial rate of hybridization is predicted to be slower for the Mirror Lake population than for other *C. pecosensis* populations.

Hybridization and subsequent introgression between *C. pecosensis* and *C. variegatus* has been extremely rapid in the Pecos River. Hybrids have not only replaced *C. pecosensis* throughout most of its historic range (Echelle and Connor 1989; Childs et al. 1996), but have also extended their range beyond that of the parental species (Echelle and Connor 1989; Echelle et al. 1997). The ecological conditions prevailing in the river when the two species first came into contact are not known. Echelle and Connor (1989) suggest that a relatively small number of *C. variegatus* were introduced at a time when the *C. pecosensis* population was at a low density. Such conditions would favor a breeding system based on a dominance hierarchy rather than territoriality (Soltz 1974). In such a case, the more aggressive *C. variegatus* males would have a competitive advantage, could control prime breeding sites, and obtain a disproportionate number of matings. Under these conditions hybridization would be extremely rapid. In the laboratory, hybrids are not only fully viable, but vigorous; they have a higher swimming endurance (an indicator of vigor) than *C. pecosensis* males (Rosenfield et al. 2004). However, under ecological conditions favoring higher population density, greater competition for breeding sites, and a territorial breeding system, other factors, such as the pursuit of alternative reproductive tactics, including satellite behavior by small males (primarily *C. pecosensis*), and interpopulation

variation in male competitiveness, may limit the rate of introgression.

Our experiments have shown an initial competitive and mating advantage of *C. variegatus* males, which must have been present during the initial contact of the two congeners in the Pecos River (Rosenfield and Kodric-Brown 2003). Rates of hybridization and introgression, however, presumably depend on local ecological conditions and behavioral attributes of *C. pecosensis* populations.

The results of this study indicate, however, that the pattern of rapid hybridization and introgression between the *C. pecosensis* and *C. variegatus* genomes in the Pecos River may not hold for all populations of *C. pecosensis*. In particular, large, stable populations, such as those in Mirror Lake, may be more resistant to hybridization. Mirror Lake is a large, deep lake that supports a dense, stable population of pupfish (Kodric-Brown 1977). During the summer months at the peak of the breeding season, breeding substrates are limiting and competition for them is intense. Thus this population is under strong sexual selection favoring aggressive, competitive males. Figure Eight Lake, which is located in the same geographic area of the Pecos River flood plain and is less than 500 m from Mirror Lake, consists of two small, shallow lakes separated by a narrow dike. Each part of the lake supports a small population of pupfish. These two populations are not only isolated, but also subjected to occasional population crashes, so that competition for breeding substrate is less intense. Thus interpopulation variation in male behavior, shaped by sexual selection, may be important in determining the extent and rate of hybridization between these two congeners.

Clearly, additional studies of *C. pecosensis* populations differing in size, stability, and density are needed to determine the relationship between population size, relative competitive ability, male reproductive success, and resistance (or susceptibility) to hybridization with *C. variegatus*. Unfortunately, there are few remaining populations of pure *C. pecosensis*, and most of these are in small springs with few individuals.

The results of this study underscore the importance of population-level differences in behavior when interpreting patterns of hybridization and introgression in native populations.

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