Asymmetries in premating isolating mechanisms in a sympatric species flock of pupfish (*Cyprinodon*)

Astrid Kodric-Brown and Rhiannon J.D. West

Department of Biology, University of New Mexico, 167 Castetter Hall, MSC03 2020, Albuquerque, NM 87131-0001, USA

We examine cues used in assortative mating in a sympatric species flock of pupfishes (*Cyprinodon*) from San Salvador Island, The Bahamas. The species are morphologically, genetically, and ecologically distinct. We focus on 2 species, a generalist detritivore and a predator/scale-eater. Experiments show that premating isolation is based primarily on visual cues and is asymmetrical. It is well developed in the less abundant scale-eater but is much less well developed in the more abundant detritivore. Responses of detritivore females from 3 lakes to conspecific and scale-eater males suggest that abundance of the predator also affects development of premating isolating mechanisms in the prey species. These results highlight the importance of frequency-dependent selection and predators in the evolution of premating isolating mechanisms.

Key words: **detritivore, frequency-dependent selection, olfactory cues, scale-eater, sympatric speciation, visual cues.**

Sympatric speciation is the divergence of 1 species into 2 or more lineages without geographic isolation (Coyne 2007). Recent studies have focused on behavioral and ecological mechanisms underlying this process of divergence. Disruptive selection to occupy distinct ecological niches can lead to assortative mating and evolutionary divergence, culminating in genetically distinct species. There is general agreement about the importance of processes, such as trophic differentiation to exploit different diets, with corresponding ecological, morphological, physiological, behavioral, and genetic divergence (Jiggins et al. 2001; Schliewen et al. 2001; Barluenga et al. 2006; Maan and Cummings 2008; Elmer et al. 2010 a,b; Rudh et al. 2011). The role of sexual selection in sympatric speciation is still debated because assortative mating by itself does not necessarily result in speciation (Arnegard and Kondrashov 2004; Leithen et al. 2012). However, sexual selection may facilitate differentiation if it leads to assortative mating based on traits such as diet, color patterns, body size, or habitat (McKaye 1986; Jiggins et al. 2001; Herder et al. 2008; Maan and Seehausen 2011; Weissing et al. 2011).

Although there are many studies on female mating preferences for male traits (Rosenthal and Evans 1998; Lehtonen et al. 2007; Kozak et al. 2008; Kahn et al. 2010; Kekalainen et al. 2010; Young et al. 2010; Mendelson and Williams 2011; Robinson et al. 2011), relatively few of them have addressed mate choice in sympatric

species (but see: McKaye 1986; Strecker and Kodric-Brown 1999, 2000; Jiggins et al. 2001; Rafferty and Boughman 2006; Maan and Cummings 2008; Seehausen et al. 2008; Elmer et al. 2009; Rudh et al. 2011). Especially relevant are traits that function in both ecological differentiation and mate choice (Gavrilets 2003; reviewed by Servedio et al. 2011). If females identify conspecific males based on traits associated with ecological characteristics (e.g., diet and habitat), then a genetic association between ecological and mating traits can potentially lead to rapid speciation (Ward et al. 2004; Reynolds and Fitzpatrick 2007; Rudh et al. 2011; Merrill et al. 2012).

In contrast to previous studies, we focused on 2 relatively unstudied processes, frequency-dependent selection and effects of predation by a closely related sister species. We quantified premating isolation and examined the sensory cues used to identify conspecifics in 2 sympatric species of pupfish (*Cyprinodon*) in 3 lakes in The Bahamas. We focused on the behavior of females, which choose mates and oviposit on lek-like territories. We compared premating isolation in the less abundant predator species (scale-eater), a trophic specialist, with that of the more abundant generalist prey species (detritivore) to determine whether the strength of premating isolation is frequency dependent and better developed in the trophic specialist than in the generalist. With high frequencies of conspecifics, probabilities of encountering individuals of another species are low, so selection for cues promoting assortative mating should be relaxed. However, if frequencies of conspecifics are low and encounter rates with heterospecifics are high, then selection for cues promoting assortative mating should be strong

Address correspondence to A. Kodric-Brown. E-mail: kodric@unm.edu.

(Strecker and Kodric-Brown 1999, 2000). This suggests that premating isolating mechanisms will be stronger in the rarer species in sympatric species flocks.

We were also able to examine the role of predators in the development of premating isolation in their prey, when both species are members of a sympatric species flock. A predatory sister species should exert strong divergent selection on the prey species because the prey should not only avoid it as a potential predator but also as a potential mate. Thus, predation should reinforce assortative mating in the prey species. Nothing is known about the effect of such predators in premating isolation because the sympatric species flocks studied to date do not include a predatory sister species (e.g., sticklebacks: Schluter 2000; cichlids: Seehausen 2006; but see pupfish: Humphries and Miller 1981).

The system

The pupfish species complex on San Salvador Island (24°0′N, 74°40′W) provides a unique opportunity to study the behavioral mechanisms of speciation, especially processes that drive the evolution of reproductive isolation. Because one of the species is a predator (scale-eater), it also provides an opportunity to examine the role of predation on the development of reproductive isolation in the prey (detritivore). This is the only case of scale-eating in *Cyprinodon*. Scale-eating is rare, and most species flocks of freshwater fish have at most a few scale-eating species (Fryer et al. 1955; Fryer and Iles 1972; Sazima 1983).

The species flock consists of 3 morphologically and genetically distinct and as yet undescribed species of pupfish (Cyprinodontidae:*Cyprinodon*) that occur sympatrically in some of the inland lakes on San Salvador Island, The Bahamas. The 3 species represent a monophyletic clade (Martin and Wainwright 2011). The likely ancestor is *Cyprinodon variegatus*, which is widely distributed and ranges from the Atlantic coast of North America throughout the Gulf of Mexico, the Caribbean, and The Bahamas. The 3 species are of recent origin because the inland lakes were dry as recently as 6000 years ago (Pacheco and Foradas 1986; Milliken et al. 2008). The lakes are hypersaline and have a depauperate ichthyofauna consisting of pupfish (*Cyprinodon* spp.), mosquito fish (*Gambusia hubbsi*), and silverside (*Atherinomorus stipes*) (Barton and Barton 2008). The 3 species of pupfish differ in diet and exhibit corresponding morphological specializations (Holtmeier 2001; Martin and Wainwright 2011). The most abundant species in all lakes is the detritivore, which feeds on detritus and algae. The scale-eater is a predator and consumes scales and fin parts of other pupfish, mostly the abundant detritivore. The molluscivore feeds on hard-shelled invertebrates. The scale-eater and the molluscivore are less abundant than the detritivore and occur only in a few lakes (Holtmeier 2001; Barton and Barton 2008).

Based on microsatellite variation, the 3 species are genetically distinct, and hybrids are rare in the field, suggesting that there is a high level of assortative mating (Turner et al. 2008). Postmating isolation is not complete, however, because viable hybrids are produced in the laboratory (Holtmeier 2001; Martin and Wainwright 2013). The detritivore and scale-eater differ in morphology, behavior, and male breeding coloration (Figure 1A,B). Breeding scale-eater males acquire a nearly black coloration on the body and fins. Breeding detritivore males display the typical color patterns seen in most pupfishes—blue iridescence on the nape and reddish orange fins and belly. However, as in most pupfishes, male breeding coloration is variable and can be turned on or off almost instantaneously (Kodric-Brown 1998; Barton and Barton 2008).

Males of both species establish territories, but they differ in location and types of substrates. Scale-eaters establish "leks," clusters of territories with well-defined boundaries on heterogeneous substrates, primarily rocky outcrops covered with algae and mangrove oysters, whereas detritivore males defend more dispersed territories with poorly defined boundaries over sandy or muddy substrate and vegetation. The differences in breeding habitat are only qualitative and partial, however, and females of both species have ready access to breeding territories of heterospecific and conspecific males. Male courtship of scale-eaters consists of a series of rapid darting movements toward the female, whereas detritivores show the typical courtship behavior of most pupfish
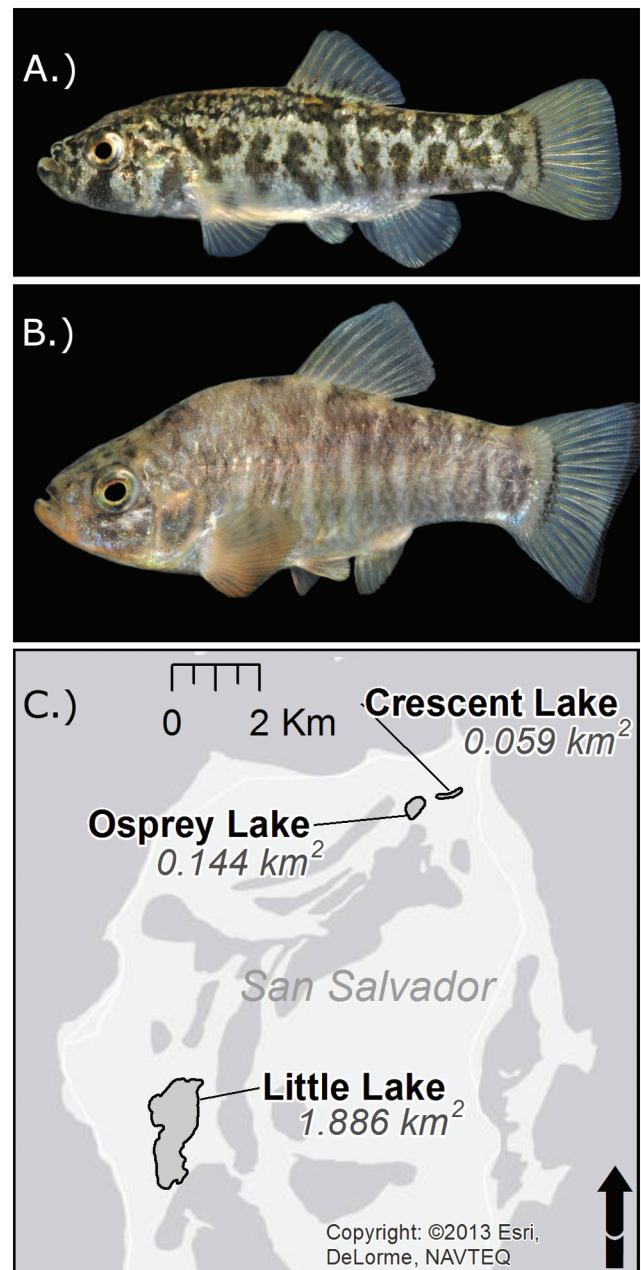

Figure 1

(A) Scale-eater male from Crescent Lake. (B) Detritivore male from Crescent Lake. (C) Map of the north end of San Salvador Island, The Bahamas, showing the location of Crescent Lake, Osprey Lake, and Little Lake.

species, circling below a female and guiding her toward the substrate of their territory. The third species, the molluscivore, is rarely collected and occurs in deeper water (Barton and Barton 2008). Little is known about their biology and interactions with the other 2 species.

MATERIALS AND METHODS

We determined the presence and strength of premating isolating mechanisms in wild-caught scale-eaters and detritivores from 3 lakes (Crescent, Osprey, and Little Lake; Figure 1C) by quantifying female preferences for conspecific males. The lakes are shallow, with a maximum depth of 4.5 m, hypersaline (more than 35 ppt), have similar habitats (including both rocky outcrops and sandy/muddy areas) but differ in size and relative abundance of scale-eaters (Holtmeier 2001; Barton and Barton 2008; Turner et al. 2008). In each lake, both species were collected with minnow traps baited with crushed mangrove oysters. Trapping efforts at each lake were standardized by sampling 10 different sites for 80 minnow trap days at each site. In order to adequately sample all habitats, we placed traps in open areas favored by the detritivores and on, or adjacent to, rocky outcrops where scale-eater males established their territories. Fish were placed in buckets and transported to the Gerace Research Center, and the water in the buckets was gradually diluted with sea water. After 24 h of acclimation, fish were transferred to concrete holding tanks filled with sea water. Species were kept in separate holding tanks, but sexes of each species were kept together. After completion of the trials, fish were returned to the locality where they were collected.

We examined the role of visual and olfactory cues using a binary choice design. We used 41 scale-eaters and 41 detritivores (25 females and 16 males of each species) from Little Lake (10–27 May 2011), 32 scale-eaters (22 females and 10 males) and 36 detritivores (22 females and 14 males) from Osprey Lake (27 May–6 June 2011), and 41 scale-eaters and 41 detritivores (25 females and 16 males of each species) from Crescent Lake (7–20 May 2012). All fish were adults, and males were in breeding coloration.

The size distribution of scale-eaters and detritivores was similar (scale-eater males: 22.6–34.6 mm, females: 23.8–33.9 mm; detritivore males: 23.7–33.7 mm, females: 23.5–36.1 mm). Each female was used once and tested for both visual and olfactory mate choice. However, some male scale-eaters from Osprey Lake were used more than once in novel combinations with different females.

Visual cues

A pool (91.5 cm in diameter) was filled to a depth of 10 cm with ~65.8 L of sea water. Two clear 7.6 L Ziploc® bags (33.02 × 39.68 cm) were suspended from the ceiling, filled with 4 L of sea water to the level of the water line in the pool, and positioned 10 cm from the walls of the pool. Males of each species were size matched within 1.62 mm for trials using scale-eating females and 2.34 mm for trials using detritivore females. Two male detritivores were placed into 1 bag and 2 male scale-eaters were placed into the other bag. The males remained in their respective bags for 24 h and were only removed once for feeding. Air stones were used to oxygenate the males' water except during the visual trials. A female was placed in the pool equidistantly from the 2 sets of males. The trial began when the female swam freely around the pool and visited both sets of males. Her behavior was recorded for 30 min with a pair of stopwatches. Female visual preference for males of each species was quantified as the time she spent actively touching

or swimming within 1 cm of the bags. We videotaped each set of males for 15 min to quantify time spent actively swimming, courtship displays, and agonistic behavior (chases and fights). Courtship displays consisted of a male orienting and facing the female, often touching the partition next to the female, swimming up and down or side to side with exaggerated movements of the pectoral fins, and closely following the female's movements. Fighting involved 2 males circling each other and occasionally biting the opponent.

Male behavior

We recorded the behavior of each set of males during visual trials with a Panasonic camcorder (HDC-TM80) placed on a tripod and a visual field that encompassed the container with the male and a 5 cm area around it. To determine whether males of each species differed in courtship of conspecific and heterospecific females, we divided the time males spent courting a female by the total time the female was actively investigating males of each species. We also analyzed the proportion of time each set of males was active (swimming, courting, and chasing).

Olfactory cues

Olfactory trials tested whether females of each species could recognize 1) the odors of conspecific males and 2) discriminate between the odors of conspecific and heterospecific males. Each part of the trial lasted 15 min. A 22 L aquarium (PETCO Bookshelf aquarium, 60 cm long × 20.65 cm wide) was filled with 20 L of sea water. Two lines were drawn on the front of the aquarium, delineating 3 "compartments." The 2 outer compartments were 17 cm wide and the middle one was 26 cm wide. A 1 L capacity (Tyco Healthcare "Kendall Kangaroo" IV) intravenous bag was suspended from the ceiling over each end of the aquarium. In the first part of the trial, one of the IV bags was filled with 200 mL of sea water (control) and the other with water in which 2 conspecific males were kept for 24 h during the visual trials (see above). We used these bags because we could accurately calibrate the drip rate of the "male stimulus" water and dispose of them after use. The IV bags were calibrated to deliver 1 drop per second (45 mL during each trial). A female was introduced into the central "compartment" of the tank. A trial began when the female started to swim and explored all parts of the aquarium. Females of each species were first presented with water from conspecific males and seawater. At the end of the trial, the female was moved into another tank, allowed to acclimate, and given a choice between water from conspecific males and water from heterospecific males. The position of the IV drip that delivered water from conspecific males was changed between the first and second part of the trial to control for any potential side bias.

Statistical analysis

Visual and Olfactory trials

To determine whether visual and olfactory preferences were correlated, we used the same females and the same males in both the visual and olfactory trials. Because the data on visual and olfactory preferences were normally distributed in all trials, no transformations were required. Results of the visual and olfactory trials were analyzed with Lawley-Hotelling MANOVAS to assess the effects of species, lake, and lake-by-species interaction on time females spent associating with conspecifics and heterospecific males. Significant effects were then examined with post hoc Tukey's HSD tests. To determine whether female preferences were consistent between the visual and olfactory trials, MANOVAs with female species, lakes, and a lake-by-species interaction were performed on the difference between time a

female spent assessing conspecific males in visual and olfactory trials. Post hoc Tukey's HSD tests were used to examine significant effects. Data on male activity during visual trials failed normality tests, even after transformation; a Kruskal–Wallis test was used to determine whether male activity differed between males, between species of females, or between lakes. Post hoc Bonferroni corrected ($\alpha = 0.002$) Mann–Whitney tests were used to examine pairwise comparisons. All data were analyzed with Minitab 16 software.

RESULTS

In all 3 lakes, scale-eaters were less abundant than detritivores. The ratio of scale-eaters to detritivores was 1:185 in Little Lake and 1:189 in Osprey Lake. Relative abundance of scale-eaters was an order of magnitude higher in Crescent Lake where the ratio of scale-eaters to detritivores was 1:19. To obtain sufficient scale-eaters for behavioral trials, additional collecting was done on or near rocky outcrops where scale-eater breeding territories were concentrated.

Visual trials

Visual preferences of all females differed between species ($F_{2136} = 4.857$, $P < 0.009$) and lakes ($F_{4272} = 11.977$, $P < 0.000$), and there was a significant interaction between species and lakes ($F_{4272} = 3.991$, $P < 0.004$). Scale-eater females from all 3 lakes spent a significantly greater proportion of each trial with conspecific males (Figure 2). Strength of visual preferences of scale-eater females for conspecific males differed between lakes. Scale-eater females from Little Lake and Crescent Lake spent significantly more time with conspecific males than those from Osprey Lake (Figure 2).

Visual preferences of detritivore females for conspecific males differed between lakes. Only detritivore females from the smallest lake, Crescent, spent significantly more time with conspecific males (Figure 2).

Male behavior

Males differed in their activity ($H_{11} = 37.18$, $P = 0.0001$). Males of both species spent most of their time courting, followed by swimming and chasing. Overall levels of activity (courting, chasing, and swimming) differed between lakes but not species (Table 1). Both scale-eater and detritivore males from Crescent Lake were more active than males from Little Lake ($P < 0.001$) and Osprey Lake ($P < 0.000$; Table 1). Scale-eater males spent more time courting scale-eater females than detritivore females ($P < 0.001$). Detritivore males divided their time equally courting detritivore and scale-eater females (Table 1).

Olfactory trials

A MANOVA on all females from the 3 lakes showed a significant effect of species-by-lake interaction ($F_{8262} = 3.02$, $P = 0.003$) but not of species ($F_{4131} = 0.68$, $P = 0.61$) or lake ($F_{8262} = 1.73$, $P = 0.09$). Scale-eater females did not discriminate between the odors of conspecific males, heterospecific males, or water (Figure 3). In Osprey Lake, detritivore females preferred the odor of conspecific males to heterospecific males (Figure 3).

DISCUSSION

Comparisons of breeding behaviors and mate choices in Mexican and San Salvador Island species flocks, the only 2 well-studied examples of sympatric speciation in pupfish, provide unique insights into the roles of ecological factors and sexual selection in the formation of isolating mechanisms. Both sympatric species flocks formed within the last 12 000 years (Covich and Stuiver 1974; Milliken et al. 2008) and occur in shallow saline lakes with few competitors (Humphries and Miller 1981; Turner et al. 2008). Both species flocks are monophyletic, descended from morphologically and ecologically similar sister species (San Salvador Island: *C. variegatus*, Mexico: *Cyprinodon artifrons*; Martin and Wainwright 2011), and both show genetic and trophic divergence among members of the species flock (Holtmeier 2001; Horstkotte and Strecker 2005; Strecker 2006). Comparisons of these 2 species flocks provide insights into 3 aspects of the formation of isolating mechanisms in sympatric populations.

Different signals

The 2 species flocks differ in the signals that are used in mate recognition and result in assortative mating. Species isolation in San Salvadoran pupfishes is based on both visual and olfactory cues.
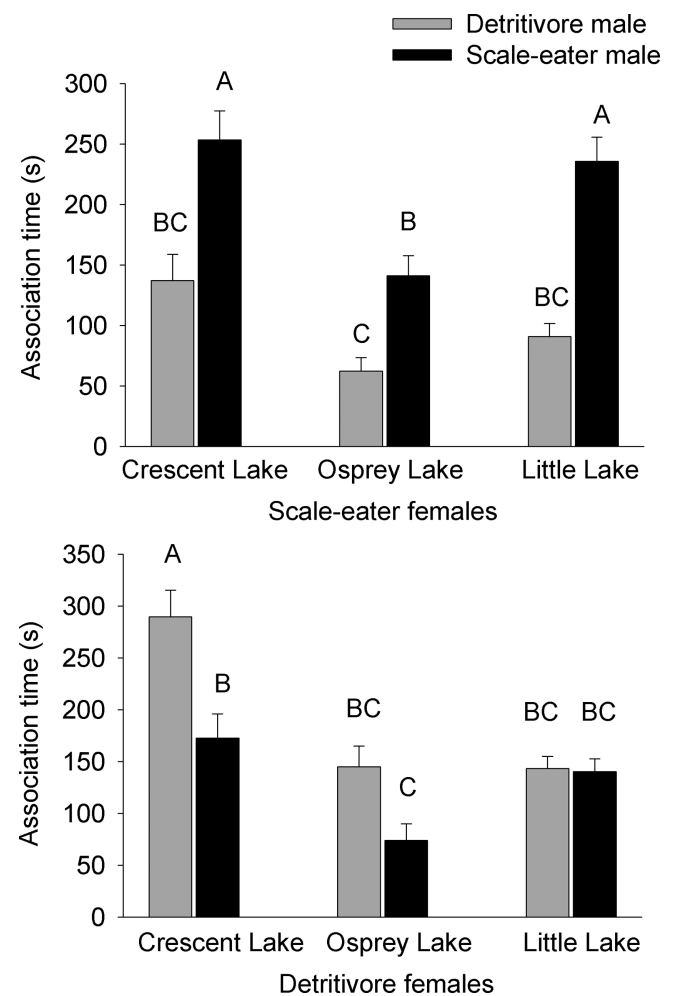

Figure 2

Association time (s) of scale-eater females and detritivore females from Crescent Lake, Osprey Lake, and Little Lake with conspecific and heterospecific males in visual trials. Pairwise comparisons that do not share a letter are significant at $P < 0.05$. Letters ABC refer to pairwise comparisons of female preferences for conspecific males within and between lakes; thus preferences of scale-eater females were similar in Crescent and Little Lake (A, $P > 0.05$) but differed from preferences of females in Osprey Lake (B, $P < 0.05$).

Table 1

Behavior of scale-eater and detritivore males toward conspecific and heterospecific females during visual trials in Little Lake, Osprey Lake, and Crescent Lake

Behavior	Comparisons	N	Median	U	P value
Court, chase, and swim	Scale-eater males vs.	393	0.048	15 0723	0.2922
	Detritivore males	390	0.064		
Court, chase, and swim	Both male species: Crescent Lake vs.	273	0.093	85 178	**0.0001**
	Little Lake	297	0.039		
Court, chase, and swim	Both male species: Crescent Lake vs.	273	0.093	73 852	**0.0000**
	Osprey Lake	213	0.021		
Court, chase, and swim	Both male species: Little Lake vs.	297	0.039	77 506	0.2416
	Osprey Lake	213	0.021		
Court	Scale-eater male/scale-eater female vs.	69	0.240	5268.5	**0.0010**
	Scale-eater male/detritivore female	62	0.082		
	Detritivore male/detritivore female vs.	62	0.168	4580.5	0.5569
	Detritivore male/scale-eater female	68	0.238		

Data were converted to proportion of time during a 15-min trial. Comparisons are for species between lakes and for species within lakes. Results are Bonferroni corrected Mann–Whitney U post hoc pairwise comparisons significant at $P < 0.002$. Significant differences are in bold.
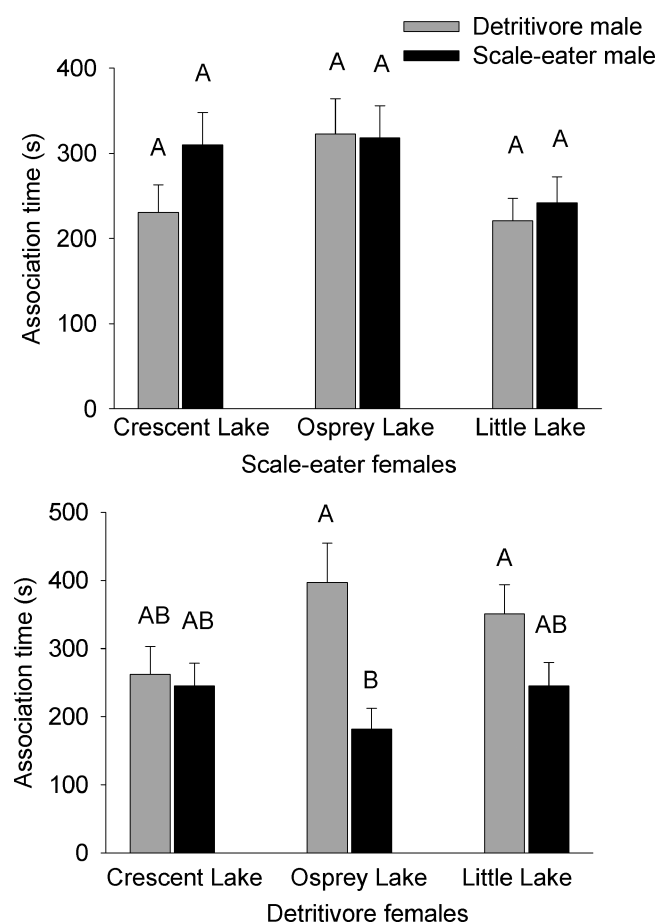

Figure 3

Association time (s) of scale-eater females and detritivore females from Crescent Lake, Osprey Lake, and Little Lake with conspecific and heterospecific males in olfactory trials. Pairwise comparisons that do not share a letter are significant at $P < 0.05$. Letters A and B refer to pairwise comparisons of female preferences for conspecific males within and between lakes.

Scale-eaters and detritivores differ in body shape, jaw morphology, and breeding coloration (Figure 1A,B; Martin and Wainwright 2011). In all 3 lakes, scale-eater females showed a strong preference for conspecific males based on visual but not olfactory cues.

In detritivores, both visual and olfactory cues were used to identify conspecifics: Visual cues in Crescent Lake and olfactory cues in Osprey Lake (Figures 2 and 3). Strength of preference for conspecific males also varied for detritivore females, from strong (Crescent and Osprey) to nonexistent (Little Lake). Thus, the 2 species differ not only in the development of premating isolating mechanisms but also in the sensory modalities used to identify conspecifics.

In 2 of the 3 species of Mexican pupfish (*Cyprinodon labiosus* and *Cyprinodon maya*) examined to date, premating isolation is based on olfactory cues that reflect differences in diet, rather than on visual cues (Strecker and Kodric-Brown 1999, 2000; Kodric-Brown and Strecker 2001). Thus, the only 2 cases of sympatric speciation in pupfish do not show parallel evolution, a familiar paradigm of many other species flocks (Schluter 2000; Langerhans and DeWitt 2004; Landry et al. 2007; Elmer et al. 2010a,b). Diversification in both systems is based on trophic divergence, but resource partitioning differs for the top predators. On San Salvador Island, the top predator is a scale-eater, similar in size to the other pupfish species, whereas in the Mexican species flock it is a specialist (*C. maya*), feeding on mollusks and other fish (Horstkotte and Strecker 2005) and is much larger than the other pupfishes. Superficially similar ecological conditions have resulted in sympatric formation of species flocks with different trophic niches.

Premating isolation is asymmetrical

Premating isolating mechanisms are well developed in the scale-eater, a trophic specialist, but reduced or absent in the detritivore. An experimental study of hybrids generated from crosses and backcrosses between detritivores and scale-eaters showed that hybridization resulted in reduced growth and lower survival of scale-eater phenotypes compared with the detritivore phenotypes (Martin and Wainwright 2013). The scale-eater species appear to have specialized coevolved traits adapted for their specialized trophic niche. There is strong selection for positive assortative mating in scale-eaters because their complex of traits is lost after hybridization.

Our results suggest that frequency-dependent processes also contribute to the development of premating isolation. Because of their specialized trophic niche, scale-eaters are less abundant than detritivores in all 3 lakes. Stronger preferences for conspecifics in scale-eaters than in detritivores suggest that selection for positive assortative mating is stronger in the less abundant scale-eaters than in the more abundant detritivores.

Comparisons with the Mexican species flock provide further insights into the importance of relative abundance and frequency-dependent selection in the development of premating isolation and reinforcement of assortative mating. A molecular phylogeny of the *Cyprinodon* clade suggests that frequency-dependent selection accentuates the rate of morphological diversification in the San Salvador Island and Mexican sympatric species flocks (Martin and Wainwright 2011, 2013). The highest rates of morphological diversification occurred in the 2 trophic specialists (scale-eater and *C. maya*) with small population sizes. Given selection for positive assortative mating is required to preserve the specialized traits of predators, such selection should be much stronger on the rarer species. The much more common detritivore species would encounter and mate with conspecifics frequently by chance alone, even in the absence of assortative mating. The rare trophically specialized species, however, would require cues to selectively find and mate with conspecifics and avoid hybridization. The evolution and maintenance of rare, trophically specialized species in sympatric species flocks appear to depend on the coevolution of strong conspecific mating preferences along with ecological traits related to feeding niches.

Predators facilitate premating isolation

Abundance of scale-eaters and the proximity of breeding areas of the 2 species in the 2 smaller lakes, Crescent and Osprey, may reinforce premating isolation based on visual and/or olfactory cues in detritivores, which appear to actively avoid scale-eaters. It is difficult to generalize from other studies on the effects of predators in reinforcing diversification in sympatric species (Rundle et al. 2003; Vamosi 2005; Nosil and Crespi 2006) because in the San Salvador pupfishes the predator (scale-eater) is a member of the species flock and is closely related to the prey (the detritivore). More than 40% of detritivores in our samples showed signs of scale removal by scale-eaters (personal observation). Thus, the impact of scale-eaters on their prey may be substantial and may select for recognition of conspecifics and assortative mating in detritivores in Crescent Lake and Osprey Lake.

The results of this study on the development of premating isolation in sympatric pupfishes support several assumptions of models of sympatric speciation. Strong reproductive isolation requires substantial phenotypic divergence and assortative mating (Bolnick and Kirkpatrick 2012); cues used in assortative mating are associated with ecological factors that reflect trophic specialization (Servedio et al. 2011). Under these conditions, premating isolation can be established quickly and leads to speciation. Our results also agree with findings of empirical studies of other sympatric closely related species, which show that conspecific mating is based on color patterns (e.g., cichlids: Seehausen et al. 2008; poison frogs: Maan and Cummings 2008) or chemical cues (e.g., sticklebacks: Ward et al. 2004; Rafferty and Boughman 2006). Other characteristics of this pupfish species flock seem not to have been addressed in theoretical or empirical studies of sympatric speciation. For example, our results highlight the importance of frequency dependence in the development of premating isolation and of asymmetries in the extent of premating isolation, which result from strong directional selection on trophic specialists and relaxed selection on generalists.

In summary, our results show that females use visual cues to identify conspecific males. Female preferences are asymmetrical and frequency dependent. They are well developed in the less abundant trophic specialists (scale-eaters) and absent or less well developed

in the more abundant generalists (detritivores). Higher abundance of predatory scale-eaters reinforces the development of premating isolation in the prey species. Thus, divergence among sympatric species can occur even when mating preferences are strong in one species and weak or absent in another. The take-home message of our study is that multiple processes can contribute to speciation in sympatry, and we have identified 2 of them: Frequency-dependent selection and predation.

REFERENCES

Arnegard ME, Kondrashov AS. 2004. Sympatric speciation by sexual selection alone is unlikely. Evolution. 58:222–237.

Barluenga M, Stoelting KN, Salzburger W, Muschick M, Meyer A. 2006. Sympatric speciation in Nicaraguan crater lake cichlid fish. Nature. 439:719–723.

Barton M, Barton C. 2008. Pupfishes of the Bahamas. J Am Killifish Assoc. 41:161–171.

Bolnick DI, Kirkpatrick M. 2012. The relationship between intraspecific assortative mating and reproductive isolation between divergent populations. Curr Zool. 58:482–492.

Covich A, Stuiver M. 1974. Changes in the oxygen 18 as a measure of long-term fluctuations in tropical lake levels and molluscan populations. Limnol Oceanogr. 19:682–691.

Coyne JA. 2007. Sympatric speciation. Curr Biol. 17:R787–R788.

Elmer KR, Kusche H, Lehtonen TK, Meyer A. 2010a. Local variation and parallel evolution: morphological and genetic diversity across a species complex of neotropical crater lake cichlid fishes. Philos Trans R Soc Lond B Biol Sci. 365:1763–1782.

Elmer KR, Lehtonen TK, Kautt AF, Harrod C, Meyer A. 2010b. Rapid sympatric ecological differentiation of crater lake cichlid fishes within historic times. BMC Biol. 8:60.

Elmer KR, Lehtonen TK, Meyer A. 2009. Color assortative mating contributes to sympatric divergence of neotropical cichlid fish. Evolution. 63:2750–2757.

Fryer G, Iles TD. 1972. The cichlid fishes of the great lakes of Africa: their biology and evolution. Edinburgh (UK): Oliver and Boyd.

Fryer GP, Greenwood H, Trevavas F. 1955. Scale-eating habits of African cichlid fishes. Nature. 175:1089–1090.

Gavrilets S. 2003. Perspective: models of speciation: what have we learned in 40 years? Evolution. 57:2197–2215.

Herder F, Pfaender J, Schliewen UK. 2008. Adaptive sympatric speciation of polychromatic "roundfin" sailfin silverside fish in Lake Matano (Sulawesi). Evolution. 62:2178–2195.

Holtmeier CL. 2001. Heterochrony, maternal effects, and phenotypic variation among sympatric pupfishes. Evolution. 55:330–338.

Horstkotte J, Strecker U. 2005. Trophic divergence in the phylogenetically young Cyprinodon species flock (Cyprinodontidae, Teleostei) from Laguna Chichancanab (Mexico). Biol J Linn Soc. 85:125–134.

Humphries JM, Miller RR. 1981. A remarkable species flock of pupfishes, genus Cyprinodon, from Yucatan, Mexico. Copeia. 1981:52–64.

Jiggins CD, Naisbit RE, Coe RL, Mallet J. 2001. Reproductive isolation caused by colour pattern mimicry. Nature. 411:302–305.

Kahn AT, Mautz B, Jennions MD. 2010. Females prefer to associate with males with longer intromittent organs in mosquitofish. Biol Lett. 6:55–58.

Kekalainen J, Valkama H, Huuskonen H, Taskinen J. 2010. Multiple sexual ornamentation signals male quality and predicts female preference in minnows. Ethology. 116:895–903.

Kodric-Brown A. 1998. Sexual dichromatism and temporary color changes in the reproduction of fishes. Amer Zool. 38:70–81.

Kodric-Brown A, Strecker U. 2001. Responses of Cyprinodon maya and C. labiosus females to visual and olfactory cues of conspecific and heterospecific males. Biol J Linn Soc. 74:541–548.

Kozak HI, Cirino LA, Ptacek MB. 2008. Female mating preferences for male morphological traits used in species and mate recognition in the Mexican sailfin mollies, *Poecilia velifera* and *Poecilia petenensis*. Behav Ecol. 19:463–474.

Landry L, Vincent WF, Bernatchez L. 2007. Parallel evolution of lake whitefish dwarf ecotypes in association with limnological features of their adaptive landscape. J Evol Biol. 20:971–984.

Langerhans RB, DeWitt TJ. 2004. Shared and unique features of evolutionary diversification. Biol. J Linn Soc.74:541–548.

Leithen K, Gonigle M, Mazzucco R, Otto SP, Dieckmann U. 2012. Sexual selection enables long-term coexistence despite ecological equivalence. Nature. 484: 506–509.

Lehtonen TK, Rintakoski S, Lindström K. 2007. Mate preference for multiple cues: interplay between male and nest size in the sand goby, *Pomatoschistus minutus*. Behav Ecol.18:696–700.

Maan ME, Cummings ME. 2008. Female preferences for aposematic signal components in a polymorphic poison frog. Evolution. 62:2334–2345.

Maan ME, Seehausen O. 2011. Ecology, sexual selection and speciation. Ecol Lett. 14:591–602.

Martin CH, Wainwright PC. 2011. Trophic novelty is linked to exceptional rates of morphological diversification in two adaptive radiations of Cyprinodon pupfish. Evolution. 65:2197–2212.

Martin CH, Wainwright PC. 2013. Multiple fitness peaks on the adaptive landscape drive adaptive radiation in the wild. Science. 339:208–211.

McKaye KR. 1986. Mate choice and size assortative pairing by the cichlid fishes of Lake Jiloa, Nicaragua. J Fish Biol. 29:135–150.

Mendelson TC, Williams TH. 2011. Female preference for male coloration may explain behavioural isolation in sympatric darters. Anim Behav. 82:683–689.

Merrill RM, Wallbank RWR, Bull V, Salazar PCA, Mallet J, Stevens M, Jiggins CD. 2012. Disruptive ecological selection on a mating cue. Proc R Soc B. 278:4907–4913.

Milliken KT, Anderson JB, Rodriguez AB. 2008. A new composite Holocene sea-level curve for the northern Gulf of Mexico. In: Anderson JB, Rodriguez AB, editors. Response of Gulf Coast Estuaries to sea-level rise and climate change. Boulder (CO): Geological Society of America (Special Paper 443). p. 1–11.

Minitab 16 Statistical Software 2010. [Computer software]. State College (PA): Minitab, Inc. (www.minitab.com).

Nosil P, Crespi BJ. 2006. Experimental evidence that predation promotes divergence in adaptive radiation. Proc Natl Acad Sci USA. 103:9090–9095.

Pacheco PJ, Foradas JG. 1986. Holocene environmental changes in the interior karst region of San Salvador, Bahamas; the Granny Lake pollen record. Proc Symp Geo Bah. 3:115–122.

Rafferty NE, Boughman JW. 2006. Olfactory mate recognition in a sympatric species pair of three-spined sticklebacks. Behav Ecol. 17:965–970.

Reynolds RG, Fitzpatrick BM. 2007. Assortative mating in poison-dart frogs based on an ecologically important trait. Evolution. 61:2253–2259.

Robinson DM, Tudor MS, Morris MR. 2011. Female preference and the evolution of an exaggerated male ornament: the shape of the preference function matters. Anim Behav. 81:1015–1021.

Rosenthal GG, Evans CS. 1998. Female preference for swords in *Xiphophorus helleri* reflects a bias for large apparent size. Proc Natl Acad Sci USA. 95:4431–4436.

Rudh A, Rogell B, Håstad O, Qvarnström A. 2011. Rapid population divergence linked with co-variation between coloration and sexual display in strawberry poison frogs. Evolution. 65:1271–1282.

Rundle HD, Vamosi SM, Schluter D. 2003. Experimental test of predation's effect on divergent selection during character displacement in sticklebacks. Proc Natl Acad Sci USA. 100:14943–14948.

Sazima I. 1983. Scale-eating in characoids and other fishes. Env Biol Fish. 9:87–101.

Schliewen U, Rassmann K, Markmann M, Markert J, Kocher T, Tautz D. 2001. Genetic and ecological divergence of a monophyletic cichlid species pair under fully sympatric conditions in Lake Ejagham, Cameroon. Mol Ecol. 10:1471–1488.

Schluter D. 2000. The ecology of adaptive radiation. Oxford (UK): Oxford University Press.

Seehausen O. 2006. African cichlid fish: a model system in adaptive radiation research. Proc Biol Sci. 273:1987–1998.

Seehausen O, Terai Y, Magalhaes IS, Carleton KL, Mrosso HD, Miyagi R, van der Sluijs I, Schneider MV, Maan ME, Tachida H, et al. 2008. Speciation through sensory drive in cichlid fish. Nature. 455:620–626.

Servedio MR, Van Doorn GS, Kopp M, Frame AM, Nosil P. 2011. Magic traits in speciation: 'magic' but not rare? Trends Ecol Evol. 26:389–397.

Strecker U. 2006. Genetic differentiation and reproductive isolation in a Cyprinodon fish species flock from Laguna Chichancanab, Mexico. Mol Phylogenet Evol. 39:865–872.

Strecker U, Kodric-Brown A. 1999. Mate recognition systems in a species flock of Mexican pupfish. J Evol Biol. 12:927–935.

Strecker U, Kodric-Brown A. 2000. Mating preferences in a species flock of Mexican pupfishes (*Cyprinodon*, Teleostei). Biol J Linn Soc. 71:677–687.

Turner BJ, Duvernell DD, Bunt TM, Barton MG. 2008. Reproductive isolation among endemic pupfishes (*Cyprinodon*) on San Salvador Island, Bahamas: microsatellite evidence. Biol J Linn Soc. 95:566–582.

Vamosi SM. 2005. On the role of enemies in divergence and diversification of prey: a review and synthesis. Can J Zool. 83:894–910.

Ward AJW, Hart PJB, Krause J. 2004. The effects of habitat- and diet-based cues on association preferences in three-spined sticklebacks. Behav Ecol. 15:925–929.

Weissing FJ, Edelaar P, van Doorn GS. 2011. Adaptive speciation theory: a conceptual review. Behav Ecol Sociobiol. 65:461–480.

Young MJ, Simmons LW, Evans JP. 2010. Pre- and post-mating sexual selection both favor large males in rainbowfish. Behav Ecol Sociobiol. 64:915–925.