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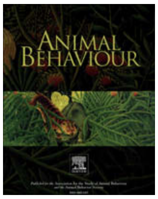

Guppy males distinguish between familiar and unfamiliar females of a distantly related species

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Because production of gametes is costly in many species, selection is expected to favour mate recognition behaviours that reduce the probability of wasted mating attempts. Male Trinidadian guppies, *Poecilia reticulata*, can learn to distinguish between conspecific females and between conspecific and congeneric females (*P. picta*) and make adaptive mating decisions on the basis of this information. In Mexico, however, introduced guppy males direct seemingly nonadaptive sexual behaviour to females of the distantly related *Skiffia bilineata* (Goodeidae). We asked whether prolonged exposure would lead to recognition and thus to a reduction of the level of male sexual behaviour experienced by *S. bilineata* females. Pairs of male guppies interacted freely with two female *P. reticulata*, two female *S. bilineata*, or one female of each species for 14 days. During trials, males encountered both familiar and unfamiliar females of the species they were trained with, and courtship towards these was quantified. We found that although male guppies habituated to familiar *S. bilineata* females, they persisted in their mating attempts towards unfamiliar ones. As with female guppies in previous studies, male guppies courted novel *S. bilineata* females and shunned those previously encountered. Our finding that guppy males recognize familiar *S. bilineata* females, but do not generalize this response, means that these endangered goodeids are vulnerable to persistent harassment.

Social experience with conspecific and heterospecific individuals can modify the sexual behaviour of some species (Galef & White 2000; Field & Waite 2004). It is now abundantly clear that fish can learn to make subtle distinctions between individuals, and that they modify their behaviour accordingly (Griffiths & Ward 2006). Learned discrimination of individuals typically arises as a result of repeated interactions, such as those that occur within fish shoals (Wootton 1991; Griffiths & Magurran 1997). The benefits of recognition include improved foraging efficiency (Laland & Williams 1997; Swaney et al. 2001), greater social cohesion (Chivers et al. 1995) and more adaptive mate choice (Kelley et al. 1999; Ward et al. 2007). Recognition that is based on previous experience, rather than on phenotype, is often termed familiarity recognition (Griffiths & Ward 2006). Two mechanisms have been invoked to explain this phenomenon. Familiarity recognition may arise when fish learn to identify a particular set of cues, such as odour (Ward et al. 2004, 2005). When this occurs, all individuals that share the trait or cue are treated in qualitatively the same way. Alternatively, recognition might depend on individual characteristics such as colour pattern. This type of individual recognition appears to underpin familiarity recognition among conspecifics in guppies, *Poecilia reticulata* (Griffiths & Magurran 1997, 1998). Although familiarity has been extensively documented in single species groups, fish can also become familiar with heterospecific individuals within mixed-species assemblages (Ward et al. 2003).

The observation that familiarity recognition is not limited to conspecifics suggests that it may play a role in learned species recognition. For example, a study where two sympatric species of three-spined sticklebacks (*Gasterosteus* spp.) were raised in social environments of variable species composition showed that fish adjust their shoaling preferences based on social experience and that this learning process may influence species recognition (Kozak & Boughman 2008). In a classic experiment, Haskins & Haskins (1949) found that male guppies exposed to conspecific and heterospecific females initially do not discriminate between *P. reticulata*, *P. picta* or *P. vivipara* females, but following repeated exposure direct significantly more gonopodial contacts to their own species. Magurran & Ramnarine (2004) confirmed that male guppies take approximately 6 days to distinguish heterospecific (*P. picta*) and

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conspecific females. Males in sympatric populations are an exception to the pattern since here species recognition is innate (Magurran & Ramnarine 2005). Learned recognition of *P. picta* females by male guppies appears to be linked to general cues, such as odour or appearance, rather than to individual recognition.

Male guppies are small, colourful poeciliid fish that are sexually very active; they persistently court and attempt copulations even though females are receptive for only a few days after parturition (Liley 1966). Sexual harassment is intense and can even result in males courting other conspecific males when females are absent (Field & Waite 2004). Male sexual behaviours are directed not only to congeneric females, as is the case with *P. picta*, but also towards heterospecific females of the nonsister family Goodeidae (Valero et al. 2008). *Poecilia reticulata* has been introduced to many tropical and subtropical countries, frequently with adverse consequences for native fish (Man & Hodgkiss 1981; Juliano et al. 1989; Allen 1991). Sexual harassment of heterospecific females of the family Goodeidae in Mexico could place an additional burden on endangered populations. Mating attempts outside the species boundary appear to be widespread in the animal kingdom (Wilson 1975; Mortenson & Follis 1997; Groning & Hochkirch 2008). However, mating outside the genus boundary is far less common, and can be costly. In one insect example sexual harassment by male *Margus obscurator* (Coreidae, Heteroptera) on female *Neacoryphus bicrucis* (Lygaeidae, Heteroptera) reduces female fecundity by 40–50% (McLain & Pratt 1999). In other species, the cost is due to physical stress as a result of large size differences between the participants. One graphic case is the prolonged attempts by a large male Antarctic fur seal, *Arctocephalus gazella*, to mount and thrust an adult king penguin, *Aptenodytes patagonicus* (de Bruyn et al. 2008) which nearly died of asphixiation.

Guppies are now found in multiple sites in Mexico (Gesundheit & Macías Garcia 2007), including some of the last few remaining localities of endangered Goodeinae species (De La Vega-Salazar et al. 2003). The Mexican Goodeinae (family Goodeidae, Order Cyprinodontiformes) is a clade of highly endangered (owing, among other factors, to landcover transformation, Mercado-Silva et al. 2002; Domínguez-Domínguez et al. 2006) livebearing fishes that resemble Poeciliids in their use of relatively shallow freshwater environments. As a result, male guppies will have many opportunities to attempt matings with goodeine females, and to learn to recognize them. Given the importance of social experience and learning in shaping the sexual behaviour of vertebrates (reviewed in Galef & White 2000; Woodson 2002) we predicted that male guppies would show familiarity recognition of heterospecific goodeine females.

A recent study found that male guppies continue to harass *Skiffia bilineata* females over a period of 3 days (Valero et al. 2008). However 3 days is insufficient for learned recognition of either heterospecifics (Magurran & Ramnarine 2004) or conspecifics (Griffiths & Magurran 1997). Here we tested the hypothesis that familiarity extends to heterospecifics from a nonsister family. We expected that, given sufficient familiarization time, the frequency of male sexual behaviours by male *P. reticulata* towards heterospecific *S. bilineata* females will decrease. Moreover, as with the learned discrimination of *P. picta* females, we predicted that this familiarity recognition will be based on species-specific (general) cues rather than individual ones.

METHODS

We used descendants of wild-caught individuals collected under licence from the secretaria de Medio Ambiente y Recursos Naturales (SEMARNAT) in October 2005. The geographical origin of the guppies is unknown; however, the fish used were of similar appearance as Trinidadian wild types. Fish had been kept in outdoor ponds at the Instituto de Ecologia, Universidad Nacional Autonoma de Mexico, and, until the beginning of the experiment, had no experience of seeing heterospecifics. We collected experimental fish from ponds with a seine net and took them to the laboratory, where they were housed in 40-litre tanks fitted with aeration and filters. The fish were kept under a 12:12 h photoperiod, at 24 °C, and were fed on flaked fish food twice daily. The tanks were illuminated with 20 W General Electric Daylight fluorescent tubes, which provided a peak of near-UV light below 400 nm. Acetate covers with 80% transmittance between 380 and 650 nm ensured that near-UV light was available during trials (but wavelengths below 360 nm would have been absent).

To increase male motivation to court or attempt copulation, we used pairs of male guppies as focal subjects. These male pairs were matched for size and randomly assigned to one of two treatments: the one-species or the two-species treatment. In the one-species treatment, males were housed with either two female *S. bilineata*, or two female *P. reticulata*, also matched for size (to avoid the potential effects of male bias for larger females; *S. bilineata*: familiar: standard length $= 27.3 \pm 1.5$ mm; unfamiliar: 27.7 ± 1.5 mm; $t_{35} = -0.38$, $P = 0.7$); in the two-species treatment, males were housed with one female *S. bilineata* and one female *P. reticulata*. Digital photographs of the fish pairs were used to measure standard length of all individuals with the aid of Image Tool shareware (http://ddsdx.uthscsa.edu/dig/itdesc.html; Table 1). First, fish were gently transferred to a small tank conditioned with a few drops of antistress solution (Pentabiocare, BIOMAA, Mexico city, Mexico). Second, a white plastic screen was introduced to use as a contrasting background and to manoeuvre the fish gently to the front of the tank to take the photograph. Fish were then returned to their assigned tanks. In the tanks fish could freely interact with each other, but had no visual contact with fish from nearby tanks. We replicated the one-species treatment 40 times (20 times for the *S. bilineata* condition, and 20 for the *P. reticulata* condition), and the two-species treatment 20 times.

The setup was maintained for 14 days to allow males in each treatment to habituate to the females (habituation phase). On the 15th, 16th and 17th day, we tested all males for familiarity acquisition by presenting them consecutively with familiar and unfamiliar females of the same species that they had seen during the habituation phase. On a test day we gently transferred a pair of males to an experimental tank in which a pair of females (familiar or unfamiliar to the males) had already been placed (Fig. 1). We began recording behaviour simultaneously for both males within 5 min or as soon as either male was displaying to a female or attempting to copulate. Sigmoid displays and sneaky matings directed to the females were tallied using recording sheets. After 10 min of behavioural recording we transferred the males to the nearby tank (Fig. 1), which already contained a pair of females (familiar or unfamiliar to the males, depending on the first session), and repeated the procedure. Familiar and unfamiliar females were presented to the males in a balanced order throughout the experiment. Males were individually identifiable and could be recognized with the aid of previously drawn sketches (Houde 1997).

Table 1
Mean standard length (mm) ± SD (*N*) of fish whose digital images allowed measuring standard length with precision

Treatment	Species	Females	Males
One species	*S. bilineata*	27.54±6.43 (37)	16.89±2.37 (36)
	P. reticulata	29.32±3.94 (39)	16.25±2.16 (38)
Two species	*S. bilineata*	24.37±5.89 (20)	15.81±1.78 (36)
	P. reticulata	23.73±6.08 (20)	

All males were *P. reticulata.*