Does sexual selection evolve following introduction to new environments?

Laura K. Easty [a,1,2], Amy K. Schwartz [b,*,1], Swanne P. Gordon [c,3], Andrew P. Hendry [a,2]

[a] Redpath Museum and Department of Biology, McGill University
[b] Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow
[c] Department of Biology, University of California, Riverside

The rate of evolution of mating preferences and mate signalling traits can influence local adaptation and diversification under environmental change. However, the rate of evolution of female preferences has not been directly examined in natural populations. An opportunity to do so arose through the introduction of high-predation Trinidadian guppies, Poecilia reticulata, from the Yarra River into high- and low-predation environments in the Damier River. Nine years (13–26 guppy generations) after the introduction, we tested whether female preferences for key aspects of male colour differed between the rivers and between introduced high- and low-predation populations. Based on two independent laboratory experiments, we conclude that little divergence in female preferences has apparently occurred between any of the populations, or between predation regimes. In combination with previous work, these results suggest that the evolution of guppy colour and female preferences are influenced by factors in addition to just predation, and that female preferences may generally take longer to evolve than other types of traits, particularly in populations that experience weak or fluctuating sexual selection gradients.
© 2011 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Many studies have documented adaptive phenotypic changes in natural populations experiencing environmental change, that is, 'contemporary evolution' occurring over approximately 100 generations or less (reviewed in Hendry & Kinnison 1999; Reznick & Ghalambor 2001; Stockwell et al. 2003; Svensson & Gosden 2007). Relatively few existing studies have examined traits relevant to sexual selection (e.g. male signals and female preferences), but it has nevertheless been suggested that these traits might evolve relatively slowly (Candolin & Heuschele 2008). However, it has also been argued that strong sexual selection has the potential to promote rapid evolutionary change (Lorch et al. 2003; Svensson & Gosden 2007; Hollis et al. 2009), although further uncertainty arises when the traits are also under natural selection (Tanaka 1996; Pfennig 2008). For example, female preference for male widowbirds, Euplectes progne, promotes longer tail feathers (Andersson 1982); however,

increased tail length increases mortality risk in flight during heavy rains (Savalli 1995).

There are many ways in which environmental change might cause the evolution of male sexual signals and female preferences. First, male signals might evolve owing to changes in sexual selection caused either by shifts in female preferences (Andersson 1994) or by changes in the signalling environments that render different signals more effective (e.g. water absorption properties, Seehausen et al. 1997; Boughman 2001; Candolin et al. 2007). Second, changes in natural selection can alter the costs of a given signal, such as fluctuations in predation risk (Endler 1980; Svensson & Friberg 2007) or in the resources necessary to express the signals (Lindström et al. 2009). Third, mating preferences might evolve because of changes in the value of different signals (Hegyi et al. 2006) or changes in the costs of expressing a given preference (Godin & Briggs 1996). Fourth, because preferences and secondary sexual traits should evolve in parallel (e.g. Houde & Endler 1990), changes in female preferences should cause reciprocal evolution in secondary male traits and vice versa.

Even with these many reasons to expect that sexual signals and preferences should evolve in response to environmental change (see also van der Sluijs et al. 2011), several factors might limit such responses. For example, female preferences might have low heritability, and although the exact genetic nature of female preferences

* Correspondence: A. K. Schwartz, Institute of Biodiversity, Animal Health and Comparative Medicine, Graham Kerr Building, University of Glasgow, Glasgow G12 8QQ, U.K.
E-mail address: amy.schwartz@glasgow.ac.uk (A. K. Schwartz).
[1] Authors contributed equally.
[2] L. K. Easty and A. P. Hendry are at the Redpath Museum, McGill University, 859 Sherbrooke Street West, Montreal, Quebec H3A 2K6, Canada.
[3] S. P. Gordon is at the Department of Biology, University of California Riverside, 2480 Life Science, Riverside, CA 92521, U.S.A.

is still generally unclear, if this were the case then it would limit the extent to which selection could produce an evolutionary response (Chenoweth & Blows 2006; Qvarnström et al. 2006; Hegyi et al. 2010). In addition, asymmetric mating isolation is common, in which females from two diverging populations share a preference for males from one population, despite experiencing different adaptive pressures (e.g. Zouros & Dentremont 1980; Endler & Houde 1995; Deering & Scriber 2002; Nickel & Civetta 2009). Such conserved preferences might evolve for reasons independent of environment, such as good genes benefits (Watt et al. 2001) or pre-existing sensory biases (Ryan & Rand 1993). Alternatively, preferences could be in the process of evolving, but, owing to fluctuations in the strength of selection, have yet to diverge enough to be detected as different (Cornwallis & Uller 2010).

The evolution of female preferences would ideally be measured by tracking independent replicate populations exposed to different environmental pressures. This has been done in laboratory studies, which have found that female preferences sometimes evolve and other times do not (Rice & Hostert 1993; Higgie et al. 2000; Rundle et al. 2005; Kwan & Rundle 2010). While laboratory studies tell us what might be possible in principle, they do not reflect the complexities of natural environments. We therefore performed an analogous study using introduced guppy, *Poecilia reticulata*, populations in Trinidad. Given that the same populations had previously shown only slight change in male coloration following introduction (Karim et al. 2007), we were also afforded an opportunity to examine the relationship between changing female preferences and their corresponding male traits.

Study System

Male coloration in guppies is frequently used as an example of the interaction between natural and sexual selection. That is, colourful males are thought to be at a reproductive advantage owing to female preference, but at a survival disadvantage owing to predation (Endler 1980; Kodric-Brown 1985; Houde 1987; Endler & Houde 1995; Brooks & Endler 2001a). The expectation is that populations subject to high predation should be less colourful than those subject to low predation. This hypothesis was supported through studies taking advantage of the fact that Trinidadian rivers can be crudely divided into (1) downstream high-predation environments, where guppies coexist with strong predatory fishes that prey on all size classes of guppies, and, separated by waterfalls, (2) upstream low-predation environments, where guppies coexist with only weak piscivores that are gape limited and can therefore prey only on small, immature size classes (Endler 1978; Reznick et al. 1996). Matching the above expectation, males in low-predation environments are often more colourful than males in high-predation environments (Endler 1980; Magurran & Ramnarine 2005; Millar et al. 2006). This inference was then strengthened through an experimental introduction when Endler (1980) introduced 200 high-predation fish from the Aripo River into a low-predation environment in the same river that had previously contained no guppies. Sampling 2 years later, he found that the high-predation males introduced into a low-predation environment had evolved more conspicuous coloration.

More recent findings on similar guppy translocations have not always matched those given above. One such example, and the basis of our experimental study, was in the Damier River. This river, located on the north slope of the Northern Range Mountains of Trinidad, was guppy free in the early 1990s. In 1996, D.N. Reznick introduced approximately 200 fish from a high-predation site on the Yarra River into a low-predation site above a barrier waterfall in the Damier (Fig. 1). Qualitative surveys a year later revealed that guppies had become established at the

low-predation site in the Damier and had also spread downstream over the barrier waterfall to colonize the high-predation site. In samples from 2004, however, little genetic change in male colour had taken place (Karim et al. 2007). This contrast between the strong colour change in Endler's (1980) experiment and the results in the Damier introduction was striking considering the methods were similar: the descendants of 200 introduced fish and their source populations assayed with approximate colour analysis methods (see Methods). Moreover, the elapsed time in Endler's (1980) experiment was shorter (2 years in the Aripo versus 9 years in the Damier), and so time constraints in the Damier were not a likely cause of the difference. Subsequent studies of other experimental introductions have also found limited evolution of these same colour pattern elements (Kemp et al. 2009; Weese et al. 2010), although more subtle changes (spectral properties of some spots) were detected (Kemp et al. 2008). These variable results in studies of male colour evolution motivated our investigation of the contemporary evolution of secondary sexual traits and female preferences.

A working hypothesis is that female preferences in low-predation environments do not consistently favour greater male colour. Although female guppies from low-predation environments frequently show stronger preferences for colourful males (Stoner & Breden 1988; Houde & Endler 1990; Endler & Houde 1995; Godin & Briggs 1996), geographical variation in female preferences is prevalent even within a given predation regime (e.g. Endler & Houde 1995; Brooks & Endler 2001a; Schwartz & Hendry 2007; see Appendix Table A1 for a summary). Perhaps the introduced Aripo females in Endler's (1980) experiment initially preferred more colourful males, whereas the introduced Damier females did not. Supporting this idea, previous work has found that Yarra females from the ancestral population do not prefer more colourful males (Schwartz & Hendry 2007; but see Houde & Hankes 1997), and so the evolution of colour in the Damier may first require the evolution of female preferences. Such evolution of female preferences and male
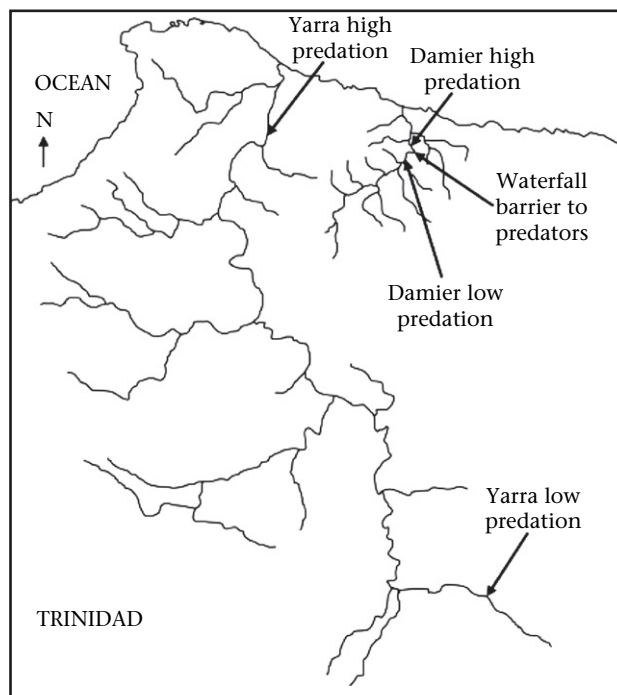

Figure 1. Map of the study sites in Trinidad. In 1996, 200 fish from the Yarra high-predation site were transplanted to the Damier low-predation site, from which the Damier high-predation site was then naturally colonized.

colour could occur owing to good genes benefits (Houde & Torio 1992), sensory biases (Rodd et al. 2002; Grether et al. 2005) or runaway coevolution (Houde & Endler 1990). Here we tested whether female preferences have evolved in the Damier, by examining population-level female preference functions in the ancestral Yarra high-predation population, the derived Damier high- and low-predation populations and a reference Yarra low-predation population (Fig. 1).

METHODS

Experimental Populations and Laboratory Rearing

We caught 15–20 pregnant females by hand nets from each of the four study populations (Fig. 1) in Trinidad and transported them to our laboratory where they were kept in population-specific tanks. Tanks were visually inspected daily for signs of pregnancy. When a female appeared ready to give birth, she was placed into a plastic pregnancy box, which floated in a 9.1-litre tank for a maximum of 24 h or until the fish demonstrated signs of stress. Because females will cannibalize their young in captivity, these boxes are essential to separate the offspring from their mothers upon birth. Once offspring were born, females were immediately returned to the population tank, and offspring were removed from the box and reared in their birth tank in family groups. Adult fish were fed Tetramin Pro flake food (Tetra) daily and offspring were fed live brine shrimp twice a day.

Offspring families were once again reared in their own aquaria until the sex of individuals could be determined. Two males from the middle of the size distribution in each brood were then isolated and placed into individual aquaria, with one male being fed a 'low-food' diet and the other a 'high-food' diet (following Reznick 1983). The low end of the diet was maintained within the natural range of energy requirements and diet levels by regularly weighing fish and adjusting food quantities appropriately. This diet manipulation was performed to control for the potential plastic influence of resource levels on the expression of male colour (e.g. Grether et al. 2001).

Offspring were regularly monitored for signs of sexual maturity and males were removed from the tank once the first indications of a gonopodium or a colour patch appeared. Each male was checked daily under a microscope to see if it was fully sexually mature, as indicated by a fully formed gonopodium (a curved hook at the distal tip of the third and fourth anal fin rays; Winemiller et al. 1990). Once the offspring matured, they were mated within each population in a randomized design that excluded brother–sister pairings, by placing an individual male and individual female in a 9.1-litre aquarium containing an airstone and java moss (which provides refuge for any offspring born prior to isolating the female in a pregnancy box). The resulting offspring of these crosses constituted the experimental fish.

Each of the resulting F2 broods was then isolated and reared in its own 22.7-litre aquarium, where offspring were monitored daily for developing males, which, based on the presence of gonopodia and the first indications of colour, were immediately removed, thus ensuring the females remained virgins. We used virgins for the experiments because they are more likely to be receptive to males during mating trials (Baerends et al. 1955; Liley 1966), which facilitates quantification of preferences (Houde 1997). The males were held in population-specific tanks with nonexperimental females from their own population, which ensured that the experimental males gained mating experience and exhibited normal courtship behaviour (Farr 1980; Price & Rodd 2006). Males were then individually isolated in the experimental aquarium for at least 1 h prior to the trials described below, to allow for acclimation and to avoid erroneous results in males that may have recently courted, as they generally cease normal sexual activity for 30 min or more following copulation (personal observation; Endler & Houde 1995).

Mate Choice Trials

We employed a 'no-choice' design that paired single males with single females to examine male–female interactions (e.g. Dugatkin 1992; Houde 1997; Nosil et al. 2002, 2003; Rutstein et al. 2007; Schwartz & Hendry 2007). Although a 'no-choice' design might not represent typical conditions in the wild, it does reveal intrinsic female preferences while eliminating potentially confounding intrasexual interactions (Dugatkin & Godin 1992; Houde 1997), and therefore seemed to be the most appropriate design.

The trials took place in 9.1-litre aquaria covered on three sides with black paper (to eliminate visual disturbances) and illuminated by an overhead full-spectrum fluorescent bulb (Vita-Lite 40 W, which approximates the colour spectrum of full sunlight; Duro-Test Canada, Toronto, ON, Canada). After a female was introduced into an aquarium with the male, a given trial lasted a minimum of 20 min. Trials continued for longer (up to 30 min) if the male continued to court the female. All trials were recorded with a video camera (Canon XL1-S) and then transferred to DVD for analysis.

We used two experimental designs that allowed complementary inferences. In the first ('home population') design, males and females were paired within their own populations (i.e. no cross-population mating trials were conducted), and each fish was used only once. Ten trials were performed for each population, generating a total of 40 trials that used a total of 40 males and 40 females. Females from each population thus interacted only with males from their own population, and so differences in preference functions between populations were potentially influenced by between-population variation in both female preferences and male traits/behaviours. We consider this design useful for understanding the nature of sexual selection operating within each population.

In the second experimental design ('standard-male population'), female responses from all four populations were compared when tested with the ancestral male population (Yarra high). Here, a single Yarra high-predation male was tested in random order with each of four females: one Yarra low-predation female, one Yarra high-predation female, one Damier low-predation female and one Damier high-predation female. This procedure was then repeated nine more times, each time with a new male and a new random sequence of new females from the four populations. The result was a total of 40 trials that used a total of 10 males and 40 females. This design allowed us to control for any individual variation in male traits or behaviour and, by comparing responses for each individual male, it further allowed an assessment of any potential evolution of preferences and relative male mating success, independent of the specific colour elements examined.

Quantifying Male Colour and Female Preference

After the trial(s) for a given male was completed, he was anaesthetized with MS-222 and photographed (Nikon Coolpix F995) on his left side on a standard, grid-ruled background. The pictures were then analysed digitally (Scion Image Software: www.scioncorp.com) to determine the size of the male (surface area) and the number and size (surface area) of spots of different colours on the body (excluding fins). Colour categories were black, orange, violet-blue and green. The total area of a given colour on a male depends on his size, and so we also calculated the relative area of each colour as the total area of a given colour divided by the total area of the male.

A female's response to a given male was estimated as the intensity of her responses to his displays. Guppy courtship usually involves a sigmoidal display, in which a male places himself in front of

a female, arches his body, extends his fins and performs several jerking vibrations (Baerends et al. 1955; Liley 1966; Houde 1997). Females may respond to a given male display in several ways: no response (score = 0), turning and orienting her body towards the male (1), gliding towards him (2), circling him (3), receiving an attempted copulation (4) or allowing full copulation (5) (Houde 1997). Following full copulation, a male's body will jerk over an interval of several minutes, and he will closely guard the female (Baerends et al. 1955; Pilastro et al. 2007). Using this scheme, we scored each response of each female to each male display over the course of each trial.

The scores for female responses were combined in a given trial to yield an overall estimate of a given female's preference for a given male (Houde 1997; Schwartz & Hendry 2007). This was done by first calculating the total (cumulative) response (TR) for a given trial as the sum of the female's responses to all of the male's displays. TR was then standardized as a fraction of the maximum total response (MR) that a female could have shown given the number of male displays in that trial. MR was calculated assuming an attempted copulation (score = 4) after each male display followed by full copulation on the last display (score = 5): i.e. MR = ((Number of displays−1) × 4) + 5. This standardization yielded a 'fractional intensity of response' (FIR = TR/MR) for a given female to a given male over the course of a trial. Note that FIR controls for variation between trials in the frequency of male displays. This FIR is very similar to Houde's (1987, 1997) Fractional Response (FR) metric, in which all scores of '2' and higher are summed and divided by the number of displays. The added benefit of using FIR is an increase in specific information about a female's willingness to mate rather than responding to a cue. Female preference functions were calculated for each population as a linear regression of FIR or FR on each male trait (e.g. Houde & Endler 1990; Endler & Houde 1995; Brooks & Endler 2001a; Gamble et al. 2003; Syriatowicz & Brooks 2004; Schwartz & Hendry 2007). We obtained similar results for FR and FIR (results not shown), and so we here only report the latter.

Statistical Analysis

Variation in male colour was evaluated by multivariate analysis of covariance (MANCOVA) including all four colour categories (orange, violet-blue, green and black) as response variables and 'River' (Yarra and Damier) and 'Predation' (high and low) as fixed factors. Body area was included as a covariate to control for possible allometric effects of body size on the relative area of colour.

Variation in female preference functions in the 'home population' trials was analysed in a similar manner as above where the response variable was FIR, male colours were included as covariates, and River and Predation were included as fixed factors. We also ran the same models for each colour trait individually and obtained similar results. A significant interaction term between colour and either Predation or River would indicate divergence in preference functions between female populations. The contemporary evolution of female preference would be indicated if preference functions differed between the Yarra high-predation (ancestral) population and the Damier (derived) populations. Natural selection would be inferred as a cause of this preference divergence if differences between predation regimes in the Damier were in the same direction as differences between predation regimes in the Yarra. Variation in FIR in the 'standard-male population' trials was determined with a linear mixed model with male identity as a random factor and female population as a fixed factor. All analyses were performed in R version 2.13 (The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org).

Ethical Note

All experiments, fish rearing and breeding conditions complied with institutional and government guidelines and were performed with the approval of the Canadian Council for Animal Care (CCAC) and McGill University's Animal Care Committee (Protocol no. 4570). Import and export permits of guppies were provided by the Department of Fisheries and Agriculture, Trinidad, W.I. Fish were transported both within and from Trinidad to McGill University (Montreal, Canada) in oxygenated 2-litre water bottles at low densities (maximum 10 fish per bottle). To keep ammonia and disease levels in the water low during transport, fish were not fed for 24 h prior to travel and two tablets of TankBuddies (Jungle Laboratories, http://www.junglelabs.com) were added to each bottle. There was minimal mortality during transport and no visible signs of stress upon arrival.

Adult fish were kept in 22.7-litre population-specific aquaria with 15–20 fish per tank. Each tank contained artificial plants, a gravel substrate bottom and a sponge filter. Water temperature was maintained between 22 and 24 °C under a 12:12 h light:dark cycle with timed full-spectrum fluorescent bulbs above aquaria (Duro-Test Canada). All fish were monitored twice a day for health. Upon signs of disease or stress (e.g. lack of feeding, clamped fins, vertical swimming) fish were immediately quarantined and medicated appropriately until recovery.

Behavioural observations and video recordings were conducted in a small observation room that was sheltered from disturbance. Individual fish were placed in tanks in the observation room 24 h in advance of recording to acclimate to any subtle temperature differences. For all mating trials, the experimenter was situated behind a black cloth with a small hole cut out for the camera lens. There were no signs of stress during mating trials. Following the trial, both males and females were returned to mixed-sex stock population tanks where they remained until their natural deaths. These tanks were maintained at either equal or female-biased sex ratios to minimize harassment of males to females and potential aggression and interference among males. Males were immediately anaesthetized following mating trials for photography. This process was generally completed within 1–2 min, and resulted in no mortality. Males were observed in a well-aerated recovery tank until they resumed normal swimming behaviour.

RESULTS

Male Colour

Predation environment affected only the relative area of orange, but not universally as indicated by the significant interaction with River (Table 1). Specifically, low-predation males in the Yarra were substantially more orange than high-predation males in the Yarra, whereas high- and low-predation males did not differ in the Damier (Fig. 2). Males from the Damier river generally had larger violet-blue spots than males in the Yarra (Fig. 2), although this trend did not approach significance (Table 1).

Female Preference Functions

There were no significant effects of Predation, River or their interaction in the home population trials (Table 2), indicating that female responsiveness (FIR) did not differ in general between the populations. Overall, there was no further evidence of divergence between populations in preferences for particular colour elements, but instead females from all populations discriminated against males with more violet-blue (indicated by the significant main effect of this trait, Table 2). Preferences for orange did appear to

Table 1
Variation in the relative area of male colour ($N = 20$ per population) between rivers (Yarra and Damier) and predation environments (high or low)

	df	River	Predation	River*Predation
Orange	1, 36	1.01 (0.32)	2.45 (0.12)	**9.29 (0.004)**
Black	1, 36	1.10 (0.30)	2.9×10^{-5} (0.97)	0.76 (0.39)
Green	1, 36	1.08 (0.31)	1.77 (0.19)	0.09 (0.77)
Violet-blue	1, 36	3.86 (0.06)	0.16 (0.69)	0.97 (0.33)
All colours	4, 32	0.91 (0.59)	0.85 (0.27)	0.75 (0.05)

Values are partial F statistics from the MANCOVA, or the Wilks's λ for the full model (last row). P values are in parentheses; significant effect is in bold.

differ in direction between the rivers, however (Fig. 3). Owing to the importance of orange in previous studies of guppy mate choice and colour divergence, we examined this trend further by isolating orange as the only colour covariate in the model. Indeed, the Orange*River interaction was the only significant effect ($F_{1,32} = 5.09$, $P = 0.03$), in which Yarra females discriminated against males that were more orange ($R^2 = 0.22$, $F_{1,19} = 5.17$, $P = 0.04$) and Damier females tended to prefer males that were more orange, although the slope was not significant ($R^2 = 0.15$, $F_{1,19} = 3.3$, $P = 0.09$).

There were no population differences evident in the mean response of females when all four populations were tested with ancestral (Yarra high-predation) males (ANCOVA: $F_{3,36} = 1.17$, $P = 0.33$), although Yarra high-predation females clearly showed the highest overall response to these males (Fig. 4).

DISCUSSION

We have examined to what extent contemporary evolution occurred following the experimental introduction of high-predation guppies into the Damier River. In a previous paper, (Karim et al. 2007) we showed that the introduced guppies have not experienced noteworthy evolution in the sizes and numbers of colour spots on males. This negative result appears in contradiction to the large changes in these same aspects of male colour that occurred in Endler's (1980) introduction in the Aripo River. The differences between these results led us to consider the potential role of female preferences in constraining the evolution of male colour, specifically that the evolution of increased male colour in the Damier would first require the evolution of increased female preferences for more colour. This last possibility was the motivation behind the present study.

We found hints that female preferences for some male colours might have begun to evolve in the Damier River. In particular, females from both populations appeared to show an increased preference for orange (Fig. 3); however, this trend was not significant when all male colours were considered together. In addition, no preference divergence was seen between predator regimes. These results suggest that the evolution of female preference for these particular aspects of colour, if indeed it is occurring in the Damier, is slow and is the result of selective factors other than predation.

Why has Preference Evolution been so Modest?

It is possible that noteworthy preference evolution has occurred in the Damier, but our experimental design and implementation were not sufficient to confirm it. Potential limitations included (1) the restricted range of phenotypes (particularly if they all surpassed a threshold for acceptance by females), (2) the relatively low sample size, and (3) the sequential mate choice design (particularly if female preferences are relative). Furthermore, preference evolution may have occurred in ways that we did not attempt to quantify. In particular, our questions were related to the direction of colour
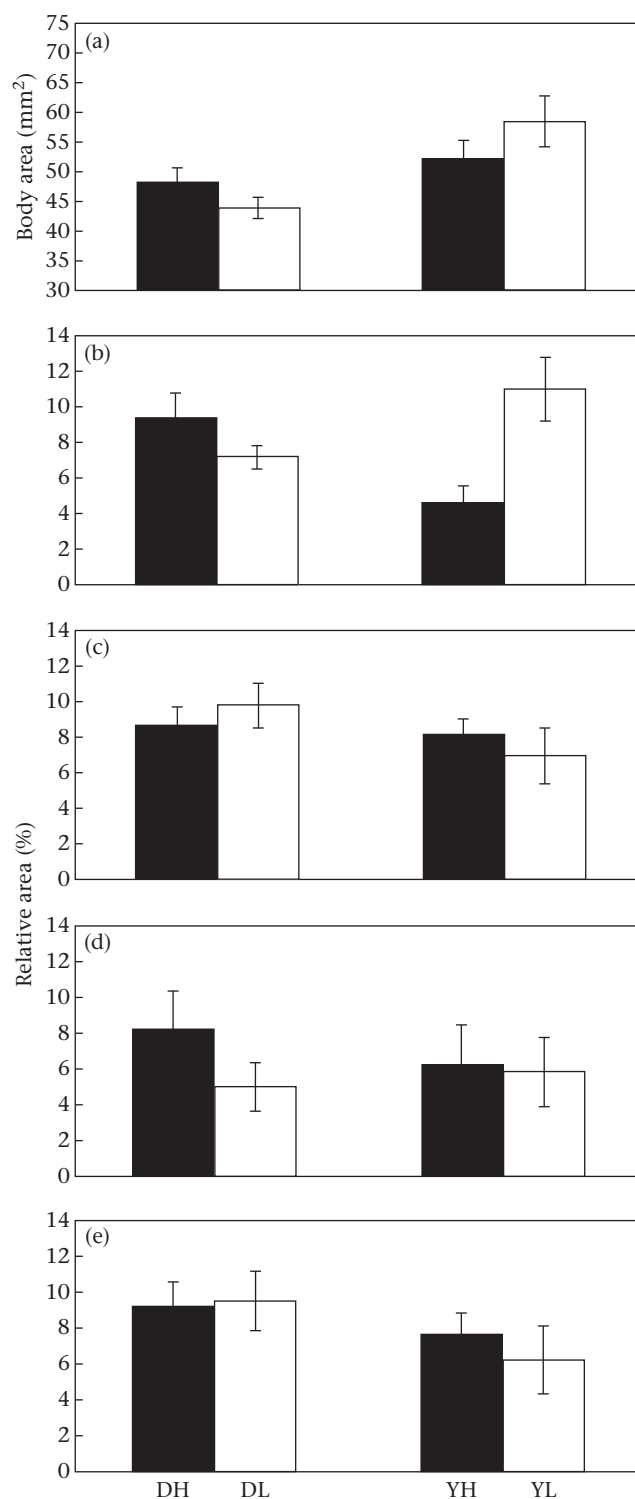

Figure 2. Aspects of male colour and body size by population: Damier high-predation (DH), Damier low-predation (DL), Yarra high-predation (YH) and Yarra low-predation (YL). High-predation populations are indicated by solid bars; low-predation ones by open bars. (a) Body area, (b) relative area of orange, (c) relative area of black, (d) relative area of green and (e) relative area of violet-blue. The mean relative area of each colour (%) is shown for each population, along with standard errors of population means.

evolution, and so we used linear functions (Brooks & Endler 2001a). Alternatively, evolution could have occurred in the nonlinear components of preference functions, as seen in other studies using quadratic regression or projection pursuit regression (see Blows et al. 2003). It is beyond our present study to examine

Table 2
Variation in within-population female preference functions ($N = 10$ trials per population, $df = 20$) for four male colour traits (relative area) between rivers (Yarra and Damier) and predation environments (high or low)

Term	F	P
River	1.12	0.3
Predation	1.21	0.28
Predation*River	0.84	0.37
Orange	0.72	0.4
Black	0.06	0.8
Green	0.05	0.82
Violet-blue	**4.8**	**0.03**
River*Orange	1.38	0.25
River*Black	0.42	0.52
River*Green	0.22	0.64
River*Violet-blue	0.27	0.61
Predation*Orange	0.19	0.66
Predation*Black	1.43	0.25
Predation*Green	0.69	0.41
Predation*Violet-blue	0.35	0.56

Significant effect is in bold.

these components here, and so we focus on the overall direction of evolution, using a linear model.

On the other hand, weak linear preference functions may in fact reflect true variation and/or plasticity of mate choice in guppies. A more formal comparison of our study to previous work shows that significant preferences, and preference differences, have been documented based on methods and sample sizes similar to ours. Figure 5 shows the relationship between sample size and effect size (calculated as Hedges' d statistic with appropriate transformations; Cooper & Hedges 1994) in a number of studies that quantified female preferences in the laboratory (see Appendix Table A1 for details). In general, variance across studies is lower when sample sizes are larger, and effect sizes tend to decrease with increasing sample size ($\beta = -0.02$, $F_{1,40} = 7.49$, $P = 0.009$); however, sample size explains only approximately 14% of the overall variation in effect sizes. Therefore, while a larger sample size would certainly have been better, studies of similar sample size have found much larger effects, and our results were consistent across two independent experiments. This leads us to suggest that our study design and implementation should not have prevented us from detecting reasonable preference changes had they occurred.

Another possibility is that preference evolution is proceeding, but is doing so too slowly to have been detected in the short time since the introduction. It is hard to evaluate this possibility because few studies have examined the time course of the evolution of female preferences in populations experiencing environmental change (Candolin & Heuschele 2008). However, we do know that life history traits have diverged between our study populations over the same timeframe and considerable trait divergence is present over similar timeframes in other guppy introductions (Endler 1980; Magurran et al. 1992; O'Steen et al. 2002; Arendt & Reznick 2005). Thus, the slow (or absent) evolution documented here is most likely to be related to the nature of female preferences, which might in turn be one of the factors contributing to the lack of observed colour evolution.

Finally, female preferences might not diverge at all, which might be the case if they are not subject to strong divergent selection or if they lack appropriate genetic variation. We have no information on the strength of divergent selection acting on female preferences, although the four populations we studied certainly do differ in a number of environmental factors thought to cause divergent selection on a variety of guppy traits (Gordon et al. 2009). As for limited genetic variation, we are sceptical because (1) 200 introduced individuals would be likely to harbour considerable genetic variation, particularly given multiple mating and sperm storage (Kobayashi & Iwamatsu 2002; Pitcher et al. 2003; Becher & Magurran 2004); (2) the population was robust a year after the introduction (D.N. Reznick, personal observation); and (3) Damier guppies are very variable for male colour and female preference (Karim et al. 2007; present study). More generally, sexually selected traits, including male colour (e.g. Brooks & Endler 2001b), tend to harbour considerable genetic variation (Svensson & Gosden 2007; Ahuja & Singh 2008). However, the amount of genetic variation for female preferences is less certain (Godin & Dugatkin 1995; Brooks & Endler 2001b; Brooks 2002; Lindholm & Breden 2002). In some cases, it appears to be conspicuously absent (Hall et al. 2004), whereas in other cases it should be sufficient to support further evolution (Godin & Dugatkin 1995). In analysing our results, we assumed that there was sufficient genetic variation to allow for evolution to occur; however, further studies investigating the genetic components of preferences would improve future interpretations.
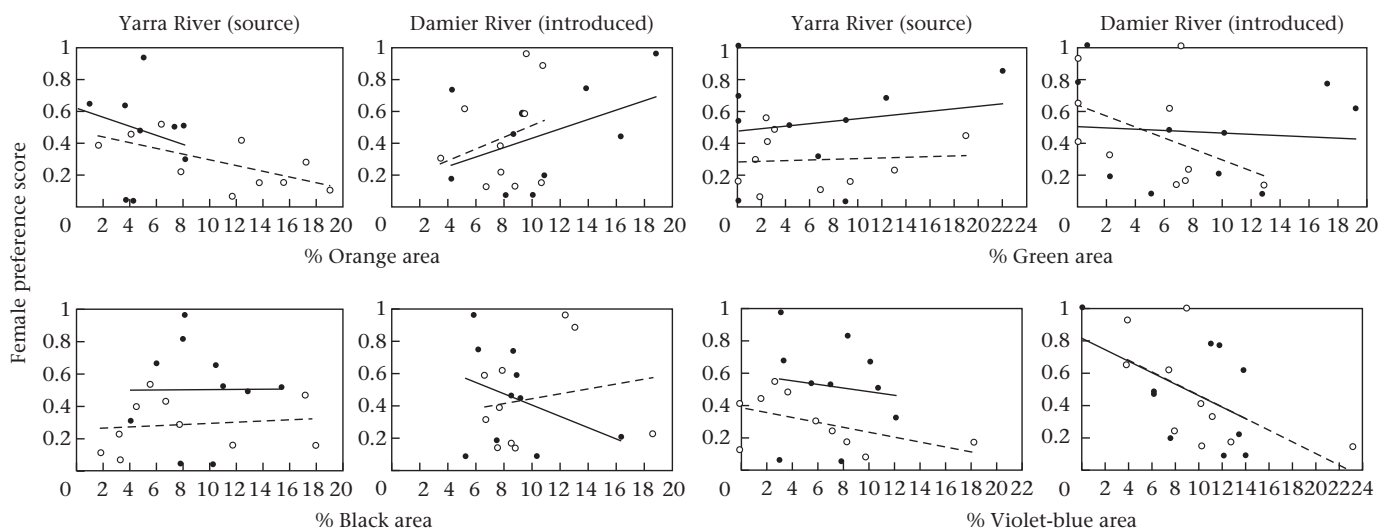

Figure 3. Population-level female preference functions in the home population trials as determined by linear regression of female preference score on each male colour category (percentage of colour on body). Preference functions for the two types of population in each river are shown together, where the high-predation populations are indicated by solid symbols and lines and the low-predation populations by open symbols and dashed lines.
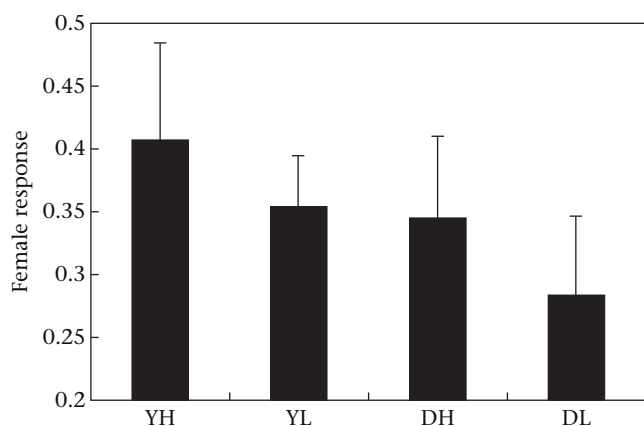
Figure 4. Variation in mean + SE female response to the same 10 Yarra-high (ancestor) males among the four female populations: Yarra high-predation (YH), Yarra low-predation (YL), Damier high-predation (DH) and Damier low-predation (DL).

Considering all of the above arguments, we suggest that the lack of noteworthy preference evolution in the present study might be caused by some combination of the relative insensitivity of the preference assays, high variation in preferences between females within a given population, and only small changes in female preferences. It is possible that the lack of change could be the result of limited genetic variation in female preferences, weak divergent selection or differences in the influence of selection on males and females (e.g. Delcourt et al. 2009). Further studies that directly examine the mode of inheritance of female preference and its genetic covariance with male colour elements (Houde 1992; Lindholm & Breden 2002) would allow for a better understanding of the degree to which these traits can evolve in response to environmental stimuli.

Summary and Implications

No study of populations experiencing environmental change has examined the time course of evolution in both sexually selected traits and female preferences, although several have examined elements of

this problem (Candolin et al. 2007; Gosden & Svensson 2008; Hegyi et al. 2010). Our earlier work on Damier guppies showed that key aspects of male colour do not always increase when high-predation guppies are introduced into low-predation environments (Karim et al. 2007; Fig. 2), and this result has been reinforced by other recent studies (e.g. Kemp et al. 2008, 2009). Therefore, divergence in male colour between predation environments might be less consistent than divergence in other types of traits, such as life history and behaviour (Endler 1995; Reznick et al. 1997; Magurran & Ramnarine 2005; Gordon et al. 2009). A likely reason is that the evolution of male colour requires that the introduced females initially prefer greater colour or that they quickly evolve in that direction, and both of these conditions might sometimes be lacking.

Although some studies have examined the contemporary evolution of female preference in the laboratory, none appears to have tested for such evolution in nature. We did so here, and found that female preferences did not necessarily evolve quickly, or at least not to an extent that was statistically detectable using a common laboratory methodology. This result suggests potential constraints on the rapidity of preference evolution, such as limited genetic variation between females (e.g. Hall et al. 2004). Furthermore, the lack of colour and preference evolution is in contrast to survival and life history trait divergence in the same populations over the same timeframe (Gordon et al. 2009). This suggests something particular to preference evolution, which would support recent assertions that traits related to sexual selection may evolve fundamentally differently to traits directly linked to viability selection (Svensson & Gosden 2007; Candolin & Heuschele 2008).

Together, these results highlight the complexities in both measuring and understanding how elements of sexual selection evolve when environments change. Although the extent to which mate preferences respond to habitat alterations can theoretically influence the potential for adaptation and persistence, this topic has only recently begun to be explored (Candolin & Heuschele 2008; van der Sluijs et al. 2011). Owing to the strong context dependence in the evolution of mating traits, insight in this field will benefit from integrating behavioural, ecological and genetic components of trait−preference coevolution.

Acknowledgments

Figure 5. Relationship between effect size and sample size across laboratory studies of guppy mate choice (see Appendix Table A1 for details of data included). Because different effect sizes are reported in the original studies, these were calculated to a standard metric, Hedges' *d* statistic (Cooper & Hedges 1994). Data points from the current study are circled.

References

Ahuja, A. & Singh, R. S. 2008. Variation and evolution of male sex combs in *Drosophila*: nature of selection responses and theories of genetic variation for sexual traits. *Genetics*, **179**, 503−509.

Andersson, S. 1982. Female choice selects for extreme tail length in a widowbird. *Nature*, **299**, 818−820.

Andersson, S. 1994. Costs of sexual advertising in the lekking Jackson widowbird. *Condor*, **96**, 1−10.

Archard, G. A., Cuthill, I. C. & Partridge, J. C. 2006. Condition-dependent mate choice in the guppy: a role for short-term food restriction? *Behaviour*, **143**, 1317−1340.

Arendt, J. D. & Reznick, D. N. 2005. Evolution of juvenile growth rates in female guppies (*Poecilia reticulata*): predator regime or resource level? *Proceedings of the Royal Society B*, **272**, 333−337.
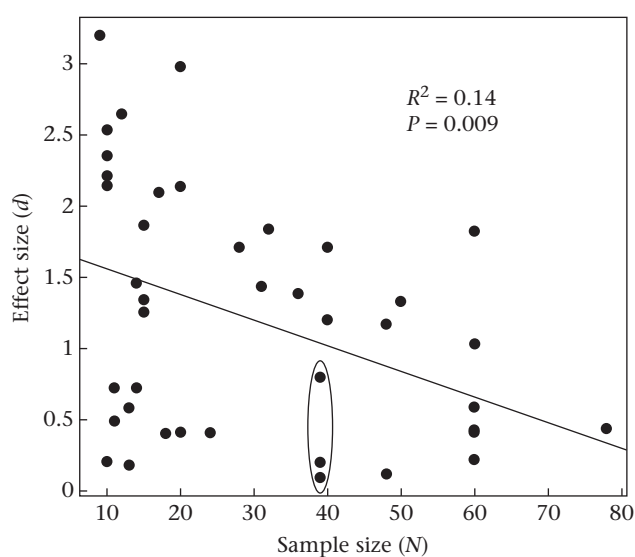
Baerends, G. P., Brouwer, R. & Waterbolk, H. T. J. 1955. Ethological studies on *Lebistes reticulatus* (Peters). 1. An analysis of the male courtship pattern. *Behaviour*, **8**, 249–334.

Becher, S. A. & Magurran, A. E. 2004. Multiple mating and reproductive skew in Trinidadian guppies. *Proceedings of the Royal Society B*, **271**, 1009–1014.

Bischoff, R. J., Gould, J. L. & Rubenstein, D. I. 1985. Tail size and female choice in the guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology*, **17**, 252–255.

Blows, M. W., Brooks, R. & Kraft, P. G. 2003. Exploring complex fitness surfaces: multiple ornamentation and polymorphism in male guppies. *Evolution*, **57**, 1622–1630.

Boughman, J. W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature*, **411**, 944–948.

Breden, F. & Stoner, G. 1987. Male predation risk determines female preference in the Trinidad guppy. *Nature*, **329**, 831–833.

Brooks, R. 2002. Variation in female mate choice within guppy populations, population divergence, multiple ornaments and the maintenance of polymorphism. *Genetica*, **116**, 343–358.

Brooks, R. & Endler, J. 2001a. Female guppies agree to differ: phenotypic and genetic variation in mate-choice behavior and the consequences for sexual selection. *Evolution*, **55**, 1644–1655.

Brooks, R. & Endler, J. 2001b. Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). *Evolution*, **55**, 1002–1015.

Candolin, U. & Heuschele, J. 2008. Is sexual selection beneficial during adaptation to environmental change? *Trends in Ecology & Evolution*, **23**, 446–452.

Candolin, U., Salesto, T. & Evers, M. 2007. Changed environmental conditions weaken sexual selection in sticklebacks. *Journal of Evolutionary Biology*, **20**, 233–239.

Chenoweth, S. F. & Blows, M. W. 2006. Dissecting the complex genetic basis of mate choice. *Nature Reviews Genetics*, **7**, 681–692.

Cooper, H. & Hedges, L. V. 1994. *The Handbook of Research Synthesis*. New York: Russell Sage Foundation.

Cornwallis, C. K. & Uller, T. 2010. Towards an evolutionary ecology of sexual traits. *Trends in Ecology & Evolution*, **25**, 145–152.

Deering, M. D. & Scriber, J. M. 2002. Field bioassays show heterospecific mating preference asymmetry between hybridizing North American *Papilio* butterfly species (Lepidoptera: Papilionidae). *Journal of Ethology*, **20**, 25–33.

Delcourt, M., Blows, M. W. & Rundle, H. D. 2009. Sexually antagonistic genetic variance for fitness in an ancestral and a novel environment. *Proceedings of the Royal Society B*, **276**, 2009–2014.

Dugatkin, L. A. 1992. Sexual selection and imitation: females copy the mate choice of others. *American Naturalist*, **139**, 1384–1389.

Dugatkin, L. A. & Godin, J.-G. 1992. Reversal of female mate choice by copying in the male guppy (*Poecilia reticulata*). *Proceedings of the Royal Society B*, **249**, 179–184.

Endler, J. A. 1978. A predator's view of animal color patterns. *Evolutionary Biology*, **11**, 319–364.

Endler, J. A. 1980. Natural selection on color patterns in *Poecilia reticulata*. *Evolution*, **34**, 76–91.

Endler, J. A. 1995. Multiple trait coevolution and environmental gradients in guppies. *Trends in Ecology & Evolution*, **10**, 22–29.

Endler, J. A. & Houde, A. E. 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution*, **49**, 456–468.

Farr, J. 1980. The effects of sexual experience and female receptivity on courtship-rape decisions in male guppies, *Poecilia reticulata* (Pisces: Poeciliidae). *Animal Behaviour*, **28**, 1195–1201.

Gamble, S., Lindholm, A. K., Endler, J. A. & Brooks, R. 2003. Environmental variation and the maintenance of polymorphism: the effect of ambient light spectrum on mating behaviour and sexual selection in guppies. *Ecology Letters*, **6**, 463–472.

Godin, J. G. J. & Briggs, S. E. 1996. Female mate choice under predation risk in the guppy. *Animal Behaviour*, **51**, 117–130.

Godin, J. G. J. & Dugatkin, L. A. 1995. Variability and repeatability of female mating preference in the guppy. *Animal Behaviour*, **49**, 1427–1433.

Godin, J.-G. J., Herdman, E. J. E. & Dugatkin, L. A. 2005. Social influences on female mate choice in the guppy, *Poecilia reticulata*: generalized and repeatable trait-copying behaviour. *Animal Behaviour*, **69**, 999–1005.

Gong, A. & Gibson, R. M. 1996. Reversal of a female preference after visual exposure to a predator in the guppy, *Poecilia reticulata*. *Animal Behaviour*, **52**, 1007–1015.

Gordon, S. P., Reznick, D. N., Kinnison, M. T., Bryant, M. J., Weese, D. J., Rasanen, K., Millar, N. P. & Hendry, A. P. 2009. Adaptive changes in life history and survival following a new guppy introduction. *American Naturalist*, **174**, 34–45.

Gosden, T. P. & Svensson, E. I. 2008. Spatial and temporal dynamics in a sexual selection mosaic. *Evolution*, **62**, 845–856.

Grether, G. F., Millie, D. F., Bryant, M. J., Reznick, D. N. & Mayea, W. 2001. Rain-forest canopy cover, resource availability, and life history evolution in guppies. *Ecology*, **82**, 1546–1559.

Grether, G. F., Kolluru, G. R., Rodd, H. F., de la Cerda, J. & Shimazaki, K. 2005. Carotenoid availability affects the development of a colour-based mate preference and the sensory bias to which it is genetically linked. *Proceedings of the Royal Society B*, **272**, 2181–2188.

Hall, M., Lindholm, A. K. & Brooks, R. 2004. Direct selection on male attractiveness and female preference fails to produce a response. *BMC Evolutionary Biology*, **4**, 1.

Hegyi, G., Torok, J., Toth, L., Garamszegi, L. Z. & Rosivall, B. 2006. Rapid temporal change in the expression and age-related information content of a sexually-selected trait. *Journal of Evolutionary Biology*, **19**, 228–238.

Hegyi, G., Herenyi, M., Wilson, A. J., Garamszegi, L. Z., Rosivali, B., Eens, M. & Torok, J. 2010. Breeding experience and the heritability of female mate choice in collared flycatchers. *PLoS One*, **5**, e13855.

Hendry, A. P. & Kinnison, M. T. 1999. Perspective: the pace of modern life: measuring rates of contemporary microevolution. *Evolution*, **53**, 1637–1653.

Higgie, M., Chenoweth, S. & Blows, M. W. 2000. Natural selection and the reinforcement of mate recognition. *Science*, **290**, 519–521.

Hollis, B., Fierst, J. L. & Houle, D. 2009. Sexual selection accelerates the elimination of a deleterious mutant in *Drosophila melanogaster*. *Evolution*, **63**, 324–333.

Houde, A. E. 1987. Mate choice based upon naturally occurring color-pattern variation in a guppy population. *Evolution*, **41**, 1–10.

Houde, A. E. 1988. Genetic difference in female choice between two guppy populations. *Animal Behaviour*, **36**, 510–516.

Houde, A. E. 1992. Sex-linked heritability of a sexually selected character in a natural population of *Poecilia reticulata* (Pisces, Poeciliidae (guppies)). *Heredity*, **69**, 229–235.

Houde, A. E. 1997. *Sex, Color and Mate Choice in Guppies*. Princeton, New Jersey: Princeton University Press.

Houde, A. E. & Endler, J. A. 1990. Correlated evolution of female mating preferences and male color patterns in the guppy *Poecilia reticulata*. *Science*, **248**, 1405–1408.

Houde, A. E. & Hankes, M. A. 1997. Evolutionary mismatch of mating preferences and male colour patterns in guppies. *Animal Behaviour*, **53**, 343–351.

Houde, A. E. & Torio, A. J. 1992. Effect of parasitic infection on male colour pattern and female choice in guppies. *Behavioral Ecology*, **3**, 346–351.

Karim, N., Gordon, S. P., Schwartz, A. K. & Hendry, A. P. 2007. Not déjà vu all over again: male guppy colour in a new experimental introduction. *Journal of Evolutionary Biology*, **20**, 1339–1350.

Kemp, D. J., Reznick, D. N. & Grether, G. F. 2008. Ornamental evolution in Trini-dadian guppies (*Poecilia reticulata*): insights from sensory processing-based analyses of entire color patterns. *Biological Journal of the Linnean Society*, **95**, 734–747.

Kemp, D. J., Reznick, D. N., Grether, G. F. & Endler, J. A. 2009. Predicting the direction of ornament evolution in Trinidadian guppies (*Poecilia reticulata*). *Proceedings of the Royal Society B*, **276**, 4335–4343.

Kennedy, C. E. J., Endler, J. A., Poynton, S. L. & McMinn, H. 1987. Parasite load predicts mate choice in guppies. *Behavioral Ecology and Sociobiology*, **21**, 291–295.

Kobayashi, H. & Iwamatsu, T. 2002. Fine structure of the storage micropocket of spermatozoa in the ovary of the guppy *Poecilia reticulata*. *Zoological Science*, **19**, 545–555.

Kodric-Brown, A. 1985. Female preference and sexual selection for male coloration in the guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology*, **17**, 199–205.

Kodric-Brown, A. 1989. Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. *Behavioral Ecology and Sociobiology*, **25**, 393–401.

Kodric-Brown, A. & Johnson, S. C. 2002. Ultraviolet reflectance patterns of male guppies enhance their attractiveness to females. *Animal Behaviour*, **63**, 391–396.

Kwan, L. & Rundle, H. D. 2010. Adaptation to desiccation fails to generate pre- and postmating isolation in replicate *Drosophila melanogaster* laboratory populations. *Evolution*, **64**, 710–723.

Liley, N. R. 1966. Ethological isolating mechanisms in four sympatric species of poeciliid fishes. *Behaviour, Supplement*, **13**, 1–197.

Lindholm, A. & Breden, F. 2002. Sex chromosomes and sexual selection in poeciliid fishes. *American Naturalist*, **160**, S214–S222.

Lindström, J., Pike, T. W., Blount, J. D. & Metcalfe, N. B. 2009. Optimization of resource allocation can explain the temporal dynamics and honesty of sexual signals. *American Naturalist*, **174**, 515–525.

Lorch, P. D., Proulx, S., Rowe, L. & Day, T. 2003. Condition-dependent sexual selection can accelerate adaptation. *Evolutionary Ecology Research*, **5**, 867–881.

Magurran, A. E. & Ramnarine, I. W. 2005. Evolution of mate discrimination in a fish. *Current Biology*, **15**, R867–R868.

Magurran, A. E., Seghers, B. H., Carvalho, G. R. & Shaw, P. W. 1992. Behavioral consequences of an artificial introduction of guppies (*Poecilia reticulata*) in N-Trinidad: evidence for the evolution of antipredator behavior in the wild. *Proceedings of the Royal Society B*, **248**, 117–122.

Millar, N. P., Reznick, D. N., Kinnison, M. T. & Hendry, A. P. 2006. Disentangling the selective factors that act on male color in wild guppies. *Oikos*, **113**, 1–12.

Nickel, D. & Civetta, A. 2009. An X chromosome effect responsible for reproductive isolation between male *Drosophila virilis* and heterospecific females. *Genome*, **52**, 49–56.

Nosil, P., Crespi, B. & Sandoval, C. P. 2002. Host-plant adaptation drives the parallel evolution of reproductive isolation. *Nature*, **417**, 440–443.

Nosil, P., Crespi, B. & Sandoval, C. P. 2003. Reproductive isolation driven by the combined effects of ecological adaptation and reinforcement. *Proceedings of the Royal Society B*, **270**, 1911–1918.

O'Steen, S., Cullum, A. J. & Bennett, A. F. 2002. Rapid evolution of escape ability in Trinidadian guppies (*Poecilia reticulata*). *Evolution*, **56**, 776–784.

Pfennig, K. S. 2008. Population differences in condition-dependent sexual selection may promote divergence in non-sexual traits. *Evolutionary Ecology Research*, **10**, 763–773.

Pilastro, A., Mandelli, M., Gasparini, C., Dadda, M. & Bisazza, A. 2007. Copulation duration, insemination efficiency and male attractiveness in guppies. *Animal Behaviour*, **74**, 321–328.

Pitcher, T. E., Neff, B. D., Rodd, F. H. & Rowe, L. 2003. Multiple mating and sequential mate choice in guppies: females trade up. *Proceedings of the Royal Society B*, **270**, 1623–1629.

Price, A. C. & Rodd, F. H. 2006. The effect of social environment on male-male competition in guppies (*Poecilia reticulata*). *Ethology*, **112**, 22–32.

Qvarnström, A., Brommer, J. E. & Gustafsson, L. 2006. Testing the genetics underlying the co-evolution of mate choice and ornament in the wild. *Nature*, **441**, 84–86.

Reznick, D. N. 1983. The structure of guppy life histories: the trade-off between growth and reproduction. *Ecology*, **64**, 862–873.

Reznick, D. N. & Ghalambor, C. K. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica*, **112**, 183–198.

Reznick, D. N., Butler, M. J., Rodd, F. H. & Ross, P. 1996. Life-history evolution in guppies (*Poecilia reticulata*) 6. Differential mortality as a mechanism for natural selection. *Evolution*, **50**, 1651–1660.

Reznick, D. N., Shaw, F. H., Rodd, F. H. & Shaw, R. G. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science*, **275**, 1934–1937.

Rice, W. R. & Hostert, E. E. 1993. Laboratory experiments on speciation: what have we learned in 40 years? *Evolution*, **47**, 1637–1653.

Rodd, F. H., Hughes, K. A., Grether, G. F. & Baril, C. T. 2002. A possible non-sexual origin of mate preference: are male guppies mimicking fruit? *Proceedings of the Royal Society B*, **269**, 475–481.

Rosenqvist, G. & Houde, A. 1997. Prior exposure to male phenotypes influences mate choice in the guppy, *Poecilia reticulata*. *Behavioral Ecology*, **8**, 194–198.

Rundle, H. D., Chenoweth, S. F., Doughty, P. & Blows, M. W. 2005. Divergent selection and the evolution of signal traits and mating preferences. *PLoS Biology*, **3**, e368.

Rutstein, A. N., Brazill-Boast, J. & Griffith, S. C. 2007. Evaluating mate choice in the zebra fish. *Animal Behaviour*, **74**, 1277–1284.

Ryan, M. J. & Rand, A. S. 1993. Sexual selection and signal evolution: the ghosts of biases past. *Philosophical Transactions of the Royal Society B*, **340**, 187–195.

Savalli, U. M. 1995. Does rainfall constrain the evolution of tail length in widow-birds? *Ethology Ecology and Evolution*, **7**, 379–385.

Schwartz, A. K. & Hendry, A. P. 2007. A test for the parallel co-evolution of male colour and female preference in Trinidadian guppies (*Poecilia reticulata*). *Evolutionary Ecology Research*, **9**, 71–90.

Seehausen, O., van Alphen, J. M. M. & Witte, F. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science*, **277**, 1808–1811.

Shohet, A. J. & Watt, P. J. 2009. Female guppies *Poecilia reticulata* prefer males that can learn fast. *Journal of Fish Biology*, **75**, 1323–1330.

van der Sluijs, I., Gray, S. M., Amorim, M. C. P., Barber, I., Candolin, U., Hendry, A. P., Krahe, R., Maan, M. E., Utne-Palm, A. C. & Wagner, H.-J., et al. 2011. Communication in troubled waters: responses of fish communication systems to changing environments. *Evolutionary Ecology*, **25**, 623–640.

Smith, E. J., Partridge, J. C., Parsons, K. N., White, E. M., Cuthill, I. C., Bennet, A. T. D. & Church, S. C. 2002. Ultraviolet vision and mate choice in the guppy (*Poecilia reticulata*). *Behavioral Ecology*, **13**, 11–19.

Stockwell, C. A., Hendry, A. P. & Kinnison, M. T. 2003. Contemporary evolution meets conservation biology. *Trends in Ecology & Evolution*, **18**, 94–101.

Stoner, G. & Breden, F. 1988. Phenotypic differentiation in female preference related to geographic variation in male predation risk in the Trinidad guppy *Poecilia reticulata*. *Behavioral Ecology and Sociobiology*, **22**, 285–292.

Svensson, E. I. & Friberg, M. 2007. Selective predation on wing morphology in sympatric damselflies. *American Naturalist*, **170**, 101–112.

Svensson, E. I. & Gosden, T. P. 2007. Contemporary evolution of secondary sexual traits in the wild. *Functional Ecology*, **21**, 422–433.

Syriatowicz, A. & Brooks, R. 2004. Sexual responsiveness is condition-dependent in female guppies, but preference functions are not. *BMC Ecology*, **4**, 5.

Tanaka, Y. 1996. Sexual selection enhances population extinction in a changing environment. *Journal of Theoretical Biology*, **180**, 197–206.

Watt, P. J., Shohet, A. J. & Renshaw, K. 2001. Female choice for good genes and sex-biased broods in guppies. *Journal of Fish Biology*, **59**, 843–850.

Weese, D. J., Gordon, S. P., Hendry, A. P. & Kinnison, M. K. 2010. Spatiotemporal variation in linear natural selection on body color in wild guppies (*Poecilia reticulata*). *Evolution*, **64**, 1802–1815.

White, E. M., Partridge, J. C. & Church, S. C. 2003. Ultraviolet dermal reflexion and mate choice in the guppy, *Poecilia reticulata*. *Animal Behaviour*, **65**, 693–700.

Winemiller, K. O., Leslie, M. & Roch, R. 1990. Phenotypic variation in male guppies from natural inland populations: an additional test of Haskins' sexual selection/predation hypothesis. *Environmental Biology of Fishes*, **29**, 179–191.

Zajitschek, S. R. K. & Brooks, R. C. 2008. Distinguishing the effects of familiarity, relatedness, and color pattern rarity on attractiveness and measuring their effects on sexual selection in guppies (*Poecilia reticulata*). *American Naturalist*, **172**, 843–854.

Zouros, E. & Dentremont, C. J. 1980. Sexual isolation among population sof *Drosophila mojavensis*: response to pressure from a related species. *Evolution*, **34**, 421–430.

Appendix

Table A1
Summary table of previous studies of guppy mate choice

Source	Population	Sample size (N females)	Design	Female response	Male trait(s) measured	Result	Test	Effect size	P
Present study	Lower Yarra; Limon (Yarra LP); Upper Damier; Lower Damier	40	No-choice	Fraction and intensity of response to displays	Orange Black Green Violet-blue	No difference in preferences between populations Preference for less violet-blue colour	ANCOVA	See Table 2	See Table 2
Bischoff et al. 1985	Unknown	1. 32 2. 94 3. 120 (20 females used repeatedly)	2-choice	Time	Tail size	Preference for larger-tailed males	Binomial tests	Not given	1. <0.002 2. <0.002 3. <0.005
Kodric-Brown 1985	Maculatus, Armatus, ornamental	20	2-choice	Time	6 Red-yellow Black Blue-green Iridescent White 'Showiness'	Positive selection for 2/6 (carotenoids & iridescence)	Spearman rank coefficient (r_S)	Carotenoids: 0.83 Iridescence: 0.73	<0.01 <0.01

(continued on next page)

Table A1 (continued)

Source	Population	Sample size (N females)	Design	Female response	Male trait(s) measured	Result	Test	Effect size	P
Breden & Stoner 1987	Aripo (HP), Arima (HP), Arima (LP)	$N=60$	2-choice	Time	Bright vs drab model males	Low-predation preference for bright; high-predation preference for drab	Nested ANOVA (F)	48.97	0.02
Houde 1987	Paria	(1) 10 (2) 50 (3) 32	(1) No-choice (2) Group tanks (3) Group tanks	(1) Mating speed (2) 'Move towards' nondisplaying males (3) Fraction response to displays	Relative orange area	Most orange males preferred in all three experiments	(1) Friedman's test & Page's test (2) ANCOVA (3) ANCOVA	(1) $\chi^2=14.43$ & $L=283.5$ (2) $F=21.69$ (3) $F=26.16$	(1) 0.002 & <0.001 (2) 0.0001 (3) 0.0001
Kennedy et al. 1987	Paria	16	2-choice	Time	Number of internal parasites	Females prefer least parasitized males	Binomial test	14/16	0.007
Stoner & Breden 1988	Aripo, Arima, El Cedro	28 High predation 28 Low predation	2-choice	Time	Brightness	Low-predation females prefer brighter male	Binomial	Not provided	HP>0.05 LP<0.001
Houde 1988	Paria Aripo	24 24	Group tanks	Fraction response to displays	Relative orange area	Paria females prefer more orange males from Paria only; Aripo females show no discrimination with either male population	Regression coefficient (r^2)	Paria females: $r^2=0.43$ Aripo females: $r^2=0.04$	Paria: <0.001 Aripo: 0.25
Kodric-Brown 1989	Paria & Aripo mixed	31	(1) 2-choice	(1) Time	High/low carotenoid diet; Area, hue & brightness of carotenoid spots	Females prefer high-carotenoid (=brighter) male	Wilcoxon rank	$F=15.49$	0.0005
Houde & Endler 1990	(1) Paria (2) Arima (3) Orupuche 1 (4) Orupuche 4 (5) Guanapo (6) Quare (7) Marianne	(1)36 (2) 14 (3) 15 (4) 14 (5) 11 (6) 11 (7) 13	Group tanks	Fraction response to displays	Relative orange area	3/7 female populations show preference for orange males	Kruskal–Wallis test for differences in preference between female populations	(1) 16.8 (2) 6.9 (3) 6.3 (4) 1.7 (5) 1.3 (6) 0.6 (7) 0.1	(1) <0.01 (2) <0.05 (3) <0.05 (4) >0.1 (5) >0.1 (6) >0.1 (7) >0.1
Godin & Dugatkin 1995	Quare	$N=40$	2-choice	Time	'Bright' vs 'drab' (number & size of melanin, carotenoid and iridescent spots)	Bright male preferred in two experiments	Paired t test	$t=5.34$ $t=3.75$	<0.001 <0.001
Endler & Houde 1995	(a) Limon (LP) (b) Marianne (LP) (c) Paria (LP) (d) Madamas (LP) (e) Arima (LP) (f) Quare (LP) (g) Guanapo (MP) (h) Quare (MP) (i) Orupuche 1 (HP) (j) Orupuche 4 (HP) (k) Rio Grande (HP)	(a) 60 (b) 107 (c) 406 (d) 54 (e) 130 (f) 114 (g) 84 (h) 72 (i) 113 (j) 114 (k) 66	Group tanks	Fraction response to displays	Total colour pattern based on seven colour elements	Significant preferences for male colour in 10/11 populations	Multiple regression (R^2)	(a) 0.53 (2) (b) 0.30 (6) (c) 0.16 (2) (d) 0.50 (2) (e) 0.09 (4) (f) 0.17 (3) (g) 0.15 (4) (h) 0.18 (2) (i) 0.12 (4) (j) 0.03 (2) (k) 0.31 (5)	(a) <0.0001 (b) <0.0001 (c) <0.0001 (d) <0.0001 (e) <0.01 (f) <0.0001 (g) <0.001 (h) <0.01 (i) 0.001 (j) >0.05 (k) <0.0001
Gong & Gibson 1996	Paria	$N=62$	2-choice	Time	Orange	Preference for more orange male	Binomial	Not given	<0.0001

Study	Location	Sample size	Choice type	Response measure	Traits	Result	Test	Statistic	P
Rosenqvist & Houde 1997	Paria	(1) High orange males: 18 (2) Low orange males: 18 (3) Mixed males: 17	2-choice	Time	Orange	(1) No preference (2) No preference (3) Preference for orange males	G test	(1) 2.04 (2) 0.22 (3) 11.25	(1) >0.05 (2) >0.05 (3) <0.001
Brooks & Endler 2001a,b	Alligator Creek, Australia	$N=251$	Multiple choice	Time	8 Univariate colour traits 5 Composite colour traits	Positive directional selection for 3/13 traits measured	Spearman correlation	Range −0.097–0.198 Significant ($P<0.05$) effects: Tail area: 0.191 Orange chroma: 0.185 Colour contrast: 0.198	Not given
Kodric-Brown & Johnson 2002	Jemez, USA	1.20			UV		Wilcoxon signed-ranks	$Z=4.7$	1. <0.001
Smith et al. 2002	Lower Tacarigua (HP)	9	2-choice	Time	UV	Preference for males in UV+ filter	ANOVA	$F=20.46$	<0.01
Pitcher et al. 2003	Paria ('showy males') Guanapo ('plain males')	48	No choice – sequential	Fraction response to displays	Orange	No preference for orange initially (2) Preference for second male only evident when he was more orange than the first	Geometric mean linear regression	(1) $F=0.17$ (2) $F=16.1$	(1) 0.71 (2) <0.001
White et al. 2003	Lower Tacarigua (HP)	10	2-choice	Time	UV in orange pigments	No preference	(1) t test	$t=-0.31$	0.76
Godin et al. 2005	Paria	$N=68$	2-choice	Time	Orange	Preference for more orange male	Binomial (Z)	1.94	<0.03
Archard et al. 2006	Tacarigua (HP)	(1) $N=15$ (2) $N=14$	2-choice (repeated 3x per treatment group)	Time	Orange Black spots Black stripes Orange tail	(1) Preference for orange in 2/6 trials (2) No effect of male colour on female preference	Stepwise regression (F)	Only significant values provided: (1) $F=5.55$	0.036
Zajitschek & Brooks 2008	Alligator Creek, Australia	531	No choice	Fraction response	(1)Familiarity, relatedness and rarity of colour pattern (2) Body size, tail size, black, orange, fuzzy black, iridescence	(1) Rare phenotypes more attractive (2) Directional selection for small tails	(1) GLM (2) Linear regression	(1) $F=19.05$ (2) $\beta=-0.162$	1. <0.0001 2. <0.05
Shohet & Watt 2009	Stock population: mixed (unknown) origin	27	2-choice	Relative count near males	Learning ability Orange Body size	Preference for increased learning rate (1) but not orange (2) or length (3)	GLM	Not given	1. <0.01 2. >0.05 3. >0.05

Sample size refers to the number of individuals used per trial group and therefore the *N* for statistical analyses.