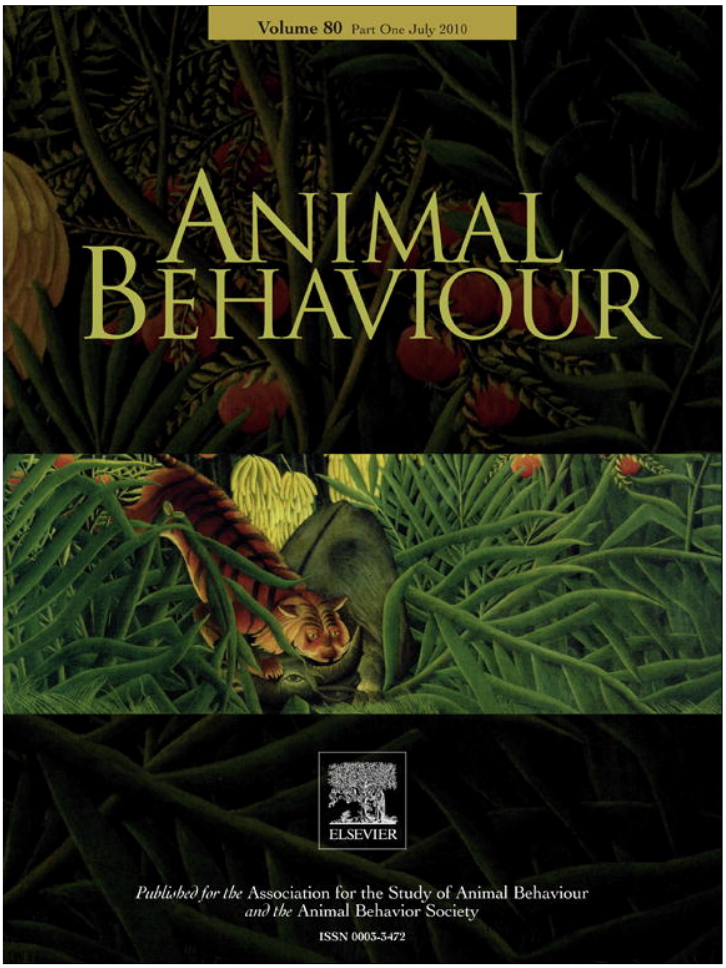

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Female mating preferences, lighting environment, and a test of the sensory bias hypothesis in the bluefin killifish

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Sensory drive proposes that environmental conditions affect signalling dynamics and the evolution of signals and receivers. For visual systems, delineating the effects of lighting on mating preferences is difficult because lighting conditions can affect preferences via three mechanisms: (1) genetic differentiation in mating preferences can result from selection under different lighting conditions, (2) development under different lighting conditions can alter the visual system and presumably female mating preferences and (3) lighting conditions can immediately alter colour perception by filtering wavelengths and altering visual backgrounds. We teased apart these effects by crossing bluefin killifish within and between a spring population and a swamp population that differed in lighting environment. We divided offspring between clear and tea-stained rearing environments (developmental plasticity) and measured preference under tea and clear conditions (immediate effects). We found genetic differentiation: spring offspring showed a strong preference for red males. We also found a three-way interaction between genetics, developmental plasticity and immediate effects on preference for blue males: swamp offspring had the highest levels of preference for blue males when raised and tested under tea-stained conditions. Thus, the environment experienced during development and the immediate conditions during mate selection interact with genetics to determine preference. We also tested the sensory bias hypothesis, which predicts that mating preferences evolve as a correlated response to selection on nonmating behaviours such as foraging. The relationship between mating and foraging preferences was weak and provided little support for sensory bias.

Sensory drive describes the manner in which female mating preferences and male secondary sex traits evolve over time placing heavy emphasis on the idea that the environmental conditions under which signalling occurs can have critically important effects on the evolution of signals, sensory systems and female mating preferences (Endler 1992, 1993). According to sensory drive (Fig. 1), males possess traits that act as signals. These signals are given at various times and places and must travel through the environment and be detected by females' sensory systems (and nervous systems), which determine females' mating preferences. Comparative studies have supported some of the basic tenets of sensory drive in visual signalling systems by demonstrating strong correlations between various aspects of male secondary sex traits and environmental lighting conditions (Marchetti 1993; Boughman 2001; Fuller 2002; Leal & Fleishman 2002; Gomez & Thery 2004, 2007; Stuart-Fox et al. 2007; Seehausen et al. 2008) and by demonstrating strong correlations between aspects of the visual system and lighting conditions (Lythgoe et al. 1994; Boughman 2001; Cummings & Partridge 2001; Fuller et al. 2003, 2004; Terai et al. 2006; Seehausen et al. 2008).

Determining the exact effects of lighting environment on the strength and direction of female mating preference is difficult because lighting environments can affect female mating preferences by at least three major mechanisms. First, differences in lighting environment can result in the evolution (i.e. genetic differentiation) of female mating preferences (Endler & Houde 1995; Boughman 2002). Mating preferences can diverge among lighting environments because of alterations in search costs for various colour patterns (direct selection on female mating preference), alterations in the fitness of different colour patterns (indirect selection of female mating preference via Fisherian or good genes processes), or because of pleiotropic effects of adaptation of the visual system in different environments (sensory bias). However, female mating preferences and the direction of sexual selection may differ because of environmental effects (i.e. plasticity). The fact that females occur and develop under different environmental lighting conditions raises the possibility that phenotypic plasticity leads to differences in preference among populations.

Phenotypic plasticity via the lighting environment can take two distinct forms. First, the lighting environment can have immediate

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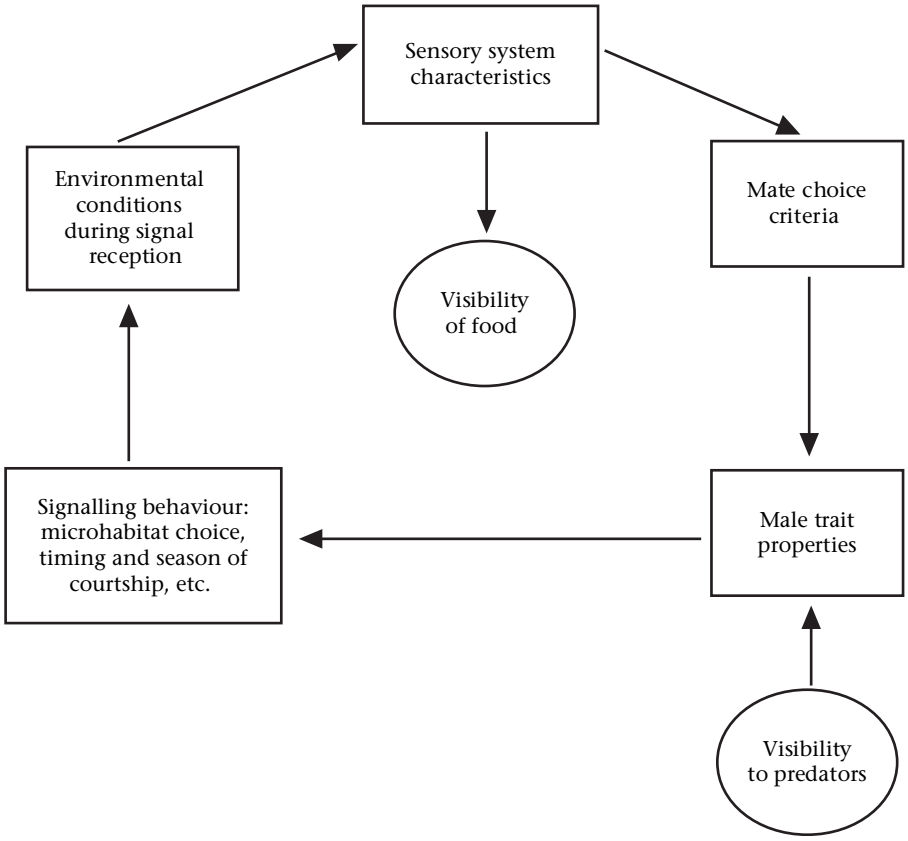

Figure 1. Sensory drive as redrawn from Endler (1992) showing the process of signalling with respect to environmental conditions, which indicates that mating behaviours can share a common sensory system with nonmating behaviours such as foraging behaviour.

effects on the transmission of signals through the environment and on their perceived brightness and contrast to the female (Endler 1990, 1991; Partridge & Cummings 1999). A variety of empirical studies using light filters have shown that altering the distribution of wavelengths results in changes in female mating preferences (Long & Houde 1989; Milinski & Bakker 1990; Evans & Norris 1996; Cummings et al. 2003; Rick et al. 2006). Second, the lighting environment can have long-term effects via developmental plasticity of the visual system. Several studies have raised animals under different experimental lighting conditions and demonstrated variation in important aspects of vision such as oil droplets (Hart et al. 2006), retinal filters (Cronin et al. 2001; Cronin & Caldwell 2002; Cheroske et al. 2003, 2006), lens properties (Kroger et al. 2001b; Schartau et al. 2009), relative abundance of cone cells (Shand et al. 2008), opsin expression (Fuller et al. 2005a), and the strength of connections between photoreceptor cells and neurons (Kroger et al. 2001a; Wagner & Kroger 2005). These changes in the visual system can presumably affect female mating preferences. Hence, the lighting environment can have three unique effects on female mating preferences. It can result in genetic differentiation among populations; it can have direct plastic effects on the perception of colour patterns through immediate effects on signal propagation; it can have long-term plastic effects on the development of the retina that affect mating preferences. There are also four possible interactions between the three main effects (genetics $\times$ developmental plasticity, genetics $\times$ immediate effects of lighting environment, developmental plasticity $\times$ immediate effects of lighting environment, and genetics $\times$ developmental plasticity $\times$ immediate effects of lighting environment). Our first goal in this study was to tease apart these sources of variation on female mating preferences in the bluefin killifish.

Our second goal in this study was to test the sensory bias hypothesis for the evolution of female mating preferences. One of the long-standing debates in evolutionary biology is why females evolve preferences for males with costly secondary sex characters (Kirkpatrick 1987; Kirkpatrick & Ryan 1991; Andersson 1994; Arnqvist & Rowe 2005; Kokko et al. 2006). The problem is that if females mate with males bearing costly secondary sex characters (and those characters are heritable), then their sons will express those traits and have to bear the associated costs. A variety of models have examined the conditions under which females are expected to evolve preferences for males with costly secondary sex characters (i.e. Fisherian runaway, good genes, etc.: Lande 1981; Kirkpatrick 1982; Pomiankowski 1987; Iwasa & Pomiankowski 1991; Pomiankowski et al. 1991; Pomiankowski & Iwasa 1993; Houle & Kondrashov 2002). These models are neutral with respect to the sensory environment, but all of the models include cost and benefit parameters that can vary with environmental conditions (see Schluter & Price 1993).

The sensory bias model states that female mating preferences evolve as correlated responses to natural selection on nonmating behaviours (e.g. foraging) that share a common sensory system (Kirkpatrick & Ryan 1991; Fuller et al. 2005b; Fuller 2009). Figure 1 shows this scenario, where both foraging preferences and mating preferences rely on the same sensory system. One often proposed example is that a selected preference for red food could cause a change in the visual system (or brain) and result in females being more likely to mate with red males (Fernandez & Morris 2007). The critical assumption with this model is that there are strong correlations between behaviours that share a common sensory system. While such correlations have been demonstrated between populations of guppies (Rodd et al. 2002; Grether et al. 2005), no such

correlations have been found within populations, and there have been no examinations of the degree to which such correlations vary with environmental conditions (see Schlichting & Pigliucci 1998 for environmentally variable genetic correlations).

The third goal of this study was to examine the relationship between opsin expression and female mating preferences. Opsins provide a convenient assay for visual sensitivity. Photoreceptor cells (rods and cones) contain photopigment, which is sensitive to various wavelengths of light, allowing animals to detect light levels and discern colour. Opsins play a critical role in determining the spectral sensitivity of photopigment. Photopigment consists of combining a vitamin A molecule (either the 11-cis-retinal or 11-cis-dehydroretinal) with an opsin protein (Wald 1968). Different opsin proteins vary in the way that they bind to the vitamin A molecule, which leads to different types of photopigment that vary in their spectral absorbance. There is a large literature on the evolution of opsins (reviewed in: Yokoyama 1999; Horth 2007; Osorio & Vorobyev 2008; Hofmann & Carleton 2009), and a variety of claims has been made concerning opsins and mate choice. For example, a large number of long-wavelength-sensitive (LWS) opsin variants have been found in guppies, leading to the hypothesis that these are important in maintaining high levels of male colour pattern polymorphism (Hoffmann et al. 2007; Weadick & Chang 2007; Ward et al. 2008). In cichlids, variation in LWS spectral tuning may contribute to speciation in two closely related species (Seehausen et al. 2008). In bluefin killifish, both the frequency of different male colour patterns and the relative expression of various opsin genes differ between spring and swamp habitats, and the implication is that variation in opsin expression is important in sexual selection (Fuller 2002; Fuller et al. 2004). Yet, the direct links between mating preference and opsin expression are unclear. Here, we examine the extent to which mating preferences are correlated with opsin expression in the bluefin killifish, *Lucania goodei*.

Lucania goodei is a compelling system within which to examine the relative effects of genetics and environment on female mating preferences. *Lucania goodei* is a small freshwater fundulid that occurs under a wide range of lighting environments ranging from tea-stained swamps, which have reduced transmission of ultraviolet and blue (hereafter UV/blue) wavelengths, to crystal clear springs, which have high transmission of UV/blue wavelengths (Fuller 2002). Supplementary Fig. S1a shows an irradiance spectrum from a spring population and a swamp population. Both male coloration and visual properties vary across populations in relation to lighting conditions (Fuller 2002; Fuller et al. 2003, 2004).

Males with blue anal fins are more abundant in tea-stained swamps, whereas males with red anal fins (and to a lesser extent, males with yellow anal fins) are more abundant in clear springs (Fuller 2002). Supplementary Fig. S1b shows reflectance spectra for blue, yellow and red males. There is genetic and environmental variation as well as an interaction between genetic and environmental variation in male colour pattern expression. Yellow versus red is controlled in large part by a single locus, where yellow is dominant over red (Fuller & Travis 2004). The expression of yellow versus red can be masked by the expression of blue. In general, males are more likely to express blue coloration when raised under swamp conditions, but there is heritable variation in male response to environmental lighting conditions (Fuller & Travis 2004). There is no evidence for different mating strategies between blue, yellow and red colour morphs (McGhee et al. 2007; McGhee & Travis 2010).

Visual properties of animals also differ between spring and swamp habitats. Swamp animals are less sensitive to UV/blue wavelengths and have fewer UV and violet cones than animals from spring populations (Fuller et al. 2003). These differences in cone frequency match differences in expression of opsins (Fuller et al. 2004). In the bluefin killifish, there are five main cone classes: ultraviolet (UV), violet, blue, yellow and red, which are maximally sensitive at 359, 405, 455, 537 and 568 nm. Supplementary Fig. S1c shows the absorption spectra for the UV, violet, blue, yellow and red cones. These five cone classes express different types of photopigment, which express different types of opsins (Yokoyama et al. 2007). In combination with 11-cis-retinal (derived from vitamin A1), the genes produce the following pigments: SWS1—UV photopigment (maximum absorbance (λ_{max}) = 359 nm); SWS2B—violet photopigment (λ_{max} = 405 nm); SWS2A—blue photopigment (λ_{max} = 455 nm); RH2—yellow photopigment (λ_{max} = 539 nm); and LWS—red photopigment (λ_{max} = 573 nm, for *L. goodei*). Comparisons among populations have shown that animals from the spring population, which have a higher frequency of UV and violet cones, also have higher expression of SWS1 and SWS2B opsins (Fuller et al. 2004). In contrast, swamp animals, which have a higher frequency of yellow and red cones, also have a higher frequency of RH2 and LWS opsins. Differences between individuals in opsin expression reflect qualitative differences in cone abundances (i.e. more versus less), but are not precise parameter estimates for actual cone abundances. In particular, the frequency of blue cones in the retina (8%) does not match the relative SWS2A opsin expression (0.1%), which is probably an experimental artefact of poor reverse transcription efficiency of this gene (Fuller et al. 2004).

At the population level, there is both genetic and environmental variation in opsin expression in *L. goodei.* A previous study found genetic variation within a population in opsin expression as well as developmental plasticity, where animals raised in clear water conditions had higher expression of SWS1 and SWS2B (which correspond to ultraviolet and violet photopigment) and animals raised in tea-stained water had higher expression of RH2 and LWS (which correspond to yellow and red photopigment) (Fuller et al. 2005a). The extent to which variation in opsin expression has meaningful effects on female mating preference is unknown.

The current study is part of a larger project examining genetic differentiation, developmental plasticity, and the immediate effects of lighting environment on the visual system and on a suite of visually based behaviours (foraging preference, mating preference, male competition, overall sexual selection). In an earlier paper, we described the effects of genetics and developmental plasticity on opsin expression and the effects of genetics and developmental plasticity and immediate effects of lighting environment on foraging behaviour (Fuller et al., in press). Here we report on the extent to which female mating preferences vary as a function of lighting environment and the extent to which they are correlated with foraging preference and opsin expression.

METHODS

The goals of this project were threefold. The first was to examine the effects of lighting environment on female mating preferences via three unique effects: genetic differentiation due to a history of selection in different lighting habitats, developmental plasticity of the visual system, and the immediate effects of lighting environment. The second goal was to examine the relationship between opsin expression and female mating preferences. The third goal was to determine the relationship between female mating preferences and foraging preferences as denoted by pecking preferences for different coloured dots. To do this, we created a series of crosses within and between a spring and a swamp population and then reared half the offspring in clear water (which mimics springs) and the other half in tea-stained water (which mimics swamps). The animals were raised to adulthood and then female mating

preferences, pecking preferences for variously coloured dots, and expression of opsin genes were measured.

Breeding Design and Animal Husbandry

The breeding design and animal husbandry are explained in depth elsewhere (Fuller et al., in press). Briefly, we crossed animals within and between a spring population (Upper Bridge, Wakulla River drainage, Wakulla County, FL, U.S.A.) and a swamp population (26-Mile Bend, Everglades drainage, Broward County, FL, U.S.A.). Animals were collected May–June 2005 with dipnets and minnow seines and were transported to the Mission Road Greenhouse at Florida State University, Tallahassee. Throughout this paper, the term 'cross' refers to the among-population effects. We established four crosses within and between spring and swamp animals (swamp ♀ × swamp ♂, spring ♀ × spring ♂, swamp ♀ × spring ♂, spring ♀ × swamp ♂). The rationale for using hybrid crosses was to generate a range of phenotypes that were intermediate between spring and swamp populations.

For each type of cross, we used eight different males with specific colour patterns. Specifically, we used two males that were red on the rear portion of the dorsal fin and red on the anal fin (R/R), two males that were yellow on the rear portion of the dorsal fin and yellow on the anal fin (Y/Y), two males that were red on the rear portion of the dorsal fin and blue on the anal fin (R/B), and two males that were yellow on the rear portion of the dorsal fin and blue on the anal fin (Y/B) for each type of cross. These colour patterns were available for both populations, although blue males were very rare in the spring population and were difficult to find. We used males with specific colour patterns with the hope of maximizing genetic variance as a function of sire and increasing our effect sizes. Preliminary analyses found no effects of sire colour pattern, and we do not consider this effect any further.

Each male was spawned with two females. Males were placed with each female on alternate days. This gave females 1 day to spawn and 1 day to recover. We collected eggs until we received a maximum of 100 eggs, although some individual crosses produced fewer eggs. Occasionally, a dam or a sire died after having produced enough eggs for one of the treatments. We retained the animals from these incomplete families and raised them to adulthood. We also replaced the original sire (or dam). If a sire died before both families were complete, we restarted the entire cross. If a dam died, we obtained a replacement female and crossed her with the original sire. Offspring were never pooled between different sires or dams.

Eggs were raised in tubs until they hatched. The resulting juvenile fish were transferred to 110-litre tanks. Half the offspring were raised under clear water conditions and the other half were raised under tea-stained conditions. Fish were maintained in their rearing environments for 1.5–2 years. Each tank was given a unique blind code so that experimentation was done blind with respect to cross. For both water treatments we used Nutrafin P-Clear (R.C. Hagen, Mansfield, MA, U.S.A.) to remove suspended algae from the water column. P-Clear causes algae to clump and drop to the bottom of the tank. Tea-stained water was created by adding Nestea instant, decaffeinated, no sugar, no lemon tea (Glendale, CA, U.S.A.) to the water column. This technique has been used in previous experiments and mimics swamp water conditions quite well (Fuller & Travis 2004; Fuller et al. 2005a). All tanks were housed in the Florida State University (FSU) Mission Road Greenhouse in Tallahassee, where they were exposed to natural sunlight.

The fish were moved to the University of Illinois in August 2006. Each family was placed in its own bucket and transferred to a greenhouse at the University of Illinois. We lost a handful of fish families in this move. Ultimately, we had 35 sires, 76 dams and 134 tanks of fish. Supplementary Table S1 describes the crosses, sample sizes and rearing environments in detail. At the University of Illinois, the fish were also kept in a greenhouse where they were exposed to natural sunlight. At the University of Illinois, we used dechlorinated city water in our tanks. We used UV water filters to eliminate algae from the water column instead of P-Clear. All other husbandry conditions were identical to those at FSU.

Female Mating Preferences

We used a one-way mate choice assay to measure female mating preferences. With this assay, a female is placed in an aquarium with a male and allowed to spawn over a period of time. The number of eggs spawned is taken as a measure of female mating preference. This approach has been successfully used in studies of species recognition between *L. goodei* and its closest relative, *L. parva* (Fuller et al. 2007). In this study, we used one-way choice tests to measure the mating preferences of the offspring resulting from our crosses.

Females were tested with three male colour patterns (red, yellow and blue anal fins) under two water conditions (clear and tea-stained water), resulting in the six mating treatments. For each trial, a female was placed in a 5-gallon (19-litre) tank at 0800 hours with a male and allowed to spawn for 4 h. At the end of 4 h, the spawning substrate was removed, and the resulting eggs were counted. We inferred female mating preference by comparing the number of eggs that each female laid when paired with a male of each colour pattern under each testing environment.

We tested individual females under the six mating treatments over a 2-week period. Gravid females were identified, measured and isolated on a Friday. Females were then allowed to mate on the following Monday, Wednesday and Friday mornings for the next 2 weeks. We gave females 1–2 days with no males between trials to increase the females' probability of the mating. During this 2-week period, females were individually housed in 19-litre aquaria using the same lighting environments in which they had been reared. Thus, females were only in the alternate lighting environment when they were tested under those conditions. In other words, females that were reared in clear water were always in clear water except during the three 4 h periods when they were tested under tea-stained conditions (and vice versa for females reared in tea-stained water). There were slight deviations in clutch size as a function of time (mean ± SE clutch size: Monday: 5.92 ± 0.28 eggs; Wednesday: 4.06 ± 0.22 eggs; Friday: 5.07 ± 0.28 eggs), but we randomized the presentation of males to control for this effect.

We were careful not to use males from either the Wakulla Upper Bridge or Everglades populations in our mating treatments. Instead, we used tester males from three other populations. We collected males from Blue Springs (Santa Fe/Suwanee drainage), St Mark's National Wildlife Refuge (St Mark's/Wakulla Drainage) and Delk's Bluff (St John's Drainage). For each set of males, we size-matched six males (2 males with red anal fins, 2 males with yellow anal fins, and 2 males with blue anal fins) within 2 mm and placed each male in a 5-gallon aquarium with either clear or tea-stained water. Each female was tested with one red, one yellow and one blue male each week, but the order was randomized. We also randomized the females among the sets of males. In most cases, our randomization resulted in replicate females from a given tank being tested with different sets of males. Preliminary analyses indicated no significant difference between the various tester sets, so we did not consider this effect further. We also randomized the order of the water treatments. We tested two to three females from each tank of fish. Females that failed to lay any eggs across the six trials were replaced. Occasionally, a female died during the 2 weeks of mating

trials. These females were excluded from analysis, and another female from the same tank was measured instead.

An initial analysis of the data suggested that females spawned more eggs in the tea-stained testing environment than in the clear testing environment. We therefore determined the proportion of eggs spawned with red, yellow and blue males in clear water and the proportion of eggs spawned with red, yellow and blue males in tea-stained water separately. We considered each female in each testing environment a separate observation. We excluded observations where females failed to spawn a minimum of four eggs between the three males in a given testing environment. We then calculated the means for each tank of fish in each of the two testing environments. Originally, there were 134 tanks of fish, although one tank did not have a sufficient number of females to be used. There were another five tanks where the females did not spawn at least four eggs in the two testing environments. Of the remaining 128 tanks, there were 16 tanks where female(s) spawned in only one of the two testing environments, resulting in 240 observations. For the remaining 112 tanks, one to three females spawned a minimum of four eggs in both the clear and the tea-stained testing environments. This resulted in 240 observations ((112 tanks $\times$ 2 testing environments) + (16 $\times$ 1 testing environment) = 240).

We used general linear models to determine the effects of cross (i.e. among-population genetic effects), rearing environment, testing environment, sires and dams, and the appropriate interactions. The full model utilized the nested nature of the data. We considered cross, rearing environment, testing environment and their interactions to be fixed effects. We considered dams and sires and their interactions to be random effects. We tested for the following nested, random effects: sire(cross), sire*rearing(cross*rearing), sire*testing(cross*testing), sire*rearing*testing(cross*rearing*testing), dam(sire*cross), dam*rearing(sire*cross*rearing), dam*testing(sire*cross*testing). Because we performed the analysis on the means of each tank of fish in each testing environment, we could not include the effect of dam*rearing*testing because this was the level at which our data were structured.

The nested nature of the experiment required that the appropriate error terms be used in calculating *F* ratios. We tested the effect of cross using the sire(cross) mean square as the error term. The effects of rearing and cross*rearing were tested using the sire*rearing(cross*rearing) mean square as the error term. The effects of testing and cross*testing were tested used sire*testing(cross*testing) mean square as the error term. The effect of cross*rearing*testing was tested using sire*rearing*testing(cross*rearing*testing) mean square as the error term. The effects of sire(cross), sire*rearing(cross*rearing) and sire*testing(cross*testing) were tested using dam(sire*cross), dam*rearing(sire*cross*rearing) and dam*testing(sire*cross*testing) mean squares as error terms, respectively. The effects of sire*rearing*testing(cross*rearing*testing), dam(sire*cross), dam*rearing(sire*cross*rearing) and dam*testing(sire*cross*testing) were all tested using the mean square error term. We analysed the data using multiple approaches. Our results were robust regardless of whether we used the full nested nature of the experimental design, or whether we tested the main effects (cross, rearing, testing) and their interactions using tanks of fish measured in each testing environment as the level of observation. We performed these analyses using SAS Proc GLM v 9.1 (SAS Institute, Inc., Cary, NC, U.S.A.).

Penny Peck Preferences

We tested for evidence of sensory bias based on correlations between mating preferences and preferences for like-coloured objects ('penny pecking preference'). The effects of cross, rearing environment, testing environment and their interactions on penny pecking preferences are presented elsewhere (Fuller et al., in press). We briefly describe the methodology of the penny peck preferences below.

We measured preferences of bluefin killifish to peck at red, orange, yellow, green, blue, black and white dots from May to June 2007. For each colour, we brushed acrylic paint onto overhead transparencies and allowed them to dry. We used a hole-punch to create coloured discs (6 mm diameter). These discs were attached to a petri dish, colour side up, using silicone. Each petri dish had the seven different coloured dots arranged in a circle. We used several petri dishes and varied the location of the discs such that their locations (and nearest neighbours) on the petri dish varied. Reflectance spectra for the dots are shown in Fuller et al. (in press).

The behavioural assay involved dropping the petri dish in the tank, letting it sink to the bottom, and counting the number of pecks at the red, orange, yellow, green, blue, black and white dots over a 2 min period. The fish generally approached the petri dish and pecked at the dots in a characteristic manner similar to when they approach and eat food. We performed the behavioural assays in the stock tanks, which contained multiple animals. Ideally, we would have tested single individuals isolated in testing aquaria. Preliminary investigations indicated that isolated individuals were too timid to peck at the petri dishes. Also, small groups of fish placed in testing aquaria required 2–3 weeks of acclimation before they would peck. The pecking rate was much higher for animals tested in their stock tanks, so we performed the assays in the stock tanks.

Animals were tested in both clear and tea-stained water. We refer to this as the testing environment. To change the testing environment from clear to tea-stained, we simply added instant tea to the water. To change the testing environment from tea-stained to clear, we emptied the tank and filled it with clear water. We measured penny peck preferences in both original and alternate environments. Animals were in the alternate environment for 1–2 weeks. After assays were completed, we returned the water conditions to their original state (i.e. tea or clear). The relative transmission varied between clear and tea-stained water, with tea-stained water having reduced transmission between 340 and 498 nm (Fuller et al., in press).

We tried to measure peck preferences on two separate occasions under each testing environment for each family of fish (four measurements total between the two testing treatments). However, the fish did not perform pecks in all trials. If the fish did not peck at the dots, then we tested them again 2–3 days later. If the fish did not peck after four separate attempts on 4 separate days, then we abandoned the particular testing environment and tested the fish in the alternate testing environment. Some families only performed pecks in a single trial (in a given testing environment), whereas others performed pecks in two trials. For each tank in each testing environment, we calculated the total number of pecks at the red, orange, yellow, green, blue, white and black dots pooled across the successful trials. From this, we calculated the proportion of pecks at each of the coloured dots. For the analysis, we only considered a tank in a given testing environment if the fish performed at least five total pecks. All trials were performed before noon.

To test the sensory bias hypothesis, we calculated the spearman correlations between mating preferences and pecking preferences for like-coloured objects (e.g. red mating preference and red pecking preference). We measured these correlations at several levels. We used both the dams*rearing environment*testing environment means and the sire*rearing environment*testing environment means. The rational for using the sire*rearing*testing means was that this would reduce the error variance because the means are calculated using multiple observations (i.e. there were multiple observations for dams and 1–2 dams per sire). For both data sets, we calculated the correlations for the complete data set as well as

a data set that included only the hybrid crosses (i.e. spring ♂ × swamp ♀ and swamp ♂ × spring ♀ crosses). The rationale for examining only the hybrids was that this would reduce fixed effects within the spring and swamp populations. For example, one population might have high levels of both red foraging and red mating preference with little causal relationship between the two behaviours.

To examine the overall relationship between pecking and mating preferences, we used canonical correlations analysis. Canonical correlation creates linear combinations for each of the two data sets (mating preference and pecking preference) that maximize the correlations between the two sets of canonical variables. The mating preference data were the proportion of eggs laid with red, yellow and blue males. The pecking data included the proportion of pecks at red, orange, yellow, green and blue dots for each tank of fish measured in each testing environment. We excluded the proportion of pecks at white and black dots because of the large number of zeroes in the data set. We calculated the first two canonical variates for the two data sets. Two was the maximum number of canonical variates that could be calculated because there were three variables for the mating preference data.

We performed this analysis using the four data sets used in the Spearman correlation analysis (i.e. dams*rearing*testing environment means and sire*rearing*testing environment means, for all crosses and for hybrids only). We used canonical correlation analysis (1) to test for a correlation between mating preference and pecking preference and (2) to determine the total amount of standardized variance in mating preference that could be accounted for by the two canonical variates of pecking. For both the canonical correlations analysis and the Spearman correlation analysis, the sample sizes were reduced from the GLM analysis because some animals did not peck (and some females showed no preference) in one or both of the testing environments (see Supplementary Table S1).

Opsin Expression

We use opsin expression to infer qualitative differences in the visual system. We initially measured opsin expression for four to six adult animals (two to three individuals of each sex) from each treatment combination. For each individual, we obtained cDNA by reverse transcribing RNA isolated from eye tissue. To control for circadian rhythms, we euthanized individuals between 1200 and 1400 hours. To control for variation among days, removal and euthanization of animals and isolation of RNA occurred on different days for a given tank. RNA extractions took place in January–March 2007. Each tissue sample was given a unique blind code. We created primers and probes that were unique to each opsin gene (see Fuller et al. 2004). *Lucania goodei* has at least two LWS loci (genbank accession numbers AY296741, AY296740). Preliminary evidence indicates that these opsin proteins do not differ in their spectral properties (N. Blows & S. Yokoyama, personal communication). These two loci have large regions of identical sequence, and we were able to design primers and probes that were common to both alleles.

For each quantitative, real-time PCR (qRT-PCR) reaction, we placed 2 μl of cDNA mixture in a 10 μl reaction with the appropriate primers, probes and taqman mix. For each individual, we performed three replicate reactions for each of the five opsins. The amount of fluorescence was monitored over 40 cycles (94 °C for 15 s, 55 °C for 30 s, 65 °C for 1 min) using the ABI Prism 7700 Sequence Detection System at the University of Illinois. We examined the three replicate reactions for each opsin for each individual and discarded any apparent outliers. Again, our samples were assigned blind codes, so this was done without knowledge of treatment. The SWS2A opsin had very low critical cycle numbers (meaning that it contributed very little to the total pool of opsin cDNA) and ran poorly on some plates, so we excluded it from this analysis. We then determined the average critical cycle number (i.e. the cycle number when the fluorescence exceeded a threshold set close to the background fluorescence) for each individual. Relative opsin expression was calculated as a fraction of total opsin genes for an individual according to the following equation.

$$\frac{T_i}{T_{\text{all}}} = \frac{\frac{1}{(1+E_i)^{C_{ti}}}}{\sum \frac{1}{(1+E_i)^{C_{ti}}}}.$$

T_i/T_{all} is the proportional gene expression for a given gene i. E_i is the PCR efficiency for each primer/probe set, and C_{ti} is the average critical cycle number for each gene. PCR efficiencies were quantified previously with a multigene construct (Fuller et al. 2004).

Preliminary analyses indicated a large effect of PCR plate on opsin expression. We performed an analysis of each opsin for each individual that considered the effects of plate and tank in SAS Proc Mixed. We calculated the least square means for each tank and used these values in this analysis. We initially analysed gene expression on four to six individuals per family, but some of our plates failed, with the result that there were some tanks with missing data.

Similar to the previous analysis, we took a two-pronged approach to discerning the relationship between opsin expression and mating preference. First, we used Spearman correlations to examine the relationship between female mating preferences and opsin expression at several levels. We examined the relationship between female mating preferences and opsin expression separately for the tea-stained and clear water testing environments. This is akin to asking whether vision physiology predicts mating preference in each of the testing environments. We calculated Spearman correlations between preferences for red, yellow and blue males, and the relative expression of SWS1, SWS2B, RH2 and LWS opsins. We originally performed the analysis using both the dams*rearing environment*testing environment means and the sire*rearing environment*testing environment means. We obtained nearly identical results with the two data sets. We present the results for the analysis at the level of sire.

Finally, we used canonical correlations analysis to determine the overall relationship between mating preferences and opsin expression. Again, canonical correlations analysis creates linear combinations for each of two data sets that maximize the correlation between the two data sets. The mating preference data set contained the proportion of eggs laid with red, yellow and blue males in each testing environment. The opsin data set contained the relative expression of SWS1, SWS2B, RH2 and LWS opsins. We tested for a significant correlation between the mating preference and opsin expression data sets, and we determined the proportion of standardized variation in mating preference that could be accounted for by opsin expression. Correlation analyses were performed with Proc Corr, and canonical correlations analyses were performed with Proc Cancorr in SAS v 9.1 (SAS Institute, Cary, NC, U.S.A.).

RESULTS

Genetics, Rearing Environment and Testing Environment

Genetic differentiation in female mating preferences among populations was indicated by the significant effect of cross on red, yellow and blue preference (Table 1, Fig. 2). Females from conspecific spring crosses (spring ♂ × spring ♀) spawned more eggs with red males than females from conspecific swamp crosses (swamp ♂ × swamp ♀) or hybrid crosses with swamp females (spring ♂ × swamp ♀). Females from conspecific swamp crosses spawned more eggs with blue males than did females from the hybrid cross

Table 1
Effects of cross, rearing environment, testing environment, sire nested within cross, dams nested within sires, and their interactions on mating preferences of female bluefin killifish as measured by the proportion of eggs spawned with red, yellow and blue males

Effect	*df*	MS	*F*	*P*	Error term
Red preference					
Cross	**3**	**0.190**	**3.90**	**0.018**	Sire(cross)
Rearing environment (RE)	1	0.139	2.00	0.167	Sire*RE(cross*RE)
Testing environment (TE)	1	0.015	0.19	0.665	Sire*TE(cross*TE)
Cross*RE	3	0.029	0.43	0.736	Sire*RE(cross*RE)
Cross*TE	3	0.060	0.79	0.511	Sire*TE(cross*TE)
Cross*RE*TE	3	0.174	2.36	0.097	Sire*RE*TE(cross*RE*TE)
Sire(cross)	31	0.049	0.77	0.775	Dam(sire*cross)
Sire*RE(cross*RE)	30	0.069	1.45	0.202	Dam*RE(sire*cross*RE)
Sire*TE(cross*TE)	31	0.076	0.94	0.565	Dam*TE(sire*cross*TE)
Sire*RE*TE(cross*RE*TE)	24	0.074	1.12	0.434	Mean square error (MSE)
Dam(sire*cross)	39	0.063	0.96	0.563	MSE
Dam*RE(sire*cross*RE)	19	0.048	0.73	0.741	MSE
Dam*TE(sire*cross*TE)	34	0.081	1.23	0.362	MSE
Mean square error	12	0.066			
Yellow preference					
Cross	**3**	**0.217**	**4.18**	**0.013**	Sire(cross)
Rearing environment (RE)	1	0.050	0.82	0.373	Sire*RE(cross*RE)
Testing environment (TE)	1	0.014	0.23	0.637	Sire*TE(cross*TE)
Cross*RE	3	0.051	0.83	0.487	Sire*RE(cross*RE)
Cross*TE	3	0.045	0.74	0.538	Sire*TE(cross*TE)
Cross*RE*TE	3	0.063	0.88	0.464	Sire*RE*TE(cross*RE*TE)
Sire(cross)	31	0.052	0.56	0.951	Dam(sire*cross)
Sire*RE(cross*RE)	**30**	**0.061**	**2.25**	**0.034**	Dam*RE(sire*cross*RE)
Sire*TE(cross*TE)	31	0.061	0.71	0.835	Dam*TE(sire*cross*TE)
Sire*RE*TE(cross*RE*TE)	24	0.071	1.20	0.379	MSE
Dam(sire*cross)	39	0.093	1.56	0.204	MSE
Dam*RE(sire*cross*RE)	19	0.027	0.46	0.936	MSE
Dam*TE(sire*cross*TE)	34	0.087	1.47	0.243	MSE
Mean square error	12	0.059			
Blue preference					
Cross	**3**	**0.088**	**3.23**	**0.036**	Sire(cross)
Rearing environment (RE)	1	0.022	0.33	0.569	Sire*RE(cross*RE)
Testing environment (TE)	1	0.057	1.40	0.245	Sire*TE(cross*TE)
Cross*RE	3	0.005	0.08	0.970	Sire*RE(cross*RE)
Cross*TE	3	0.002	0.04	0.990	Sire*TE(cross*TE)
Cross*RE*TE	**3**	**0.163**	**3.38**	**0.035**	Sire*RE*TE(cross*RE*TE)
Sire(cross)	31	0.027	0.56	0.950	Dam(sire*cross)
Sire*RE(cross*RE)	30	0.066	1.43	0.208	Dam*RE(sire*cross*RE)
Sire*TE(cross*TE)	31	0.041	0.66	0.879	Dam*TE(sire*cross*TE)
Sire*RE*TE(cross*RE*TE)	24	0.048	1.95	0.115	MSE
Dam(sire*cross)	39	0.049	1.96	0.105	MSE
Dam*RE(sire*cross*RE)	19	0.046	1.86	0.137	MSE
Dam*TE(sire*cross*TE)	**34**	**0.062**	**2.49**	**0.047**	MSE
Mean square error	12	0.025			

Statistically significant effects are listed in bold. Error term denotes the mean square used in the denominator and the corresponding degrees of freedom.

with swamp females (spring ♂ × swamp ♀). Females from hybrid crosses between spring ♂ × swamp ♀ spawned more eggs with yellow males than females from all other crosses.

The environments in which animals were reared and tested affected mating preferences, but these effects depended on cross type. There was a three-way interaction between cross, rearing environment and testing environment that accounted for a significant amount of variation in the preference for blue males (Table 1, Fig. 3c). This interaction was driven by the striking preference of females from conspecific swamp crosses for blue males when raised and tested under tea-stained water. Hence, blue males were only attractive to swamp females that had been raised in tea-stained water and when viewed under tea-stained conditions. Females from conspecific spring crosses also tended to have their highest levels of preference for red males when reared and tested under clear water conditions, although the interaction between cross, rearing environment and testing environment was not

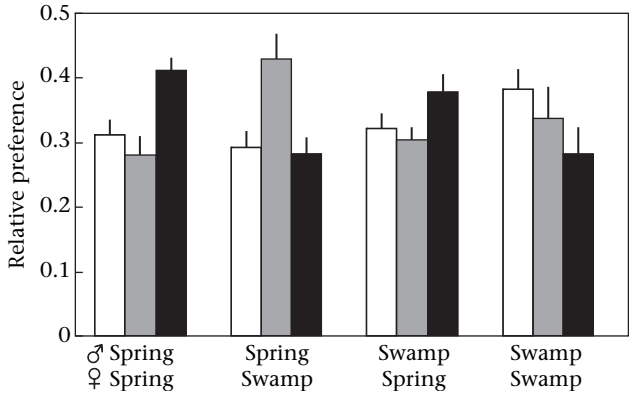

Figure 2. Effects of cross on level of female preference for blue (□), yellow (▒) and red (■) bluefin killifish males. Means and standard errors were calculated using overall sire means.

significant ($P = 0.097$; Fig. 3a). Genetic variation within populations in phenotypic plasticity was indicated for both yellow and blue mating preferences. For yellow mating preference, there was a significant interaction between sires and rearing environment. For blue mating preference, there was a significant interaction between dams and testing environment.

Mating and Pecking Preference

The relationship between pecking preference and mating preference was weak. At the level of dam*rearing*testing, there was no support for the sensory bias hypothesis. The correlations between mating and pecking preference for like-coloured objects were positive, but not statistically significant for red or yellow (Table 2). The correlation between blue pecking and mating preference was negative. This negative correlation was statistically significant when only hybrids were considered.

The relationship between pecking and mating preferences was slightly stronger when analysed at the level of sires (Table 2). The positive correlation between yellow mating and pecking preference was statistically significant for the full data set and also when only hybrids were considered. However, the negative correlation between blue mating and blue pecking preference also strengthened. None of the correlations remained statistically significant after a sequential Bonferroni correction for 12 tests.

More importantly, pecking preferences accounted for little of the variation in mating preferences. Canonical correlations analysis provided little evidence that pecking and mating preferences were related to one another. The hypothesis that the first canonical correlation (i.e. the correlation between the first canonical variate of the two data sets) was 0 could not be rejected for any of the four data sets (dams*rearing environment*testing environment: all crosses: $F_{10,380} = 1.06$, $P = 0.39$; hybrids only: $F_{10,192} = 0.75$, $P = 0.68$; sires*rearing*testing environment: all crosses: $F_{10,242} = 0.97$, $P = 0.47$; hybrids only: $F_{10,116} = 0.89$, $P = 0.54$). Furthermore, the proportion of the standardized variance in mating preference accounted for by pecking preference was less than 8% in all four tests.

Mating Preference and Opsin Expression

The relationship between mating preference and opsin expression was also weak. Of 48 correlations examined, only two were statistically significant (Table 3). Neither of these correlations remained significant after a sequential Bonferroni adjustment.

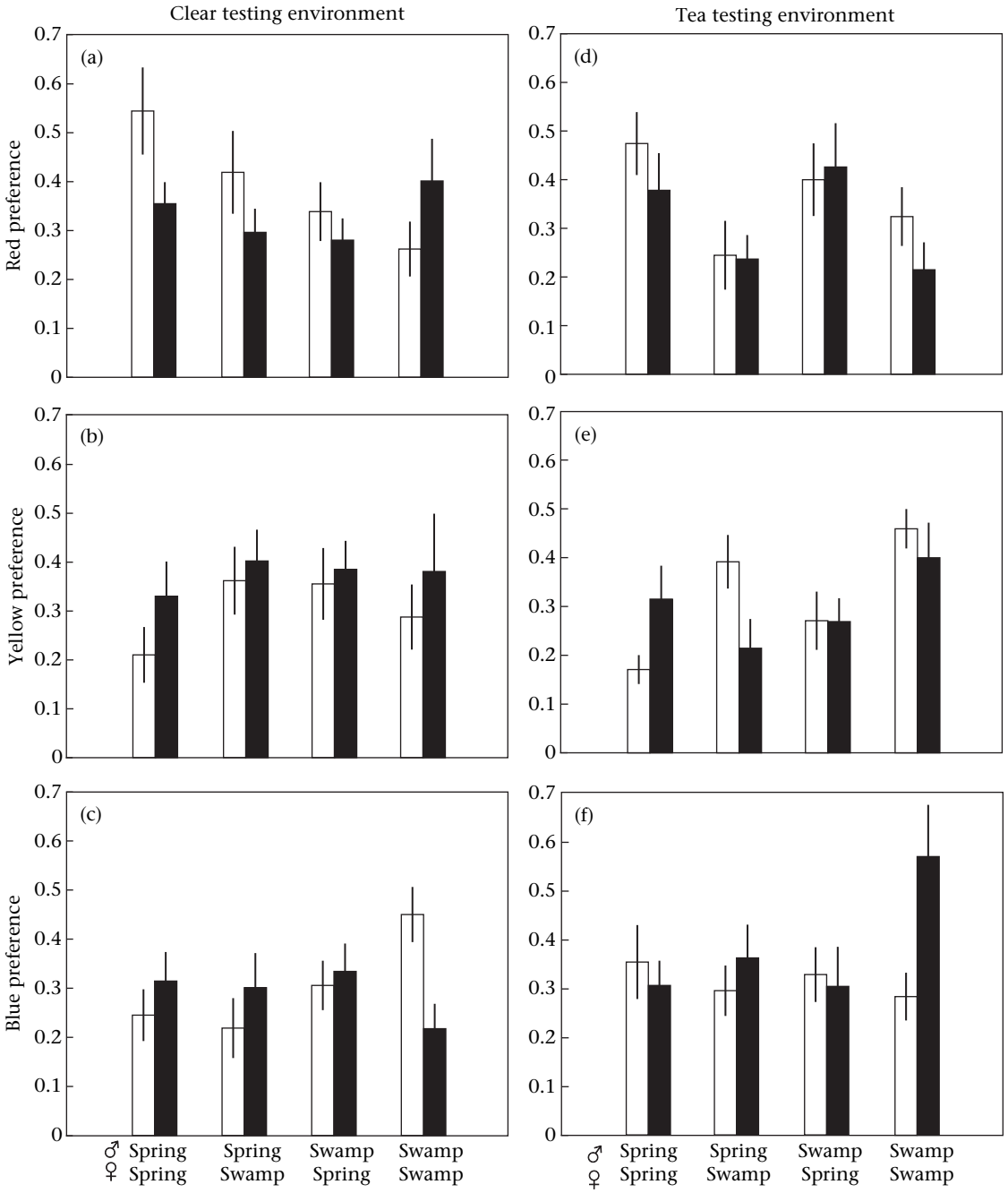

Figure 3. Three-way interaction between testing environment, rearing environment and cross for bluefin killifish. Mean female preferences for (a) red, (b) yellow and (c) blue males as a function of rearing environment and cross when in the clear testing environment. Mean female preferences for (d) red, (e) yellow and (f) blue males as a function of rearing environment and cross when in the tea testing environment. Means and standard errors were calculated using the sire*rearing environment*testing environment means. □: clear rearing environment; ■: tea rearing environment.

Preference for red males was negatively correlated with RH2 expression within the hybrid data set in the clear water testing environment and negatively correlated with LWS expression for the complete data set in the tea-stained testing environment.

The canonical correlations analysis also indicated little relationship between mating preference and opsin expression. Specifically, the analysis could not reject the hypothesis that the correlation between the two data sets differed from zero (clear testing environment: all crosses: $F_{8,120} = 1.06$, $P = 0.40$; hybrids only: $F_{8,58} = 1.19$, $P = 0.3210$; tea testing environment: all crosses: $F_{8,124} = 0.53$, $P = 0.83$; hybrids only: $F_{8,58} = 0.56$, $P = 0.8065$). For the full data set, the two canonical variates accounted for less than 8% of the variation for both testing environments (Table 3). When the data set was restricted to hybrids, the two canonical opsin variates accounted for 13.3% of the standardized variation in preference in the clear testing environment but only 7.5% of the standardized variation in preference in the tea-stained testing environment.

DISCUSSION

This study shows that lighting environments influence female mating preferences via multiple mechanisms in *L. goodei*. Female mating preferences emerged as a result of genetic differentiation

Table 2
Spearman correlations between mating preference and pecking preference of bluefin killifish for like-coloured objects and the proportion of standardized variance in preference accounted for by pecking behaviour according to canonical correlations analysis using dam*rearing*testing means and sire*rearing*testing means

	Dam*rearing*testing		Sire*rearing*testing	
	All $N = 197$	Only hybrids $N = 103$	All $N = 128$	Only hybrids $N = 65$
Red mating–red pecking	0.114 (0.110)	0.107 (0.283)	0.029 (0.746)	0.025 (0.846)
Yellow mating–yellow pecking	0.095 (0.184)	0.134 (0.178)	**0.195 (0.028)**	**0.249 (0.045)**
Blue mating–blue pecking	−0.027 (0.705)	**−0.204 (0.039)**	−0.066 (0.456)	**−0.354 (0.004)**
Standardized variance in mating preference accounted for pecks	2.9%	4.0%	3.9%	7.4%

Uncorrected probability values are listed in parentheses. Values in bold denote statistically significant unadjusted *P* values.

(overall preference of spring females for red males and of swamp females for blue males), but also as a complex interaction between genetics, developmental plasticity and immediate effects of lighting environment (swamp females preferred blue males when reared and tested in tea-stained water). These results suggest that the strength and direction of sexual selection is sensitive to lighting environment and that this involves both developmental plasticity of the visual system and the immediate effects of the lighting environment on the perception of male colour patterns. While there have been studies showing genetic differentiation in female mating preference (Endler & Houde 1995; Gray & McKinnon 2007) and studies showing that different lighting environments can alter female mating preferences (Long & Houde 1989), this is the first study to systematically examine variation in all three components.

Lighting environments can affect female mating preferences via multiple mechanisms that act at different timescales. Long-term rearing under different lighting environments can affect the development of the visual system (Wagner & Kroger 2005; Fuller et al., in press), whereas the lighting conditions that animals are viewed under can have immediate effects on their perception of those colour patterns. Within the behavioural plasticity and behavioural syndromes literature, there are calls to determine the long-term plastic effects of rearing environment on behaviour versus the near-term effects of the 'contextual' environment on behaviour (Stamps & Groothuis 2009). This study provides a clear example of the effects of long-term and short-term environmental effects on animal behaviour and also illustrates the fact that various types of plasticity can interact with one another.

The implications of these plastic effects are that female mating preferences (and the direction of sexual selection) may be highly variable over space and time. Variation in lighting conditions among populations will result in different colour patterns being favoured in different populations. Dispersal between different habitats may result in increased levels of within-population phenotypic variation in female mating preferences. There are high levels of variation in lighting conditions among populations of *L. goodei*, and this variation is correlated with differences in vision physiology and male colour morph abundance (Fuller 2002; Fuller et al. 2004; Fuller & Travis 2004). The two drainages used in this study (Wakulla and Everglades) have relatively low spatial variation in lighting environment. However, the Suwanee/Santa Fe and the St John's are river systems whose headwaters originate in swamps but have clear water springs that connect to the main channel. In these systems, particularly during wet years when the main channel becomes tea-stained with the influx of tannins, fish may be able to move between clear and tea-stained habitats.

Temporal variation in lighting conditions may cause the direction of sexual selection within a population to vary over time. Also, particularly high levels of temporal variation may result in high levels of variation in female mating preference within populations. Water clarity also varies temporally in Florida. During wet years, the influx of tannins causes the waters to be more tea-stained, as noted above. In contrast, during dry years, the water is clearer because there is little incoming organic material and bacterial decomposition reduces the standing levels of dissolved organic material in the water (B. Fugate, personal communication). The extent to which this temporal variation in lighting environment is important for *L. goodei* depends on the degree to which the retinas of these fish can quickly reorganize. If the retinas can reorganize over a short period (i.e. quickly track visual habitats), then variation in female mating preferences within populations may be low. If the retinas require longer periods to reorganize, or if there are critical

Table 3
Spearman correlations between relative opsin expression and female mating preferences in bluefin killifish measured in clear testing environments and in tea testing environments based on means calculated at the level of sire*rearing, and the proportion of standardized variance in mating preference accounted for by the first two canonical variates for opsin expression

	Clear testing		Tea testing	
	All cross types $N = 66$	Hybrids only $N = 35$	All cross types $N = 68$	Hybrids only $N = 35$
Red pref–SWS1	0.033 (0.795)	0.172 (0.322)	0.184 (0.134)	0.029 (0.869)
Red pref–SWS2B	0.067 (0.595)	0.150 (0.391)	0.075 (0.541)	−0.098 (0.577)
Red pref–RH2	−0.159 (0.201)	**−0.337 (0.048)**	0.154 (0.211)	0.163 (0.349)
Red pref–LWS	0.143 (0.252)	0.218 (0.209)	**−0.258 (0.033)**	−0.142 (0.414)
Yellow pref–SWS1	−0.008 (0.949)	−0.165 (0.344)	0.026 (0.833)	0.113 (0.516)
Yellow pref–SWS2B	0.046 (0.715)	0.009 (0.957)	−0.029 (0.813)	0.194 (0.265)
Yellow pref–RH2	0.194 (0.118)	0.211 (0.224)	−0.042 (0.733)	0.008 (0.964)
Yellow pref–LWS	−0.229 (0.065)	−0.191 (0.271)	0.054 (0.660)	−0.098 (0.575)
Blue pref–SWS1	0.033 (0.795)	−0.104 (0.553)	−0.101 (0.411)	−0.004 (0.980)
Blue pref–SWS2B	0.067 (0.595)	−0.068 (0.696)	−0.101 (0.411)	0.016 (0.928)
Blue pref–RH2	−0.159 (0.201)	0.120 (0.491)	−0.125 (0.311)	−0.219 (0.207)
Blue pref–LWS	0.143 (0.252)	0.030 (0.863)	0.159 (0.195)	0.183 (0.294)
Standardized variance in mating preference accounted for by opsins	6.8%	13.3%	3.8%	7.5%

Unadjusted *P* values are listed in parentheses. Values in bold denote statistically significant unadjusted *P* values.

periods when the retinas are most responsive to lighting environments, then variation in lighting environment during development may cause appreciable variation in female mating preferences.

Our finding that females from conspecific spring crosses preferred red males is consistent with census data showing that red males are more abundant in spring populations than in swamps (Fuller 2002). However, this finding is at odds with some of our previous work on mating preferences. McGhee et al. (2007) measured mating preferences of spring females in clear water and found no overall preference for red males over yellow males. Furthermore, multiple studies involving spring females choosing between yellow and red males have found no evidence of an overall female mating preference using dichotomous choice tests in clear water (R. C. Fuller, unpublished data). However, Fuller & Johnson (2009) performed a study testing for negative frequency dependence between yellow and red males using males and females from the spring population. In that study, animals were allowed to mate freely in stock tanks containing various ratios of red to yellow males. While that study found no evidence for negative frequency dependence, it did find a slight mating advantage for red males. We have no explanation as to why these different experimental approaches produced different results. A recent meta-analysis by Bell et al. (2009) suggests that mating preferences have lower repeatability than other types of behaviours.

Our finding that females from conspecific swamp crosses preferred blue males is also consistent with census data showing that blue males are more common in swamp habitats (Fuller 2002). The particularly interesting pattern is the degree of plasticity in blue male mating preference among females from conspecific swamp crosses. Previous work on the genetics of the male colour pattern indicates that the expression of blue coloration in males is also very plastic and that males are more likely to express blue coloration when reared under tea-stained conditions (Fuller & Travis 2004). Males from conspecific swamp crosses also have higher levels of plasticity in blue colour pattern expression than do males from conspecific spring crosses (R. C. Fuller, unpublished data). Swamp males are particularly plastic and are quite likely to express blue coloration under the conditions when blue should be favoured via female choice.

In terms of fitness, we have no explanation for why spring females preferred red males and swamp females preferred blue males. However, in terms of visual ecology, the results are striking and suggest that female mating preferences favour males that contrast with their visual backgrounds. Red preference was highest among females from conspecific spring crosses. Spring populations have high transmission of UV/blue wavelengths and a large amount of blue backscatter. In contrast, blue preference was highest among swamp females, particularly when females were raised and tested in tea-stained water. This is ironic because tea-stained water results in lower expression of SWS1 and SWS2B opsins (which is correlated with lower abundance of UV and violet cone cells). Tea-stained water also filters out the UV and blue wavelengths. Other fish species have provided strong support for the idea that female mating preferences favour males that contrast with their visual backgrounds. In three-spine sticklebacks, *Gasterosteus aculeatus*, males with red bellies are much more common in clear, blue-shifted waters (Reimchen 1989; Boughman 2001; Scott 2001). The hypothesis is that red coloration creates high contrast against a background high in blue backscatter but has lower contrast in tea-stained waters (Boughman 2001; Lewandowski & Boughman 2008). In *Telmatherina sarasinorum*, blue and yellow male colour morphs are found most frequently in visual habitats that maximize their contrast with background conditions (Gray et al. 2008). However, this pattern is not universal. Work in cichlids generally indicates a scenario where red males are more common in deeper habitats, which are relatively high in red wavelengths, whereas blue males are more abundant in shallow habitats, which are relatively high in blue wavelengths (Seehausen et al. 2008). Similarly, Endler & Houde (1995) found that female guppies from 'orange-shifted' waters (i.e. more tannin stained) had stronger preferences for males with orange and black coloration. It is unclear why some fish use colours that match the predominant wavelengths of light in their environments and other fish use colours that contrast with the predominant wavelength of light.

Mating Preferences, Pecking Preference and Sensory Bias

The sensory bias hypothesis predicts that selection on nonmating behaviours results in correlated responses in mating preferences that are mediated by a shared sensory system and underlying pleiotropic effects (Kirkpatrick & Ryan 1991; Fuller et al. 2005b). One hypothesis that arises from this is that there should be strong correlations between preferences for like-coloured objects (e.g. red food and red mates; Fuller 2009). The evidence for this was weak. Table 4 summarizes the effects of cross, rearing environment, testing environment and their interactions on pecking preferences (Fuller et al., in press) and on mating preferences (this study) as well as the effects of rearing environment, cross and their interaction on opsin expression (Fuller et al., in press). The first point to emerge is that pecking preferences and mating preferences were not affected by the same treatments. Pecking preferences were strongly affected by rearing environment, testing environment and their interaction, whereas mating preferences were affected by cross and the interaction between cross, rearing and testing environment. Similarly, there was no evidence for strong, positive correlations between like-coloured objects (red pecking preferences and red mating preferences, etc.) that would indicate that female mating preferences evolve to appreciable levels as correlated responses to selection on nonmating behaviours such as foraging. Instead, the results suggest that the two behaviours are independent of one another and that lighting environments affect these behaviours through different mechanisms.

Proponents of sensory bias argue that sensory bias acts in conjunction with other models of sexual selection and that low correlations may be sufficient in this scenario. A number of models of sexual selection can result in the evolution of higher levels of female mating preferences and male secondary sex traits provided that female mating preferences are variable and greater than zero (Lande 1981; Kirkpatrick 1982; Pomiankowski 1987; Payne & Pagel 2001). The idea is that sensory bias could essentially 'get the ball rolling' and result in the evolution of low levels of female mating preferences that differ from zero, which are then subsequently elaborated through other evolutionary mechanisms (Ryan & Rand

Table 4
Overview of results for opsin expression, mating preferences and pecking preferences of bluefin killifish as a function of rearing environment, testing environment and cross

Statistical effects expression	Opsin expression	Mating preferences	Pecking preference
Rearing environment (RE)	Yes	No	Yes
Testing environment (TE)	NA	No	Yes
RE × TE	NA	No	Yes
Cross	Yes	Yes	No
Cross × RE	No	No	No
Cross × TE	NA	No	No
Cross × RE × TE	NA	Yes	No
Reference	Fuller et al. (in press)	This study	Fuller et al. (in press)

NA = not applicable.

1993; Phelps & Ryan 2000; Jennions & Brook 2001; Ryan et al. 2001; Arnqvist 2006). In this study, the signs of the correlation coefficients between mating and pecking preferences were positive for both red and yellow. When the analysis was performed at the level of sires, the correlation between yellow mating and yellow pecking preference was statistically significant for both the full data set and the data set that was limited to hybrids, although these results were not significant after a sequential Bonferroni correction. Performing quantitative genetic studies on behaviour requires large sample sizes, and this is particularly the case when trying to test the hypothesis that low-level genetic correlations are significantly different from zero. This study clearly lacks the power to determine whether low-level genetic correlations between mating and pecking preferences are significantly different from zero. However, the data at hand suggest that these relationships are not strong.

Mating Preferences and Opsin Expression

The weak correlations between female mating preferences and opsin expression highlight the fact that opsin expression does not directly equate with female mating preference. There were no overall correlations between opsin expression and female mating preference that remained statistically significant after a sequential Bonferroni correction. Again, this is not surprising given that opsin expression and female mating preferences were influenced by different treatment effects (Table 4). The relative expression of SWS1, SWS2B and LWS were strongly influenced by rearing environment (Fuller et al., in press), where SWS1 and SWS2B had higher levels of expression in clear water conditions and LWS had lower levels of expression in tea-stained conditions. Only SWS1 was significantly affected by cross, where animals from spring parents had higher levels of SWS1 expression than animals from swamp parents. Hence, the highest levels of SWS1 expression were found in spring animals that had been raised in clear water, yet these animals preferred red males. The highest levels of LWS expression were found in animals raised in tea-stained water. Yet, at least in the case of animals from swamp crosses, these animals were more likely to prefer blue males. This mismatch between opsin expression and mating preference supports the hypothesis that animals possess visual systems that are attuned to the distribution of wavelengths in their environments and that females prefer males with colour patterns that contrast with their visual backgrounds.

This is not to say that there is no role for opsins in the evolution and expression of female mating preferences. Females can only respond to cues that they can perceive. The spectral sensitivities of the cone cells determine the wavelengths of light to which they can respond. Shifts in opsin genetic sequences may alter spectral sensitivities (Yokoyama 1997), as can shifts in chromophore usage (i.e. whether the opsin is attached to 11-cis-retinal or 11-cis-3,4-dehydroretinal; Partridge & Cummings 1999). Similarly, the filtering properties of structures such as ellipsosomes (Flamarique & Harosi 2000) and the general wiring of the retina may affect female mating preferences. Sophisticated models of animal vision also use data on contrasts between the excitation of different cone classes, and the magnitudes of these contrasts may be affected by the relative cone abundances (Vorobyev & Osorio 1998). Still, the idea that higher LWS expression leads to higher preference for red males or that higher SWS1 or SWS2B expression leads to higher levels of preference for blue males is overly simplistic. Obviously, animals possess central nervous systems that allow them to assign value to the cues they perceive in their environments. The relative importance of neural processing in the peripheral visual system versus the importance of neural processing in the brain on female mating preferences is unknown and has important implications for where we should place our effort in determining the physiological mechanisms of animal behaviour.

In conclusion, this study shows that lighting environments can affect female mating preferences via a diversity of mechanisms. The offspring of spring parents preferred red males over yellow and blue males. Females of swamp parents preferred blue males and this was particularly so when females were reared and tested under tea-stained conditions. The latter result suggests that the high abundance of blue males in swamps may be attributable to the genetic properties of females in those populations, the developmental plasticity induced by living in tea-stained water, and the immediate effects of tea-stained water on the perception of blue males. An earlier study using these same families and treatments found effects of lighting environments on foraging preferences (as inferred by a pecking test) and opsin expression (Fuller et al., in press). Despite the fact that lighting environments affected all three traits (mating preference, pecking preference and opsin expression), there was little relationship among them. Hence, lighting environments greatly affect mating and pecking preferences as well as opsin expression, but they do so by independent mechanisms.

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Supplementary Material

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