

A possible non-sexual origin of mate preference: are male guppies mimicking fruit?

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In most animals, the origins of mating preferences are not clear. The ‘sensory-bias’ hypothesis proposes that biases in female sensory or neural systems are important in triggering sexual selection and in determining which male traits will become elaborated into sexual ornaments. Subsequently, other mechanisms can evolve for discriminating between high- and low-quality mates. Female guppies (*Poecilia reticulata*) generally show a preference for males with larger, more chromatic orange spots. It has been proposed that this preference originated because it enabled females to obtain high-quality mates. We present evidence for an alternative hypothesis, that the origin of the preference is a pleiotropic effect of a sensory bias for the colour orange, which might have arisen in the context of food detection. In field and laboratory experiments, adult guppies of both sexes were more responsive to orange-coloured objects than to objects of other colours, even outside a mating context. Across populations, variation in attraction to orange objects explained 94% of the inter-population variation in female mate preference for orange coloration on males. This is one of the first studies to show both an association between a potential trigger of a mate-choice preference and a sexually selected trait, and also that an innate attraction to a coloured inanimate object explains almost all of the observed variation in female mate choice. These results support the ‘sensory-bias’ hypothesis for the evolution of mating preferences.

Keywords: sensory bias; mate choice; guppies; *Poecilia reticulata*

1. INTRODUCTION

Organisms assess a wide variety of traits when choosing a mate (Andersson 1994). These traits may directly or indirectly reveal the quality of a mate (Kirkpatrick & Ryan 1991; Maynard Smith 1991; Rowe & Houle 1996). There is evidence that a number of mechanisms have been involved in the evolution of mate preferences (good genes and Fisherian models; Andersson 1994), however the origins of the preferences are more contentious (Enquist & Arak 1993; Marler & Ryan 1997; Endler & Basolo 1998; Payne & Pagel 2001). Evidence is now accumulating that mate-choice criteria are sometimes derived from pre-existing biases of the sensory system (Basolo 1990, 1995; Christy 1995; Phelps & Ryan 1998; Proctor 1991, 1992; Ryan 1990, 1998; Ryan & Rand 1993; West-Eberhard 1984). For example, in a well-documented case, male water mites mimic prey in order to attract the attention of females (Proctor 1991, 1992). It has been suggested that males may be mimicking food resources in other invertebrates (spiders (Clark & Uetz 1992), *Xylocopa* bees (West-Eberhard 1984), *Photuris* fireflies and oriental fruit moths (Christy 1995)) and that a pre-existing bias may also explain the correspondence between food preferences and plumage coloration in several avian species (Dücker 1963).

Here, we test the pleiotropic sensory-bias hypothesis in guppies (*Poecilia reticulata*) (Family: Poeciliidae) and ask whether sensory bias can explain geographical variation in mate preference. These small freshwater fishes have become a model system for studying mate-preference evolution, in part because male sexual traits and female mate preferences vary geographically (Endler 1980, 1995; Endler & Houde 1995; Houde 1997). However, the mate-choice decisions of females from many populations are influenced by the carotenoid-containing orange spots of males (Houde 1987; Kodric-Brown 1989; Nicoletto 1991; Endler & Houde 1995; Grether 2000). While the size of orange spots is genetically controlled (Houde 1992), the chroma (colour saturation) is condition dependent and depends on carotenoid ingestion (Grether 2000; Kodric-Brown 1989) and parasite load (Houde & Torio 1992). These findings support the hypothesis of Endler (1980) that female guppies use the orange spots as indicators of male foraging ability and health. However, a new study shows that female preferences do not covary with geographical variation in carotenoid limitation as this hypothesis predicts (Grether 2000). Why, then, has the preference for carotenoid coloration evolved?

We have observed that both male and female guppies in natural populations are attracted to and voraciously consume orange-coloured food items, including orange-coloured fruit. The bright orange fruits produced by the cabrehash tree (Elaeocarpaceae: *Sloanea laurifolia* (Williams & Cheesman 1929)) and another unidentified

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tree species appear to be preferred, but apparently rare, food sources for guppies in Trinidad where food is limited (Reznick *et al.* 2001). The fleshy aril of the *S. laurifolia* fruit is a good source of protein, sugar (Keeler-Wolf 1988) and carotenoids (D. F. Millie, personal communication) and is consumed by fishes and frugivorous birds (Keeler-Wolf 1988). We have observed that, soon after these ripened fruits fall into a pool, most or all of the guppies present begin feeding on them with considerable competition for access to the fruits. *Sloanea laurifolia* is distributed, like guppies, in northeastern South America and on the islands of Trinidad and Tobago (Smith 1954). In Trinidad, it grows on both the north- and south-facing slopes of the Northern Range (Marshall 1939), areas where guppies have been studied intensively.

Our observations of guppies foraging on these fruits suggested a possible non-sexual origin of the female mate preference for orange males; if the visual system of guppies is tuned to detect orange food items preferentially, the mate preference for males with larger or more chromatic orange spots might be a pleiotropic effect of selection in a foraging context. At least two testable predictions can be made from this hypothesis:

- (i) males as well as females should be attracted to orange objects outside the mating context; and
- (ii) variation among populations in attraction to orange objects should explain some of the inter-population variation in female mate preferences.

2. MATERIAL AND METHODS

(a) *Field study*

We measured the attraction of guppies to different colours in natural populations in the Northern Range in Trinidad during the dry season (March–April) in 1996 and 1999. These tests were done in pools in small streams, separated by riffles, or along edges of quiet stretches of rivers. We used small, painted discs to measure responsiveness to colour at these sites. In 1996, the discs were made from thin, transparent plastic painted with Liquitex acrylic paint (see below and table 1). In 1999, to make a more durable facsimile of those discs, Trinidadian dimes (1.6 cm in diameter) were thickly painted with the same acrylic paint, ensuring that all metal was covered.

For each trial, a single coloured disc was placed on a leaf retrieved from the bottom of one of the pools in the stream (black discs were placed on a light-coloured leaf, the other colours were all placed on the same dark leaf) and covered with a small rock. Particulate matter was allowed to settle and the fishes to return to the area before the rock was removed and the observation session was started. Each observation session (i.e. period during which a specific colour was tested) ran for 5 min.

We recorded the sex, stage class (adult versus juvenile) and behaviour of individuals swimming near the disc, including approaches to within 2.5 cm of the disc, and pecking (nibbling) at the disc. We distinguished between approaches and pecks because approaches might assay attraction from a distance while pecks might assay motivation to eat an object. The density of guppies in a pool probably influenced the number of approaches to a particular disc, potentially increasing the noise in the data and making the statistical analyses conservative. We attempted to mitigate this source of bias by placing the disc only in pools where at least six individuals were present during a preliminary

scan and by placing it in a section of the pool where guppies were present before the observation was started. For the majority of the tests, many individual guppies visited the discs. A different pool was used for each observation of each different colour and colours were tested in random order, so this procedure should have avoided confounding density with colour. Replicates for each site were made by moving up- or downstream to a new pool or section of stream.

Tests were performed in five low-predation sites and three high-predation sites. Low-predation sites were: Quare River tributaries I, II and VII, a tributary to the Marianne River, and a tributary of the Arima River, Tripp Trace. The predators present were the small killifish (*Rivulus hartii*) or the prawn (*Macrobrachium crenulatum*) that do not appear to be serious predators of guppies (Endler 1978, 1983; Reznick *et al.* 1996). Our high-predation populations were in the lower Quare, Ceniza and Mausica Rivers (see Reznick *et al.* (1996) for localities). High-predation sites are those where guppies co-occur with the pike cichlid (*Crenicichla alta*) and males tend to have fewer, smaller spots (Endler 1978, 1983).

For most sites, we tested each of the five colours at least twice for a total of 88 tests. At three sites (Mausica and Quare Tributaries I and VII), each colour was tested three or four times. At two sites (Ceniza and Marianne), because of inclement weather, we were only able to test each colour once. The data were later summarized for analysis using the OBSERVER program (v. 2.01, 1989–1991) (Noldus 1991). The OBSERVER tabulated the frequencies of each behaviour–identity combination observed in each colour trial. For all classes of guppies, the dependent variables, ‘total number of approaches within 2.5 cm of the disc’ and ‘total number of pecks of the disc’, were transformed using the formula $\sqrt{x + 3/8}$ (Zar 1996). This produced residuals that were normally distributed and homoscedastic. When the total number of approaches or pecks to a particular colour at all sites was less than three, data for that colour-class combination were excluded from the analysis. All statistical tests were performed in JMP (SAS Institute, Inc. 1997). For brevity, we report the effects of site (population) only if the site main effect or any interactions involving site were significant.

(b) *Laboratory experiment*

We used 149 second-generation laboratory descendants of guppies from six populations in the Northern Range of Trinidad to measure attraction to coloured discs and female mate preference. Experimental fish were derived from two sites in each of three river drainages in Trinidad: the Marianne, Paria and Quare river drainages. Each of the six sites was separated from each other by multiple barriers to gene flow, including two or more waterfalls. All sites were low-predation sites. One hundred and forty one wild females contributed offspring to the F₁ generation (15–27 per population). The number of male founders was probably much larger since females mate multiply in the wild and store sperm (Kelly *et al.* 1999; Houde 1997). In the F₁ generation, the sexes were separated before males matured. After sexual maturity, unrelated F₁ fishes were paired to produce outbred F₂ offspring that were used in the mate-choice and colour-attraction tests. Fishes to be used in the coloured-disc experiment were reared from birth to maturity on brown-coloured flake food. For more than one week prior to the start of the tests, they were fed orangey-brown *Artemia* nauplii and Tetramin flake food (greens, browns and reds) and housed in single-sex aquaria (38 l) (7–12 non-virgin fishes per aquaria, two aquaria per population).

Table 1. Spectral parameters of the coloured discs.

(P_T and D_{max} are guppy-specific estimates of perceived brightness and chroma, respectively. Hue is measured in degrees (0–359), with pure red, yellow, green and blue at 0, 90, 180 and 270, respectively. Chroma is a measure of colour saturation ranging from 0 to 1. R_T is the percentage of the white standard's total reflectance. Reflectance spectra are shown in figure 1. See § 2 for further details.)

colour	material	P_T	D_{max}	hue	chroma	R_T
red	plastic	0.27	0.80	11.87	0.69	18.93
red	metal	0.21	0.89	12.44	0.74	18.84
orange	plastic	0.47	0.91	29.58	0.64	24.76
orange	metal	0.50	0.94	38.60	0.61	22.88
yellow	plastic	1.28	0.95	52.33	0.48	41.41
yellow	metal	1.49	0.97	57.46	0.49	46.91
green	plastic	0.66	0.91	163.82	0.44	11.40
green	metal	0.69	0.93	163.09	0.49	11.92
black	plastic	0.12	0.48	253.06	0.27	2.07
black	metal	0.12	0.59	245.93	0.18	2.12
white	plastic	3.63	0.82	75.25	0.05	82.02
blue	plastic	0.18	0.41	266.78	0.49	2.77
purple	plastic	1.50	0.60	303.02	0.22	34.11

Attraction to coloured discs was measured by recording numbers of approaches to and pecks at coloured discs using simultaneous-choice tests on individual fishes. Discs were constructed by painting a thick layer of Liquitex acrylic paint on a thin sheet of plastic (overhead transparency) and using a hole punch to cut 1.3 cm diameter discs from the sheets. The discs were then glued, plastic side up, onto cover slips. Each fish was presented with eight different coloured discs (see following text and table 1) that were evenly spaced across the bottom of a large test tank (90 cm × 30 cm × 45 cm). The bottom of the tank was covered with natural-coloured gravel and the right- and left-hand sides of the tank were covered with beige paper. We established zones around discs by burying a clear plastic dish (12 cm in diameter) under each disc. Only the rim of the dish was exposed, providing an inconspicuous boundary around each colour. A line was drawn on the side of the tank, 6 cm above the gravel, to delimit the upper boundary of a cylindrical zone around each coloured disc. All observations were conducted with this line at eye level. The tank was illuminated with four incandescent light bulbs and one full spectrum fluorescent bulb spanning the full length of the tank; all lights were suspended above and slightly behind the tank. These bulbs were the only source of lighting during the tests. At the beginning of each test, all eight coloured discs were collected and randomly assigned a position in the aquarium using a random-number generator. We placed one guppy in a small, clear plastic tank, half filled with water. This tank was floated on the surface of the water in the test tank for a 5 min acclimatization period. The individual was then released and we recorded the number of approaches into each colour zone for 7 min. We used the event-recording program ACTION RECORDER (L. Watts, University of York, York, UK) to record the fish's entry and exit from each zone and to record the number of times that the fish pecked the discs.

We also measured total time spent within each zone (duration). However, approaches and durations were not independent and results for both were very similar, so we only present results for the frequency of approaches. The three individuals that did not enter any colour zones were deleted from these analyses. Number of approaches to the zones was

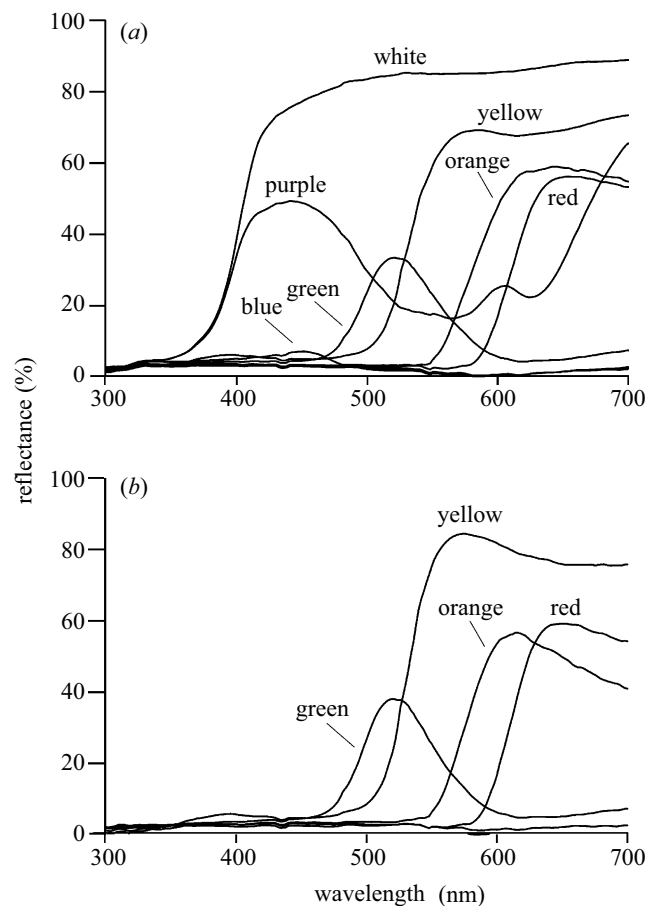


Figure 1. Reflectance spectra of the coloured discs used in (a) the first field study and in the laboratory experiment (clear plastic) and (b) the second field study (metal coins).

transformed using natural logarithms to meet the ANOVA assumptions. Number of pecks and the frequency and duration of visits to the colour zones were analysed using a repeated-measures analysis of variance (SAS Institute, Inc. 1989) with the eight different colours entered as the repeated measure. We used the contrast option to compare the response to orange with responses to the other colours. The independent variables in the analysis were sex, site and tank. The tank was nested within the sex and site variables. Interactions amongst these variables were also considered. The F values were adjusted using the Huynh-Feldt estimator (SAS Institute, Inc. 1989). All conclusions regarding the laboratory study remain unchanged after correction for multiple tests. There are 27 p values in the laboratory analyses so we would only expect 1.3 tests to be significant by chance alone.

We measured mate preferences within the same populations by giving females a choice between males raised on diets with three different levels of carotenoid pigments (<0.16, 13.84, or 199.02 ppm) that caused males to express different levels of orange chroma (see Grether (2000) for further details).

(c) Coloured discs

The following Liquitex acrylic pigments were used to create the coloured discs: red (naphthol crimson, munsell hue (MH) = 6.0R), orange (indo orange red (perinone orange), MH = 9.5R), yellow (brilliant yellow cadmium yellow medium hue, MH = 1.3Y), white (titanium white, MH = white), green (green light, permanent (mixture), MH = 1.2G), blue

(ultramarine blue, MH = 8.3BP), black (ivory black, MH = black) and purple (brilliant purple (mixture), MH = 1.8 P).

Reflectance of the coloured discs (figure 1) was measured with a diode-array spectroradiometer (Ocean Optics S-2000 optimized for ultraviolet (UV) and visible light, with a PX-2 xenon strobe and R-400 reflectance probe). Reflectance spectra were calculated by reference to a white standard (Oriol Spectralon) and processed to yield estimates of quantum catch by each of the four classes of guppy photoreceptor cones (see Endler 1991). Absorbance functions for guppy cones were calculated from the published λ_{\max} values of 389 (UV), 410 (s), 465 (m), and 543 nm (l) (Archer & Lythgoe 1990) using equations and parameters provided in Stavenga *et al.* (1993) and an optical density of 0.3 (based on table 11.1 in Nicol & Somiya (1989)). The photon catch for each cone class was estimated from $P_{ij} = \sum_k r_{jk} a_k t_k s_{jk}$ where i refers to the cone class (UV, s, m, or l), r_{jk} is the mean reflectance of orange spot j at wavelength k , a_k is the ambient irradiance at wavelength k above the observation aquarium, t_k is the transmission fraction through 0.25 m of clear water at wavelength k (from fig. 5 in Endler (1991)) and s_{jk} is the spectral sensitivity or absorbance of cone class j at wavelength k . In the absence of information on how the visual system of guppies weighs input from different cone classes, the total cone photon catch $P_T = \sum P_i$ provides the best estimate of perceived brightness (Endler 1991). Perceived chroma (D_{\max}) was estimated as the maximum relative cone contrast (Endler 1991). For comparative purposes, non-species specific estimates of hue, chroma and brightness (R_T) were calculated using the segment classification method (Endler 1990) for the human visible spectrum (400–700 nm).

3. RESULTS

We tested our first prediction, that males as well as females should be attracted to orange objects outside the mating context, in both field and laboratory experiments. First, we measured the attraction of guppies to different colours in natural populations in the Northern Range in Trinidad. We found that guppies of all age and sex classes showed a strong attraction to the orange-coloured discs. At seven of the eight sites, adult females approached and pecked orange discs significantly more often than discs of any other colour, except red (approaches: orange versus all colours except red: $F_{1,48} > 19.5$, $p < 0.0001$, orange versus red: $F_{7,48} = 1.57$, $p > 0.19$; pecks: orange versus all colours except red: $F_{1,48} = 41.2$, $p < 0.0001$, orange versus red: $F_{1,29} = 2.9$, $p > 0.10$). At the Ceniza site, females were more likely to approach the green disc than the orange one ($F_{1,48} = 12.85$, $p < 0.0001$). However, there was variation among the sites in the frequency at which adult females approached the orange disc ($F_{1,48} > 43.0$, $p < 0.0001$), even when the site showing the lowest response to orange was excluded ($F_{1,48} = 11.95$, $p < 0.0012$). Across all sites, adult females pecked the orange disc 117 times, the red disc 68 times and the others much less frequently (black, three times; green, seven times; and yellow, three times). Adult males approached orange discs significantly more often than discs of any other colour ($F_{1,34} = 24.9$, $p < 0.0001$) and were more likely to peck the orange or red discs than discs of other colours (over all populations, 88 of 91 pecks were of orange or red discs and there was no overall difference in how often males pecked orange versus red ($F_{1,13} = 2.25$, $p > 0.15$)). Juveniles pecked only orange or red discs (with

the exception of one peck at a green disc at one site) and did not discriminate between orange and red ($F_{1,20} = 0.02$, $p > 0.90$). Juveniles showed no significant pattern in approaches ($F_{3,33} = 0.46$, $p > 0.70$). We found no indication of a difference between guppies from high- versus low-predation populations; however, only three high-predation sites were examined.

We also used a common garden experiment to measure genetically based variation among populations in attraction to coloured discs, and then compared disc attraction to female preference for carotenoid coloration on males. In general, adult guppies showed a strong attraction to orange discs in the laboratory study. Females from all populations pecked at orange discs significantly more often than the other colours (pecks of orange versus all other colours (excluding the 27 females that did not peck any disc): $F_{1,36} > 9.06$, $p < 0.0047$). The site by colour interaction was not significant ($F_{10,62} = 0.93$, $p > 0.49$). Females approached orange significantly more often than all other colours except red (orange versus red: $F_{1,62} = 0.17$, $p > 0.67$; orange versus the other colours: $F_{1,62} = 15.44$, $p < 0.0002$). Males pecked orange and red more often than all other colours (pecks of orange versus all colours except red: $F_{1,59} > 30.9$, $p < 0.0001$; orange versus red: $F_{1,59} = 3.08$, $p > 0.084$; 45 males did not peck at any colour). Males approached orange, red, yellow, and green discs equally frequently ($F_{1,59} < 1.62$, $p > 0.20$) and the other colours significantly less often (orange versus black, white, blue, and purple: $F_{1,59} > 4.79$, $p < 0.03$). Overall, females showed a greater relative interest in orange than males, while males showed a greater relative interest in yellow, green and white (frequency of approaches: $F_{1,121} > 4.61$, $p < 0.034$). It is improbable that our laboratory fishes would have learned to associate only the colours orange and red with food, since they were raised on multicoloured food.

A second prediction that can be made from the hypothesis that attraction to orange food items has contributed to the mate preference for carotenoid coloration is that, across populations, the strength of this preference should be positively correlated with attraction to orange objects in both sexes. In support of this prediction, we found a strong and highly significant correlation between female mate preference and female pecking behaviour (figure 2). Remarkably, this association accounted for 94% of the variation among populations in the strength of the female mate preference for carotenoid coloration in males ($n = 6$, $p = 0.0004$). We also found a strong and significant correlation between female mate preference and male pecks of the orange discs, accounting for 74% of the variation in female mate preference ($n = 6$, $p = 0.0249$). These correlations for females (0.97) and males (0.86) are statistically indistinguishable ($t = 0.472$, $p > 0.5$) (Sokal & Rohlf 1995). There was a significant correlation between male and female pecks of the orange discs ($r = 0.88$, $n = 6$, $p = 0.018$). One possible explanation for the association between female preference and pecks is that the pecks of the discs reflect a tendency to investigate novel objects. This seems improbable because only six females and five males pecked a disc that was not red or orange, and all of those fishes, except one of the females, also pecked the orange or red disc. Neither female nor male pecks of the red disc correlated well with female preference slopes

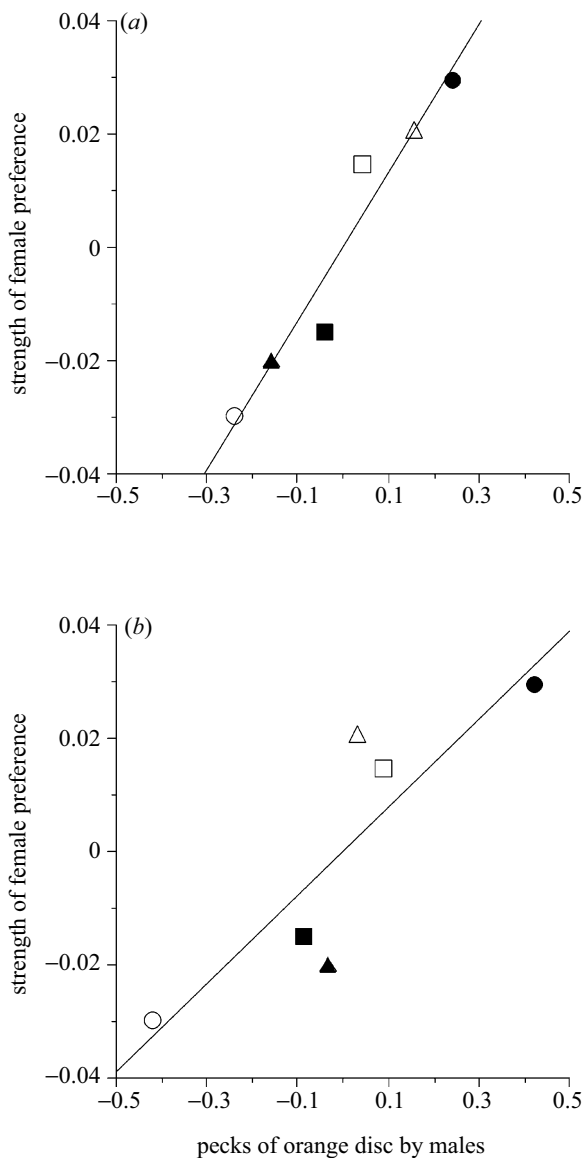


Figure 2. Relationship between the strength of the female mate preference for carotenoid coloration and the rate at which guppies of both sexes ((a) females, (b) males) pecked orange discs. Female-preference strength was measured as the slope of male attractiveness on the log carotenoid concentration of male diet; male attractiveness was measured as the proportion of a male's courtship (sigmoid) displays that elicited a sexual (glide) response from a female (see Grether (2000) for further details). The values plotted on both axes are residuals from river drainage means, to control for genetic variation among drainages. River drainage is indicated by the shape of the symbol (circle, Paria; square, Marianne; triangle, Quare). Sites with relatively open (closed) forest canopies in their respective drainages are indicated by unfilled (filled) symbols. For further information on these sites, including GPS coordinates, see Grether *et al.* (2001).

(females $r = -0.47$, $p = 0.38$; males $r = 0.51$, $p = 0.33$). Interestingly, there was a negative correlation between male and female pecks of the red disc ($r = -0.94$, $p = 0.0024$). Finally, we also asked whether there was an association between mean female pecks of the orange discs and the mean area of orange coloration on males from the

same population; there was not ($r = -0.05$, $p = 0.93$, $n = 6$).

4. DISCUSSION

We infer that guppies of both sexes are innately attracted to orange, and sometimes red, objects. Attraction of guppies to these colours may have evolved for detecting rare food sources (such as the cabrehash fruit). If so, the female mate preference for carotenoid coloration may have arisen as a pleiotropic side-effect of foraging behaviour. The reverse scenario (attraction to orange objects arising as a side-effect of the female mate preference for carotenoid coloration) seems less plausible because males, as well as females, were attracted to orange objects. Although it could be argued that male attraction to orange objects is the result of a genetic correlation between the sexes, this seems improbable because females from one population (Tripp Trace) apparently lack mate preference for carotenoid coloration (Endler & Houde 1995), but still showed a strong attraction to inanimate orange objects. This indicates that females can be attracted to orange objects without a corresponding mate preference. If attraction to orange objects arose as a side-effect of the female mate preference, it would be difficult to explain why an attraction to orange objects persists in both males and females after the mate preference for orange coloration is lost.

Our observations therefore suggest that female mate preference for orange males resulted initially from a genetic correlation with foraging behaviour. The inter-population variation in attraction to orange discs may have resulted from genetic drift, variation in the environment including the availability of orange-coloured food or resources in general, or variation in the transmission properties of the water (water colour varies from clear to tea coloured; Endler 1992; Endler & Houde 1995) that could influence the visual sensitivities of guppies. Previous work has revealed variation in the visual sensitivities of guppies, such that some individuals would perceive orange as more chromatic and others would see red as more chromatic (Archer *et al.* 1987; Archer & Lythgoe 1990; Endler *et al.* 2001) and that the spectral sensitivity functions are heritable and respond rapidly to selection (Endler *et al.* 2001). If, as our results suggest, the female preference for carotenoid coloration is genetically correlated with orange attraction and orange attraction is an adaptation for foraging, this constraint could explain why the female preference for carotenoid coloration appears not to have evolved through the indicator process (Grether 2000). It is also possible that, originally, attraction to orange discs was similar across all populations and then some form of direct selection on mate preferences (e.g. sensory drive) or the Fisherian process caused divergence among populations in female preference for carotenoid coloration. In this case, the attraction to orange objects could have been enhanced or decreased through genetic correlation with female preference.

Basolo and Endler (Basolo 1990, 1995; Endler & Basolo 1998) present four criteria for demonstrating that a male trait evolved as a result of a pre-existing female bias for that trait: female choice must be based on variation in the trait; the trait must be derived, that is, absent (or in

a primitive form) in the ancestral clade; the preference for the trait must be ancestral; and finally, there must be evidence for a bias in the sensory or neural system that matches the direction of the preferences. Meeting the first criterion, Endler & Houde (1995) have shown that, for female guppies from most populations, mate choice is based on variation in the trait (area of orange). Meeting the fourth, we have shown that there is a bias in the sensory or neural system that matches the direction of the preference; guppies are attracted to the colours orange and red, even outside the contexts of food and mates.

To meet the second and third criteria of Basolo and Endler (Basolo 1990, 1995; Endler & Basolo 1998), it must be demonstrated that the trait is derived in the ancestral clade and that preference for the trait is ancestral, so that females in other species of the clade will prefer the trait, even though males of those species lack it. To conduct these tests, male coloration and female preference must be mapped onto a phylogeny of the clade. Although, to our knowledge, this has not yet been attempted for the genus *Poecilia*, evidence is accumulating that a mate preference for orange coloration exists even in poeciliids that do not normally have orange coloration. Schlupp *et al.* (1999) found that females from two related genera (*Poecilia latipinna* and *P. mexicana*) preferred males of their own species with a novel, orange-coloured spot in the dorsal fin (one male had a tumour in the dorsal fin, the other had a unique area of orange coloration). A. Lindholm & F. Breden (unpublished manuscript) also found in *P. parae*, a species with five naturally occurring male morphs, that females preferred the red-striped morph over four other non-red morphs. However, Breden & Bertrand (1999) found no evidence for female mate choice based on orange coloration in *P. picta*, a species in which males have orange spots. These data suggest that a sensory bias favouring orange-red coloration exists in the genus *Poecilia* but is sometimes lost, perhaps when ecological conditions (e.g. fruit availability) do not favour its maintenance. To Basolo and Endler's list of criteria (Basolo 1990, 1995; Endler & Basolo 1998), we propose this additional condition for the pleiotropic mate preferences with an ecologically based origin: we would only expect those preferences to arise where the relevant ecological conditions (e.g. rarity of a preferred food item) exist. This new condition incorporates the geographical and ecological components of this mechanism with the phylogenetic components suggested by Basolo and Endler. Of course it is possible, if direct selection on mate preference begins to play a role, that ecological (e.g. foraging) selection may no longer be necessary to maintain the preference.

Our results suggest that the mate preference of female guppies for orange coloration on males has arisen as a result of a sensory bias for orange-coloured objects, perhaps orange-coloured fruit. The correlation of the response to orange objects with mating preference suggests a causal relationship; however, it remains for further study to determine to what extent the geographical variation in female preference for carotenoid coloration is a result of natural selection on foraging behaviour and to what extent, if any, this mate preference has been co-opted by sexual-selection mechanisms.

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REFERENCES

- Andersson, M. 1994 *Sexual selection*. Princeton University Press.
- Archer, S. N. & Lythgoe, J. N. 1990 The visual pigment basis for cone polymorphism in the guppy, *Poecilia reticulata*. *Vis. Res.* **30**, 225–233.
- Archer, S. N., Endler, J. A., Lythgoe, J. N. & Partridge, J. C. 1987 Visual pigment polymorphism in the guppy *Poecilia reticulata*. *Vis. Res.* **27**, 1243–1252.
- Basolo, A. L. 1990 Female preference predates the evolution of the sword in swordtail fish. *Science* **250**, 808–810.
- Basolo, A. L. 1995 Phylogenetic evidence for the role of a pre-existing bias in sexual selection. *Proc. R. Soc. Lond. B* **259**, 307–311.
- Breden, F. & Bertrand, M. 1999 A test for female attraction to male orange coloration in *Poecilia picta*. *Environ. Biol. Fishes* **55**, 449–453.
- Christy, J. H. 1995 Mimicry, mate choice, and the sensory trap hypothesis. *Am. Nat.* **146**, 171–181.
- Clark, D. L. & Uetz, G. W. 1992 Morph-independent mate selection in a dimorphic jumping spider: demonstration of movement bias in female choice using video-controlled courtship behaviour. *Anim. Behav.* **43**, 247–254.
- Dücker, G. 1963 Spontane Bevorzugung art eigener Farben bei Vögeln. *Z. Tierpsychol.* **20**, 43–65.
- Endler, J. A. 1978 A predator's view of animal color patterns. *Evol. Biol.* **11**, 319–364.
- Endler, J. A. 1980 Natural selection on color pattern in *Poecilia reticulata*. *Evolution* **34**, 76–91.
- Endler, J. A. 1983 Natural and sexual selection on color patterns in poeciliid fishes. *Environ. Biol. Fishes* **9**, 173–190.
- Endler, J. A. 1990 On the measurement and classification of colour in studies of animal colour patterns. *Biol. J. Linn. Soc.* **41**, 315–352.
- Endler, J. A. 1991 Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. *Vis. Res.* **31**, 587–608.
- Endler, J. A. 1992 Signals, signal conditions, and the direction of evolution. *Am. Nat.* **129**, S125–S153.
- Endler, J. A. 1995 Multiple-trait coevolution and environmental gradients in guppies. *Trends Ecol. Evol.* **10**, 22–29.
- Endler, J. A. & Basolo, A. L. 1998 Sensory ecology, receiver biases and sexual selection. *Trends Ecol. Evol.* **13**, 415–420.
- Endler, J. A. & Houde, A. E. 1995 Geographic variation in female preferences for male traits for *Poecilia reticulata*. *Evolution* **49**, 456–468.
- Endler, J. A., Basolo, A., Glowacki, S. & Zerr, J. 2001 Variation in response to artificial selection for light sensitivity in guppies (*Poecilia reticulata*). *Am. Nat.* **158**, 36–48.
- Enquist, M. & Arak, A. 1993 Selection of exaggerated male traits by female aesthetic senses. *Nature* **361**, 446–448.

- Grether, G. F. 2000 Carotenoid limitation and mate preference evolution: a test of the indicator hypothesis in guppies (*Poecilia reticulata*). *Evolution* **54**, 1712–1724.
- 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.
- Houde, A. E. 1987 Mate choice based upon naturally occurring color-pattern variation in a guppy population. *Evolution* **41**, 1–10.
- Houde, A. E. 1992 Sex-linked heritability of a sexually selected character in a natural population of *Poecilia reticulata*. *Heredity* **69**, 229–235.
- Houde, A. E. 1997 *Sex, color, and mate choice in guppies*. Monographs in *Behavioral Ecology*. Princeton University Press.
- Houde, A. E. & Torio, A. J. 1992 Effect of parasitic infection on male color pattern and female choice in guppies. *Behav. Ecol.* **3**, 346–351.
- Keeler-Wolf, T. 1988 Fruit and consumer differences in three species of trees shared by Trinidad and Tobago. *Biotropica* **20**, 38–48.
- Kelly, C. D., Godin, J.-G. J. & Wright, J. M. 1999 Geographical variation in multiple paternity within natural populations of the guppy (*Poecilia reticulata*). *Proc. R. Soc. Lond. B* **266**, 2403–2408. (DOI 10.1098/rspb.1999.0938.)
- Kirkpatrick, M. & Ryan, M. J. 1991 The evolution of mating preferences and the lek paradox. *Nature* **350**, 33–38.
- Kodric-Brown, A. 1989 Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. *Behav. Ecol. Sociobiol.* **25**, 393–401.
- Marler, C. A. & Ryan, M. J. 1997 Origin and maintenance of a female mating preference. *Evolution* **51**, 1244–1248.
- Marshall, R. C. 1939 *Silviculture of the trees of Trinidad and Tobago, British West Indies*. London: Oxford University Press.
- Maynard Smith, J. 1991 Theories of sexual selection. *Trends Ecol. Evol.* **6**, 146–151.
- Nicol, J. A. C. & Somiya, H. 1989 *The eyes of fishes*. Oxford University Press.
- Nicoletto, P. F. 1991 The relationship between male ornamentation and swimming performance in the guppy, *Poecilia reticulata*. *Behav. Ecol. Sociobiol.* **28**, 365–370.
- Noldus, L. P. J. J. 1991 The OBSERVER: a software system for collection and analysis of observational data. *Behav. Res. Meth. Instrum. Comput.* **23**, 415–429.
- Payne, R. J. H. & Pagel, M. 2001 Inferring the origins of state-dependent courtship traits. *Am. Nat.* **157**, 42–50.
- Phelps, S. M. & Ryan, M. J. 1998 Neural networks predict response biases of female tungara frogs. *Proc. R. Soc. Lond. B* **265**, 279–285. (DOI 10.1098/rspb.1998.0293.)
- Proctor, H. C. 1991 Courtship in the water mite *Neumania papillator*: males capitalize on female adaptations for predation. *Anim. Behav.* **42**, 589–598.
- Proctor, H. C. 1992 Sensory exploitation and the evolution of male mating behaviour: a cladistic test using water mites *Acari Parasitengona*. *Anim. Behav.* **44**, 745–752.
- Reznick, D. N., Butler IV, 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 IV, D. N., Butler IV, M. J. & Rodd, F. H. 2001 Life history evolution in guppies 7: the comparative ecology of high and low predation environments. *Am. Nat.* **157**, 126–140.
- Rowe, L. & Houle, D. 1996 The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. B* **263**, 1415–1421.
- Ryan, M. J. 1990 Sexual selection, sensory systems and sensory exploitation. *Oxf. Surv. Evol. Biol.* **7**, 157–195.
- Ryan, M. J. 1998 Sexual selection, receiver biases, and the evolution of sex differences. *Science* **281**, 1999–2003.
- Ryan, M. J. & Rand, A. S. 1993 Sexual selection and signal evolution: the ghost of biases past. *Phil. Trans. R. Soc. Lond. B* **340**, 187–195.
- SAS Institute, Inc. 1989 *SAS/STAT user's guide*, v. 6. Cary, NC: SAS Institute, Inc.
- SAS Institute, Inc. 1997 *JMP statistical discovery software*. SAS Institute, Inc.
- Schlupp, I., Waschulewski, M. & Ryan, M. J. 1999 Female preferences for naturally-occurring novel male traits. *Behaviour* **136**, 519–527.
- Smith, C. E. 1954 The New World species of *Sloanea*. *Contrib. Gray Herb. Harv. Univ.* **175**, 1–114.
- Sokal, R. R. & Rohlf, F. J. 1995 *Biometry: the principles and practice of statistics in biological research*. New York: Freeman.
- Stavenga, D. G., Smits, R. P. & Hoenders, B. J. 1993 Simple exponential functions describing the absorbance bands of visual pigment spectra. *Vis. Res.* **33**, 1011–1017.
- West-Eberhard, M. J. 1984 Sexual selection, competitive communication and species-specific signals in insects. In *Insect communication* (ed. T. Lewis). New York: Academic.
- Williams, R. D. & Cheesman, E. E. 1929 Flora of Trinidad and Tobago. *Dep. Agric. Trinidad Tobago* **1**, 108–111.
- Zar, J. H. 1996 *Biostatistical analysis*. Englewood Cliffs, NJ: Prentice-Hall.

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