# Sex-linked heritability of a sexually selected character in a natural population of *Poecilia reticulata* (Pisces: Poeciliidae) (guppies)

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The relative extent of orange-pigmented spots in the colour patterns of male guppies, *Poecilia reticulata*, has previously been shown to be a basis for female choice. Parent–offspring and half-sib breeding experiments and selection experiments were conducted to determine if heritability of this character is maintained in the face of apparent sexual selection. The results demonstrate strong heritability of orange area. High father–son regression slopes, strong response to selection and a relatively small dam component of variance indicate that this character has Y-linked inheritance. This result for a quantitative character is in agreement with earlier work showing Y-linked inheritance of particular colour pattern elements in guppies. The high heritability of orange area suggests that this trait is probably not an indicator of fitness variation as postulated by some 'good genes' models for the evolution of female mate choice but that it does have the potential for further evolutionary change and elaboration.

**Keywords:** colour pattern, guppy, heritability, *Poecilia reticulata*, sex-linkage, sexual selection.

## Introduction

Models for the elaboration of secondary sexual characters and for the evolution and maintenance of mate choice in non-resource based polygynous mating systems require heritable variation in male characters on which choice is based. In quantitative studies, sexual selection appears to be particularly strong relative to other forms of natural selection (Endler, 1986), and may tend to deplete additive genetic variance for sexually selected characters (Williams, 1975; Maynard Smith, 1978; Falconer, 1981). Whether heritability of such characters can be maintained by mutation-selection balance is unresolved theoretically (Lande, 1981; Turelli, 1984). On the other hand, Kodric-Brown & Brown (1984) argue that characters with low heritability are most likely to be used as cues in 'adaptive' mate choice (see discussion below). Empirical data are therefore needed to determine if sufficient additive genetic variance in sexually selected characters is maintained so that the potential for rapid change persists, or if characters with low heritability can be a basis for female choice. Significant heritability for sexually selected characters of males has been documented in several species (e.g. Carson & Teramoto, 1984;

McLain, 1987; Simmons, 1987; Hedrick, 1988; Moore 1989). This paper reports on data on the heritability of a colour pattern character in guppies (*Poecilia reticulata*) which is known to be used as a cue in mate choice.

The guppy is notable for extreme variation in male colour patterns both among individuals and among populations (Haskins *et al.*, 1961; Endler, 1978; 1983), and differences in colour pattern affect mate choice by females (Endler, 1983; Kodric-Brown, 1985; Houde, 1987, 1988a, b; Long & Houde, 1989; Houde & Endler, 1990). In particular, females appear to use the relative area of orange-pigmented spots in male colour patterns as a cue for mate choice and discriminate against males with relatively little orange. Individual male guppies vary dramatically in orange area (Endler, 1978, 1983; Houde, 1987), this work sought to determine how much of this variation was heritable. The presence or absence of certain elements of guppy colour patterns are known to be under sex-linked genetic control (Winge, 1927; Haskins *et al.*, 1961) but the genetic basis for continuous variation in relative amounts of colour has not been examined. Data are presented on the heritability of orange area in guppies from parent–offspring regressions, sib analysis, and selection experiments.

## Methods

### Origin and rearing of fish

The guppy is a live-bearing poeciliid fish native to streams and rivers of Trinidad and adjacent parts of South America. Fertilization is internal, and young develop within the mother for 3–4 weeks before they are born. Colour patterns, which are expressed only in males, begin to develop around the time of sexual muturation (8–10 weeks) and are fully expressed about 4 weeks after pigmentation first appears.

Guppies used in these experiments were either wild-caught individuals collected from a low-predation locality in the Paria River of Trinidad, or were first or second generation laboratory-reared descendents of wild-caught fish. For one of the parent–offspring analyses, laboratory-reared Paria males were mated to females descended from individuals collected at a low predation locality in Trinidad's Aripo river.

Most of the experimental fish were obtained by isolating single pregnant females in 8-litre aquaria, allowing them to give birth and rearing their young. Experimental matings were conducted by placing a virgin female in an aquarium with a known male and allowing them to interact freely for at least 1 week before removing the male. Young males and females were separated at 4–6 weeks of age using MS-222 anaesthesia (a solution of tricaine methanesulphonate; 40 mg in 150 ml water), and were reared to maturity in single-sex groups of up to 12 individuals in 8-litre aquaria or in 10-litre sections of 40-litre aquaria. The fact that full-sib families were reared together means that the common environment could have contributed to resemblance between brothers. All fish were fed twice daily, once with newly hatched brine shrimp nauplii, and once with liver paste. Fish were housed at 22–27°C with a 12-h light, 12-h dark photoperiod.

### Scoring colour patterns

Colour patterns of young males were scored within 4 weeks of sexual maturity (judged by morphological development of the gonopodium, the modified anal fin of guppies used to transfer sperm). Colour patterns were recorded by anaesthetizing males in MS-222 and photographing them as soon as they stopped moving. The fish were anaesthetized as lightly as possible because in heavily anaesthetized fish, black spots and lines in the colour pattern increase in size and may obscure parts of orange spots. Fish were photographed with Kodak Ektachrome 160 slide film under tungsten light.

Slides were projected onto paper and colour patterns were traced, noting the colour of each spot. The area of orange spots was then calculated using a digitizing tablet. The total body area of the fish excluding the fins was also recorded. Analyses are based on the relative area of orange in the colour pattern (henceforth, orange area), calculated as the total area of all orange spots divided by body area. The orange area of Paria guppies varies from 0 in fish with no orange pigment to 0.30 or more in fish with the largest orange area (Houde, 1987) and averages about 0.15 in most samples.

### Parent–offspring regressions

The inheritance of orange area was examined by plotting orange area of fathers against the mean orange area of their sons in three separate analyses (Falconer, 1981). In all cases offspring means were based on 1–4 sons per family. The three analyses (Table 1) differed in the origin of parents as follows: (i) fathers were Paria males wild-caught as adults, mated to laboratory-reared Paria females, (ii) fathers were laboratory-reared Paria males mated to laboratory-reared Paria females, (iii) fathers were laboratory-reared Paria males mated to laboratory-reared Aripo females. The matings for analysis (ii) and (iii) were set up as part of another experiment: fathers were chosen by taking matched pairs of full brothers with nearly identical colour patterns and simultaneously mating one brother to a Paria female (analysis 2) and the other to an Aripo female (analysis 3). The regression analyses are therefore directly comparable. Aripo guppies have little orange in their colour patterns relative to Paria guppies (see Houde, 1988a), but express other colour pattern elements absent in the Paria population. Comparison of results using Paria vs. Aripo females should therefore give insight into the female contribution to colour patterns of their sons.

### Sib analyses

Two separate half-sib/full-sib analyses were carried out. Sample sizes were very small in both so conclu-

**Table 1** Regressions of relative area of orange of sons on fathers. See test for explanation of origins of parents for the four analyses. All fathers are the Paria population

| Analysis | Fathers | Mothers | $n$ | slope | s.e. |
|---|---|---|---|---|---|
| 1 | Wild-caught | Paria | 38 | 0.54 | 0.15 |
| 2 | Laboratory-reared* | Paria | 35 | 0.71 | 0.07 |
| 3 | Laboratory-reared* | Aripo | 37 | 0.38 | 0.08 |

*Matched pairs of brothers (see text).

sions must be based on qualitative rather than quantitative results. In the first analysis, six males were each mated to two females and up to four sons were scored from each family. In the other, three males were each mated to four (two males) or six (one male) females, and up to six sons were scored from each family. Sire and dam variance components (Falconer, 1991) were estimated from the unbalanced data and significance tests were carried out using synthetic mean squares (Sokal & Rohlf, 1981: 302–308). Sample sizes were too small for estimates of standard errors of variance components to be meaningful (Turner & Young, 1969).

### Selection experiment

Artificial selection for increased and decreased orange area was imposed on two pairs of high and low selection lines. The first pair of lines (replicate 1) was initiated by selecting, by eye, the 20 males with greatest orange area and the 20 males with least orange area from a pool of 65 males collected from the Paria River as juveniles. Each group of males was mass mated to 20 randomly chosen females, also captured as juveniles, in separate wading pools. Replicate 2 was initiated in a similar way except that there were only 40 males in the initial pool, so all were used for either the high or the low line, and the females were laboratory-reared descendants of wild-caught individuals. The four selection lines were subsequently maintained separately. The parents for every generation were kept in the wading pools for 6–8 weeks while the females gave birth to young. New-born young were removed from the pools as they appeared and placed in 8-litre aquaria in groups of 12 and were reared to maturity as described above. A total of at least 100 young were collected for the subsequent generation in each line. All mature males were scored for orange area when mature and the 20 males with the greatest or least orange area (depending on selection line) were chosen to be the fathers of the next generation and were mass mated to 20 randomly chosen females from the same selection line. The total number of males from which selected males were drawn averaged about 40 in any given line and generation (range: 33–51). Four generations of selection were imposed on the replicate 1 lines, three generations on replicate 2 lines. Selection differentials and the response to selection were estimated for each line in each generation (Falconer, 1981). Note that mass mating of parents in each generation allows sexual selection to occur, which could increase or reduce the observed response to selection.

## Results

All of the analyses revealed strong, significant heritability of orange area. They all also suggest that this character may be largely Y-linked, with little contribution by mothers to offspring colour patterns in the Paria river population.

### Parent–offspring regressions

Regression slopes were substantial and significant in all three analyses, indicating significant heritability (Table 1, Fig. 1). Heritability for autosomally inherited characters is estimated as twice the parent–offspring regression slope when only one parent is measured (Falconer, 1981). This gives heritability estimates considerably greater than 1.0 in 2 of the 3 analyses, which suggests some Y-linked contribution to orange area. If the trait is completely Y-linked, the appropriate estimate of heritability is the regression slope itself. The slopes given in Table 1 therefore provide minimum estimates of heritability. The use of Aripo mothers in analysis 3 led to a significant reduction in regression slope relative to analysis 2 in which all parents were from the Paria population ($t = 3.13$, 68 d.f., $P < 0.01$). The regression slope was slightly less when wild-caught fathers were used in analysis 1, relative to analysis 2 with laboratory-reared fathers but the difference is not statistically significant ($t = 1.07$, 69 d.f., $P > 0.2$).
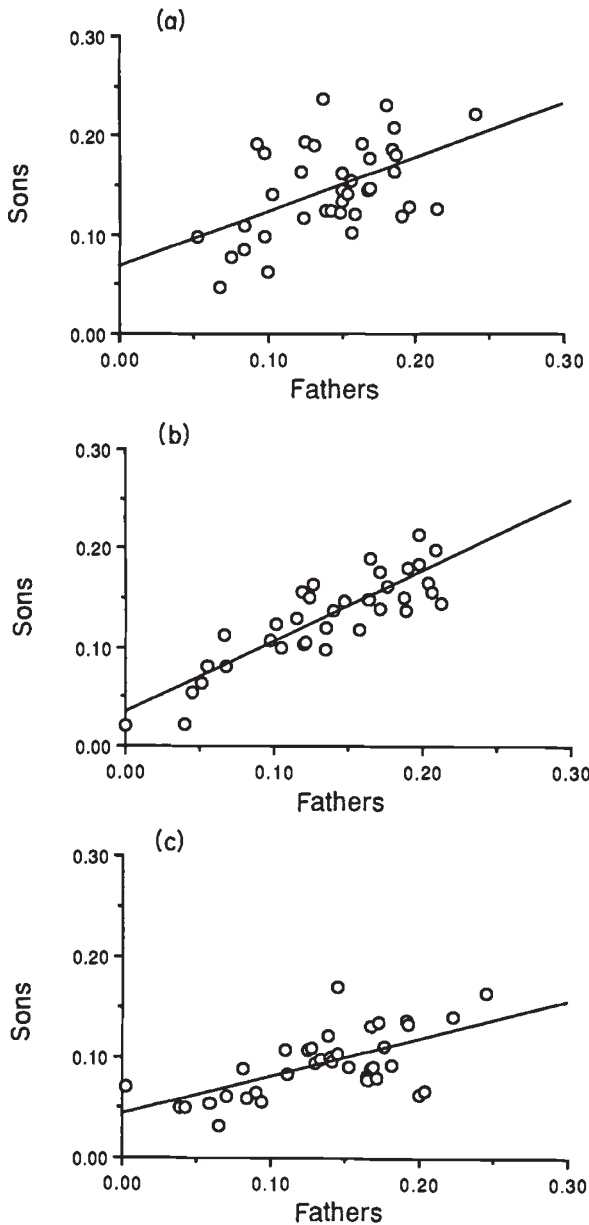
### Sib analyses

In both experiments the sire component of variance was highly significant and accounted for the majority of the total variation (Table 2). Mothers made no significant contribution to orange area of sons in experiment 1, but made a small but significant contribution in experiment 2. As in the regression analyses, these data are consistent with Y-linked determination of orange area. Under complete Y-linkage, the appropriate heritability estimate would be the sire variance component itself, expressed as a fraction of total variance, so the values given in Table 2 (88 and 89%) represent minimum heritability estimates.

### Selection experiment

There was a strong divergence between high and low selection lines in both replicates of the selection experiment (Fig. 2). Slopes of regressions of response to selection on the cumulative selection differential are given for each line in Table 3. Heritability is normally estimated as twice the slope of such regressions when

**Fig. 1** Plots of orange area of fathers against average orange area of up to four sons for analyses with (a) wild-caught fathers from the Paria population mated to laboratory-reared Paria mothers (analysis 1, Table 1); (b) laboratory-reared Paria father and mothers (analysis 2, Table 1); (c) laboratory-reared Paria fathers and Aripo mothers (analysis 3, Table 1). Linear regression lines are shown; slopes are given in Table 1.

selection is imposed on only one sex (Falconer, 1981). This yields heritability estimates greater than 1.0 in three of four lines. As in the parent–offspring and sib analyses, the slopes themselves can be used as minimum heritability estimates under the assumption of complete Y-linkage. For a completely Y-linked trait, selec-

tion simply results in a reduction in the population of different Y-chromosomes. The low slope for the high line in replicate 1 seems inconsistent with the other results presented here, and may have resulted from a founder effect or other random fluctuation in the small experimental population.

## Discussion

Several different experiments and different approaches all demonstrate that orange area in Paria guppies is strongly heritable and indicate that this character is determined largely by Y-linked genes. Previous work (Winge, 1927; Haskins et al., 1961) has demonstrated that particular colour pattern elements in guppies have Y-linked inheritance. My results are consistent with previous evidence for Y-linkage even though variation in orange area is continuous and is not necessarily related to particular polymorphic colour pattern elements described previously (e.g. Winge, 1927). Male guppies do appear to resemble their fathers strongly in the shape and placement of orange spots as well as in total area. Sexual selection favouring conspicuous colour patterns in males in conjunction with selective predation, favouring crypsis in females as well as males, is probably responsible for Y-linked inheritance of colour patterns (Fisher, 1930).

Haskins et al. (1961) induced expression of colour patterns (including orange spots) in Paria females with testosterone treatment. This demonstrates some degree of X-linked or autosomal inheritance of the colour pattern, consistent with the significant dam variance component in one of the sib analyses discussed here. However, the fact that males usually resemble their fathers in almost all details of the colour pattern suggests that the X-linked or autosomal genes, which contribute to orange spots in treated females, may not normally be expressed in males and that orange area in males may be mainly the result of expression of the Y-linked genes. Even if not expressed in males, X-linked colour pattern genes could contribute to variation in orange area through occasional recombination with the Y chromosome (Winge, 1927; Haskins et al., 1961).

Table 1 reveals considerable variation in heritability estimated by the four different parent–offspring regressions. The relatively low father–son regression slope in the case where Aripo females were used as mothers (analysis 3) suggests that Aripo females may contribute more to offspring colour patterns, at least in this cross, than do Paria females. Indeed, the hybrid offspring in this experiment had colour pattern elements characteristic of the Aripo population (e.g. silver streaks) which often seemed to be expressed to the exclusion of

**Table 2** Results of half-sib breeding experiments

| Experiment | Source of variation | d.f. | MS | F-test | Variance component (%) |
|---|---|---|---|---|---|
| | Fathers | 5 | 253.3 | $P<0.001$ | 40.5 (88%) |
| 1 | Mothers within fathers | 6 | 4.6 | ns | −0.4 ( 0%) |
| | Error | 25 | 5.7 | | 5.7 (12%) |
| | Fathers | 2 | 1489.0 | $P<0.001$ | 84.5 (89%) |
| 2 | Mothers within fathers | 11 | 21.7 | $P<0.001$ | 4.3 ( 5%) |
| | Error | 40 | 5.6 | | 5.6 ( 6%) |
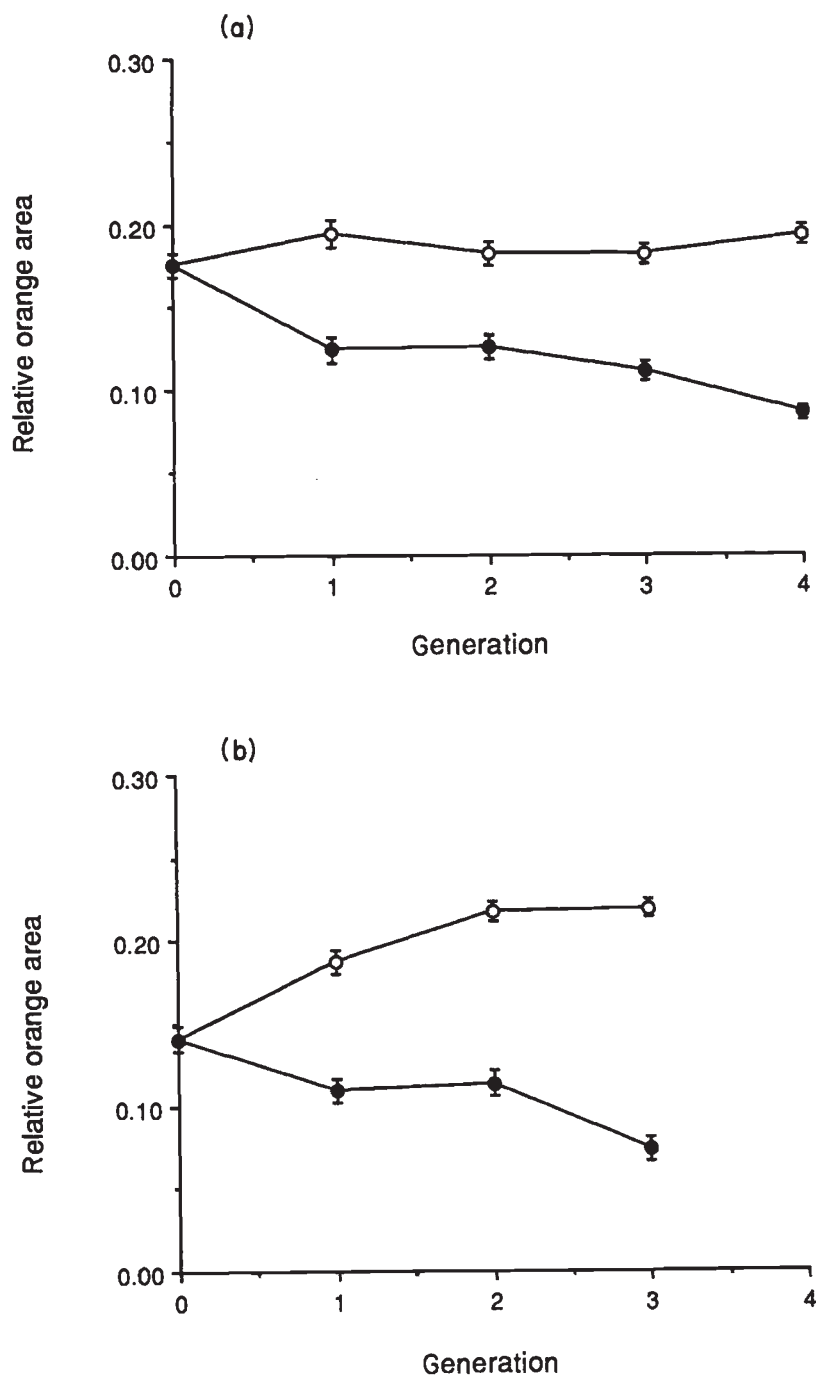


Fig. 2 Response to artificial selection on relative orange area in two pairs of selection lines. (a) Responses to four generations of selection for increased and decreased orange area in replicate 1. (b) Response to three generations of selection for increased and decreased orange area in replicate 2. Standard error bars are shown. (—○—) High, (—●—) low.

**Table 3** Slopes of regressions of response to selection on cumulative selection differential for each selection line. This is a measure of realized heritability, however, slopes are not doubled (see text)

| Replicate | High lines | Low lines |
|---|---|---|
| 1 | 0.10 | 0.54 |
| 2 | 0.75 | 0.56 |

orange spots. The relatively low regression slope when wild caught males were used as fathers (analysis 1) suggests that heritability of orange area in wild populations may not be as high as that implied by analysis 2, involving laboratory-reared males. Wild-caught males varied much more in age when their colour patterns were scored than did laboratory-reared individuals. A fraction of males develop additional orange spots relatively late in life (personal observation). Such age-related changes could result in an additional component of variation in orange area in wild-caught samples of males. Even with the more variable wild-caught fathers, additive genetic variance in orange area appears to be substantial.

Orange area provides an example of a male character in which genetic variation is maintained despite evident sexual selection by female choice. Other studies have documented significant, moderate to high heritability for sexually selected traits (e.g. Carson & Teramoto, 1984; McLain, 1987; Simmons, 1987; Hedrick, 1988; Moore, 1989; see also Cade, 1984). Heritability of preferred characters in males indicates the potential for further evolutionary change in the system and possible further elaboration of the characters. High heritability of orange area is probably most consistent with 'non-adaptive' models for the joint evolution of female preferences and male characters such as that of Lande (1981) but 'adaptive' models (e.g. Kodric-Brown & Brown, 1984) are not necessarily ruled out. Adaptive, or 'good genes' models argue that characters used in mate choice reflect the overall health and vigour of males. Kodric-Brown & Brown (1984) argue that a high degree of environmental variation in such characters means that they should have low heritability, however, there could be additional additive genetic variation in male traits that is unrelated to health and vigour. In the case of orange area in guppies, there seems to be such a large fraction of directly heritable additive genetic variation that there is little potential for this character to reflect health and vigour. The brightness of orange colour in guppy colour patterns is more likely than spot area to be a direct indicator of condition (Kodric-Brown, 1989; Nicolleto, 1991; A. E. Houde & A. J. Torio, unpublished observations).

Brightness of orange colour in large spots might be a better indicator of condition than in small ones, favouring a preference for greater orange area. Alternatively, the preference based on orange area might be an incidental effect of female preferences originally evolved in the context of variation in the brightness of orange spots.

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