

---

# Effects of genetics and light environment on colour expression in threespine sticklebacks

EVA LEWANDOWSKI† and JANETTE BOUGHMAN\*

Department of Zoology, University of Wisconsin, Madison, WI 53706, USA

Received 5 December 2007; accepted for publication 31 October 2007

---

The genetic basis of traits that are under sexual selection and that are involved in recognizing conspecific mates is poorly known, even in systems in which the phenotypic basis of these traits has been well studied. In the present study, we investigate genetic and environmental influences on nuptial colour, which plays important roles in sexual selection and sexual isolation in species pairs of limnetic and benthic threespine sticklebacks (*Gasterosteus aculeatus* species complex). Previous work demonstrated that colour differences among species correlate to differences in the ambient light prevalent in their mating habitat. Red fish are found in clear water and black fish in red-shifted habitats. We used a paternal half-sib split-clutch design to investigate the genetic and environmental basis of nuptial colour. We found genetic differences between a red and a black stickleback population in the expression of both red and black nuptial colour. In addition, the light environment influenced colour expression, and genotype by environment interactions were also present. We found evidence for both phenotypic and genetic correlations between our colour traits; some of these correlations are in opposite directions for our red and black populations. These results suggest that both genetic change and phenotypic plasticity underlie the correlation of male colour with light environment. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, **94**, 663–673.

**ADDITIONAL KEYWORDS:** *Gasterosteus aculeatus* – variation – genotype by environment interaction – nuptial colour – phenotypic plasticity.

---

## INTRODUCTION

Determining whether there is a genetic basis for sexually selected traits and those involved in sexual isolation is important for understanding the evolution of such traits. The evolutionary dynamics of these traits can depend on their genetic architecture and genetic correlations with other traits under selection. Evolutionary change can also depend on the extent to which trait expression is influenced by environmental factors. Genetic and environmental factors, as well as the nature of selection acting on a trait, determine the extent to which genetic variation is maintained, and sexual selection can result in evolutionary change only if variation among males has a genetic basis. Yet,

such variation is expected to be eroded under consistently strong directional sexual selection. Therefore, establishing the presence of genetic variation in sexually selected traits and explaining its maintenance is an important endeavor. When the same traits that are under sexual selection within a species are also involved in sexual isolation between species, such studies can also give us insight into the selective and genetic mechanisms underlying speciation. The genetics of sexual isolation has been studied fairly extensively in model systems such as *Drosophila* (Coyne & Charlesworth, 1997; Noor *et al.*, 2001; Moehring *et al.*, 2004). However, similar studies on non-model systems are in their infancy, and even fewer studies have investigated the genetics of traits involved in both sexual selection and sexual isolation (Kronforst *et al.*, 2006). Quantitative genetic experiments can reveal the quantitative genetic architecture of sexually selected traits and the extent to which genetics and environment influence their

---

\*Corresponding author. E-mail: jboughman@wisc.edu

†Current address: Kellogg Biological Station and Department of Zoology, Michigan State University, Hickory Corners, MI 49060, USA.

expression (Shaw, 1996; Brooks & Endler, 2001; Rodriguez & Greenfield, 2003).

Colour is often involved in both sexual selection and sexual isolation, making it an intriguing trait to study in these contexts. Several studies have found that colour differences between populations have a genetic basis. However, evidence that the environment influences colour expression through effects of diet and environment has also emerged (Grether, Hudon & Millie, 1999; McGraw *et al.*, 2001; Hill, Inouye & Montgomerie, 2002). Genotype by environment interactions may also be involved (Fuller & Travis, 2004). Therefore, differences between populations in colour may be due to genetic differences, environmental differences, or to genetically different populations responding to the environment in dissimilar ways.

Male threespine sticklebacks use throat, eye, and body colour as visual signals to attract choosy females during courtship. In laboratory and field experiments, females have been shown to base their choice of mates, in part, on the amount and intensity of nuptial colour, which shows extensive variation (McLennan & McPhail, 1990; Bakker & Milinski, 1993; Kraak, Bakker & Mundwiler, 1999). Earlier studies showed that variation within a population in red nuptial colour is influenced by both genetics (Bakker & Milinski, 1993) and various environmental factors, including parasite levels (Milinski & Bakker, 1990) and diet (Frischknecht, 1993).

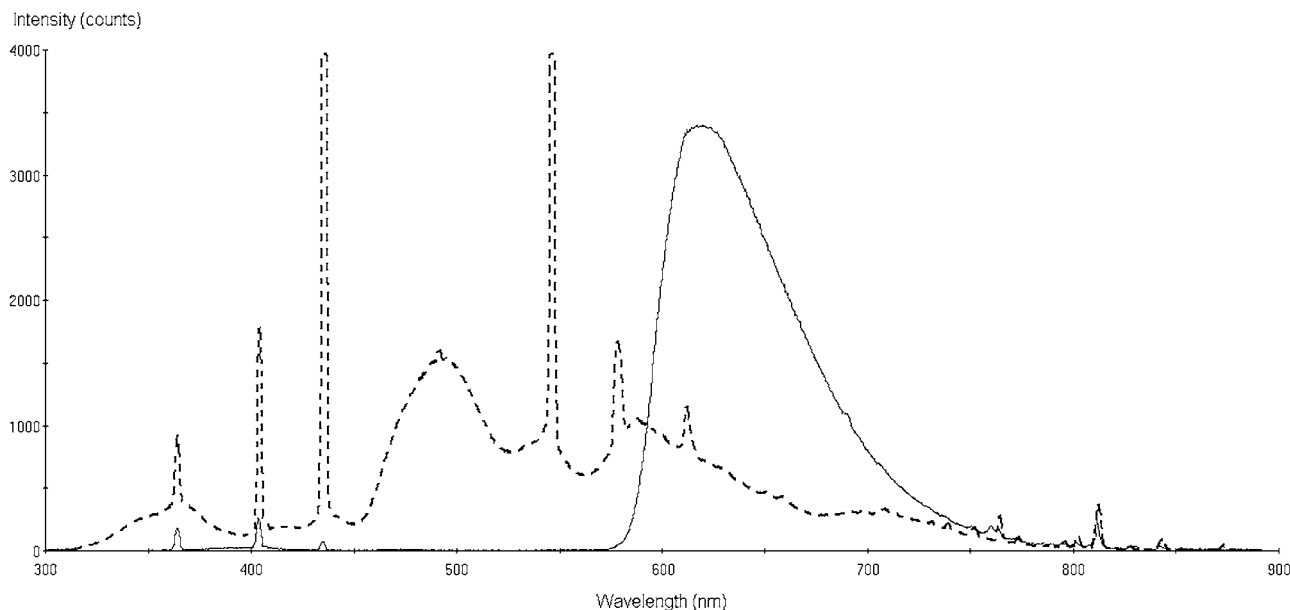
There is also a great deal of variation in the expression of male nuptial colour between populations (McPhail, 1984; McLennan & McPhail, 1989; Boughman, 2001). In the majority of stickleback populations, sexually mature males develop a mosaic of bright red throat patches, blue eyes, and blue or green flanks. However, in some populations, males express melanic colour instead of the more common mosaic colour pattern. In these populations, male throats are black instead of red, and they have dark or black bodies (Reimchen, 1989; Scott, 2001). These melanic males are generally found in waters that appear red-shifted or tea-stained due to the presence of tannins, where the ambient light environment is dominated by long wavelengths (Reimchen, 1989; Boughman, 2001; Scott, 2001). By contrast, populations that express the more typical mosaic colour pattern occur in waters which are less red-shifted. Therefore, the light environment may partly explain population differences in colour, likely because of how it affects colour contrast. Red contrasts with the background in full spectrum light; whereas black contrasts with the background in red-shifted light. Conspicuous colours are likely to be favored by selection because they are easier for females to see. This pattern of colour expression is consistent with predictions from sensory drive (Endler, 1992; Boughman, 2002).

The differences in colour among stickleback populations could have arisen either through a plastic response to light environment, or because selection has led to genetic differences between populations. Differentiating between these possibilities is important to understanding the causes of divergence in colour, and how this divergence might facilitate speciation. In the present study, we used a paternal half-sib, split-clutch design to determine if there are genetic and environmental effects on expression of male nuptial colour for a red and a black population. We focused on the benthic species because colour differs among populations of benthics. We manipulated light environment to imitate water colour in clear and red-shifted lakes. Paternal half-sibs with either red-throated or black-throated fathers were raised under either full spectrum or red-shifted light. At maturity, we measured various components of male nuptial colour. This design allowed us to investigate genetic and environmental effects of colour variation at several levels. First, we determined whether there is a genetic basis to throat, body, and eye colour traits by making comparisons both within and between our black and red populations. Second, we also estimated maternal effects by looking at dam nested within sire. Next, we investigated the effect of light environment by comparing half-sibs raised in the full spectrum and red-shifted light. Finally, we explored the potential for genotype by environment interactions ( $G \times E$ ) by estimating the interaction of light treatment with each of our genetic effects. We also investigated phenotypic and genetic correlations between colour traits and asked if those correlations were similar for our black and red populations.

## MATERIAL AND METHODS

### ARTIFICIAL CROSSES

Sires and dams included both laboratory-reared and wild-caught fish, captured with minnow traps, from the benthic regions of two British Columbia lakes. Paxton benthic males express red colour, and Enos benthic males express black colour. We crossed three red males and five black males each to two females randomly chosen from the same population as the males, giving six red and ten black half-sib families. We chose sires in full reproductive mode with good colour expression. We performed artificial crosses by sacrificing males in MS-222, removing their testes, and extracting sperm. We gently squeezed the females to obtain their eggs and spread the sperm over the eggs. We split each clutch and placed one half in a room with full spectrum light and the other half in a room with red-shifted light. Thus, each male had two half-clutches in full spectrum and two in



**Figure 1.** Spectral distribution of the ambient light in both the full spectrum (dashed line) and red-shifted (solid line) light environments. Spectra were recorded with an Ocean Optics S2000 spectrophotometer.

red-shifted light. We measured colour on male offspring reared in the laboratory.

#### REARING ENVIRONMENT AND CONDITIONS

We placed egg masses in aerated egg cups within 110-L tanks, where they remained after hatching, except families consisting of eight or fewer individuals, which were moved to 38 liter tanks. We equalized densities for all families at  $0.2 \text{ fish L}^{-1}$ . Males and females from the same family were housed together. Fish were fed brine shrimp as juveniles and Chironomid larvae as adults. Both diets provide a reasonable supply of carotenoids.

To produce a red-shifted light environment, we covered fluorescent lights (40-W Econo-watt and Philips Natural Sunshine) with theatrical gels (GamProducts JR270). This eliminated wavelengths of light below 575 nm, and created a peak at 617 nm, which is similar to natural red-shifted habitats (Boughman, 2001). In the full spectrum environment, we used the same fluorescent lights and added an ultraviolet-producing light (40 watt ESU Reptile Super UV Daylight). We used neutral density filters (GamProducts Neutral Density JR1516 0.6) to equalize light intensity in both treatments. The full spectrum environment included light wavelengths from 315 nm. The maximum wavelength of light in both treatments was 876 nm (Fig. 1).

To induce reproductive condition and colour expression, we manipulated the light cycle and temperature

to simulate the seasons. For 5 months, both rooms were maintained under a 12 : 12 h light/dark cycle at  $15.6^\circ\text{C}$ . We decreased light over the next month by shifting to a 8 : 16 h light/dark cycle and maintained this cycle for 40 days to simulate winter. We simulated spring by increasing light over the next 2 months by shifting to a 18 : 6 h light/dark cycle, which was equivalent to the natural daylight hours of the fish's native range during the breeding season. We also increased the temperature, with 2 months at  $16.7^\circ\text{C}$  and three at  $17.5^\circ\text{C}$ . Exposure to gravid females helps to bring males into reproductive condition (Bakker & Milinski, 1993; Rush *et al.*, 2003); thus, after 9 months, a gravid female inside a clear jar was placed in each tank for 5 min every 3 days for a period of 30 days.

#### MEASURING COLOUR

We collected colour data only from sons showing body, throat, and eye colour. For each male, we measured seven components of colour. We scored four measures of throat colour: the area expressing red, the intensity of red, the intensity of black, and a red index that we calculated by summing the red intensity and red area scores for each male. A multiplicative index of intensity and area gave similar results to the additive index. The other three measures were overall body darkness, body brightness, and the intensity of eye colour. We based the scores for both black throat intensity and body darkness on the number and

extent of melanophores. The body darkness score also took into account the hue of the body colour, such as dark blue or pale green. Body brightness was based on the area and intensity of green and blue body colour. We scored each colour component on a six-point scale, from zero to five. A score of '0' represented no colour, and a score of '5' represented the most extreme colour. We measured sires in the same way. We are aware that human vision is not strictly equivalent to stickleback vision; however, this technique of hand scoring has been used in previous stickleback studies (Boughman, 2001; Rowe, Baube & Phillips, 2006).

#### STATISTICAL ANALYSIS

##### *Estimating genetic and environmental effects*

Although our experimental design was initially balanced, due to loss of fish from some groups, our final data set was not. We also measured unequal numbers of sons from each treatment group due to biased sex ratios and variation in family size (2–11 sons per half-sib family, mean = 9). The final design of the experiment included ten black and six red half-sib families and 144 sons, including 77 from black and 67 from red sires. Therefore, we used type three sums of squares and estimated degrees of freedom using Satterthwaite's approximation.

We used mixed model analysis of variance (Proc Mixed in SAS; SAS Institute Inc.) to estimate genetic and environmental effects for each of the colour measures. We treated population colour and sire nested within population as fixed effects because we selected sires based on their level of expression of colour traits. We considered dam nested within sire as a random effect because we randomly selected females from each male's population. Light treatment, the interaction between light and population, and the interaction between light and sire were considered as fixed effects. We tested the effect of sires, population, light environment, and the interaction of light by population by using the interaction between light and sire within population as the error term [light  $\times$  sire(pop)]. Other effects were tested over experimental error. We estimated family means within light treatment by calculating least square means for half-sibs (from both dams within each sire) for each light treatment separately.

##### *Correlations among colour traits*

We also calculated phenotypic and genetic correlations among colour traits. Phenotypic correlations allow us to determine the extent to which expression of one colour trait is related to other colour traits. We investigated genetic correlations to gain insight into whether traits are free to evolve independently or should be expected to evolve in a correlated fashion.

To estimate phenotypic correlations, we calculated Pearson correlation coefficients separately for red and black populations across light treatments using individual sons as the replicates ( $N = 77$  for black and  $N = 67$  for red populations). To estimate genetic correlations, we used least square mean estimates for half-sibs calculated separately for each light treatment. We calculated Pearson correlations using these family means as the replicates ( $N = 5$  for black and  $N = 3$  for red populations).

## RESULTS

### GENETIC AND ENVIRONMENTAL EFFECTS ON COLOUR EXPRESSION

We found significant genetic and environmental effects on colour expression, as well as some genotype by environment interactions (Table 1; Fig. 2). Genetic effects were substantial at the population level for red and black measures of throat colour and marginally significant for body darkness, suggesting that our red and black populations differ in the genetic basis of red and black nuptial colour. We found slightly weaker genetic effects within population for red area, red index, and black intensity (Table 1; Fig. 2). There was no evidence of genetic effects for the intensity of eye colour or body brightness either between or within populations. We also found evidence of maternal effects for all measures except red index and black intensity (Table 1). These could include both maternal genetic and environmental effects. We cannot partition variation due to these two sources with our experimental design.

Light environment affected all traits except red intensity, red index, and eye intensity. In red-shifted light, measures of red and black on the throat and body darkness had higher scores; whereas body brightness and eye intensity tended to have lower scores in the red-shifted environment (Fig. 2).

We also found evidence for  $G \times E$  at both the between and within population levels (Table 1; Fig. 2); although within population effects were stronger. Sires within populations showed significant variation in their response to the light environment. Red index, body darkness, and body brightness had significant  $G \times E$  interactions; whereas interactions for red intensity, black intensity, and eye intensity were marginally significant. There were changes in rank for families in both red and black populations for most colour measures. Therefore, sires with the highest scoring sons in full spectrum light did not necessarily have high scoring sons in red-shifted light. In addition to the within population  $G \times E$ , red and black populations responded marginally differently to light treatment for red intensity and body darkness.

**Table 1.** Mixed model analysis of variance for colour phenotypes

Effect	d.f.	Red index	Red area	Red intensity	Black intensity	Body darkness	Body brightness	Eye intensity	Effect type
<b>Population colour</b>	1	8.4*	44.4**	30.1**	18.8**	5.1#	1.8	1.6	G
<b>Sire(pop)</b>	6	2.9	4.5*	4.7*	4.1#	2.2	1.9	1.4	G
<b>Light treatment</b>	1	0.0	8.9*	2.6	14.8*	21.1**	10.3*	1.9	E
<b>Light × colour</b>	1	3.9	2.4	4.6#	0.0	5.4#	0.2	1.2	G × E
Light × sire(pop)	5	3.9**	1.6	2.0#	2.0#	4.7***	2.8*	2.2#	G × E
Dam(sire (pop))	6	1.4	3.4**	2.4*	0.8	9.6***	2.8*	3.3**	G + E
Light × sire(pop) error¶	5	0.19	0.16	0.35	1.67	1.63	1.55	1.62	
Experimental error¶	125	0.05	0.10	0.18	0.82	0.35	0.56	0.76	
$R^2$		0.55	0.60	0.57	0.59	0.68	0.46	0.34	

# $P < 0.10$ , \* $P < 0.05$ , \*\* $P < 0.005$ , \*\*\* $P < 0.0005$ . ¶Values shown are MS error.

Values shown are  $F$ -ratios. Terms in bold are tested over light × sire(pop) as the error term and other terms are tested over experimental error. Effect type is indicated for genetic effects (G), environmental effects (E), genotype by environment interaction (G × E), and genetic plus maternal effects (G + E). See text for details of analyses.

d.f., degrees of freedom.

#### CORRELATIONS AMONG COLOUR TRAITS

In general, related traits showed positive phenotypic correlations for both red and black populations (Table 2). The three measures of red throat colour were positively correlated, as were the two measures of black intensity. Also, eye intensity and body brightness were positively correlated. However, body brightness was negatively correlated with black intensity and body darkness. Moreover, the phenotypic correlations of red with black measures differed for the two populations. The red population showed positive phenotypic correlations between red throat colour and body darkness (Table 2), indicating that the reddest males also had the darkest bodies. By contrast, the black population showed negative phenotypic correlations between red throat colour and black intensity, and a weaker negative correlation between red on the throat and body darkness. Furthermore, black intensity was significantly negatively correlated with eye intensity for the black but positively correlated for the red population. This indicates that in the black but not the red population, the blackest, darkest males showed the least expression of red. Thus, we found that many phenotypic correlations were in opposite directions for red and black populations.

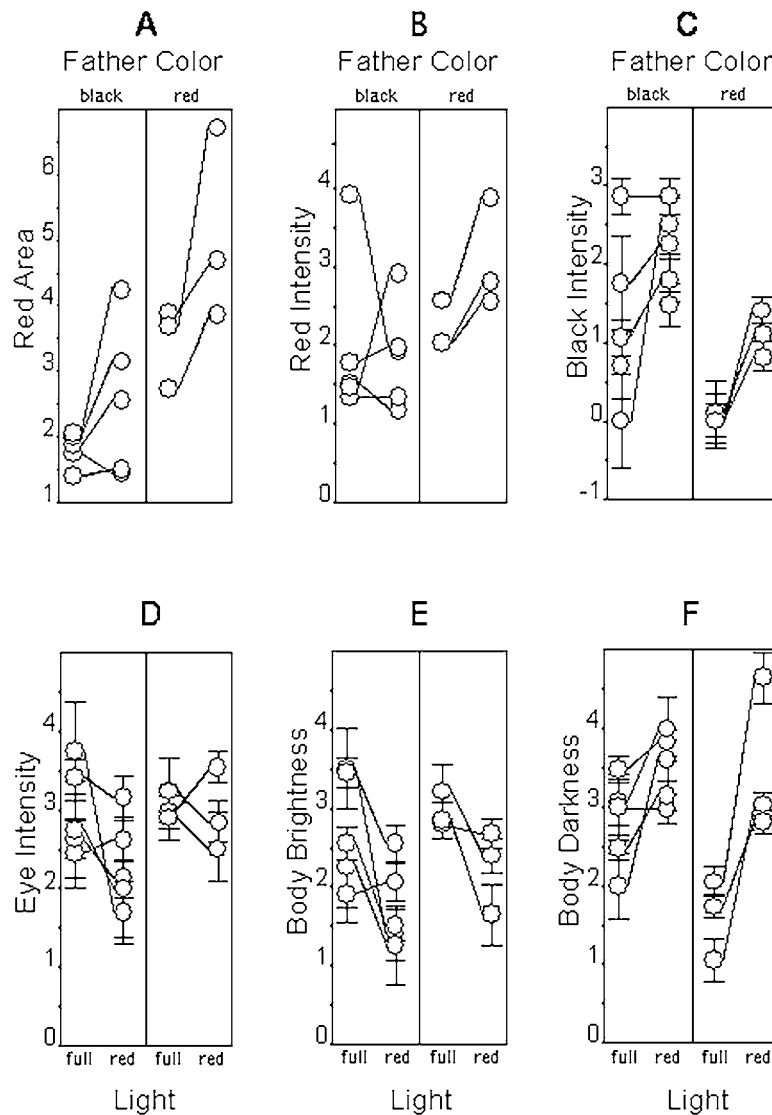
Genetic correlations were similar to, but overall weaker than phenotypic correlations (Table 3), and there were few significant differences between the black and red populations. Related traits tended to be positively genetically correlated; however, black intensity and body darkness had a significant positive genetic correlation only for the black population (Table 3).

#### DISCUSSION

Our results indicate that differences in nuptial colour between the red and black populations of sticklebacks are due to the combined effect of genes and light environment. Thus, both genetic evolution and phenotypic plasticity appear to underlie the correlation between male colour and light environment found previously (Reimchen, 1989; Boughman, 2001; Scott, 2001).

Genetic effects were significant at both the within and between population levels. We found substantial genetic effects between populations for all red and black measures. We also found significant genetic effects within populations for red and marginal effects for black throat colour. This corroborates and extends earlier work that showed genetic variation for red throat colour within a population (Bakker & Milinski, 1993). A larger sample size would improve our power and the precision of our estimates; nonetheless, it is clear that the genetics of colour differs for the red and black populations.

Environmental effects were also found for both throat and body colour. The importance of light environment is further highlighted because the substantial phenotypic differences in colour expression found between our populations in their native light environments are reduced between fish reared in the alternative environments. In the present study, some fish from the red population expressed nearly as much black in red-shifted light as many fish from the black population expressed in full spectrum light. Both red and black fish were blacker under red light, which provides colour contrast and may ensure that the male is detected. In addition, both populations



**Figure 2.** Means  $\pm$  standard error of colour measures for black and red families raised under full and red light. Lines connect sibs in each light environment. A, red area; B, red intensity; C, black intensity; D, intensity of eye colour; E, body brightness; F, body darkness. Some error bars are too small to be visible.

expressed more red area in red-shifted light. This could be a way to enhance brightness contrast. Surfaces that reflect the more abundant wavelengths of light will appear brighter than those that reflect other wavelengths (Endler, 1993; McDonald, Reimchen & Hawryshyn, 1995). Therefore, red throats should appear very bright under red-shifted light, despite the lack of colour contrast. This is especially true if the red is present on the lateral surfaces of the fish (McDonald *et al.*, 1995) because females view males in this plane. These results demonstrate clearly that colour expression does respond to ambient light, but the presence of strong genetic effects shows that there are limits to this plasticity. On average, fish from the red population expressed more red, and fish from the

black population expressed more black, regardless of light environment.

By contrast to the pattern for red and black throat colour and for body darkness, body brightness responded to light environment but showed little genetic variation between and within populations. The degree of plasticity is not surprising, as body colour may be involved in background matching for crypsis (Rowe *et al.*, 2004) and may change in response to social interactions either to enhance or diminish conspicuousness or to signal dominance (Rush *et al.*, 2003).

We also have evidence of a genetic basis to the phenotypic plasticity observed. We found marginally significant  $G \times E$  between populations for red inten-

**Table 2.** Phenotypic correlations for individual sons in the red (above diagonal,  $N = 67$ ) and black (below diagonal,  $N = 77$ ) populations across light treatments

	Red index	Red area	Red intensity	Black intensity	Body darkness	Body brightness	Eye intensity
Red index		0.862***	0.964***	<b>0.135</b>	<b>0.498***</b>	<b>-0.097</b>	<b>-0.002</b>
Red area	0.910***		0.696***	<b>0.132</b>	<b>0.456***</b>	<b>-0.001</b>	<b>-0.042</b>
Red intensity	0.880***	0.610***		<b>0.131</b>	<b>0.456***</b>	0.001	0.067
Black intensity	<b>-0.338**</b>	<b>-0.201#</b>	<b>-0.373***</b>		0.506***	-0.250*	<b>0.020</b>
Body darkness	<b>-0.208#</b>	<b>-0.175</b>	<b>-0.166</b>	0.769***		-0.235*	-0.141
Body brightness	<b>0.226*</b>	<b>0.205#</b>	0.201#	-0.378***	-0.548***		0.393**
Eye intensity	<b>0.221*</b>	<b>0.198#</b>	0.197#	<b>-0.359**</b>	-0.538***	0.628***	

# $P < 0.10$ , \* $P < 0.05$ , \*\* $P < 0.005$ , \*\*\* $P < 0.0005$ .

Values in bold indicate that red and black families differ in the direction of correlation with at least one correlation coefficient significantly different from 0.

**Table 3.** Genetic correlations based on sire means (1/2 sibs) for red (above diagonal,  $N = 3$ ) and black (below diagonal,  $N = 5$ ) families averaged across light treatments

	Red index	Red area	Red intensity	Black intensity	Body darkness	Body brightness	Eye intensity
Red index		0.999*	0.996*	-0.299	0.732	-0.771	-0.976
Red area	0.889*		0.991#	-0.338	0.704	-0.745	-0.966
Red intensity	0.923*	0.646		-0.211	0.791	-0.826	-0.992#
Black intensity	-0.450	-0.256	-0.491		0.431	-0.376	0.083
Body darkness	-0.107	-0.009	-0.116	0.888*		-0.998*	-0.863
Body brightness	-0.186	-0.082	-0.286	-0.375	-0.737		0.892
Eye intensity	0.077	0.163	-0.053	-0.492	-0.766	0.965**	

# $P < 0.10$ , \* $P < 0.05$ , \*\* $P < 0.01$ .

These are narrow sense correlations

sity and body darkness. The  $G \times E$  effect for populations is expected given the previously demonstrated result that male colour matches the light environment. Therefore, this result corroborates earlier findings based on phenotypic measurements alone (Boughman, 2001) and demonstrates that there is some genetic component to this matching. With increased sample size, we might expect to see even more evidence of  $G \times E$ . We also found significant or marginally significant  $G \times E$  within populations for all colour measures except red area. The rank order of both red and black families changed across light environment for all measures of colour, (Fig. 2), demonstrating genetic variation in plasticity.

Plasticity and evolutionary change in response to light environment are in the same direction for some colour traits; fish from the black populations express more black and are darker overall than their counterparts from the red population, and fish from both populations are darker and blacker when reared in the red-shifted environment. This synergistic effect

would tend to enhance the degree of colour contrast to background light, and therefore is likely to be adaptive. A positive correlation in the direction of plastic and evolutionary responses has been found for other traits in threespine sticklebacks as well. For example, plasticity is shown when fish raised in fresh water grow to a smaller size than in the marine environment (Pritchard & Schluter, 2001; McKinnon *et al.*, 2004). Yet body size is also genetically based, as fish from populations that differ in size maintain size differences in common garden experiments (McPhail, 1977; Snyder & Dingle, 1989; Snyder & Dingle, 1990), and quantitative trait loci for body size have been found (Colosimo *et al.*, 2004). The causes and consequences of correlations between plasticity and the direction of evolutionary change would be an interesting area for further research.

The difference in direction of phenotypic correlations among red and black colour traits in red and black populations, as well as a similar but weaker pattern for genetic correlations, suggests different

mechanisms underlie their expression. Red families with high scores for red nuptial colour simultaneously had high scores for black intensity. This positive correlation suggests a mechanism that affects overall colour intensity. By contrast, black families with high scores for black intensity had little or no red nuptial colour. This negative correlation suggests that red and black are alternative nuptial colour phenotypes for these black families.

The results obtained in the present study show that there is a genetic basis to the differences in nuptial colour exhibited by the two populations under investigation. Earlier work implicated the light environment as the ultimate selective force acting on nuptial colour in these populations, due to its effects on the transmission of colour signals and on how females perceive colour (Boughman, 2001). Males display nuptial colour that contrasts with the background light to enhance conspicuousness. Moreover, changes in female perception are correlated to differences in female preference for red nuptial colour: females from populations that mate in red-shifted habitats have low sensitivity to red light and weak or no preference for red males; whereas the reverse is true for females from populations that mate in full spectrum light. The present results indicate that selection has caused divergence among the populations we studied in the genetic basis of male nuptial colour expression.

Predation against red males or lack of carotenoids in red-shifted habitats have been suggested as alternative causes of differences in red and black nuptial colour for threespine sticklebacks (Reimchen, 1989; Scott, 2001). We can rule out these explanations in the present study because we raised fish in the absence of predation and on the same diet in both light environments. We found similar levels of colour expression to that which is seen in the wild populations, suggesting that predation and carotenoid levels play a relatively minor role in colour expression for our populations. However, experiments to test these effects are needed.

Colour differences are involved in pre-mating reproductive isolation in sticklebacks (Boughman, 2001; Boughman, Rundle & Schluter, 2005). The genetic basis and differences in colour that we found should enhance colour-based pre-mating isolation. However, plasticity in colour expression suggests colour-based pre-mating isolation might be susceptible to changes in light environment. Indeed, black benthics and red limnetics from Enos Lake have recently begun hybridizing (Kraak *et al.*, 1999; Taylor *et al.*, 2006), suggesting reduced pre-mating isolation. This could be because historical differences in mating habitat appear to have been lost as water colour is no longer red-shifted (personal observation). Our findings

suggest colour differences between species could be eroded by plastic responses to changes in light, and this might be one factor contributing to increased hybridization. Hybrids would also have a mix of red and black alleles, further eroding colour differences.

#### CAUSES OF COLOUR VARIATION IN OTHER FISHES

Our findings are consistent with several other recent studies investigating the basis of variation in nuptial colour in fishes. Guppies (*Poecilia reticulata*) vary enormously in colour pattern both within and between populations (Endler & Houde, 1995), and male colour has been shown to be under genetic control (Houde, 1992; Brooks & Endler, 2001; Hughes, Rodd & Reznick, 2005). Carotenoid based orange colour shows higher heritability than structural and melanic colours (Hughes *et al.*, 2005), suggesting different underlying genetic mechanisms and differences in the extent of phenotypic plasticity for these colour traits. The intake of carotenoids through diet has been shown to affect male colour (Grether *et al.*, 1999); thus, there is also plasticity in the expression of orange colour. Houde & Torio (1992) also found plasticity in response to parasite infection. These results are consistent with expectations for allocation tradeoffs of carotenoids between immune response and signal function (Blount *et al.*, 2003; Faivre *et al.*, 2003; Grether *et al.*, 2004), and with predictions from indicator models (Hamilton & Zuk, 1982; Andersson, 1994; Grether, 2000).

The combined effects of environmental factors and genetics have also been investigated in other systems. Studies on bluefin killifish (*Lucania goodei*) have revealed causes of variation among populations in expression of blue, red, and yellow colour (Fuller, 2002). Similar to the results of the present study, variation in nuptial colour for bluefin killifish included significant genetic, light environment, and  $G \times E$  effects (Fuller & Travis, 2004). Melanic colour in fishes has also been shown to be influenced both by genetics and environment (Angus, Dass & Blanchard, 1999). Melanic forms of mosquito fish (*Gambusia holbrooki*) are different genotypes (Angus, 1989), but have temperature dependent expression of either silver or melanic body colour (Angus *et al.*, 1999; Horth, 2003).

As with sticklebacks, sensory drive has been implicated in several of these systems as well. The light environment plays a role in the evolution of colour traits in guppies (Gamble *et al.*, 2003; Grether, Kolluru & Rodd, 2005), killifish (Fuller & Travis, 2004), and swordtails (Franck, Dikomey & Schartl, 2001). Moreover, perceptual biases, as with those of the female stickleback, also play a role (Basolo, 1990; Rodd *et al.*, 2002; Fuller *et al.*, 2003).

## GENETICS OF MATING TRAITS IN OTHER TAXA

There have been a few quantitative genetic studies investigating traits involved in both sexual selection and sexual isolation in other taxa. Cuticular hydrocarbons (CHCs) function in both mate choice and mate recognition in *Drosophila* species (Coyne & Charlesworth, 1997; Blows & Allan, 1998; Etges & Jackson, 2001). A series of experiments on two *Drosophila* species from Australia found some additive genetic variance for CHCs, but suggested that selection has depleted substantial portions of genetic variation (Blows, Chenoweth & Hine, 2004). The results of the present study also suggest depletion of genetic variance within populations. These questions have also been addressed in *Laupala* crickets, in which song pulse rate isolates species (Shaw, 1996; Mendelson & Shaw, 2002; Shaw & Parsons, 2002) and song is a target of mate choice (Shaw & Herlihy, 2000). Quantitative genetic studies uncovered a polygenic basis for song pulse rate differences between two of these species (Shaw, 1996) and for female mate recognition based on these pulse rate differences (Shaw, 2000). The similarly complex genetic basis for both song and song preference would facilitate coevolution of the signaller and receiver components of this communication system.

## CONCLUSIONS

The present study takes a first step towards understanding the causes of variation of complex traits that are involved in both sexual selection and pre-mating isolation in threespine sticklebacks. We demonstrate a genetic basis to nuptial colour variation within and between a red and a black population, show that colour traits respond in a plastic manner to the light environment, and that red and black populations differ somewhat in these responses. Genetic differences between populations are likely to result from past divergent environment-dependent selection. Carotenoid and melanin based colours depend on different biochemical pathways and respond to different aspects of the environment (Griffith, Parker & Olson, 2006); thus, it is not surprising to find differences in genetics and plasticity between red and black populations. Differences in genetic mechanisms of colour should allow for continued divergence in response to local environments. Further work is necessary to obtain more detailed information on the number and identity of genes responsible for colour differences, and the magnitude and nature of their effects.

## ACKNOWLEDGMENTS

We thank C. Lee, C. Peichel, M. Ritchie, D. Fairbairn, and two anonymous reviewers for helpful comments

and suggestions on the manuscript, M. Posavi for statistical advice, and J. Hutchens for assistance measuring fish. Funding was provided by a Trewartha Undergraduate Honors Research Grant from the UW-Madison L&S Honors Program and a Bunde Fund Undergraduate Research Grant from the UW-Madison Department of Zoology to E.J.L., and by UW-Madison Graduate School funds and an NSF grant to J.W.B.

## REFERENCES

- Andersson M.** 1994. *Sexual selection*. Princeton, NJ: Princeton University Press.
- Angus RA.** 1989. Inheritance of melanistic pigmentation in the eastern mosquitofish. *Journal of Heredity* **80**: 387–392.
- Angus RA, Dass B, Blanchard PD.** 1999. Quantification of the expression of a temperature-sensitive pigment allele in sailfin mollies (*Poecilia latipinna*) by image analysis. *Pigment Cell Research* **12**: 126–130.
- Bakker TCM, Milinski M.** 1993. The advantages of being red – sexual selection in the stickleback. *Marine Behaviour and Physiology* **23**: 287–300.
- Basolo AL.** 1990. Female preference predates the evolution of the sword in swordtail fish. *Science* **250**: 808–810.
- Blount JD, Metcalfe NB, Birkhead TR, Surai PF.** 2003. Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science* **300**: 125–127.
- Blows MW, Allan RA.** 1998. Levels of mate recognition within and between two *Drosophila* species and their hybrids. *American Naturalist* **152**: 826–837.
- Blows MW, Chenoweth SF, Hine E.** 2004. Orientation of the genetic variance–covariance matrix and the fitness surface for multiple male sexually selected traits. *American Naturalist* **163**: 329–340.
- Boughman JW.** 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* **411**: 944–948.
- Boughman JW.** 2002. How sensory drive can promote speciation. *Trends in Ecology Evolution* **17**: 571–577.
- Boughman JW, Rundle HD, Schluter D.** 2005. Parallel evolution of sexual isolation in sticklebacks. *Evolution* **59**: 361–373.
- Brooks R, Endler JA.** 2001. Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). *Evolution* **55**: 1002–1015.
- Colosimo PF, Peichel CL, Nereng K, Blackman BK, Shapiro MD, Schluter D, Kingsley DM.** 2004. The genetic architecture of parallel armor plate reduction in threespine sticklebacks. *PLoS Biology* **2**: 635–641.
- Coyne JA, Charlesworth B.** 1997. Genetics of a pheromonal difference affecting sexual isolation between *Drosophila mauritiana* and *D. sechellia*. *Genetics* **145**: 1015–1030.
- Endler JA.** 1992. Signals, signal conditions, and the direction of evolution. *American Naturalist* **139**: S125–S153.
- Endler JA.** 1993. The color of light in forests and its implications. *Ecological Monographs* **63**: 1–27.

- Endler JA, Houde AE. 1995.** Geographic-variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* **49**: 456–468.
- Etges WJ, Jackson LL. 2001.** Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis* part 6 – epicuticular hydrocarbon variation in *Drosophila mojavensis* cluster species. *Journal of Chemical Ecology* **27**: 2125–2149.
- Faivre B, Gregoire A, Preault M, Cezilly F, Sorci G. 2003.** Immune activation rapidly mirrored in a secondary sexual trait. *Science* **300**: 103–103.
- Franck D, Dikomey M, Schartl M. 2001.** Selection and the maintenance of a colour pattern polymorphism in the green swordtail (*Xiphophorus helleri*). *Behaviour* **138**: 467–486.
- Frischknecht M. 1993.** The breeding coloration of male 3-spined sticklebacks (*Gasterosteus aculeatus*) as an indicator of energy investment in vigor. *Evolutionary Ecology* **7**: 439–450.
- Fuller RC. 2002.** Lighting environment predicts the relative abundance of male colour morphs in bluefin killifish (*Lucania goodei*) populations. *Proceedings of the Royal Society of London Series B, Biological Sciences* **269**: 1457–1465.
- Fuller RC, Fleishman LJ, Leal M, Travis J, Loew E. 2003.** Intraspecific variation in retinal cone distribution in the bluefin killifish, *Lucania goodei*. *Journal of Comparative Physiology A, Neuroethology Sensory Neural and Behavioral Physiology* **189**: 609–616.
- Fuller RC, Travis J. 2004.** Genetics, lighting environment, and heritable responses to lighting environment affect male color morph expression in bluefin killifish, *Lucania goodei*. *Evolution* **58**: 1086–1098.
- Gamble S, Lindholm AK, Endler JA, Brooks R. 2003.** Environmental variation and the maintenance of polymorphism: the effect of ambient light spectrum on mating behaviour and sexual selection in guppies. *Ecology Letters* **6**: 463–472.
- Grether GF. 2000.** Carotenoid limitation and mate preference evolution: a test of the indicator hypothesis in guppies (*Poecilia reticulata*). *Evolution* **54**: 1712–1724.
- Grether GF, Hudon J, Millie DF. 1999.** Carotenoid limitation of sexual coloration along an environmental gradient in guppies. *Proceedings of the Royal Society of London Series B, Biological Sciences* **266**: 1317–1322.
- Grether GF, Kasahara S, Kolluru GR, Cooper EL. 2004.** Sex-specific effects of carotenoid intake on the immunological response to allografts in guppies (*Poecilia reticulata*). *Proceedings of the Royal Society of London Series B, Biological Sciences* **271**: 45–49.
- Grether GF, Kolluru GR, Rodd FH, de la Cerda J, Shimazaki K. 2005.** Carotenoid availability affects the development of a colour-based mate preference and the sensory bias to which it is genetically linked. *Proceedings of the Royal Society of London Series B, Biological Sciences* **272**: 2181–2188.
- Griffith SC, Parker TH, Olson VA. 2006.** Melanin-versus carotenoid-based sexual signals: is the difference really so black and red? *Animal Behaviour* **71**: 749–763.
- Hamilton WD, Zuk M. 1982.** Heritable true fitness and bright birds – a role for parasites. *Science* **218**: 384–387.
- Hill GE, Inouye CY, Montgomerie R. 2002.** Dietary carotenoids predict plumage coloration in wild house finches. *Proceedings of the Royal Society of London Series B, Biological Sciences* **269**: 1119–1124.
- Horth L. 2003.** Melanic body colour and aggressive mating behaviour are correlated traits in male mosquitofish (*Gambusia hotbrooki*). *Proceedings of the Royal Society of London Series B, Biological Sciences* **270**: 1033–1040.
- Houde AE. 1992.** Sex-linked heritability of a sexually selected character in a natural population of *Poecilia reticulata* (Pisces, Poeciliidae) (Guppies). *Heredity* **69**: 229–235.
- Houde AE, Torio AJ. 1992.** Effect of parasitic infection on male color pattern and female choice in guppies. *Behavioral Ecology* **3**: 346–351.
- Hughes KA, Rodd FH, Reznick DN. 2005.** Genetic and environmental effects on secondary sex traits in guppies (*Poecilia reticulata*). *Journal of Evolutionary Biology* **18**: 35–45.
- Kraak SBM, Bakker TCM, Mundwiler B. 1999.** Sexual selection in sticklebacks in the field: correlates of reproductive, mating, and paternal success. *Behavioral Ecology* **10**: 696–706.
- Kronforst MR, Young LG, Kapan DD, McNeely C, O'Neill RJ, Gilbert LE. 2006.** Linkage of butterfly mate preference and wing color preference cue at the genomic location of wingless. *Proceedings of the National Academy of Sciences of the United States of America* **103**: 6575–6580.
- McDonald CG, Reimchen TE, Hawryshyn CW. 1995.** Nuptial colour loss and signal masking in *Gasterosteus*: an analysis using video imaging. *Behaviour* **132**: 963–977.
- McGraw KJ, Hill GE, Stradi R, Parker RS. 2001.** The influence of carotenoid acquisition and utilization on the maintenance of species-typical plumage pigmentation in male American goldfinches (*Carduelis tristis*) and northern cardinals (*Cardinalis cardinalis*). *Physiological and Biochemical Zoology* **74**: 843–852.
- McKinnon JS, Mori S, Blackman BK, David L, Kingsley DM, Jamieson L, Chou J, Schluter D. 2004.** Evidence for ecology's role in speciation. *Nature* **429**: 294–298.
- McLennan DA, McPhail JD. 1989.** Experimental investigations of the evolutionary significance of sexually dimorphic nuptial coloration in *Gasterosteus aculeatus* (L) – temporal changes in the structure of the male mosaic signal. *Canadian Journal of Zoology-Revue Canadienne de Zoologie* **67**: 1767–1777.
- McLennan DA, McPhail JD. 1990.** Experimental investigations of the evolutionary significance of sexually dimorphic nuptial coloration in *Gasterosteus aculeatus* (L) – the relationship between male color and female behavior. *Canadian Journal of Zoology-Revue Canadienne de Zoologie* **68**: 482–492.
- McPhail JD. 1977.** Inherited interpopulation differences in size at 1st reproduction in threespine stickleback, *Gasterosteus aculeatus* L. *Heredity* **38**: 53–60.
- McPhail JD. 1984.** Ecology and evolution of sympatric sticklebacks (*Gasterosteus*) – morphological and genetic-

- evidence for a species pair in Enos Lake, British Columbia. *Canadian Journal of Zoology-Revue Canadienne de Zoologie* **62**: 1402–1408.
- Mendelson TC, Shaw KL. 2002.** Genetic and behavioral components of the cryptic species boundary between *Laupala cerasina* and *L. kohalensis* (Orthoptera: Gryllidae). *Genetica* **116**: 301–310.
- Milinski M, Bakker TCM. 1990.** Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature* **344**: 330–333.
- Moehring AJ, Li JA, Schug MD, Smith SG, deAngelis M, Mackay TFC, Coyne JA. 2004.** Quantitative trait loci for sexual isolation between *Drosophila simulans* and *D. mauritiana*. *Genetics* **167**: 1265–1274.
- Noor MAF, Grams KL, Bertucci LA, Almendarez Y, Reiland J, Smith KR. 2001.** The genetics of reproductive isolation and the potential for gene exchange between *Drosophila pseudoobscura* and *D. persimilis* via backcross hybrid males. *Evolution* **55**: 512–521.
- Pritchard JR, Schluter D. 2001.** Declining interspecific competition during character displacement: summoning the ghost of competition past. *Evolutionary Ecology Research* **3**: 209–220.
- Reimchen TE. 1989.** Loss of nuptial color in threespine sticklebacks (*Gasterosteus aculeatus*). *Evolution* **43**: 450–460.
- Rodd FH, Hughes KA, Grether GF, Baril CT. 2002.** A possible non-sexual origin of mate preference: are male guppies mimicking fruit? *Proceedings of the Royal Society of London Series B, Biological Sciences* **269**: 475–481.
- Rodriguez RL, Greenfield MD. 2003.** Genetic variance and phenotypic plasticity in a component of female mate choice in an ultrasonic moth. *Evolution* **57**: 1304–1313.
- Rowe MP, Baube CL, Loew ER, Phillips JB. 2004.** Optimal mechanisms for finding and selecting mates: how threespine stickleback (*Gasterosteus aculeatus*) should encode male throat colors. *Journal of Comparative Physiology A, Neuroethology Sensory Neural and Behavioral Physiology* **190**: 241–256.
- Rowe MP, Baube CL, Phillips JB. 2006.** Trying to see red through stickleback photoreceptors: functional substitution of receptor sensitivities. *Ethology* **112**: 218–229.
- Rush VN, McKinnon JS, Abney MA, Sargent RC. 2003.** Reflectance spectra from free-swimming sticklebacks (*Gasterosteus*): social context and eye-jaw contrast. *Behaviour* **140**: 1003–1019.
- Scott RJ. 2001.** Sensory drive and nuptial colour loss in the three-spined stickleback. *Journal of Fish Biology* **59**: 1520–1528.
- Shaw KL. 1996.** Polygenic inheritance of a behavioral phenotype: Interspecific genetics of song in the Hawaiian cricket genus *Laupala*. *Evolution* **50**: 256–266.
- Shaw KL. 2000.** Interspecific genetics of mate recognition: Inheritance of female acoustic preference in Hawaiian crickets. *Evolution* **54**: 1303–1312.
- Shaw KL, Herlihy DP. 2000.** Acoustic preference functions and song variability in the Hawaiian cricket *Laupala cerasina*. *Proceedings of the Royal Society of London Series B, Biological Sciences* **267**: 577–584.
- Shaw KL, Parsons YM. 2002.** Divergence of mate recognition behavior and its consequences for genetic architectures of speciation. *American Naturalist* **159**: S61–S75.
- Snyder RJ, Dingle H. 1989.** Adaptive, genetically based differences in life-history between estuary and fresh-water threespine sticklebacks (*Gasterosteus aculeatus* L). *Canadian Journal of Zoology-Revue Canadienne de Zoologie* **67**: 2448–2454.
- Snyder RJ, Dingle H. 1990.** Effects of fresh-water and marine overwintering environments on life histories of threespine sticklebacks – evidence for adaptive variation between anadromous and resident fresh-water populations. *Oecologia* **84**: 386–390.
- Taylor EB, Boughman JW, Groenenboom M, Sniatynski M, Schluter D, Gow JL. 2006.** Speciation in reverse: morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteus aculeatus*) species pair. *Molecular Ecology* **15**: 343–355.