

Condition-dependent expression of red colour differs between stickleback species

J. W. BOUGHMAN

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

Keywords:

colour;
condition dependence;
sexual selection;
speciation;
sticklebacks.

Abstract

Sexual isolation may arise when male mating traits and female preferences differ between species. Such divergence in mating traits is likely to occur when the strength or targets of sexual selection differ. Therefore, by comparing the traits under sexual selection in closely related species and the nature of preference for those traits, we can gain insight into when sexual selection contributes to sexual isolation and how it does so. Collecting these data is no easy undertaking. To simplify this comparison, I use the presence and extent of condition dependence in traits to determine whether directional sexual selection is acting on them. Condition dependence thus serves as a signature of sexual selection. I investigate differences in sexual selection on red nuptial colour in limnetic–benthic species pairs of three-spined sticklebacks. I evaluate condition dependence by comparing the strength of the relationship between colour and condition, and the magnitude of variance in red nuptial colour to other colour traits and to nonsexual traits. I find that limnetic males have strong condition-dependent expression of red nuptial colour whereas benthic males have at most weak condition-dependent expression. Ancestral anadromous males show no condition dependence. This suggests that colour is under strong directional sexual selection only in limnetics and that this is the derived state. Moreover, I find that the strength of female preference for red is related to the extent of condition dependence. The extent of condition dependence is also associated with the importance of colour differences to mate recognition. These results show that differences between these species in the action of sexual selection underlie their sexual isolation.

Introduction

Sexual selection is thought to be involved in speciation, and there is a recent upsurge of interest in testing this idea. One of the first steps has been to investigate differences in male mating traits and female preferences between species and ask if those traits are involved in reproductive isolation. Evidence of this type is accumulating (Seehausen & van Alphen, 1999; Shaw, 2000; Boughman, 2001; Couldridge & Alexander, 2002; Masta & Maddison, 2002; Knight & Turner, 2004; Mendelson &

Shaw, 2005), although counterexamples also exist (Price, 1998; Gage *et al.*, 2002; Ritchie *et al.*, 2005b). Implicit in this approach is that sexual selection is acting in a different way in species to cause differences in mating traits. This could occur if the mode of sexual selection varies from directional (with open-ended preferences) to stabilizing (with unimodal preferences). In addition, preferences may differ in their strength (Boughman, 2001), or in the values of a specific trait preferred by females (Endler & Houde, 1995; Wells & Henry, 1998; Uy & Borgia, 2000; Naisbit *et al.*, 2001). The actual targets of preference may also differ, for example if one species focuses on colour and another on courtship behaviour. Any of these differences in sexual selection could cause divergence in mating traits, resulting in sexual isolation. Ultimately then, to assess the ability of sexual selection to

Correspondence: Janette Boughman, Department of Zoology, University of Wisconsin-Madison, WI 53706, USA.
Tel.: (608) 262 2636; fax: (608) 265 6320;
e-mail: jboughman@wisc.edu

drive speciation we need to know whether sexual selection operates differently between closely related species.

Yet, how can we do this? One promising approach is to find 'signatures' of sexual selection and compare those across populations or species. We can infer that sexual selection acts differently when species differ in the presence or magnitude of the signature. Sexual dimorphism has been used this way repeatedly to infer the action of sexual selection in speciation (Mitra *et al.*, 1996; Price, 1998; Panhuis *et al.*, 2001; Gage *et al.*, 2002; Morrow *et al.*, 2003; Gonzales Zuarth & Macias Garcia, 2006). Yet, sexual dimorphism is a fairly gross indicator that sexual selection is either present or absent in a species or clade. A potentially more sensitive signature of sexual selection is the degree of condition dependence in sexual traits. Variation in the extent of condition dependence should correlate with variation in how sexual selection is acting in a particular species. This is true because the action of sexual selection itself can generate condition dependence in male trait expression (Price *et al.*, 1993; Iwasa & Pomiankowski, 1994; Rowe & Houle, 1996; Houle & Kondrashov, 2002). Patterns of condition dependence should be able to tell us not only whether sexual selection is acting, but also details of its how it plays out in a particular species; for example, whether it varies in strength, direction, or mode.

Condition dependence can be used this way because directional sexual selection is predicted to generate strong linear condition dependence in the male traits that are the focus of mate choice (Price *et al.*, 1993; Iwasa & Pomiankowski, 1994; Rowe & Houle, 1996; Houle & Kondrashov, 2002); whereas stabilizing and disruptive sexual selection are not. Traits on which sexual selection is not acting should show little relationship between the trait and condition (Bonduriansky & Rowe, 2005). Traits under stabilizing sexual selection – when females prefer males with the mean value of a trait – should show either low condition dependence or a curvilinear relationship between the trait value and condition. Disruptive sexual selection can be caused by several processes, including alternative mating strategies, and should result in a weak or curvilinear relationship between a trait and condition, but with curvature in the opposite direction from that resulting from stabilizing sexual selection (Candolin, 1999). These expected relationships allow us to use the presence and shape of condition dependence to infer the presence of directional sexual selection. We can do this within a single species to compare several traits putatively under sexual selection. Those experiencing strong directional sexual selection are predicted to show high condition dependence whereas those experiencing no directional sexual selection are predicted to show low condition dependence (Wilkinson & Taper, 1999; Bonduriansky & Rowe, 2005).

The value of this approach for speciation studies is that the prediction should also hold when comparing the

same trait between closely related species. If species differ in the extent of condition dependence for a trait, we can infer that they also differ in the strength of sexual selection on the trait, or in the nature of sexual selection (e.g. whether it is directional or stabilizing). Open-ended and unimodal preferences are likely to differ in their capacity to cause divergence in male mating traits (Ritchie, 1996; Price, 1998; Shaw & Herlihy, 2000), and therefore, the nature of preference has consequences for speciation via sexual selection. This approach investigates a particular kind of sexual selection, which is not to say that other kinds of sexual selection are not involved in the evolution of sexual isolation; only to test the role of directional sexual selection leading to condition dependence.

I use this approach to study the contribution of sexual selection to reproductive isolation in species pairs of limnetic and benthic three-spined sticklebacks (*Gasterosteus* spp.) in which male nuptial colour and female preference for colour differ (Ridgway & McPhail, 1984; Boughman, 2001). These are good biological species because they are isolated by strong premating and postmating barriers and thus, have little gene flow. Sexual selection may have been especially important in the evolution of sexual isolation. The extent of difference in colour and preference between replicate populations of each species correlates to the degree of reproductive isolation between them, demonstrating that divergence in these traits does indeed contribute to reproductive isolation between the species (Boughman, 2001). In addition, reproductive isolation depends on different combinations of traits for the two species; body size and odour are paramount for benthics (Nagel & Schluter, 1998; Rafferty & Boughman, 2006) and a combination of colour and size for limnetics (Boughman *et al.*, 2005). We know already that female limnetics have different colour preferences than female benthics. We also know that male limnetics have more colour than male benthics. The species also differ in size. Differences in colour, size, and preference suggest that sexual selection differs between species.

If the strength or mode of sexual selection on colour differs between limnetics and benthics, the extent of condition dependence is predicted to differ. Specifically, one predicts that limnetic males will have high condition-dependent expression of red nuptial colour because limnetic females have strong directional preference for red. In contrast, benthic males will have low condition-dependent expression because benthic females have weak or no preference for red. Obtaining mates which are currently in high condition is likely to benefit female sticklebacks directly because males provide all parental care. High condition males are more likely to survive the parental phase and have been shown to have a higher probability of successfully rearing offspring than low condition males (Candolin, 2000). They are also less likely to eat eggs (Manica, 2002). Females

may also gain indirect benefits if high condition males carry good genes.

To test condition-dependent expression of red nuptial colour I test three primary predictions. I also test one prediction specific to its role in speciation. In each case I test predictions for condition dependence within each species, and then compare across species. The within-species comparisons allow me to determine whether red nuptial colour is in fact, condition dependent. The between-species comparisons allow me to determine whether sexual selection is acting differently in the species. To infer the direction of evolutionary change in condition dependence, I investigate condition-dependent expression of red nuptial colour in the ancestor, the anadromous three-spined stickleback (*Gasterosteus aculeatus*). This allows me to determine whether condition dependence has increased or decreased in the descendent species.

First, I test the prediction that traits under directional sexual selection will have higher condition dependence than traits that are not (Grafen, 1990; Price *et al.*, 1993; Iwasa & Pomiankowski, 1994; Rowe & Houle, 1996; Houle & Kondrashov, 2002; Cotton *et al.*, 2004). Therefore, to determine whether red nuptial colour is condition dependent in each species I compare the extent of condition dependence for red nuptial colour to nonsexual traits. I also compare red to other colour traits to assess the strength of sexual selection on each. Condition dependence has been shown to vary among traits in other taxa (Bonduriansky & Rowe, 2005), suggesting that sexual selection targets some of these traits and not others. Secondly, condition-dependent traits are expected to have higher phenotypic and genetic variance than traits that are not condition dependent through a process known as genic capture (Rowe & Houle, 1996). Essentially, when trait values depend on condition, genetic variance in condition translates into genetic variance in the male trait. To test this prediction, I compare the magnitude of phenotypic variance in red nuptial colour to other colour traits and to nonsexual traits. Thirdly, if female choice has been involved in the evolution of condition dependence, then the extent of condition dependence in the male trait should be related to the strength of female preference. Here, I test whether the extent of condition dependence in red nuptial colour is related to the strength of female preference for red. These three predications test whether red nuptial colour is condition dependent and whether it is under directional sexual selection in each species. Finally, if sexual isolation evolves from differences in sexual selection, then differences between species in the strength of sexual selection on a trait should be related to the trait's importance in sexual isolation. To test this prediction I ask whether differences in the importance of red nuptial colour in sexual isolation for sympatric pairs of limnetics and benthics are related to the extent of condition dependence.

Methods

Study populations

I studied several populations of sticklebacks found in coastal British Columbia, Canada. Freshwater populations included three pairs of limnetic and benthic species – from Paxton and Priest Lakes on Texada Island, and from Enos Lake on Vancouver Island. Limnetics and benthics exploit alternate foraging niches: limnetics forage in the open water on zooplankton and benthics forage in the littoral zone on invertebrates (Bentzen & McPhail, 1984; McPhail, 1984; McPhail, 1992, 1994; Schluter & McPhail, 1992). They differ in many ecological, morphological, and mating traits and exhibit strong parallel divergent evolution (Schluter & McPhail, 1992; Boughman *et al.*, 2005). All freshwater populations evolved after the anadromous stickleback, *G. aculeatus*, colonized newly formed lakes at the end of the last glacial episode approximately 10 000–15 000 years ago. I also included an anadromous population that spawns in the Nanaimo River on Vancouver Island to represent the ancestor. Patterns of genetic variation indicate that each limnetic and benthic population represents independent invasions by this marine ancestor and are thus, evolutionarily independent of one another (Taylor & McPhail, 1999, 2000). Limnetics and benthics are good biological species. Sexual isolation is quite strong limiting hybridization to 1–5% (McPhail, 1984, 1992; Gow *et al.* 2006) and those hybrids produced are selected against (Schluter, 1994; Rundle, 2002) such that long-term estimates of gene flow for sympatric pairs are on the order of 10^{-3} (Gow *et al.*, 2005). Sample sizes for each species are $n = 92$ for limnetics, $n = 82$ for benthics, and $n = 56$ for anadromous sticklebacks.

All fish used in the study were caught in the wild using minnow traps during the height of each population's breeding season (May and June) in 2001 and 2004. Paxton fish were measured on 16 and 17 May 2001 and 6 and 7 May 2004; Priest fish on 18 May 2001; Enos fish on 5 June 2001; and Nanaimo fish on 6 June 2001. Traps were set and pulled within an hour to minimize trap time for the fish. Males in breeding condition were transferred to a holding cage fashioned from a minnow trap, which was suspended in the water column near the site of capture. Individual males were taken from this holding cage to be measured. To ensure minimal changes caused by duration of capture and handling, all measurements were made at the site within 1 h of capture and each male was handled for only 5 min before being released. This rapid and consistent measurement of males was designed to minimize colour changes caused by handling and ensure that fish experienced similar conditions. Holding time was random with respect to condition. My sampling scheme yields a snapshot of males from each population and an estimate of each male's current condition during the height of the breeding season.

Indicating current condition is likely to be especially important in this system given the importance of direct benefits and previous work showing that current condition correlated with direct benefits (Candolin, 2000). Other work has also shown genetic differences in nuptial colour between populations (E.J. Lewandowski & J.W. Boughman, unpublished). Ten males from each of the limnetic and benthic populations were kept for later morphological analysis. These males were killed with an overdose of MS-222, and then frozen.

Measuring colour and nonsexual traits

I scored several aspects of male nuptial colour, focusing on reddening of the lips, throat and belly. Female choice of red nuptial colour in sticklebacks has been the focus of many studies (McLennan & McPhail, 1990; Milinski & Bakker, 1990; Bakker & Mundwiler, 1994). But male nuptial colour is not restricted to reddening of the throat. The body and eye intensify to blue or blue-green which provides high contrast to the red throat (Rush *et al.*, 2003; Rowe *et al.*, 2004). It is not clear the extent to which these other colour traits are the focus of female choice. Therefore, I measured throat, eye and body colour. I used both a scoring method and collected reflectance data. Scoring allows an estimate of both area and intensity of colour. Reflectance data cannot be used to estimate area, but is designed to estimate colour intensity (brightness and saturation) and hue. The two methods give roughly similar results for intensity measures here, and so, corroborate each other. First, I scored the area of the throat that was red (0 is no red and 5 is maximal extent of red). I also scored the intensity of throat colour (red), eye colour (blue), and body brightness (blue or silver) using a 5-point scale where 0 is no colour, 1 is very low intensity, and 5 is maximal

intensity. Because the degree of redness perceived by females may be a combination of area and intensity I calculated an index of red nuptial colour by adding scores for red area and red intensity. A multiplicative index gave similar results to this additive one. In some populations males develop black bodies and throats during the breeding season, and in many populations healthy fish have black patches on their dorsum and melanophores distributed across their bodies, darkening the fish. Therefore, I also scored body darkness on a 5-point scale where 0 is not dark (no patches, spots, or melanic skin) and 5 is very dark. All fish were scored in standard lighting conditions against a standard background, and the person scoring colour was naive to the fish's condition.

I also collected reflectance data using an Ocean Optics S2000 spectrometer and Spectralon reflectance standard (Ocean Optics Inc., Dunedin, FL, USA). Reflectance corresponds most closely to intensity scores, but cannot be used to determine overall area of colour. I illuminated the fish with an LS-1 light at 45° and positioned the detection probe at 90°. I analysed those data with principal components separately for each species. I use each individual male's values for the principal components as their reflectance measures in condition-dependent analyses. The first principal component (PC1) for limnetic, benthic, and anadromous fish accounted for 85.4%, 94.3% and 94.1% of the variance respectively and is equivalent to brightness, given that all wavelengths loaded approximately equally (Fig. 1). Because this PC reveals little about actual colour it is not analysed further. The second principal component (PC2) accounted for 9.8%, 3.0% and 3.3% of the variance respectively for limnetic, benthic and anadromous fish. This principal component appears to distinguish short from long wavelengths, and thus, blue from yellow to red.

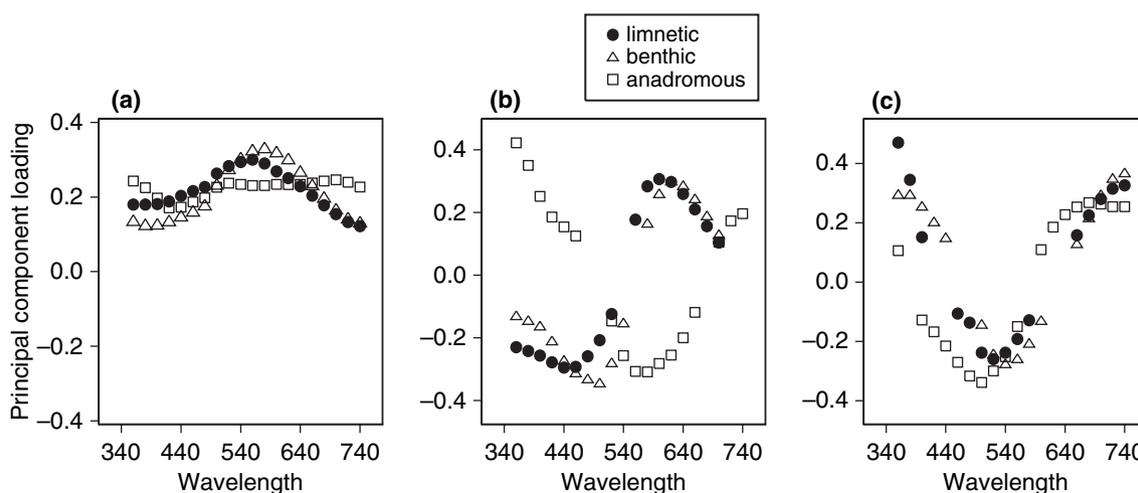


Fig. 1 Loadings for principal component analysis of reflectance data: (a) principal component 1, (b) principal component 2, and (c) principal component 3. Principal components are calculated separately for each species.

However, the pattern for limnetic and benthic sticklebacks is different than for anadromous sticklebacks. In limnetics and benthics, long wavelengths loaded positively while short wavelengths loaded negatively. In contrast, for anadromous sticklebacks the reverse is true. The third principal component (PC3) accounted for 3.9%, 2.3% and 1.9% of the variance respectively for limnetic, benthic and anadromous fish. Both short and long wavelengths loaded positively on this component and middle wavelengths loaded negatively. This component appears to distinguish middle from short and long wavelengths and thus, green from both blue and red.

I also measured two traits not thought to be involved in female choice or male competition but known to differ between species: the diameter of the eye and the length of the pelvic spine. Eye diameter may affect visual acuity (Green *et al.*, 1980; Shand, 1997) and planktivores have larger eyes than benthivores (McPhail, 1984), suggesting planktivores rely more heavily on vision in foraging. Pelvic spines help in defence against gape-limited predators (Reimchen, 1994; Reimchen & Nosil, 2002), and longer spines confer greater advantage (Reimchen, 1983; Schluter & McPhail, 1992). Limnetics have longer spines than benthics, likely resulting from selection by different predators (Reimchen, 1980). Both traits are expected to be under natural selection. Furthermore, the traits may be influenced by body condition during the growth because they form and reach full size while the fish are growing to adult size. Finally, foraging strategies may be sexually dimorphic, at least in limnetics (Bentzen & McPhail, 1984). Therefore, these traits provide a good comparison with the sexual traits. Any differences in condition dependence between these types of traits is not because of a complete lack of selection on one type or an inability of condition to affect trait magnitude, but rather to different types of selection. Eye diameter and pelvic spine length were measured on the 10 frozen males from each population. Fish were partially thawed and then photographed under standardized illumination with a Leica DC300 digital camera mounted on a Leica MZ6 dissecting scope (Leica Microsystems, Germany) fitted with a 250 mm surgical lens. Scion IMAGEJ (NIH Bethesda, MD, USA) was used to measure traits from these digital photographs, accurate to at least 0.01 mm. I do not have data on nonsexual traits for anadromous sticklebacks.

Measuring condition

To estimate body condition I measured male weight and length. I weighed males on a portable Ohaus Scout balance to the nearest 0.01 g. I took the average of two standard length measurements using vernier calipers accurate to the nearest 0.02 mm. I used regression analysis to estimate each individual's condition. I conducted linear regressions of weight on length and took the residuals from this analysis as an estimate of each

individual's condition, and call these residuals the 'condition index'. The condition index thus removes any confounding effect of length on the expression of the male trait. Because we know that there are shape differences between species I analysed each species separately. This ensures that differences in shape do not contribute to differences in estimated condition index between species. It also avoids the assumption of homogeneous slopes among groups (Garcia-Berthou, 2001). Thus, each male's condition index is the deviation of its weight from the species-specific predicted value for its length.

I explored the data and residuals both graphically and statistically to verify that the residual approach is not subject to the problems that have been identified (Darlington & Smulders, 2001; Garcia-Berthou, 2001; Green, 2001). Correcting for size using body length with least squares regression is only appropriate if the correlation between weight and length is high and the relationship linear (Green, 2001). Otherwise least squares regression will underestimate the slope, and generate large residuals for large individuals, introducing bias. Using least squares regression rather than reduced major axis regression seems reasonable for the data at hand because the correlation between length and weight is quite high within each species (r values are 0.81 for anadromous sticklebacks, 0.89 for benthics, and 0.95 for limnetics). Scatter plots, normal QQ graphs, and polynomial analyses show a strong linear relationship with no curvature and no inflation of residual size for long fish. Therefore, standard length provides a good estimate of overall body size for stickleback species. Regressing weight on length for each species separately should generate unbiased residuals that capture an individual's relative condition for its size. The natural logarithm of length and weight is often calculated to correct for allometric scaling (Kotiaho, 1999). However, when I did this I found that residuals were not distributed as evenly as without the transformation. Untransformed residuals were normally distributed and showed no trace of heteroscedasticity. Therefore, I used residuals from the regression with untransformed variables. I adjusted the degrees of freedom in all tests to account for the fact that I have calculated the slope from the data (Garcia-Berthou, 2001). This has no effect on my conclusions probably because error degrees of freedom are large.

Estimating condition dependence

To estimate condition dependence I used linear regression, regressing colour measures on condition index (species-specific residuals) for each species separately. I use two metrics to estimate the extent of condition-dependent expression of colour. The first is the slope of these lines. A slope significantly different from zero indicates that colour increases (or decreases) with condition index. However, the fit of the lines to the data and the amount of variation around those lines also indicates

how tightly colour is tied to condition index. Small deviations and a good fit indicate that colour predicts condition with high accuracy. In contrast, large deviations and a poor fit indicate that colour is a poor predictor of condition. Whether a female can use colour to select a high condition mate depends on how well condition can be predicted from colour. To estimate how well red nuptial colour predicts condition, I use R^2 from the linear regression. The second measure of condition dependence is therefore, these R^2 values. I performed regressions for red area, red intensity, red colour index, and for PC2 and PC3. I also performed similar regressions for the other colour traits (eye intensity and body darkness), and for the nonsexual traits (eye diameter and pelvic spine length) to determine whether they are condition dependent. I adjusted significance levels for the nine variables using Sidak's correction and an average correlation among variables of 0.55.

If females exert either stabilizing or disruptive selection on male nuptial colour, the relationship between colour and condition may not be linear. Curvilinear relationships could also arise if there are alternative strategies for signalling. A curvilinear relationship between red nuptial colour and condition has been found in at least one other stickleback population (Candolin, 1999). To explore the possibility of curvilinear condition dependence, I also conducted polynomial regressions using second-order terms. I did this for each species separately. I conducted similar analyses for the nonsexual traits.

Analysing female preference and condition dependence

I also assessed the relationship between the extent of condition dependence and the strength of female preference. I used estimates of the strength of female preference for red area from a previous study of these same populations that used 685 no-choice tests within and between species, and that scored male colour in similar ways (Boughman, 2001). In that study, preference was estimated as the slope of the linear regression of female response on red area, calculated separately for each population. For the present paper, I used regression to calculate condition dependence separately for each population. These analyses gave estimates for three benthic and three limnetic populations for both condition dependence and preference strength. Then, I calculated the Pearson correlation between the measures for condition dependence and female preference. I used the two metrics of condition dependence in separate tests – the slope of the regression of colour on condition, and the R^2 values from those regressions. Because the separate limnetic and benthic populations are evolutionarily independent I used each population as a replicate and thus, had six independent values for both female preference strength and condition dependence.

Analysing condition dependence and sexual isolation

Finally, I explored whether the difference between populations in the extent of condition dependence in red nuptial colour is related to the importance of colour in sexual isolation. To estimate the importance of red nuptial colour to sexual isolation I used data from an earlier study (Boughman *et al.*, 2005). That study used 143 no-choice mating trials where males and females were from different species to estimate the extent of sexual isolation. I compared male nuptial colour in trials where spawning between species did occur to where it did not. I calculated the difference in colour between these trial outcomes for each limnetic and benthic population from the three study lakes, yielding six estimates of colour-based sexual isolation. Positive values of colour-based sexual isolation indicate that females of the population rejected heterospecifics with low colour scores.

Results

Condition-dependent expression of red nuptial colour

Limnetic males have strongly positive condition-dependent expression of red nuptial colour, whereas benthic and anadromous males do not. Slope estimates indicated that the strength of the relationship between condition and red area is strongly positive for limnetics, significantly negative for benthics, and not different from zero for anadromous sticklebacks (Fig. 2; Table 1). Red area slope estimates for limnetics and benthics differ significantly ($F_{1,155} = 33.3$, $P < 0.0001$). The intensity of red colour showed a different pattern with slope direction reversed for benthics. Limnetic males still had a strongly positive slope for condition and red intensity, but benthic males had a weak and nonsignificantly positive slope, and anadromous males a nonsignificant relationship. Limnetic and benthic red intensity slopes are not significantly different ($F_{1,155} = 0.05$, $P = 0.9$). Colour index showed a pattern similar to that for red area. The relationship between condition and colour index was again strongly positive for limnetics, nonsignificantly negative for benthics, and not different from zero for anadromous sticklebacks. Limnetic and benthic red intensity slopes are significantly different ($F_{1,155} = 14.9$, $P < 0.0001$). For benthic males the area of red is significantly negatively associated with condition while the intensity of red is weakly and nonsignificantly positive, suggesting males in good condition have small areas of intense red. Anadromous males show no hint of condition dependence. Limnetic males in good condition had large areas of intense red. All three measures of red nuptial colour indicate strong condition dependence for limnetic males, as judged both by the significance of slope estimates and the proportion of variance explained.

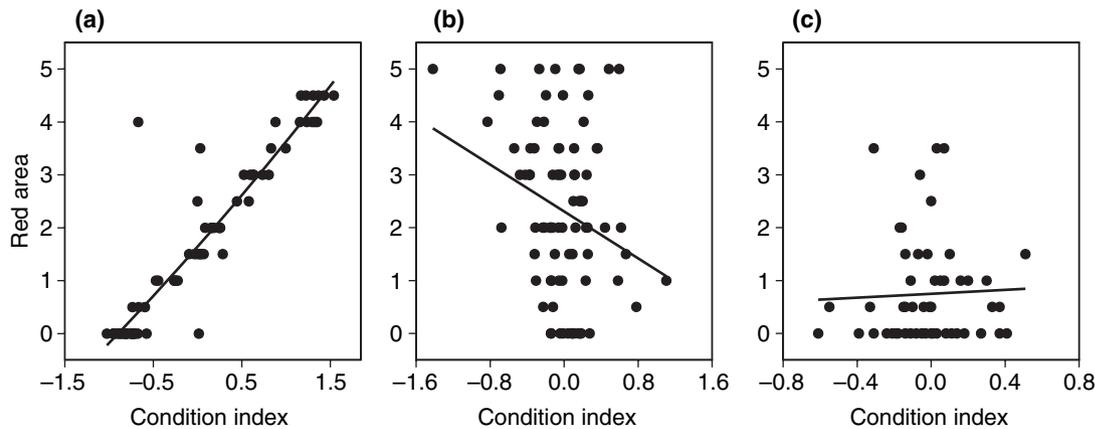


Fig. 2 Regressions of red area on condition index for (a) limnetic, (b) benthic, and (c) anadromous sticklebacks. Linear slope estimates are shown. Populations include Paxton, Priest, and Enos Lake limnetics and benthics, and Nanaimo River anadromous sticklebacks.

Table 1 Slope estimates and coefficients of determination for sexual and nonsexual traits in limnetic, benthic, and anadromous sticklebacks.

Trait type	Trait	Limnetic				Benthic				Limnetic vs. benthic		Anadromous			
		Slope	SE	<i>P</i>	<i>R</i> ²	Slope	SE	<i>P</i>	<i>R</i> ²	<i>F</i> _{1,155}	<i>P</i>	Slope	SE	<i>P</i>	<i>R</i> ²
Sexual	Red area	0.97	0.043	0.0001	0.87	-1.11	0.454	0.017	0.07	33.3	0.0001	0.18	0.302	0.76	0.002
Sexual	Red intensity	0.47	0.061	0.0001	0.44	0.41	0.263	0.12	0.03	0.05	0.95	-0.02	0.295	0.95	0.000
Sexual	Color index	1.43	0.090	0.0001	0.77	-0.70	0.654	0.29	0.14	14.9	0.001	0.18	0.863	0.85	0.002
Sexual	PC2 (red vs. blue)	1.04	0.417	0.016	0.12	1.22	0.502	0.018	0.08	1.3	0.26	0.02	0.522	0.97	0.00
Sexual	PC3 (green vs. red/blue)	-0.44	0.278	0.12	0.05	0.45	0.363	0.22	0.02	0.68	0.41	-0.57	0.386	0.15	0.04
Sexual	Eye intensity	0.06	0.091	0.52	0.007	-0.47	0.378	0.22	0.02	2.02	0.16	-0.42	0.817	0.62	0.007
Sexual	Back darkness	0.05	0.085	0.58	0.004	0.71	0.468	0.14	0.03	2.28	0.13	0.15	0.707	0.84	0.001
Nonsexual	Pelvic spine length	-0.01	0.069	0.86	0.001	-1.20	1.110	0.29	0.04	1.09					
Nonsexual	Eye diameter	0.04	0.022	0.06	0.12	-0.21	0.301	0.50	0.02	0.0					

Values shown are slope estimates, standard errors, probabilities, and *R*² calculated from linear regression of colour on condition index. The column labelled 'Limnetic vs. benthic' tests differences between limnetics and benthics using ANCOVA to test the interaction between condition and species. This is the key test of whether species differ in condition dependence. Values shown in bold are significant using Sidak's correction and an average correlation among variables of 0.55. Sample sizes for colour are limnetic = 92; benthic = 82; anadromous = 56 and for nonsexual traits are limnetic = 29; benthic = 31.

Coefficients of determination reveal that red nuptial colour is a good indicator of condition in limnetics but a poor indicator in both benthic and anadromous sticklebacks (Table 1). The coefficients of determination for limnetic red area and index are significantly higher than those for benthics ($t_4 = 52.4$, $P < 0.0001$; $t_4 = 14.8$, $P < 0.0001$ respectively) and nearly significant for red intensity ($t_4 = 2.4$, $P < 0.07$).

A pattern similar to that for red intensity was seen in the analyses of reflectance data. Limnetics and benthics had significantly positive condition dependence for PC2 (red vs. blue) whereas anadromous males did not (Table 1; Fig. 3). Therefore, high condition males had high red reflectance whereas low condition males had high blue reflectance on their throats. There are no significant differences between species in condition dependence for principal components (Table 1). This is

consistent with results from red intensity scores. Variance explained by residual analysis of principal components is low for all three species, suggesting that even for limnetic and benthic females, reflectance is a poor indicator of male condition.

None of the species showed evidence of curvilinear condition dependence (quadratic coefficients for limnetics = 0.08 ± 0.13 , $P = 0.53$; benthics = 0.92 ± 0.60 , $P = 0.13$; anadromous sticklebacks = -1.67 ± 1.74 , $P = 0.34$). Thus, relationships appear to be strictly linear.

Comparing condition dependence in sexual and nonsexual traits

First, I compare condition dependence in red nuptial colour to other colour traits thought to be involved in courtship, including body and eye colour, to test the

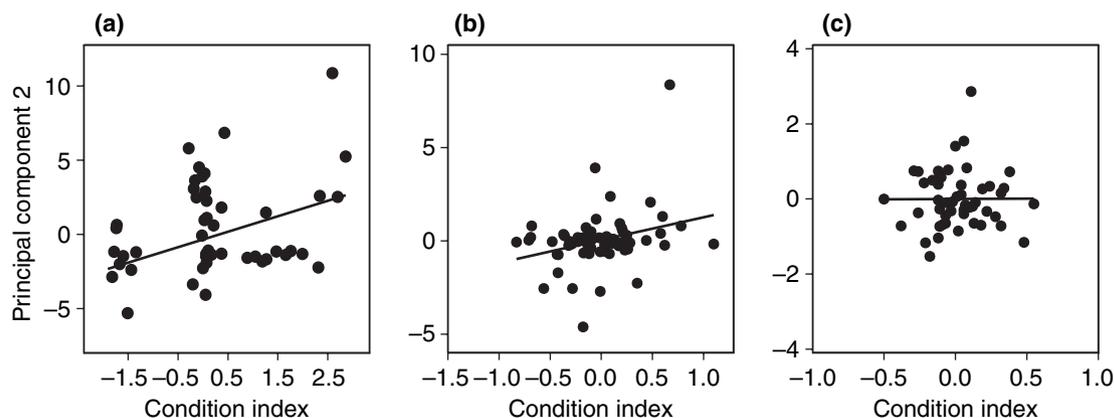


Fig. 3 Regression of PC2 on condition index for (a) limnetic, (b) benthic, and (c) anadromous sticklebacks. Linear slope estimates are shown. Populations include Paxton, Priest, and Enos Lake limnetics and benthics, and Nanaimo River anadromous sticklebacks.

Table 2 Coefficients of variation in colour and nonsexual traits for limnetic, benthic, and anadromous sticklebacks.

Species	Red area	Red intensity	Colour index	PC2	Eye intensity	Back darkness	Body length	Spine length	Eye size
Limnetic	128.5	112.6	208.0	982	41.7	59.4	60.0	17.0	3.8
Benthic	97.1	92.6	139.5	174	55.1	61.5	43.5	16.6	8.9
Anadromous	134.7	56.5	177.1	170	65.2	38.3	4.6		

hypothesis that another trait has replaced red nuptial colour as the basis for mate choice. However, neither eye nor body colour appear to be condition dependent in limnetic, benthic, or anadromous sticklebacks (Table 1). Slope estimates are near zero for limnetics, and although benthic and anadromous slope estimates are higher they remain nonsignificant, and the proportion of variance explained is extremely low for all species. Therefore, neither of these colour traits appear to be under directional sexual selection in any of the three species.

Secondly, I test a key prediction for handicap theory: that sexual traits show condition dependence while nonsexual traits do not. This prediction is borne out in the current study (Table 1). Strong positive condition dependence for limnetic red nuptial colour is not matched by condition dependence in either pelvic spine length or eye diameter in that species. The slope estimates for these traits are very near zero with low coefficients of determination. This is in marked contrast to red nuptial colour measures for limnetics. Neither do benthics show condition dependence for the nonsexual traits. Slope estimates are nonsignificantly negative and coefficients of determination are low.

Magnitude of variance in condition-dependent and nonsexual traits

The genic capture hypothesis predicts that genetic variation for condition-dependent traits is higher than for other traits. Patterns of phenotypic variation are partly

consistent with this prediction. Within species, red nuptial colour shows higher phenotypic variance than other traits (Table 2). This is especially true for limnetics, for whom measures of red nuptial colour had two to five times more variation than other colour traits, two to three times more than length, and five to 50 times more than nonsexual traits. Principal component 2 had 20, 15 and 200 times more variation than other colour, length and nonsexual traits respectively. Benthics have twice as much variation in red nuptial colour as in length, and four to 10 times more than in nonsexual traits. Principal component 2 had three, four and 10 times more variation. The between-species pattern meets predictions only weakly for colour scores but strongly for PC2 (Table 2). Benthic and anadromous fish show only a fifth the variation in PC2 as limnetics. Even though red nuptial colour does not appear to be condition dependent in benthic and anadromous sticklebacks, they do show elevated levels of variation for red nuptial colour as measured by red area and colour index; although to a lesser extent than limnetics. Benthics have 30% less variation in red area and colour index than limnetics, yet similar variation in other colour measures. Anadromous sticklebacks have high variation in red area and colour index, and very low variation in length.

Female preference evolution and condition dependence

Females are predicted to use condition-dependent traits to select mates; therefore, the strength of female

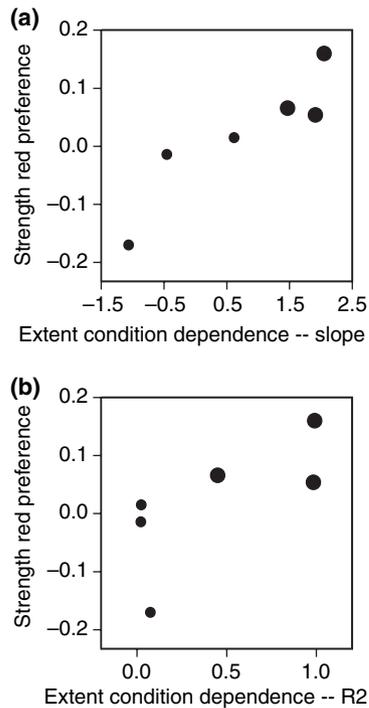


Fig. 4 Relationship between the extent of condition dependence and strength of red preference for six stickleback populations (Paxton, Priest, and Enos Lake limnetics and benthics). Large dots indicate limnetic populations and small dots are benthic populations. (a) Preference is correlated with the extent of condition dependence as estimated by the slope of residual regression; and (b) preference is weakly correlated with condition dependence as estimated by the coefficient of determination from residual regression.

preference and the extent of condition dependence in a trait should be correlated. Comparisons across limnetic and benthic populations show that the strength of preference for red area is positively correlated with the extent of condition-dependent expression in red as estimated by the slope from the residual analysis (Fig. 4a; $r = 0.90$, $N = 6$, $P = 0.015$). It is weakly and nonsignificantly correlated with condition as estimated by R^2 (Fig. 4b; $r = 0.67$, $N = 6$, $P = 0.15$). Thus female preference appears to depend on traits that indicate male condition.

Condition dependence and sexual isolation

Females may rely on condition-dependent traits to distinguish not only among high and low condition males of their own species, but also to distinguish between conspecific and heterospecific males that differ in colour expression. I compared the importance of red nuptial colour differences on the probability of spawning with sympatric heterospecifics to the extent of condition dependence as measured by the slope of residual regressions. I found a strong correlation (Fig. 5; $r = 0.96$, $N = 6$, $P < 0.0028$), indicating that reliance on colour

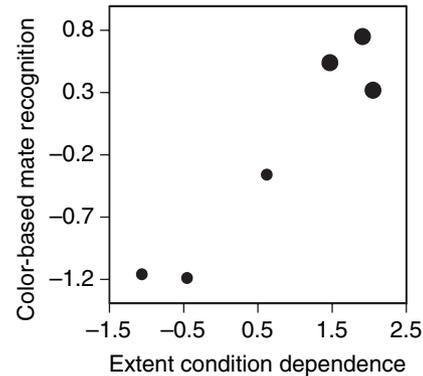


Fig. 5 Relationship between the extent of condition dependence and the importance of colour differences in sexual isolation for six stickleback populations (Paxton, Priest, and Enos Lake limnetics and benthics). Large dots indicate limnetic populations and small dots are benthic populations.

differences for sexual isolation increases in importance as condition dependence increases.

Discussion

Condition dependence within and between species

The primary finding is that stickleback species differ in the extent of condition dependence for red nuptial colour. To my knowledge, this is the first study to show that closely related species differ to this extent in condition dependence and in the direction predicted by preference differences. All three predictions from condition-dependent handicap theory indicate that red nuptial colour is under strong directional sexual selection in limnetic sticklebacks. Patterns of condition dependence suggest sexual selection on colour is weak and inconsistent for benthic and absent in anadromous sticklebacks. The lack of significant curvilinear effects for all three species indicates that only linear sexual selection is operating on red nuptial colour, and that only in limnetics. Therefore, the linear condition dependence in limnetics appears to result from open-ended preference for redder males.

Limnetic males showed strongly positive slopes for all measures of red colour, and variation in condition explained substantial variation in red nuptial colour. In addition, red nuptial colour had higher phenotypic variation than other traits, consistent with genic capture. These results are consistent with condition dependence facilitating the maintenance of variation in traits under directional sexual selection. Moreover, this strong condition dependence means that red conveys fairly accurate information to limnetic females on male condition. This is in contrast to the other measures of body colour and nonsexual traits. This pattern is consistent with these

colour traits serving distinct functions and consequently being under different forms of selection.

In contrast to limnetics, directional sexual selection does not appear to be acting strongly on red nuptial colour for benthics. This is indicated by weak and inconsistent relationships between colour measures and condition, and low variance explained. This suggests that benthic females cannot infer male condition from colour. Benthics do show an increase in variation for red nuptial colour over other traits, although it is less pronounced than for limnetics which is consistent with the weak and primarily nonsignificant effects found. Benthics also showed no condition dependence in a lab study of colour expression (Lewandowski & Boughman, unpublished), and earlier work showed that benthics have less red area than limnetics (Boughman, 2001), consistent with the idea that they may be losing this trait. Loss of colour dimorphism has been found in other species as well (Wiens, 2001).

Anadromous sticklebacks show no hint of condition dependence for any of the traits measured. Slope estimates are very shallow, and the amount of variance explained is negligible. Therefore, in anadromous sticklebacks, colour conveys almost no information on male condition. This is surprising, as studies of other anadromous populations have found that sexual selection does act on male colour (McLennan & McPhail, 1990; Ostlund Nilsson & Nilsson, 2000). However, at least one other study found little correlation between colour intensity and condition in anadromous sticklebacks (Rush *et al.*, 2003). The presence of high levels of variation in red area and colour index suggests that further work is needed on whether and how sexual selection acts on colour in anadromous sticklebacks. For example, studies are needed that identify the targets of female choice, and test whether male competition acts on colour. More work is also needed on the mechanisms controlling colour variation and condition in all stickleback populations. Experiments that manipulate condition and track concomitant changes in colour expression would be very useful to test mechanisms in all three species (similar to those done for river populations by Milinski & Bakker, 1990), and would provide an important complement to this study which compares naturally occurring variation across species. That anadromous males show no condition dependence suggests that limnetic males have gained rather than benthic males have lost condition dependence. This pattern suggests that only in limnetics is red nuptial colour under directional sexual selection, and that this is the derived state.

Evolution of condition dependence and preference for red

I also found a strong relationship between the strength of female preference for red colour across populations and the extent of condition dependence. This is consistent

with models of condition-dependent signalling (Kodric-Brown & Brown, 1984; Nur & Hasson, 1984; Lorch *et al.*, 2003) and further supports the hypothesis that sexual selection differs between the species. This correlation also indicates that female preference and condition-dependent expression of red colour have coevolved.

Distinguishing causality in such coevolutionary frameworks is difficult. It could be that red nuptial colour initially evolved condition dependence in limnetics because it is under sexual selection. This is predicted by several models (Kodric-Brown & Brown, 1984; Price *et al.*, 1993; Payne & Pagel, 2001; Lorch *et al.*, 2003). Alternatively, female preference may have initially evolved for traits that reveal potential mates' underlying condition as predicted by other models (Iwasa & Pomiankowski, 1994; Houle & Kondrashov, 2002). Unfortunately, I cannot distinguish between these hypotheses with the present data. Indeed, it is difficult to do so empirically.

We do however, have information for sticklebacks that suggests a likely evolutionary scenario. The initial preference for red could have emerged from sensory bias (Basolo, 1990; Ryan, 1990). Pre-existing bias for red colour has been found in other stickleback populations (Smith *et al.*, 2004) suggesting it may also be present in the limnetic, benthic and anadromous populations I studied. The effect of bias may be stronger in limnetics given their greater sensitivity to red light (Boughman, 2001). Sensory bias for red may have initiated an iterative, dynamic coevolutionary process between preference and condition-dependent red colour that resulted in the further exaggeration of red preference and colour and an increase in condition-dependent expression of red, in a process similar to that envisioned by Rowe & Houle (1996). This could occur as follows. The initial preference for red that arose from sensory bias may subsequently have led to increased condition dependence in red. This is because sexual selection for red colour generated by bias would push males off the natural selection optimum for colour. In these circumstances, if high condition males experienced small fitness loss relative to low condition males, then selection would cause condition to become correlated with red colour. This increasing condition dependence may have further selected for stronger red preferences, as females that preferred red males would obtain mates in higher condition. A similar scenario has been proposed for several Goodeinae species (Macias Garcia & Ramirez, 2005).

Differences in sexual selection contribute to sexual isolation

Variation between species in condition dependence implies that differences in sexual selection have contributed to sexual isolation between sympatric benthics and limnetics. Moreover, as the extent of condition

dependence increases, colour plays a larger role in sexual isolation. Condition dependence is high for all three limnetic populations and they all rely heavily on colour differences to distinguish conspecifics from heterospecifics. Two benthic populations have lower condition dependence and rely less on colour to discriminate against heterospecifics. Paxton benthics have intermediate condition dependence and preference strength, and rely to an intermediate extent on colour in sexual isolation.

Whether the differences in condition dependence and preference came first and helped to initiate speciation or evolved after the speciation process was well underway is difficult to establish. It seems likely that some ecological differentiation would have arisen early and may even have been present on secondary contact (McPhail, 1993), but unlikely that gene flow would be prevented (and thus speciation complete) without fairly strong premating isolation, given the lack of intrinsic post-mating isolation in the species pairs. Moreover, reproductive isolation has been shown to be a byproduct of divergent natural and sexual selection in the species pairs (Rundle *et al.*, 2000; Boughman, 2001; Boughman *et al.*, 2005), and premating isolation has an especially strong effect on overall reproductive isolation (Ramsey *et al.*, 2003). This makes it likely that divergence in mating traits occurred early in the process of speciation. However, additional experiments are necessary for a definitive answer.

Causes of different sexual selection between species

Why did condition dependence evolve solely in limnetics? In addition to the likely role of sensory drive, species may differ in the relative benefits and costs of red signalling, leading to different optimal signalling strategies. The cost of expressing red may differ between species because of differences in ecology, which could be either predator mediated or physiological. Predator-mediated costs are likely to differ given that limnetics and benthics are exposed to different suites of predators (Vamosi & Schluter, 2004). Physiological costs could include those associated with acquiring and assimilating carotenoids, or trading off their use for immune vs. signalling functions (Hamilton & Zuk, 1982; Lozano, 1994; Blount, 2004). A benthivorous diet may be low in carotenoids relative to the planktivorous diet (Tartarotti *et al.*, 1999; Lotocka *et al.*, 2004), putting especially strong selection on acquisition or assimilation for benthics. Allocation tradeoffs seem especially likely for limnetics because planktivores have been shown to have higher parasite loads than benthivores (Gray *et al.*, 2005; A. McColl, unpublished). If so, limnetics may need to invest more in immune function and parasite resistance than benthics, and may have higher nonsignalling demands for carotenoids. Their brighter colour indicates that they also invest more in signalling function. Therefore, selection in limnetics could act more strongly on

allocation whereas in benthics it may act more strongly on acquisition of carotenoids. Testing this hypothesis requires further work.

The benefits of signalling may also differ between species. In the red-shifted light prominent in benthic mating habitat females may not be able to distinguish bright from dull red males, especially because they have low sensitivity to red light (Boughman, 2001, 2002). This could reduce benefits for benthic males. Bright red benthic males that cannot be distinguished from dull red males may not benefit from increased mating success; however, they would still incur the costs of acquiring and assimilating carotenoids, and of trading off signalling and immune function.

Implications for speciation

I have presented several lines of evidence suggesting that differences in the action of sexual selection between species contribute to their reproductive isolation. Although a number of studies have demonstrated condition-dependent expression of colour (e.g. McGraw & Ardia, 2003; Saks *et al.*, 2003), to my knowledge this is the first study to show that condition-dependent traits are involved in speciation.

First, the differences in condition-dependent expression of colour between species strongly suggest that sexual selection acts differently on colour in limnetic than it does in benthic or anadromous sticklebacks. Such divergent sexual selection is predicted to cause male mating traits and female preferences for them to diverge, resulting in sexual isolation. Prior work demonstrated such differences in mating traits (Ridgway & McPhail, 1984; Boughman, 2001) and suggested a strong role for environment-dependent sexual selection (Boughman *et al.*, 2005). The present study indicates that differences in the nature and targets of sexual selection are at the root of those differences in mating traits. Secondly, divergence in sexual selection relates to whether and how traits are used in sexual isolation. Stickleback populations with high condition dependence of colour rely to a greater extent on colour in sexual isolation. This is likely, in part, because they rely to a greater extent on colour in mate choice. This direct relationship between sexual selection within species and sexual isolation between them is a clear evidence that divergent sexual selection has contributed to the rapid speciation of three-spined stickleback species pairs.

Acknowledgments

Thanks to M. McDonald and J. Hutchens for great help in the field and lab, to the Boughman lab, R. Snook, and four anonymous reviewers for comments that improved the manuscript, and to the National Science Foundation for support. This work was approved by the University of Wisconsin Animal Care and Use Committee.

References

- Bakker, T.C.M. & Mundwiler, B. 1994. Female mate choice and male red coloration in a natural three-spined stickleback (*Gasterosteus aculeatus*) population. *Behav. Ecol.* **5**: 74–80.
- Basolo, A.L. 1990. Female preference predates the evolution of the sword in swordtail fish. *Science* **250**: 808–810.
- Bentzen, P. & McPhail, J.D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*) – specialization for alternative trophic niches in the Enos Lake species pair. *Can. J. Zool.* **62**: 2280–2286.
- Blount, J.D. 2004. Carotenoids and life-history evolution in animals. *Arch. Biochem. Biophys.* **430**: 10–15.
- Bonduriansky, R. & Rowe, L. 2005. Sexual selection, genetic architecture, and the condition dependence of body shape in the sexually dimorphic fly *Prochyliza xanthostoma* (Piophilidae). *Evolution* **59**: 138–151.
- Boughman, J.W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* **411**: 944–948.
- Boughman, J.W. 2002. How sensory drive can promote speciation. *Trends Ecol. Evol.* **17**: 571–577.
- Boughman, J.W., Rundle, H.D. & Schluter, D. 2005. Parallel evolution of sexual isolation in sticklebacks. *Evolution* **59**: 361–373.
- Candolin, U. 1999. The relationship between signal quality and physical condition: is sexual signalling honest in the three-spined stickleback? *Anim. Behav.* **58**: 1261–1267.
- Candolin, U. 2000. Male-male competition ensures honest signaling of male parental ability in the three-spined stickleback (*Gasterosteus aculeatus*). *Behav. Ecol. Sociobiol.* **49**: 57–61.
- Cotton, S., Fowler, K. & Pomiankowski, A. 2004. Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proc. Roy. Soc. Lond. B* **271**: 771–783.
- Couldridge, V.C.K. & Alexander, G.J. 2002. Color patterns and species recognition in four closely related species of Lake Malawi cichlid. *Behav. Ecol.* **13**: 59–64.
- Darlington, R.B. & Smulders, T.V. 2001. Problems with residual analysis. *Anim. Behav.* **62**: 599–602.
- Endler, J.A. & Houde, A.E. 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* **49**: 456–468.
- Gage, M.J.G., Parker, G.A., Nylin, S. & Wiklund, C. 2002. Sexual selection and speciation in mammals, butterflies and spiders. *Proc. Roy. Soc. Lond. B* **269**: 2309–2316.
- Garcia-Berthou, E. 2001. On the misuse of residuals in ecology: testing regression residuals vs. the analysis of covariance. *J. Anim. Ecol.* **70**: 708–711.
- Gonzales Zuarth, C. & Macias Garcia, C. 2006. Phenotypic differentiation and pre-mating isolation between allopatric populations of *Girardinichthys multiradiatus*. *Proc. Roy. Soc. Lond. B* **273**: 301–307.
- Gow, J. L., Peichel, C. L. & Taylor, E. B. 2006. Contrasting hybridization rates between sympatric three-spined sticklebacks highlight the fragility of reproductive barriers between evolutionarily young species. *Molecular Ecology*, **15**: 739–752.
- Grafen, A. 1990. Biological signals as handicaps. *J. Theor. Biol.* **144**: 517–546.
- Gray, S.M., Robinson, B.W. & Parsons, K.J. 2005. Testing alternative explanations of character shifts against ecological character displacement in brook sticklebacks (*Culaea in-*
- stans*) that coexist with ninespine sticklebacks (*Pungitius pungitius*). *Oecologia* **146**: 25–35.
- Green, A.J. 2001. Mass/length residuals: measures of body condition or generators of spurious results? *Ecology* **82**: 1473–1483.
- Green, D.G., Powers, M.K. & Banks, M.S. 1980. Depth of focus, eye size and visual acuity. *Vision Res.* **20**: 827–835.
- Hamilton, W.D. & Zuk, M. 1982. Heritable true fitness and bright birds – a role for parasites. *Science* **218**: 384–387.
- Houle, D. & Kondrashov, A.S. 2002. Coevolution of costly mate choice and condition-dependent display of good genes. *Proc. Roy. Soc. Lond. B* **269**: 97–104.
- Iwasa, Y. & Pomiankowski, A. 1994. The evolution of mate preferences for multiple sexual ornaments. *Evolution* **48**: 853–867.
- Knight, M.E. & Turner, G.F. 2004. Laboratory mating trials indicate incipient speciation by sexual selection among populations of the cichlid fish *Pseudotropheus zebra* from Lake Malawi. *Proc. Roy. Soc. Lond. B* **271**: 675–680.
- Kodric-Brown, A. & Brown, J.H. 1984. Truth in advertising: the kinds of traits favored by sexual selection. *Am. Nat.* **124**: 309–323.
- Kotiaho, J.S. 1999. Estimating fitness: comparison of body condition indices revisited. *Oikos* **87**: 399–400.
- Lorch, P.D., Proulx, S., Rowe, L. & Day, T. 2003. Condition-dependent sexual selection can accelerate adaptation. *Evol. Ecol. Res.* **5**: 867–881.
- Lotocka, M., Styczynska-Jurewicz, E. & Bledzki, L.A. 2004. Changes in carotenoid composition in different developmental stages of copepods: *Pseudocalanus acuspes* Giesbrecht and *Acartia* spp. *J. Plankton Res.* **26**: 159–166.
- Lozano, G.A. 1994. Carotenoids, parasites, and sexual selection. *Oikos* **70**: 309–311.
- Macias Garcia, C. & Ramirez, E. 2005. Evidence that sensory traps can evolve into honest signals. *Nature* **434**: 501–505.
- Manica, A. 2002. Filial cannibalism in teleost fish. *Biol. Rev.* **77**: 261–277.
- Masta, S.E. & Maddison, W.P. 2002. Sexual selection driving diversification in jumping spiders. *Proc. Natl. Acad. Sci. U.S.A.* **99**: 4442–4447.
- McGraw, K.J. & Ardia, D.R. 2003. Carotenoids, immunocompetence, and the information content of sexual colors: an experimental test. *Am. Nat.* **162**: 704–712.
- McLennan, D.A. & McPhail, J.D. 1990. Experimental investigations of the evolutionary significance of sexually dimorphic nuptial coloration in *Gasterosteus aculeatus* (L) – the relationship between male color and female behavior. *Can. J. Zool.* **68**: 482–492.
- McPhail, J.D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*) – morphological and genetic evidence for a species pair in Enos Lake, British Columbia. *Can. J. Zool.* **62**: 1402–1408.
- McPhail, J.D. 1992. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*) – evidence for a species-pair in Paxton Lake, Texada Island, British Columbia. *Can. J. Zool.* **70**: 361–369.
- McPhail, J.D. 1993. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): origin of the species pairs. *Can. J. Zool.* **71**: 515–523.
- McPhail, J.D. 1994. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of south-western British Columbia. In: *The Evolutionary Biology of the Threespine*

- Stickleback* (M. A., Bell & S. A. Foster eds), pp. 399–437. Oxford University Press, Oxford.
- Mendelson, T.C. & Shaw, K.L. 2005. Sexual behaviour: rapid speciation in an arthropod. *Nature* **433**: 375–376.
- Milinski, M. & Bakker, T.C.M. 1990. Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature* **344**: 330–333.
- Mitra, S., Landel, H. & Pruett Jones, S.G. 1996. Species richness covaries with mating system in birds. *Auk* **113**: 544–551.
- Morrow, E.H., Pitcher, T.E. & Arnqvist, G. 2003. No evidence that sexual selection is an 'engine of speciation' in birds. *Ecol. Lett.* **6**: 1038.
- Nagel, L. & Schluter, D. 1998. Body size, natural selection, and speciation in sticklebacks. *Evolution*, **52**: 209–218.
- Naisbit, R.E., Jiggins, C.D. & Mallet, J. 2001. Disruptive sexual selection against hybrids contributes to speciation between *Heliconius cydno* and *Heliconius melpomene*. *Proc. Roy. Soc. Lond. B* **268**: 1849–1854.
- Nur, N. & Hasson, O. 1984. Phenotypic plasticity and the handicap principle. *J. Theor. Biol.* **110**: 275–297.
- Ostlund Nilsson, S. & Nilsson, G.E. 2000. Free choice by female sticklebacks: lack of preference for male dominance traits. *Can. J. Zool.* **78**: 1251–1258.
- Panhuis, T.M., Butlin, R.K., Zuk, M. & Tregenza, T. 2001. Sexual selection and speciation. *Trends Ecol. Evol.* **16**: 364–371.
- Payne, R.J.H. & Pagel, M. 2001. Inferring the origins of state-dependent courtship traits. *Am. Nat.* **157**: 42–50.
- Price, T.D. 1998. Sexual selection and natural selection in bird speciation. *Philos. Trans. R. Soc. Lond. B* **353**: 251–260.
- Price, T.D., Schluter, D. & Heckman, N. 1993. Sexual selection when the female directly benefits. *Biol. J. Linn. Soc.* **48**: 187–211.
- Rafferty, N. E. & Boughman, J. W. 2006. Olfactory mate recognition in a sympatric species pair of three-spined sticklebacks. *Behav. Ecol.*, **17**: 965–970.
- Ramsey, J., Bradshaw, H.D. & Schemske, D.W. 2003. Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution* **57**: 1520–1534.
- Reimchen, T.E. 1980. Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus* – an adaptation to predators. *Can. J. Zool.* **58**: 1232–1244.
- Reimchen, T.E. 1983. Structural relationships between spines and lateral plates in threespine stickleback (*Gasterosteus aculeatus*). *Evolution* **37**: 931–946.
- Reimchen, T.E. 1994. Predators and morphological evolution in threespine stickleback. In: *The Evolutionary Biology of the Threespine Stickleback* (M. A. Bell & S. A. Foster, eds), pp. 240–276. Oxford University Press, Oxford.
- Reimchen, T.E. & Nosil, P. 2002. Temporal variation in divergent selection on spine number in threespine stickleback. *Evolution* **56**: 2472–2483.
- Ridgway, M.S. & McPhail, J.D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): mate choice and reproductive isolation in the Enos Lake species pair. *Can. J. Zool.* **62**: 1813–1818.
- Ritchie, M.G. 1996. The shape of female mating preferences. *Proc. Natl. Acad. Sci. U.S.A.* **93**: 14628–14631.
- Ritchie, M.G., Saarikettu, M. & Hoikkala, A. 2005a. Variation, but no covariance, in female preference functions and male song in a natural population of *Drosophila montana*. *Anim. Behav.* **70**: 849–854.
- Ritchie, M.G., Webb, S.A., Graves, J.A., Magurran, A.E. & Macias Garcia, C. 2005b. Patterns of speciation in endemic Mexican goodeid fish: sexual conflict or early radiation? *J. Evol. Biol.* **18**: 922–929.
- Rowe, L. & Houle, D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. Roy. Soc. Lond. B* **263**: 1415–1421.
- Rowe, M.P., Baube, C.L., Loew, E.R. & Phillips, J.B. 2004. Optimal mechanisms for finding and selecting mates: how threespine stickleback (*Gasterosteus aculeatus*) should encode male throat colors. *J. Comp. Physiol. A* **190**: 241–256.
- Rundle, H.D. 2002. A test of ecologically dependent postmating isolation between sympatric sticklebacks. *Evolution* **56**: 322–329.
- Rundle, H. D., Nagel, L., Boughman, J. W. & Schluter, D. 2000. Natural selection and parallel speciation in sympatric sticklebacks. *Science*, **287**: 306–308.
- Rush, V.N., McKinnon, J.S., Abney, M.A. & Sargent, R.C. 2003. Reflectance spectra from free-swimming sticklebacks (*Gasterosteus*): social context and eye-jaw contrast. *Behaviour* **140**: 1003–1019.
- Ryan, M.J. 1990. Sexual selection, sensory systems, and sensory exploitation. *Oxf. Surv. Evol. Biol.* **7**: 156–195.
- Saks, L., Ots, I. & Horak, P. 2003. Carotenoid-based plumage coloration of male greenfinches reflects health and immunocompetence. *Oecologia* **134**: 301–307.
- Schluter, D. 1994. Experimental evidence that competition promotes divergence in adaptive radiation. *Science* **266**: 798–801.
- Schluter, D. & McPhail, J.D. 1992. Ecological character displacement and speciation in sticklebacks. *Am. Nat.* **140**: 85–108.
- Seehausen, O. & van Alphen, J.J.M. 1999. Can sympatric speciation by disruptive sexual selection explain rapid evolution of cichlid diversity in Lake Victoria? *Ecol. Lett.* **2**: 262–271.
- Shand, J. 1997. Ontogenetic changes in retinal structure and visual acuity: a comparative study of coral-reef teleosts with differing post-settlement lifestyles. *Environ. Biol. Fishes* **49**: 307–322.
- Shaw, K.L. 2000. Interspecific genetics of mate recognition: inheritance of female acoustic preference in Hawaiian crickets. *Evolution* **54**: 1303–1312.
- Shaw, K. L. & Herlihy, D. P. 2000. Acoustic preference functions and song variability in the Hawaiian cricket *Laupala cerasina*. *Proc. Roy. Soc. Lond. B* **267**: 577–584.
- Smith, C., Barber, I., Wootton, R.J. & Chittka, L. 2004. A receiver bias in the origin of three-spined stickleback mate choice. *Proc. R. Soc. Lond.* **271**: 949–955.
- Tartarotti, B., Cabrera, S., Psenner, R. & Sommaruga, R. 1999. Survivorship of cyclops *Abyssorum taticus* (Cyclopoida, Copepoda) and *Boeckella gracilipes* (Calanoida, Copepoda) under ambient levels of solar uvb radiation in two high-mountain lakes. *J. Plankton Res.* **21**: 549–560.
- Taylor, E. B. & McPhail, J. D. 1999. Evolutionary history of an adaptive radiation in species pairs of threespine sticklebacks (*Gasterosteus*): insights from mitochondrial DNA. *Biol. J. Linn. Soc.* **66**: 271–291.
- Taylor, E. B. & McPhail, J. D. 2000. Historical contingency and ecological determinism interact to prime speciation in sticklebacks, *Gasterosteus*. *Proc. Roy. Soc. Lond. B* **267**: 2375–2384.
- Uy, J.A.C. & Borgia, G. 2000. Sexual selection drives rapid divergence in bowerbird display traits. *Evolution* **54**: 273–278.

- Vamosi, S. M. & Schluter, D. 2004. Character shifts in the defensive armor of sympatric sticklebacks. *Evolution*, **58**, 376–385.
- Wells, M.L.M. & Henry, C.S. 1998. Songs, reproductive isolation, and speciation in cryptic species of insects: a case study using green lacewings. In: *Endless Forms: Species and Speciation* (D. J. Howard & S. H. Berlocher eds), pp. 217–233. Oxford Univ Press, Oxford.
- Wiens, J. J. 2001. Widespread loss of sexually selected traits: how the peacock lost its spots. *Trends Ecol & Evol*. **16**: 517–523.
- Wilkinson, G.S. & Taper, M.L. 1999. Evolution of genetic variation for condition-dependent traits in stalk-eyed flies. *Proc. Roy. Soc. Lond. B* **266**: 1685–1690.

Received: 8 December 2006; revised: 22 January 2007; accepted: 24 January 2007