

Character displacement of male nuptial colour in threespine sticklebacks (*Gasterosteus aculeatus*)

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Character displacement in signalling traits occurs when differences between species are greater in sympatry than where either species occurs alone. Finding character displacement in a signalling trait suggests that the trait has diverged as a result of interspecific interactions such as competition, aggression, predation, or reproductive interference. We tested for character displacement of male nuptial coloration between sympatric species of threespine sticklebacks (*Gasterosteus aculeatus* spp.). The sympatric pairs consist of a large 'benthic' species, which feeds on benthic invertebrates, and a smaller planktivorous 'limnetic' species. Breeding males of both species develop red throats and blue bodies, although limnetic males appear brighter. To test for character displacement, we compared the nuptial colour of benthics and limnetics from two species-pair lakes (sympatric) with that of males from three similar allopatric lakes (only one species present). We measured the intensity of blue and red coloration using reflectance spectra taken from live fish. We found that allopatric males were intermediate between limnetic and benthic males in the intensity of red colour, indicating character displacement in that trait in sympatry. By contrast, we found no evidence for character displacement in blue intensity, although it differed sharply between the species pairs in one lake (Priest). © 2007 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2007, 91, 37–48.

ADDITIONAL KEYWORDS: mating signal divergence – reflectance – reproductive isolation – speciation.

INTRODUCTION

In many taxa, the predominant differences between closely related sympatric species are in mating signals such as coloration or song type. Many processes may cause such divergence, but a role for interspecific interactions in sympatry can be inferred if the differences in mating signals are greater in sympatry than when either species occurs alone (i.e. character displacement: Brown & Wilson, 1956; Schluter, 2000a, b).

Character displacement in mating signals is usually explained by the action of reinforcement, which occurs when selection against hybrid matings causes an increase in premating isolation between the species

(Dobzhansky, 1940; Brown & Wilson, 1956; Servedio & Noor, 2003). Reinforcement is often measured as an increase in female mating discrimination in sympatry, but is also implicated in cases of character displacement in mating signals (Höbel & Gerhardt, 2003; for a review, see Servedio & Noor, 2003). There are two potential effects of reinforcement on signalling traits: we either expect female preferences to become more narrow and hone in on differences already present between species (no character displacement in signals), or we expect females to prefer more extreme males and drive divergence in male signalling traits (Noor, 1999).

Despite the attraction of reinforcement as an explanation for character displacement in mating signals, other interspecific interactions can cause the same pattern (Noor, 1999; Servedio & Noor, 2003). One such interspecific interaction is competition between species for signal space, which may drive divergence of

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signals in sympatry. For example, the acoustic signals of different frog species may interfere with each other, causing poor communication between males and females, which may lead to dispersion of signals to avoid overlap and interference in sympatry (Chek, Bogart & Lougheed, 2003). Alternatively, species may only be able to coexist in sympatry if their signals are substantially different (species sorting). Regardless of whether there has been evolution of signals in sympatry or if displacement is due to species sorting, competition for signal space causes a pattern of character displacement.

Aggressive interactions between species may also contribute to character displacement in mating signals. Divergence of plumage colour between sympatric populations of collared and pied flycatchers is driven at least partly by the fact that only dull coloured pied flycatcher males can set up territories in the presence of collared flycatcher males without being attacked (Alatolo, Gustafsson & Lundberg, 1994). Similarly, displacement of wing spot size in the damselfly *Calopteryx splendens* appears to be due to aggressive territorial interactions with the sympatric *Calopteryx virgo* (Tynkkynen, Rantala & Suhonen, 2004). *Calopteryx splendens* males with large wing spots more closely resemble *C. virgo* males, and therefore suffer more aggression than males with smaller spots.

Competition between species for resources may lead to displacement in morphological traits that then lead to divergence in mating signals as a secondary consequence. Galapagos finches are a classic example of ecological character displacement in beak morphology driven by competition for shared resources (Schluter, Price & Grant, 1985). It was recently shown that beak size and shape limit the range of songs that a species can sing, and thus divergence in beak shape has also driven divergence in song, a cue used for species recognition in finches (Podos, 2001). Ecological character displacement may also influence divergence in mating signals in a more subtle way; if displacement in habitat or diet indirectly changes the signalling environment, this in turn drives changes in mating signals (Schluter, 2000b; Boughman, 2002).

Regardless of the cause, finding character displacement of mating signals suggests that signals are not diverging by arbitrary changes in female preference or genetic drift. Instead, differences are driven in predictable directions through interspecific interactions. In the present study, we evaluate the evidence for character displacement of male nuptial coloration between sympatric species of threespine sticklebacks (*Gasterosteus aculeatus* spp.). Along the coast of British Columbia, most lakes contain one population of sticklebacks (allopatric), but several low-lying lakes support independently derived sympatric species pairs (Schluter & McPhail, 1992; Taylor & McPhail,

2000). Each pair consists of a large, benthic invertebrate feeder, the benthic species, and a small, streamlined, zooplanktivore, the limnetic species (McPhail, 1984; McPhail, 1992; Schluter & McPhail, 1992).

During the breeding season, males build nests, court females, and then care for the eggs and newly hatched fry (Whoriskey & FitzGerald, 1994). Generally, male sticklebacks develop red throats, blue or blue-green bodies, and blue irises during the breeding season. Female sticklebacks display a preference for males with the most intensely red throats (McLennan & McPhail, 1990; Milinski & Bakker, 1990), and with the largest area of red coloration (Boughman, 2001). Whether or not females have any preference for the relative area or intensity of the blue body coloration remains unknown.

We tested for character displacement of male colour by comparing the male colour (both red and blue) of benthics and limnetics from two species-pair lakes (sympatric) with that of males from three allopatric lakes. This is the same type of comparison that was previously used to detect character displacement in foraging traits (Schluter & McPhail, 1992), and armour (Vamosi & Schluter, 2004), with the assumption that the allopatric populations represent the derived solitary freshwater state. Differences in red colour between sympatric sticklebacks have already been demonstrated (McPhail, 1984; Boughman, 2001; Boughman, Rundle & Schluter, 2005). Comparison with allopatric populations in otherwise similar lakes allows us to determine whether the differences in sympatry are unusually large. They also provide a reference to determine which sympatric species departs most from the expected single-species state, and in which direction, and provide evidence that interspecific interactions were responsible for divergence.

MATERIAL AND METHODS

FISH COLLECTION

We measured the nuptial coloration of males from five lakes: two species pair lakes, Paxton Lake (49°42'N, 124°31'W) and Priest Lake (49°44'N, 124°33'W), and three allopatric lakes, Klein Lake (49°43'N, 123°58'W), Trout Lake (49°30'N, 123°52'W) and Cranby Lake (49°41'N, 124°30'W). We chose the allopatric populations that have the most similar ecological conditions to the species pair lakes (aside from containing two species). The lakes are small and contain cutthroat trout (*Oncorhynchus clarki*) as the only other fish species present. In addition, these lakes have been used in previous analyses of character displacement of other traits in the species pairs (Schluter & McPhail, 1992; Vamosi & Schluter, 2004), and are assumed to represent the expected phenotype when

only one type is present in a lake. Unfortunately, we were only able to use two of the four previously described species pairs (Schluter & McPhail, 1992; McPhail, 1993). This was because one pair (Hadley Lake) has gone extinct following an introduction of brown bullhead. A second pair (Enos Lake) is in the advanced stages of collapse via hybridization for reasons that are still unclear (Kraak, Mundwiler & Hart, 2001; Taylor *et al.*, 2006).

We collected sticklebacks using minnow traps and dip nets in May, June, and July 2003. Fish were kept in 102-L mixed-sex tanks separated by population at the University of British Columbia, Vancouver, Canada. All fish were maintained at approximately 18 °C under an 16 : 8 h light/dark cycle and fed chironomid larvae and brine shrimp (*Artemia* sp.) daily to satiation. We assessed male colour in early July 2003.

WATER COLOUR MEASUREMENT

We measured the background colour of the water within each lake using a dual channel Ocean Optics SD200 spectrometer and a 200 µm UV/VIS reflectance probe attached to a CC-3-UV cosine corrector. The sidewelling irradiance was recorded for depths of 10 cm, 50 cm, 1 m, and 2 m (Fig. 1). Sidewelling irradiance provides a measure of the colour of the background against which male fish are viewed by females (McDonald & Hawryshyn, 1995). We calculated λP_{50} for the sidewelling light at each depth. λP_{50} measures the dominant wavelength of the sidewelling spectrum, and is calculated as the wavelength that halves the area under the irradiance curve. These values were used to determine if the water colour differed significantly between lakes used in this study.

The λP_{50} values of the sidewelling light for all of the lakes used were similar to each other, and typical of blue-green lakes generally (Novales Flamarique, Hendry & Hawryshyn, 1992; McDonald & Hawryshyn, 1995). The range of λP_{50} values for the lakes (from depths of 10 cm to 2 m) were: Priest = 562–577 nm, Paxton = 561–577 nm, Cranby = 575–597 nm, Klein = 570–576 nm, and Trout = 549–558 nm. Redshifted lakes typically have λP_{50} values higher than 600 nm (Novales Flamarique *et al.*, 1992; McDonald & Hawryshyn, 1995). λP_{50} values increased slightly with depth from 10 cm to 2 m; however, there is no evidence to suggest that any of the lakes were particularly redshifted, which would cause a reduction in the visibility of the red throat coloration. There was also no correlation between λP_{50} at 1 m depth (or any other depth) and the average red score in the lakes (Pearson's $r = -0.04$, $P = 0.933$). Because the data show no association with the minor differences between lakes in water colour, we have not included them in subsequent analyses.

MALE COLOUR MEASUREMENT

Males were chosen for measurement if they displayed nuptial coloration (red throats and blue bodies and irises). Only the most colourful males from a tank were chosen for measurement at any one time. In most tanks, one male asserted himself as the dominant male (N. P. Millar, pers. observ.), an attribute most visible through behaviour and colour. The dominant male chased and bit the other males, and exhibited full nuptial coloration, whereas the other males had subdued coloration. When the dominant male was removed from the tank for a brief period (a few hours), another male became dominant and changed his behaviour and coloration. Because males were not returned to their tanks after measurement, we were able to measure all males over a period of days allowing each to develop to his maximum nuptial coloration under laboratory conditions.

Male coloration is thus highly dependent on social context. For example, male sticklebacks are capable of quickly increasing the colour saturation of their throat and iris when confronted with reproductive females or with other territorial males (Rush *et al.*, 2003). Because the males we used were not guarding nests or courting females at the time of measurement, it is possible that they were not as colourful as wild males at their peak. Nevertheless, males were able to interact with other males in dominance hierarchies as described above. Previous experiments have shown a strong correlation between the wavelength and intensity of colours that males use for both intra- and intersexual interactions (McLennan & McPhail, 1990; Rowland, 1994; Baube, 1997; Rush *et al.*, 2003). Therefore, it is likely that the most colourful males in the holding tanks were expressing similar levels of colour saturation as they would if courting females. In addition, all males were housed under the same conditions, and differences in colour that would be visible in the wild should still be present in the laboratory. Clearly, however, this experiment provides only a first step into understanding the dynamics of male colour evolution. Further colour measurement of free-swimming males (Rush *et al.*, 2003) at multiple stages of the nesting cycle and at different times in the season will be required to more fully describe the colour of males from different populations.

Fish were individually anaesthetized using carbonated water in a darkened container for 30–60 s. After the fish were anaesthetized, we took photographs and reflectance measurements (described below). Fish were then either left to recover and placed in new tanks, or were given an overdose of anaesthetic (MS-222) and preserved in 95% ethanol. All fish care and measurement complied with the University of British Columbia animal care regulations.

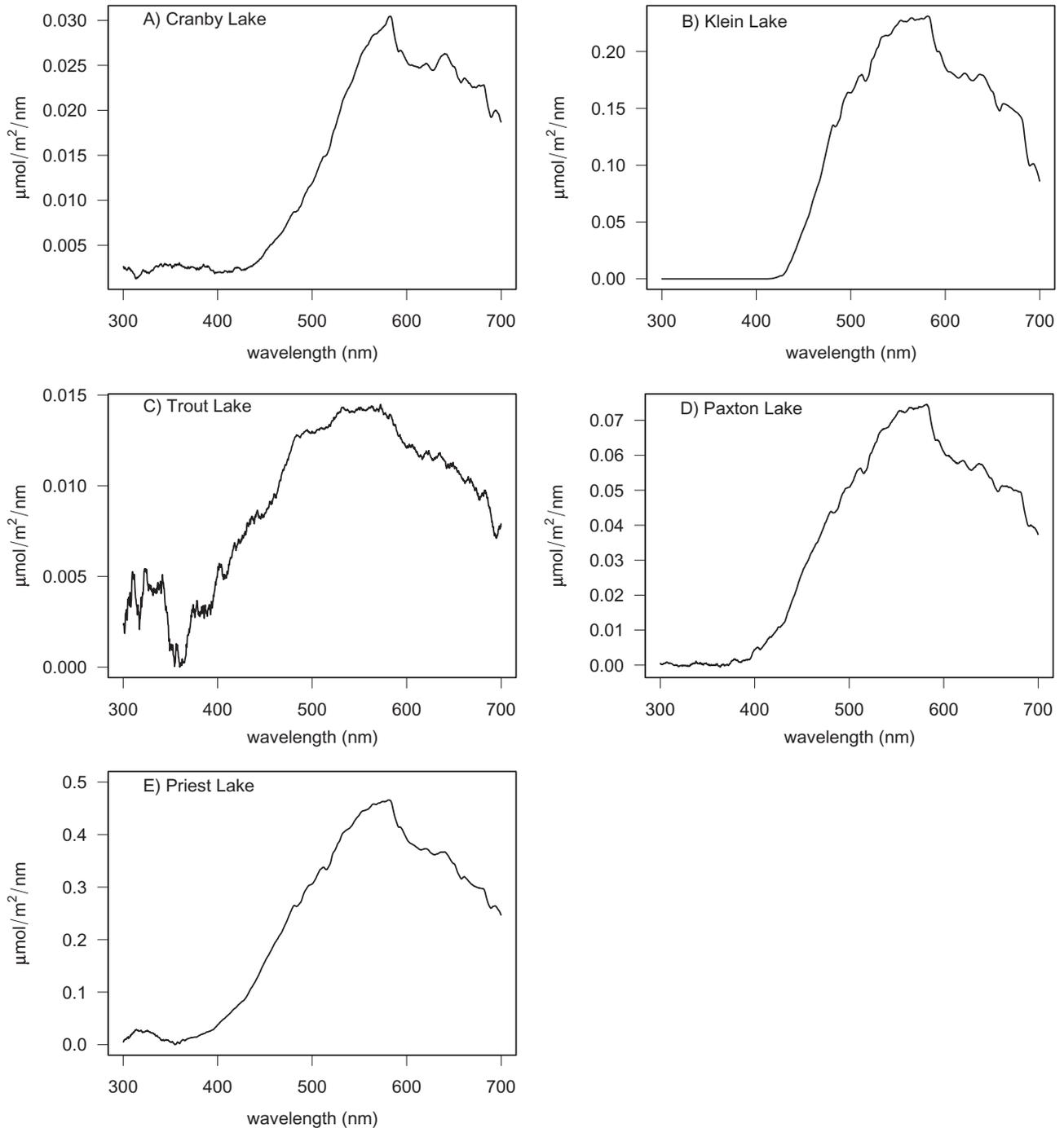


Figure 1. Sidewelling irradiance ($\mu\text{mol m}^{-2} \text{nm}^{-1}$) at 1 m depth for the lakes in the present study. Maximum irradiance is not identical for each lake because weather conditions differed when measurements were made. However, the shapes of the curves are comparable. A, Cranby Lake; B, Klein Lake; C, Trout Lake; D, Paxton Lake; E, Priest Lake.

PHOTOGRAPHY AND VISUAL ASSESSMENT OF RED AREA

We photographed the left side of males with a Nikon D1H digital SLR camera. The fish were illuminated

with four halogen lights (120 V, 50 W). We mounted all of the equipment on a frame to ensure that all components (fish, camera, and lights) maintained their relative spatial relationships with each other throughout.

The relative extent of the red throat colour was assessed by visual examination of the digital photographs on a standard computer monitor. The photographs were labelled with random numbers and assessed in that order to prevent bias resulting from measuring all fish from one population in a block, and from knowing the identity of the population. Using tpsDig (Rohlf, 2001), we measured the length of the red patch and the standard length of the fish. This allowed us to calculate the relative length of the red area on the fish by calculating the ratio of red length to total length. We measured 16 males from Klein Lake, 15 males from Trout Lake, 35 males from Cranby Lake, 30 limnetics and 39 benthics from Paxton Lake, and 25 limnetics and 28 benthics from Priest Lake.

REFLECTANCE

Because measurement of colour by eye is partly subjective, we also measured the reflectance spectra of male colours to obtain a more objective comparison (Endler, 1990). Although reflectance measurements do not provide information about the area or extent of a particular colour, they do provide objective estimates of the differences in brightness (total reflectance), saturation (intensity) and hue (colour) (Endler, 1990). To measure the reflectance spectra of two spots (throat and belly) on each live fish, we used the following equipment available from Ocean Optics: an USB 2000 spectrometer, a DT 1000 light source (200–1100 nm), and an R400-7-UV/VIS reflectance probe. We chose these spots because the throat region is typically red and the belly is typically the bluest spot on the body even on fish with little overall blue area. Each spot was measured five times per fish to minimize measurement error. We recorded percent reflectance relative to an Ocean Optics WS-1 reflectance standard at 0.38-nm intervals from approximately 300–700 nm. The probe was inserted into a custom made black polyvinyl chloride probe holder to minimize the influence of ambient light on the measurements. The probe was then held against the side of the fish at a 90° angle, and a reflectance spectrum was stored using Ocean Optics OOIBase 32 software.

We used principal components analysis (PCA) to assess differences between populations in reflectance spectra. PCA is a useful method for analysing differences in the brightness and shape of spectral data when details of the visual system of the receiver are unknown (Cuthill *et al.*, 1999; Grill & Rush, 2000). The segment analysis method of Endler (1990), which assumes trichromatic vision, provided very similar results to those presented here for the PCA on reflectance. Stickleback colours are most likely optimized for the stickleback visual system of four cone types (Rowe *et al.*, 2004), and thus we present the results of

the PCA instead of the segment method because PCA does not rely on any assumptions about the number of types of cones. Generally, the first principal component of a PCA on reflectance spectra is equivalent to total reflectance (brightness) and explains more than 90% of the variance between spectra (Cuthill *et al.*, 1999; Grill & Rush, 2000). The second and third components can be more difficult to interpret (Grill & Rush, 2000). Examination of their loadings allows for an assessment of their relationships to the original spectra. The means of the five replicate reflectance spectra for each spot and fish were used in all analyses. We reduced the amount of data in these mean spectra by calculating the median reflectance at 20 nm intervals from 310 nm to 690 nm. This provided 20 variables (wavelengths) that were used in the PCA analysis. Figures 2 and 3 show the average reflectance spectra for fish from all seven populations.

RESULTS

REFLECTANCE ANALYSIS

PCA on throat reflectance

The first principal component of the PCA on the throat reflectance spectra explained the most variance (90.3%). All of the wavelengths loaded positively for PC1 (Fig. 4A), suggesting that it explained differences in total reflectance or brightness between the spectra. The large amount of the variance explained by the first component, and its association with brightness, has been seen in other studies using PCA on reflectance spectra (Grill & Rush, 2000).

The second principal component (red score) explained 5.8% of the variance, and was associated with differences between the relative reflectance at short vs. long wavelengths (Fig. 4A). Positive values of red score represent high reflectance at long wavelengths (> 600 nm) and a lower reflectance at short wavelengths, suggesting that it is associated with variation in red intensity between males. The higher the value for red score, the greater the saturation (intensity) of the red colour because there is less reflectance at lower wavelengths to wash it out (Endler, 1990).

The third principal component explained 2.4% of the variance (Fig. 4A). A positive score for PC3 represents higher reflectance at middle wavelengths relative to short and long wavelengths. PC3 therefore represents variation in reflectance at green wavelengths. We used only the red score (PC2) in the regression analysis because the other two components were not associated with variation in red.

PCA on belly reflectance

The first principal component of the belly reflectance spectra explained 93.1% of the variance, and was

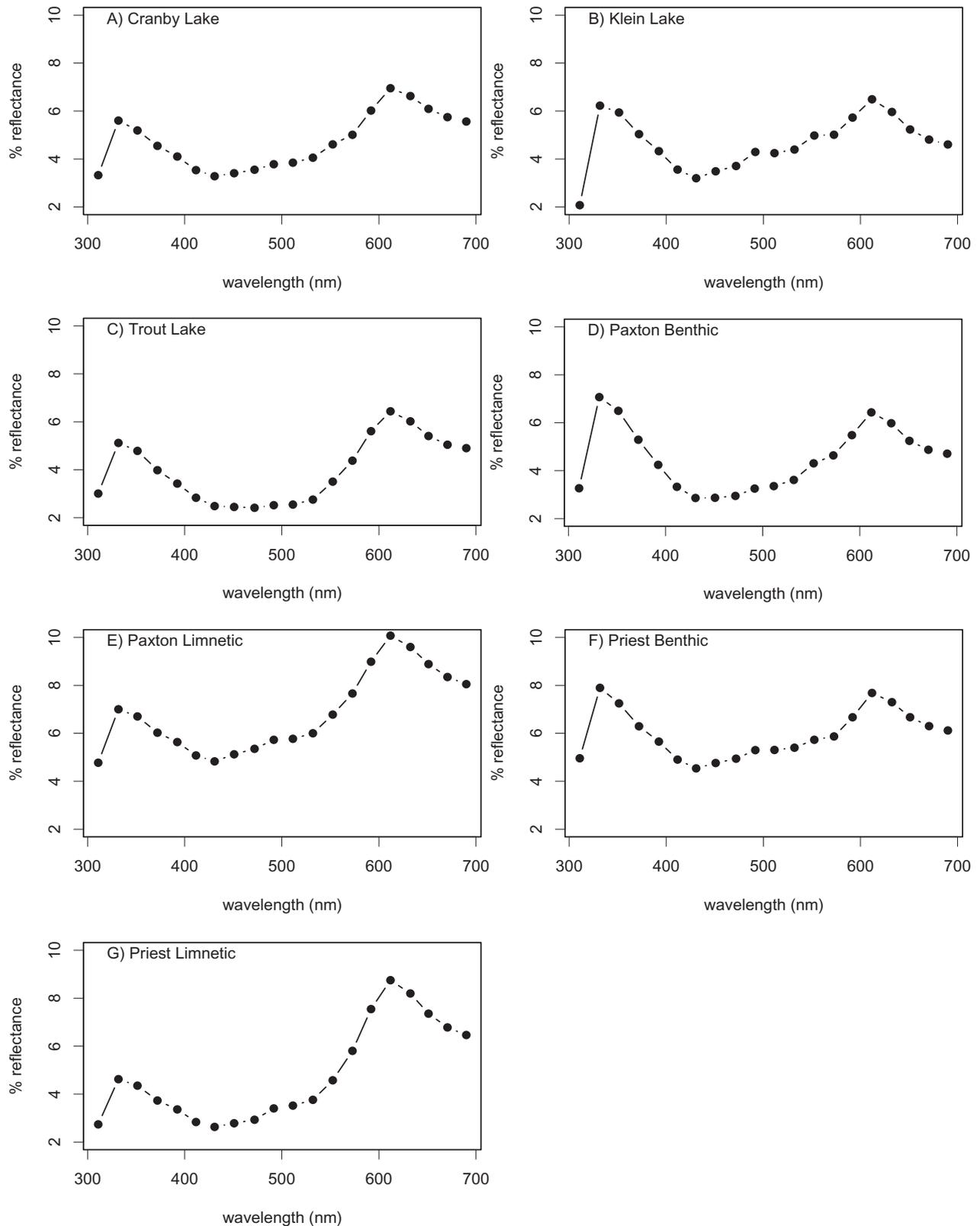


Figure 2. Average reflectance spectra of the throat region for each population. Each point represents the median reflectance over a 20 nm interval. A, Cranby; B, Klein; C, Trout; D, Paxton benthic; E, Paxton limnetic; F, Priest benthic; G, Priest limnetic.

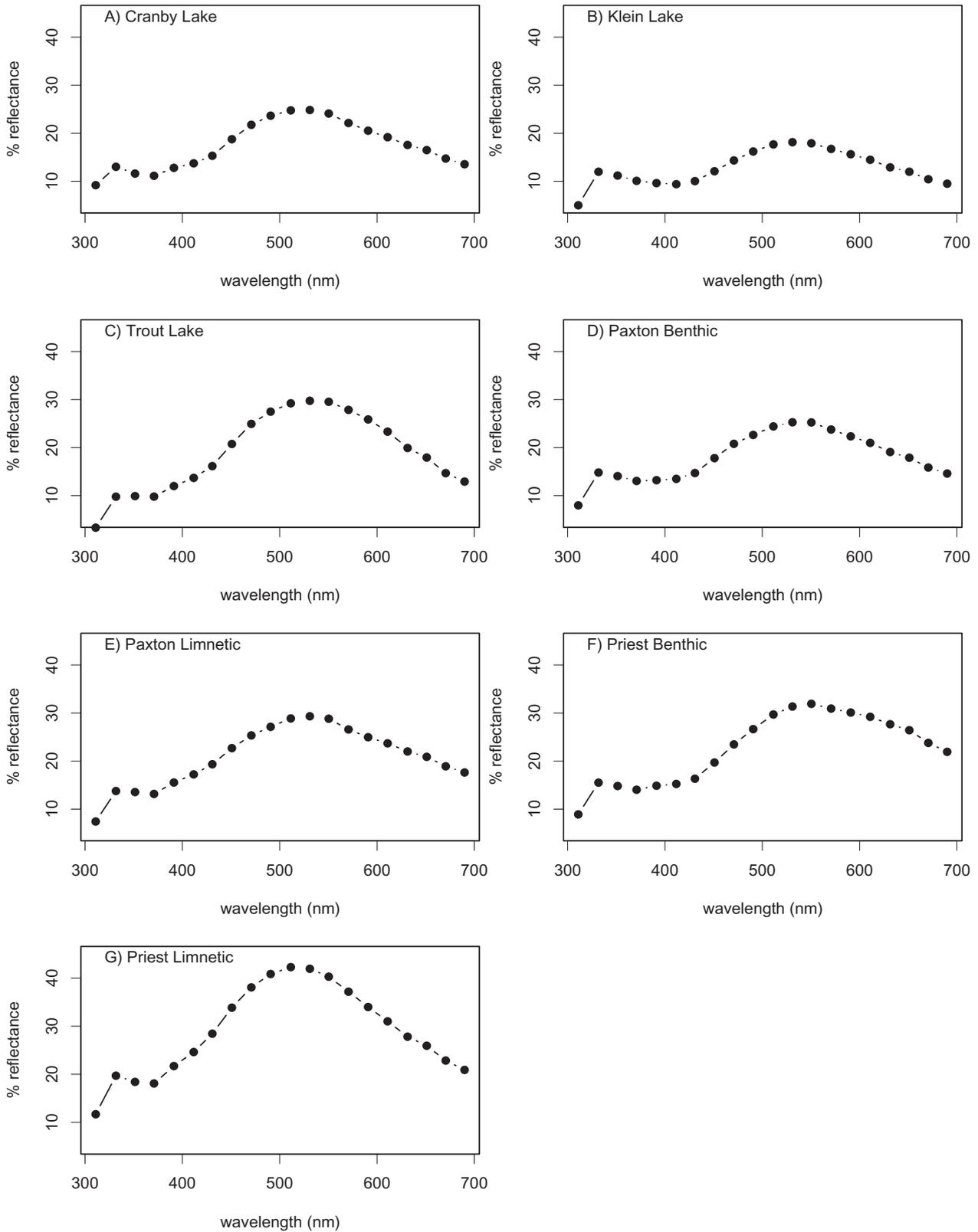


Figure 3. Average reflectance spectra of the belly region for each population. Each point represents the median reflectance over a 20 nm interval. A, Cranby; B, Klein; C, Trout; D, Paxton benthic; E, Paxton limnetic; F, Priest benthic; G, Priest limnetic.

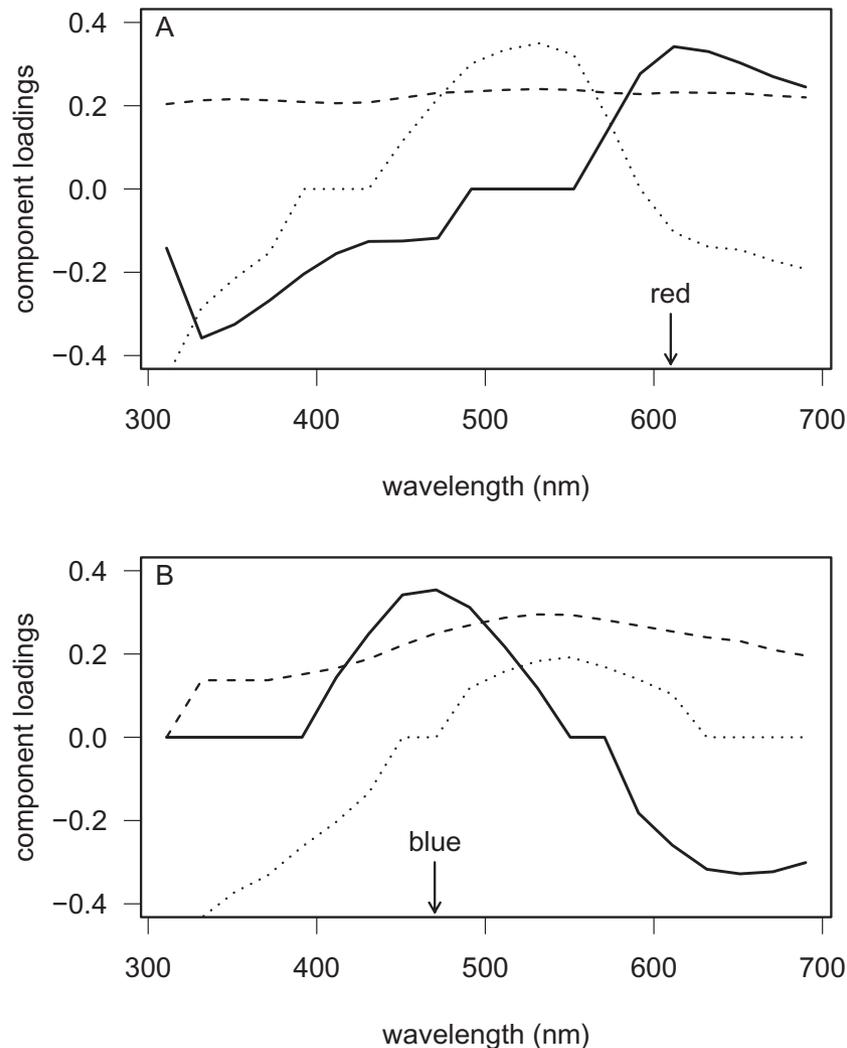


Figure 4. Component loadings for the first three principal components of the principal components analysis on (A) throat and (B) belly reflectance. The solid lines indicate the second components (red score and blue score), the dashed lines indicate the first components and the dotted lines indicate the third components.

associated with strong positive loadings across all wavelengths (Fig. 4B). Therefore, it represents differences between the reflectance spectra in total reflectance or brightness, similar to the first principal component of the throat reflectance analysis.

The second principal component (blue score) explained 4.0% of the variance between reflectance spectra (Fig. 4B). A positive score for blue score indicates high reflectance at low to medium wavelengths and lower reflectance at long wavelengths, consistent with a blue–green colour. The more positive the value for blue score, the more saturated (intense) the blue colour because reflectance at longer red wavelengths is reduced.

The third principal component explained 2.2% of the variance (Fig. 4B). A positive score for PC3 is related

to greater reflectance at mid to long wavelengths. This component is difficult to interpret but probably reflects variation between males in the background colour of their skin beneath the blue. As with the throat reflectance, we used only the second component (blue score) in further analyses due to a lack of association between the other two components and blue colour.

CHARACTER DISPLACEMENT ANALYSIS

The best test of greater differences in sympatry than in allopatry is a random effects model (Schluter & McPhail, 1992; Vamosi & Schluter, 2004). Therefore, we used a nested linear mixed-effects model to test for differences between the three types (limnetic, benthic,

and allopatric) in their means for the colour variables. The linear mixed effects model was constructed with type (L, B, or A) as the fixed main effect, with population (a random effect) nested within type. Character displacement was inferred if all three types had different means, or if two types had the same mean but the limnetics and benthics differed [e.g. (L = A) \neq B, or

L \neq (B = A)]. All statistical analyses were carried out in R (R Development Core Team, 2004).

Red

Red score differed significantly among types in the nested linear mixed effects model ($F_{2,4} = 36.79$, $P = 0.0027$) (Fig. 5A, Table 1). To determine if the benthic and allopatric types differed, we assessed whether a model in which the benthic and allopatric types were considered as one group ($F_{1,5} = 31.17$, $P = 0.0025$) would fit the data better than the full model where all three types were considered separately. The full model did fit the data significantly better than the reduced model (log likelihood ratio test (LLRT) = 4.45, $P = 0.034$), suggesting that all three types differ.

By contrast, the relative length of red did not differ significantly between types ($F_{2,4} = 5.09$, $P = 0.08$; Fig. 5B, Table 1), although there is a slight trend for the allopatric populations to have less overall red area.

Blue

Blue score ($F_{2,4} = 4.68$, $P = 0.090$) did not differ significantly between types (Fig. 5C, Table 1). Thus, in contrast to red, there is no evidence of character displacement in blue nuptial coloration. Interestingly, blue score differed greatly between benthics and limnetics in Priest Lake (Welch's t -test, $t_{37} = -5.30$, $P < 0.001$) but not Paxton Lake (Welch's t -test, $t_{47} = -1.20$, $P = 0.235$). This suggests that the dynamics of male colour evolution may differ between species pairs.

DISCUSSION

The results from both the reflectance measurements and the visual assessment of the males suggest that

Table 1. Mean \pm standard errors of the colour measurements

	Male type		
	Limnetic	Benthic	Allopatric
Red score	3.09 \pm 0.41	-2.12 \pm 0.38	-0.51 \pm 0.40
Relative red length	0.33 \pm 0.01	0.28 \pm 0.01	0.23 \pm 0.01
Blue score	5.84 \pm 2.07	-5.74 \pm 1.30	1.27 \pm 1.37

Red score and blue score are the second principal components of principal components analysis on throat and belly reflectance, respectively (for details, see text). Relative red length is measured as length of red throat area divided by standard length.

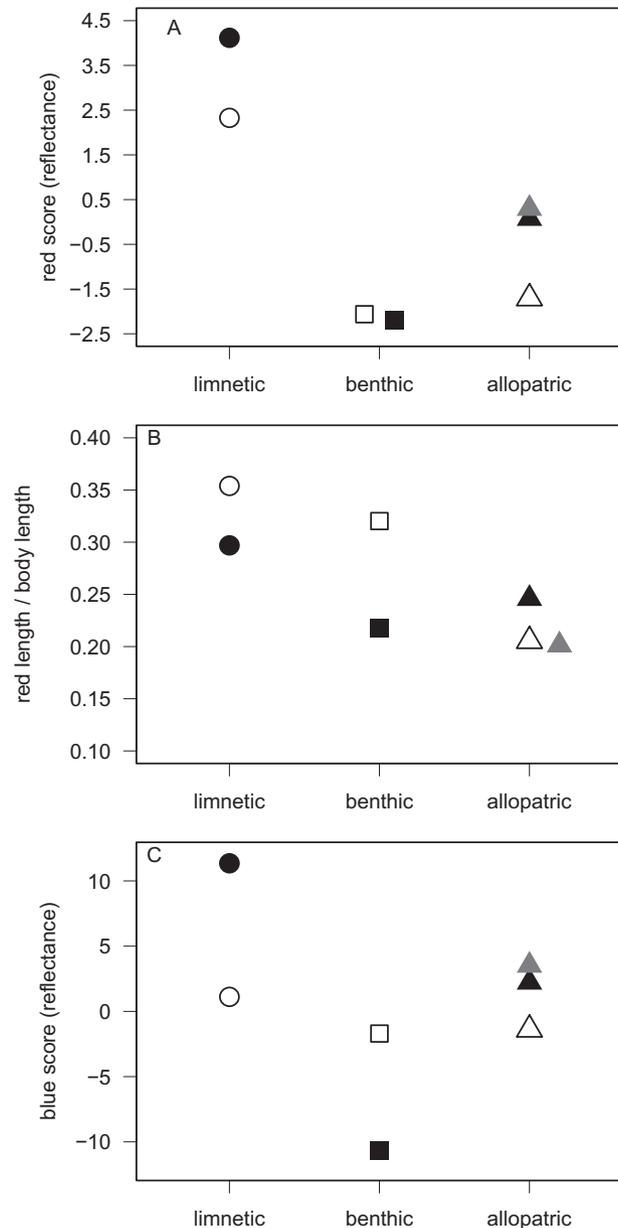


Figure 5. Population means for the colour measurements. Open circles, Paxton limnetics; black circles, Priest limnetics; open squares, Paxton benthics; black squares, Priest benthics; open triangles, Klein; black triangles, Cranby; grey triangles, Trout. A, red score; B, relative red length; C, blue score.

there is character displacement of male nuptial colour between sympatric species of threespine sticklebacks. Limnetics have more intense (saturated) red throats than the allopatric populations, whereas benthics lie below the allopatric populations. The parallel displacement of red intensity suggests that similar mechanisms are causing divergence in red between the two lakes. Interestingly, the relative amount of red does not differ significantly between types, suggesting that the mechanisms causing changes in red intensity and red area are different. For the blue coloration, there is evidence for divergence in one lake (Priest), but not the other, and there is no evidence of character displacement. This suggests that the mechanisms causing divergence in blue are not parallel between independently derived species pairs.

What interactions could drive character displacement of red in these lakes? Benthic and limnetic sticklebacks show character displacement in foraging traits driven by competition for shared resources (Schluter & McPhail, 1992; Schluter, 2000a). This ecological character displacement might influence displacement of red in several ways. First, adaptation of benthics and limnetics to different foraging environments causes a reduction in the fitness of hybrids relative to parental types (Hatfield & Schluter, 1999; Rundle, 2002). Reduced hybrid fitness could result in reinforcement and selection to strengthen premating isolation. There is evidence that both male and female sticklebacks are more discriminating when sympatric with the other species than are individuals from allopatric populations (Rundle & Schluter, 1998; Albert & Schluter, 2004), which is a predicted outcome of reinforcement.

However, for reinforcement to lead to displacement in red between the species, the preferences of benthic and limnetic females for red would have to differ, with benthic females preferring duller red males and limnetic females preferring brighter red males. A difference in preference for red could be mediated by differences in the sensitivity of females to red wavelengths, and there is some evidence that benthic females are less sensitive to red (Boughman, 2001). This observation relies on measurements of females from Enos Lake where male benthics display black nuptial coloration. In lakes where males display red (all lakes in this study), females differ only slightly in red sensitivity (Boughman, 2001). Furthermore, both benthic and limnetic females prefer the brightest red conspecific males, although the preferences of benthic females are slightly weaker (Boughman, Rundle & Schluter, 2005). It is therefore unlikely that reinforcement has caused divergence in female preference for red, leading to divergence of red in males. This remains to be tested by a comparison of sympatric female preferences for red with the preferences of allopatric females.

The second way by which ecological character displacement might influence displacement in red is through differences in diet. Differences in diet may expose benthics and limnetics to different amounts and types of carotenoids. Differences in red within a European population of sticklebacks were correlated to differences in the relative amounts of various carotenoids in the pigment cells (Wedekind *et al.*, 1998). Unfortunately, we have no data concerning the amounts and types of carotenoids available to benthics and limnetics, or if they differ in availability between the species pairs. An intermediate diet would also explain why allopatric populations are intermediate in red (Schluter & McPhail, 1992). Note, however, that differences in carotenoids are unlikely to explain differences in blue coloration in Priest Lake because blue is a structural rather than a pigment-based colour (Rowe *et al.*, 2004).

Third, character displacement in red may be a consequence of displacement in nesting habitat use. Benthic males tend to nest in more covered and vegetated areas than limnetic males, which tend to nest in open or sparsely vegetated areas (McPhail, 1994; Hatfield & Schluter, 1996; Vamosi & Schluter, 1999). The amount of light available under cover may be much less than that available in the open, leading to a reduction in the visibility of the red signal in the benthic nesting habitat. If females are less able to see red due to poor light conditions, then the expression of the male display may be reduced relative to an open water signalling environment.

A link between differences in colour between closely related species or populations and differences in the quality of light available in different signalling environments has been shown in birds (Endler & Théry, 1996; McNaught & Owens, 2002), anolis lizards (Macedonia, 2001), killifish (Fuller, 2002), and other stickleback populations (Reimchen, 1989; Boughman, 2001). There is some evidence that the colour of the water becomes redder with depth in Priest and Paxton Lakes, which would reduce the visibility of a red signal and could result in reduced expression of red in benthics if they nest at greater depths, or under cover. Further investigation is required to determine the strength of any correlation between depth, habitat, and the location of nests in benthic, limnetic, and allopatric populations.

The difference in nest site microhabitat may result due to male–male aggressive interactions. Benthic males may be better competitors for concealed nest locations, driving limnetic males into the open. In tests using other populations of sticklebacks, male size was strongly correlated with success at establishing territories (Rowland, 1989) and, because benthic males are larger, they may force limnetic males into more open nesting locations. The body size difference

between the species pairs appears to be a result of ecological character displacement in foraging ecology (Schluter & McPhail, 1992), providing another link between displacement in red and competition for shared resources.

In summary, we found evidence for character displacement of red coloration between species pairs of threespine sticklebacks. The exact cause of displacement remains unknown but it is unlikely that reinforcement has played a dominant role. This implicates other types of interspecific interactions in signal displacement, such as competition for shared resources and territorial aggression. In addition, the role of differences in blue coloration between the species pairs in Priest Lake warrants further investigation. Ultimately, we would like to know if divergence in mating signals is generally driven by divergence in female preferences, or as a consequence of environmental differences and other interactions. Understanding how these forces work together to shape phenotypes will provide much insight into how interspecific interactions can drive divergence between species.

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