

Male–male competition and nuptial-colour displacement as a diversifying force in Lake Victoria cichlid fishes

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We propose a new mechanism for diversification of male nuptial-colour patterns in the rapidly speciating cichlid fishes of Lake Victoria. Sympatric closely related species often display nuptial colours at opposite ends of the spectrum with males either blue or yellow to red. Colour polymorphisms within single populations are common too. We propose that competition between males for breeding sites promotes such colour diversification, and thereby speciation. We hypothesize that male aggression is primarily directed towards males of the common colour, and that rare colour morphs enjoy a negatively frequency-dependent fitness advantage. We test our hypothesis with a large dataset on the distributions and nuptial colorations of 52 species on 47 habitat islands in Lake Victoria, and with a smaller dataset on the within-spawning-site distributions of males with different coloration. We report that territories of males of the same colour are negatively associated on the spawning site, and that the distribution of closely related species over habitat islands is determined by nuptial coloration in the fashion predicted by our hypothesis. Whereas among unrelated species those with similar nuptial colour are positively associated, among closely related species those with similar colour are negatively associated and those with different colour are positively associated. This implies that negatively frequency-dependent selection on nuptial coloration among closely related species is a sufficiently strong force to override other effects on species distributions. We suggest that male–male competition is an important and previously neglected agent of diversification among haplochromine cichlid fishes.

Keywords: character displacement; colour evolution; divergent selection; frequency-dependent selection; sexual selection; sympatric speciation

1. INTRODUCTION

In Lake Victoria in East Africa several hundred haplochromine cichlid species coexist that are believed to have evolved from very few ancestors in less than 200 000 years (Nagl *et al.* 2000) and many probably in the last 15 000 years (Johnson *et al.* 2000; Seehausen *et al.* 2003). Alpha diversity among African lake cichlids is high with up to 60 species in a single site in Lake Victoria (Turner *et al.* 2001). Their staggering diversity has motivated many theoretical investigations of phenotypic divergence and speciation (Turner & Burrows 1995; Payne & Krakauer 1997; Van Doorn *et al.* 1998; Dieckman & Doebeli 1999; Higashi *et al.* 1999; Kondrashov & Kondrashov 1999; Kawata & Yoshimura 2000; Lande *et al.* 2001).

Many Lake Victoria cichlids are highly sexually dimorphic with colourful males and cryptically coloured females, although some have colourful females too. All are polygamous, and typically only females invest in parental care. Hypotheses for the origin of the extraordinary species diversity have drawn attention to the fact that, while

closely related species are often somewhat ecologically differentiated (Bouton *et al.* 1997; Seehausen & Bouton 1997), such differences appear minor next to their conspicuous differences in male nuptial coloration. The inference is that sexual selection on male nuptial colour is a central process in the diversification of haplochromine cichlid fishes. Most research effort along these lines has centred on mechanisms for divergence driven via changes in female preference (Dominey 1984; Turner & Burrows 1995; Payne & Krakauer 1997; Seehausen & Van Alphen 1998; Higashi *et al.* 1999; Kawata & Yoshimura 2000; Lande *et al.* 2001).

Here, we consider an alternative possibility that has not been discussed before, that competition between males for breeding sites promotes colour diversification and thereby sets the stage for speciation. The colourful male haplochromines occupy territories, which they defend aggressively against other males. Hence, there is a strong potential for intrasexual selection on male traits. Males of sympatric closely related species in Lake Victoria often display colours at opposite ends of the spectrum, with males of one species being blue and those of the other yellow to red (figure 1; Seehausen *et al.* 1997; Seehausen & Van Alphen 1999). Colour polymorphisms within populations of a single species are also common and closely resemble the differences between species, though they are somewhat more muted (figure 1; Seehausen *et al.* 1999), suggesting

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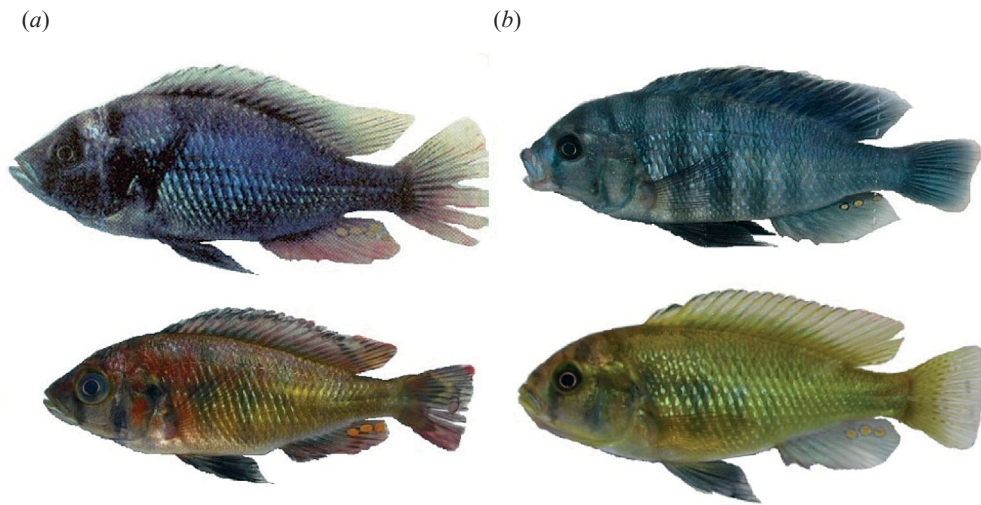


Figure 1. Representative 'blue' and 'yellow-red' male nuptial-colour types of Lake Victoria cichlids. (a) Two species: *Lithochromis rubripinnis* (top) and *Lithochromis* sp. 'red dorsum' (bottom). (b) Two nuptial-colour morphs from one population (Makobe Island) of *Neochromis omnicaeruleus*.

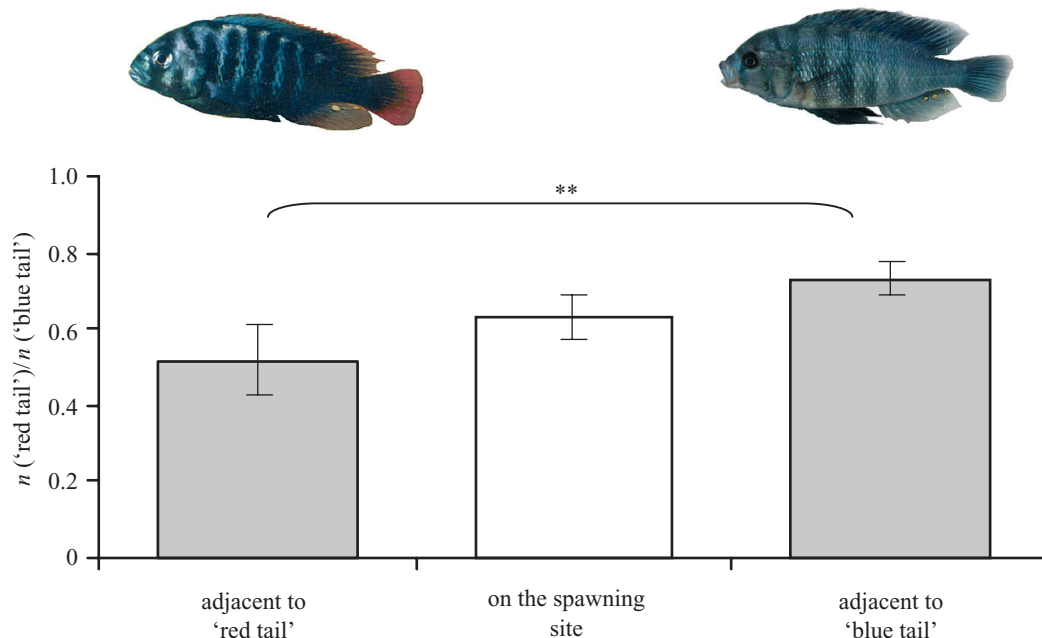


Figure 2. Under-representation of a territory owner's own coloration among the males that occupy adjacent territories. White bar, the mean ratio $n(\text{'red tail'})/n(\text{'blue tail'})$ among territorial males on a spawning site (256 m²) at Makobe Island (Lake Victoria) mapped by scuba diving. Grey bars, the same ratios among territories adjacent to territory owners with a 'red tail' (*Neochromis rufocaudalis*) or a 'blue tail' (*N. omnicaeruleus*). Based on 30 'red tail' and 48 'blue tail' males. Error bars are standard errors. Asterisks, comparison significantly different in the predicted direction at $p(\text{one-tailed}) < 0.01$.

that polymorphisms may sometimes represent incipient stages of speciation by disruptive selection on male coloration.

The hypothesis of male territoriality leading to divergence in cichlid male nuptial coloration was suggested to us by three observations. First, in the face of territorial aggression from males of the collared flycatcher, *Ficedula albicollis*, divergently coloured males of the pied flycatcher, *F. hypoleuca*, which otherwise has a similar plumage, are able to establish territories more easily (Alatalo *et al.* 1994). Second, behavioural experiments with the colour polymorphic central American Midas cichlid, *Cichlasoma citrinellum*, have demonstrated effects of colour on

aggressive interactions (Barlow 1983). Third, while keeping many different Lake Victoria cichlid species in aquaria over many years, one of us observed that it is often easier to accommodate heterospecific males of very different colours in the same aquarium than it is to accommodate heterospecific males of similar colour. This stimulates us to investigate predictions of the new hypothesis to explain the high incidence of colour polymorphism and colour diversification in sympatric cichlid species.

We suggest that males of a rare colour morph in a population or species assemblage might enjoy a fitness advantage if they elicit less aggression from males of the abundant colour morphs than do males whose colour is

common in the population or community. This could result from males evolving the ability to recognize conspecific males, which compete with them for the same set of females by virtue of their colour, or if males learn through experience to recognize the most abundant phenotypes among their competitors. It would also result if differential aggression towards like-coloured males is an incidental by-product of colour divergence that has not been selected against. We hypothesize that males possessing different colour patterns from those present in the given local cichlid community establish territories more easily than males that are similar in colour to species already present. Thus the ability of males of a given colour morph to acquire a breeding territory and/or access to females would be negatively frequency dependent.

We tested predictions of this hypothesis by testing for ‘character displacement’ in male coloration between closely related species. That is, we tested whether closely related species present in the same local site are more divergent in colour than would be expected by chance, or, equivalently, whether closely related species that are similar in colour are less likely than expected to co-occur in local sites. Such a pattern would suggest both that coexistence of species is facilitated by colour differences, and that there is negative frequency-dependent selection on male nuptial coloration. We tested the hypothesis against the alternatives that the presence or absence of species at sites is random with respect to their male colour, that species with similar colour tend to co-occur and that the probability of the co-occurrence of species is determined mainly by ecological differentiation, with colour differences being an incidental by-product.

We analysed distribution data from 52 stenotopic rock-bottom-dwelling species and 47 local (habitat island) communities to test these hypotheses. Many haplochromine species have strong habitat preferences, and migration is often restricted between habitat patches that are isolated by unsuitable habitat (Van Oppen *et al.* 1997). This is particularly true in lineages that are specialized to inhabit rocky shores, reefs and islands, a widespread but patchily distributed habitat type. Up to 28 such species coexist at any one rocky island in Lake Victoria, and species composition varies considerably even between nearby islands (Seehausen & Van Alphen 1999), making this an excellent metapopulation system in which to test predictions from ecological and evolutionary models in community biology.

Additionally we tested a prediction that the male-competition hypothesis makes on a within-site scale: that the breeding territories of males of two sympatric, closely related and ecologically and morphologically highly similar species with different male coloration are distributed such that territories of males of the same colour are less often, and territories of males of different colours are more often, adjacent than expected by chance.

2. MATERIAL AND METHODS

(a) *Distribution of nuptial coloration over local communities*

We analysed four datasets of closely related (congeneric) species of cichlid fishes endemic to Lake Victoria, collected during several years of fieldwork in the Tanzanian part of the lake (Seehausen 1996; Seehausen & Van Alphen 1999). The four

datasets are for the genera *Neochromis* Regan 1920, *Pundamilia* Seehausen & Lippitsch 1998, *Lithochromis* Lippitsch & Seehausen 1998 and *Mbipia* Lippitsch & Seehausen 1998. All of their species live exclusively over rocky bottoms. Each dataset recorded the presence or absence of all species in one genus in 46 or 47 local communities. The number of species in a local site ranged from zero to five species in each genus. Information on male breeding coloration from 321 populations allowed us to classify male colour as either blue or yellow–red (hereafter referred to as ‘red’) as defined elsewhere (Seehausen *et al.* 1999; figure 1). In one genus (*Neochromis*) males were classified differently into three types: blue, blue with red tail and blue with red ventral fins.

We used the data to investigate whether:

- (i) species were randomly distributed over communities regardless of nuptial coloration;
- (ii) species of similar colour were positively associated;
- (iii) species of similar colour were negatively associated;
- (iv) species of opposite colour were positively associated; or
- (v) species of opposite colour were negatively associated.

Note that, because the total number of species varies between islands and the number of occupied islands varies between species, support or lack thereof for patterns (ii) and (iii) is independent of and complimentary to support or lack thereof for patterns (iv) and (v).

The five possible patterns above and the four possible combinations of two patterns represent the predictions of four sets of models to explain species-distribution patterns. Pattern (i) and combinations (ii) + (iv) (related species positively associated irrespective of nuptial colour) and (iii) + (v) (related species overdispersed irrespective of nuptial colour) are null models with regard to the effects of coloration on distribution (models 1–3). Support for (ii) and (v) alone and support for both in combination suggest environmental effects or positive frequency-dependent selection on colour as overriding effects (models 4–6, environment models), e.g. (v) alone suggests that environmental effects favour one or the other colour but not both in any one site. Support for (iii) alone suggests competitive exclusion among species whose males have a similar nuptial colour (model 7, competitive-exclusion model). This provides evidence for negative frequency-dependent selection on colour but is also consistent with species of similar colour merging through selective introgression upon secondary contact (see § 4). Support for (iv) alone is consistent with negative frequency-dependent selection without competitive exclusion (model 8), whereas support for (iii) and (iv) in combination suggests negative frequency-dependent selection with competitive exclusion among species whose males have similar nuptial colour (model 9). Model 9 is predicted if the negative frequency dependence exerted by male–male competition is a diversifying force.

We applied the variance ratio method to test for associations between species occurrences in sites (Robson 1972; Schluter 1984). The method compares the variance in the total number of species among sites (s_T^2) with the sum of the variances of individual species’ occurrence among sites ($\sum \sigma_i^2$). If species are randomly distributed, the two variances should be similar and the variance ratio ($V = s_T^2 / \sum \sigma_i^2$) should be close to one. Values of V below one indicate negative species associations (species are less likely to occur together at sites than would be expected by chance), and values above one indicate positive species

associations (species co-occur more frequently than expected by chance).

V ratios were computed separately for each colour category (e.g. red and blue) to test for associations between congeneric species of similar colour. Each species was given a one if it was present at a given site and a zero if it was not. The significance of V was tested in two ways. First, we made use of the fact that the quantity $W = VN$, where N is the number of sampled sites, is approximately χ^2 distributed with N degrees of freedom. Second, we used a randomization procedure to generate a more exact distribution for V under the null hypothesis. Each iteration involved reassigning observed occurrence values (zeros and ones) of each species to sites randomly and without replacement and recalculating V . A total of 1000 iterations was performed for each test. This more exact approach always yielded lower p -values than the χ^2 approximation, suggesting that the latter is too conservative for these data. Hence we present only the randomization results. Nevertheless, we used the χ^2 approximation when combining the results of multiple tests, as described below.

A modification of the above approach was used to test for associations between congeneric species that differ in colour. For each colour we counted the number of species at each site displaying the colour. We then calculated the V statistic to measure the association between the counts for the colours. V should be less than one if species of different colours tend not to occur at the same sites, whereas V will exceed one if species of different colours tend to co-occur. The p -values were calculated using randomization.

Combined tests were also carried out using the χ^2 approximation. For example, two V statistics were calculated for a given genus having both red species and blue species. A combined test of the non-random association of similarly coloured species within the genus was obtained by summing the corresponding W values and comparing the total with a χ^2 distribution having $2N$ degrees of freedom. All calculations were carried out separately for each of the four genera and also with data pooled across all four genera.

(b) *Distribution of ecomorphology over communities*

Similar methods were applied to test associations between congeneric species with similar and different ecomorphological characteristics. For each species we measured the length and width of the lower jaw (Barel *et al.* 1977) and calculated the length–width ratio (LJL/W), which is generally a good predictor of feeding ecology (Bouton *et al.* 1997; Seehausen & Bouton 1997). We use it here as a measure of ecological similarity between species. Measurements were taken on formalin preserved fishes with a digital calliper to the nearest 0.1 mm. Within each genus we divided the range of the ratio into short broad jaws (LJL/W below the genus mean) and long narrow jaws. We then tested for species associations within and between these ecomorphological categories with the variance ratio test. Closely related species with similar morphology tend to eat similar food (Bouton *et al.* 1997). The ecomorphology dataset allows us to test whether variation in colour is associated with variation in ecomorphology and to detect potential colour \times morphology interaction effects on species–distribution patterns. If resource competition between species has an overriding effect on community composition, we would detect this in the form of negative associations between species of similar ecomorphology.

(c) *Distribution of intraspecific male colour polymorphisms over communities*

A prediction of the hypothesis of negative frequency-dependent selection on colour morphs within populations is that the likelihood of a rare morph becoming established in a population of one species is negatively correlated with the abundance in the same community of related species in which that colour predominates. To test it, we investigated associations between the distribution of red populations and the occurrence of rare red morphs in predominantly blue populations of the same genus.

(d) *Distribution of territorial males within a breeding site*

The two species *Neochromis rufocaudalis* (blue with red tail) and *N. omnicaruleus* (mostly blue with blue tail) are sympatric, fully syntopic and morphologically and ecologically extremely similar (see Bouton *et al.* (1997) and Seehausen & Bouton (1997), where the two species are referred to as *N. nigricans* and *N.* ‘blue scraper’, respectively). In the rocky littoral zone of Makobe Island (Speke Gulf, Lake Victoria) 16 quadrates of 4 m \times 4 m were established on the rocky bottom within the spawning area of the two species using a sinker rope. Four quadrates were always chosen adjacent to each other such that the 16 quadrates formed four blocks of 8 m \times 8 m. Territorial males were counted and their positions mapped with a pencil on maps prepared on PVC sheets. The centre of a territory was defined as the rock or crevice to which the territory holder was repeatedly seen to return after its forays. To test whether males of different coloration are more often, and males of the same coloration are less often, territorial neighbours than would be expected by chance, we compared the ratio of ‘red tail’ to ‘blue tail’ among owners of territories adjacent to ‘red tail’ territories with the same ratio among males adjacent to ‘blue tail’ territories, by paired t -tests with the four 8 m \times 8 m blocks as the level of replication. Distances between any two territory centres were measured on the quadrate maps, and territories were defined as adjacent when their centre points were no more than 2 m apart and not separated by intervening territories, including those of males of species of other genera.

3. RESULTS

(a) *Distribution of closely related species with regard to male nuptial coloration*

In all four genera we found V ratios below one within each colour category (table 1), which is consistent with the prediction of competitive exclusion among species with similar male nuptial coloration. Negative associations between blue species were significant in three out of the four genera (table 1). Strong trends towards negative associations were found for red-tailed *Neochromis* species and for red *Pundamilia* species. Combining the V ratios and corresponding W s for the two or three (in *Neochromis*) colour categories within each genus demonstrated significant overdispersion of male colour in the genera *Neochromis*, *Pundamilia* and *Lithochromis* and a non-significant trend in *Mbipia*. Combining the within-colour V ratios and corresponding W s of the four genera demonstrated a highly significant overall negative association between closely related species of similar male nuptial coloration (table 1). This allowed us to reject null models 1 and 2 and environment models 4 and 6.

Table 1. Tests of association (V) between species according to male nuptial colour. Negative frequency-dependent selection predicts a negative association ($V < 1$) between congeners of similar colour, but predicts a positive association ($V > 1$) between species differing in colour. (Two-tailed p -values for individual V ratios were calculated using randomization. Tests combining V ratios across groups used the χ^2 approximation. * $p \leq 0.05$, ** $p \leq 0.01$.)

	<i>Neochromis</i>	<i>Pundamilia</i>	<i>Lithochromis</i>	<i>Mbipia</i>	combined test	between genera	red morphs within genera	red morphs between genera
number of species in genus	13	20	12	7				
blue ($V(n)$)	0.677* (5)	0.621* (11)	0.538** (9 ^b)	0.962 (4)	**	1.529 (4)**	—	—
blue with red tail ($V(n)$)	0.725 (8 ^a)	—	—	—	—	—	—	—
blue with red ventral fins ($V(n)$)	0.905 (2 ^a)	—	—	—	—	—	—	—
red ($V(n)$)	—	0.785 (9)	0.930 (5 ^b)	0.895 (3)	n.s.	1.223 (3)	0.97	1.28
combined test	*	*	*	n.s.	**	*	—	—
V between colours	1.223	1.208	1.303*	1.304*	*	1.185	1.11	1.13

^a In one species (*N. omnicaeruleus*) most populations are blue without red fins, but some have a red tail, and some have red ventral fins.

^b In two species some populations are predominantly blue whereas others are predominantly red.

Species with red male coloration and species with blue male coloration tended to be positively associated in each genus. All V ratios were larger than one, in *Lithochromis* and *Mbipia* significantly so, which is consistent with the predictions of negative frequency-dependent selection on male coloration (table 1). Combining the V ratios and corresponding W s for the four genera demonstrated an overall significant positive association between closely related species of different male nuptial coloration (table 1). This allowed us further to reject null model 3, environment model 5 (general overdispersion of related species) and models 7 and 8, leaving unrejected only model 9, which is predicted by negative frequency-dependent selection on nuptial colour plus competitive exclusion of species with similar nuptial colour.

(b) Distribution of unrelated species with regard to breeding coloration

When we calculated V ratios for species of similar male colour in different genera by summing, for each genus separately, the number of species of one colour in a site, all negative associations among species of similar colour were lost and were replaced by positive associations (table 1, all V ratios greater than one), and the positive association between species of different colours was no longer significant. This suggests that the mechanisms that cause negative associations among species of similar colour and positive associations among those of different colours in closely related species are less influential among more distantly related species, and that, when these unrelated species are compared, environment has an overriding effect on male coloration.

(c) Distribution of intraspecific male nuptial-colour polymorphisms over communities

Seventeen populations in our matrix of 321 populations are polymorphic for male nuptial coloration. With a single exception, blue is the numerically dominant, and yellow-red the less abundant, morph (χ^2 -test: $p < 0.05$). In the analyses in §§ 3a and 3b the populations were considered to be composed entirely of the numerically dominant morph. This is conservative with regard to testing our hypothesis of negative frequency-dependent selection. Including the rare morph in polymorphic populations as a separate taxon strengthens the positive associations between taxa with different male colours.

The occurrence of red morphs in predominantly blue populations tends to be negatively associated with the number of congeneric red species at a site, and positively associated with the number of congeneric blue species (table 1). Just as with the species data, the direction of the association between red morphs and red species was reversed when we repeated the calculations across morphs and species of several genera (table 1). This again suggests that the mechanism that causes negative associations among taxa of similar colour overrides other, e.g. environmental, effects on colour only among closely related species.

(d) Distribution of ecomorphology over communities

Morphology was not associated with colour. In the red versus blue comparisons, seven red and 15 blue species

Table 2. Tests of association (V) between species according to jaw shape. Negative frequency-dependent selection predicts a negative association ($V < 1$) between congeners of similar jaw type, whereas it predicts a positive association ($V > 1$) between species differing in jaw type.

(Two-tailed p -values for individual V ratios were calculated using randomization. Tests combining V ratios across groups used the χ^2 approximation. * $p \leq 0.05$, ** $p \leq 0.01$.)

	<i>Neochromis</i>	<i>Pundamilia</i>	<i>Lithochromis</i>	<i>Mbipia</i>	combined test
number of species	13	20	12	7	
short jaws (V (n))	0.745 (6)	0.727 (11)	0.813 (7)	1.113 (4)	n.s.
long jaws (V (n))	1.032 (7)	1.488* (9)	1.845** (5)	0.945 (3)	**
V between jaw types	1.049	0.702**	0.667*	1.129	n.s.

had short jaws relative to the genus mean, and eight red and nine blue species had long jaws (Fisher's exact test: $p = 0.51$). In the red tail versus blue tail comparisons, four red-tail and two blue-tail species had short jaws, and two red-tail and five blue-tail species had long jaws (Fisher's exact test: $p = 0.29$). None of the associations between species of similar morphology was significantly negative (table 2), although trends towards negative associations were found among the short-jaw morphotypes in *Neochromis*, *Pundamilia* and *Lithochromis* (table 2).

By contrast, species with long narrow jaws were significantly positively associated ($V > 1$) with each other in *Pundamilia* and *Lithochromis*. Combining the V ratios and corresponding W s for the four genera confirmed this significantly positive association of closely related species with long narrow jaws (table 2). These results suggest the possibility of weak frequency-dependent competition among species with short broad jaws (mostly feeding on firmly attached benthic food), but an overriding effect of environmental variation on the distribution of species with, relative to the genus mean, long narrow jaws (which include more plankton in their diet). Between species of different jaw morphology a significantly negative association ($V < 1$) was observed in *Lithochromis* and *Pundamilia*, consistent again with a stronger environment than competition effect on the ecotype composition of local communities.

Given the absence of an association between colour and jaw shape, negative frequency-dependent selection on ecomorphology cannot explain the over-dispersion of colour. In each genus V ratios in at least one colour category were lower than the lowest V ratio among morphological categories (table 1).

(e) *Distribution of territorial males on a breeding site*

Territories of 30 male *N. rufocaudalis* ('red tail') and 51 male *N. omnicaeruleus* were mapped in a total area of 256 m². Out of the 51 *N. omnicaeruleus* 48 were blue with blue tail ('blue tail'), whereas two were yellow–red and one was orange–blotched. The yellow–red and orange–blotched males were excluded from the analysis because their colour was similarly different from 'red tail' and 'blue tail' males. 'Blue tail' was more common than 'red tail' with an overall abundance ratio n ('red tail')/ n ('blue tail') of 0.66 ± 0.11 . In agreement with predictions made by the male-competition hypothesis, this ratio was smaller among males that held territories adjacent to a 'red tail' ($0.52 \pm \text{s.d. } 0.18$) than among males that held territories adjacent to

a 'blue tail' ($0.73 \pm \text{s.d. } 0.09$) (difference: $0.21 \pm \text{s.d. } 0.11$; $t = -3.99$, $p(\text{one-tailed}) < 0.01$; figure 2).

4. DISCUSSION

The explosive adaptive radiation of haplochromine cichlids in East African lakes is associated with very frequent evolutionary transitions between conspicuously different male nuptial-colour patterns (McElroy *et al.* 1991; Deutsch 1997; Seehausen *et al.* 1997, 1999; Allender *et al.* 2003). In Lakes Victoria and Malawi closely related species with very different coloration (figure 1) are often sympatric or parapatric (Seehausen & Van Alphen 1999; Danley *et al.* 2000), and many evolutionary transitions in colour appear to have occurred in geographical proximity (Smith & Kornfield 2002; Allender *et al.* 2003). Speciation by divergent or disruptive selection on nuptial coloration has been suggested to explain such observations (Seehausen & Van Alphen 1999; Shaw *et al.* 2000; Danley & Kocher 2001; Smith & Kornfield 2002; Allender *et al.* 2003) but the possible mechanisms are only incompletely understood (Kornfield & Smith 2000; Lande *et al.* 2001; Kocher 2004).

Intraspecific polymorphisms in colour pattern are also common in Lake Victoria cichlid fishes, with differences between morphs often resembling those between closely related species (figure 1; Seehausen *et al.* 1999). In the colour polymorphic central American Midas cichlid (*Cichlasoma citrinellum*) the less common (red or 'gold') morph experiences less aggression from the more abundant (grey) morph (Barlow 1983). Kept in aquaria, males of Lake Victoria cichlids readily flush their nuptial colours upon visual exposure to other males, and males of many species are highly territorial in nature (Seehausen 1996). All these observations stimulated us to test predictions of the hypothesis that competition among males for territories and/or females exerts negative frequency-dependent selection on male coloration within populations and communities.

The observed distribution of coloration among 321 populations of rock-dwelling haplochromine cichlids over 47 rocky habitat patches in Lake Victoria is consistent with the predictions of this hypothesis: the local occurrence of species is negatively correlated with those of closely related species of similar colour but positively correlated with those of closely related species of different colours. Remarkably, we found a reversal of the negative association into a positive one among species of similar colour when we compared unrelated species, and there

was no significant association among unrelated species of different colours. Whereas closely related species differ mainly in nuptial coloration, species of different genera also differ in body shape, melanic stripe pattern and behaviour (Seehausen 1996). If variation in these characters co-determines female mate choice, mating competition among unrelated species does not rely primarily on male nuptial coloration, which would explain the absence of effects of negative frequency-dependent selection on nuptial coloration among unrelated species.

We cannot explain this strong pattern by ecological resource competition among species with similar feeding requirements because negative associations among related species of similar coloration are, in all four genera, stronger than negative associations among species of similar eco-morphology, and because eco-morphology and colour are not associated with each other. In fact there is only weak evidence for effects of resource competition on the occurrence of species with relatively short broad jaws (benthic feeders), whereas the occurrences of species with relatively long narrow jaws (more limnetic feeders) are very significantly positively correlated. Benthic feeding often involves interference competition over feeding sites (Genner *et al.* 1999), whereas to forage on plankton males often leave their territories and shoal (O. Seehausen, personal observation).

Classical reinforcement of reproductive isolation as a result of natural selection against hybrids in secondary contact zones can at present not be ruled out, but does not seem to be a likely explanation for the positive associations between haplochromine species of opposite coloration. In contrast to the flycatcher case (Saetre *et al.* 1997), no reduction in viability or fertility has been observed in interspecific hybrids of Lake Victoria cichlids in the laboratory (Seehausen *et al.* 1997), and, in contrast to many pairs of fish species in postglacial lakes (Schluter 1996, 2000), congeneric species overlap broadly in resource use (Bouton *et al.* 1997; Seehausen & Bouton 1997). However, ecological fitness reductions in hybrids remain a possibility that needs further testing. Reinforcement may also be possible by sexual selection through female choice without reduced ecological hybrid fitness (Kawata & Yoshimura 2000), a possibility that needs empirical investigation.

Our observations on the distribution of species and colour morphs over habitat islands and of the negative association between males of the same nuptial coloration within a spawning site are consistent with the hypothesis that males of the same colour compete more heavily than males of different colours, exerting negative frequency-dependent selection on nuptial colour. Such selection would result in the observed distribution pattern through local species selection among species drawn from a common species pool (species sorting). Importantly, it may also explain the evolutionary origin of colour diversity. Upon secondary contact between geographical incipient species with the same nuptial coloration, competition for territories may favour different morphs in the two newly sympatric species (i.e. the morph that is favoured by competition in one species is not the same as the morph that is favoured by competition in the other species). Further, the frequency of initially rare male colour morphs may increase within populations under negative frequency-

dependent selection, which could lead to stable polymorphisms or sympatric speciation. That the occurrence of colour morphs within populations reveals trends of association that are in the same direction as those in the species data is consistent with the idea that co-occurrence of closely related species and occurrence of colour morphs within populations are determined by similar processes.

A combination of negative frequency-dependent selection on intraspecific colour variation and species sorting during range expansion may provide a parsimonious explanation for our data. Success or failure of the establishment of morphs that arise within populations and range expansion of species by the establishment of immigrant populations are determined by the species composition of local assemblages. The mechanism could cause sexual-character displacement or speciation by reinforcement through sexual selection when the establishment of new morphs is driven by frequency-dependent competition with a heterospecific resident morph in secondary contact. It could also possibly cause sympatric speciation when the establishment of new morphs, after they have arisen by mutation, recombination or hybridization, is driven by frequency-dependent competition with the numerically dominant conspecific morph.

If our hypothesis is correct, the larger numbers of blue than red species in all genera and all communities that we studied may suggest a systematic deviation from symmetry of the frequency-dependent interaction between colour morphs, as a result of which more blue than red species would coexist in an equilibrium situation. We note that in populations of the Midas cichlid in Central American lakes, red morphs ('gold') are usually less abundant than grey morphs (Barlow 1983), and that the 'red tail' species of *Neochromis* at Makobe Island is less abundant than the 'blue tail' species.

An alternative mechanism that could explain the negative association between species of similar coloration is selective introgression, where, upon secondary contact, species of similar nuptial coloration merge, and only species of different nuptial coloration persist. However, even though $V < 1$, competitive exclusion among like-coloured species is not absolute. Species of the same nuptial colour type occur together at several sites without hybridizing. Also, selective introgression would not result in the observed positive association between species of different coloration because the numbers of species of each colour vary between sites from zero to four.

The commonness of sympatric red–blue sister species among cichlid fishes in Lake Victoria has previously been interpreted as resulting from disruptive selection through female mate choice in polychromatic visual environments where yellow or red (depending on water clarity) and blue are more conspicuous than other colours (Seehausen *et al.* 1997; Allender *et al.* 2003). Disruptive selection by female choice does indeed predict the positively associated distribution of closely related species with red and blue male nuptial coloration that we report here. However, it does not predict the negative associations between species of the same colour type that we also found, unless it acts in combination with selective introgression. Hence, the only explanation for our data other than male–male competition is a combination of sympatric speciation generating red–blue species pairs and introgression upon secondary

contact between allopatrically arisen species of the same colour.

If male coloration evolved exclusively under sexual selection by female choice, spreading of an initially rare male colour morph would be difficult unless there was a pre-existing variation in female mating preferences. Importantly, a pure female-choice model cannot account for the observation that some predominantly blue populations accommodate yellow–red male morphs at relatively high frequencies without apparently evolving reproductive isolation between the morphs (Seehausen *et al.* 1999). This observation is consistent with the suggestion that new male colour morphs can invade a population and increase to a considerable frequency without corresponding frequencies of matching female preference genotypes. Furthermore, disruptive selection by female choice would not predict the overdispersal of territories of males of the same colour within a spawning site.

(a) *A new verbal model of sympatric speciation*

Assume that female mate choice is not based exclusively on the nuptial hue of the male, but instead that females assess male quality by combining information on several male attributes. Even if nuptial hue carries more weight than any other attribute, its effect could be overridden if a male's quality in terms of other attributes conflicts with the suitability of its hue. Such attributes could be related to male condition (e.g. weight, courtship vigour, territory quality). Consequently, a male that does not display the nuptial hue preferred by the females in the population may nevertheless enjoy above-average mating success if its lower frequency and/or intensity of conflicts with other males enables it to maintain above-average physical condition and to obtain a high-quality territory.

We suggest that, if negative frequency-dependent selection on nuptial coloration as a result of male–male competition results in a strong enough advantage for rare male colour morphs that they can invade populations against the predominant female preference, this could in turn exert positive selection on rare female preference genotypes with a preference for the rare male colour, because this colour is associated with above-average fitness. Associations between genes for the new male colour and genes for the new female preference could then arise in a genetic feedback loop, as in classical Fisherian sexual selection (Lande 1982). If the association has become strong enough by the time that the frequency-dependent fitness advantage for the new male morph disappears at equilibrium frequencies, two sympatric reproductively isolated incipient species may have arisen. Long-term persistence of reproductive isolation between the incipient species requires a disadvantage of hybrid males in obtaining matings. Such a disadvantage might at least partially be mediated through male–male competition for territories (i.e. if phenotypically intermediate hybrid males received above-average amounts of aggression because they received aggression from males of both parental types).

If the establishment of colour polymorphisms under negative frequency-dependent male–male competition is typically followed by the evolution of divergent mating preferences, this might explain why only a minority of haplochromine populations contain intraspecific colour

polymorphisms without reproductive isolation between them. To test further the hypothesis of male–male competition and nuptial-colour displacement as a key diversification force in rapidly speciating cichlid fishes, experimental investigations of the effects of male coloration on aggressive competition among heterospecific and conspecific males and hybrid males are required, as well as experimental characterization of female mate-choice rules and mathematical modelling of the effects of interactions between intra- and intersexual selection on the fitness of, and gene flow between, nuptial-colour morphs.

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