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Parasite-mediated sexual selection and species divergence in Lake Victoria cichlid fish

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We investigate the role of parasite-mediated sexual selection in the divergence of two species of Lake Victoria cichlids. *Pundamilia pundamilia* and *Pundamilia nyererei* represent a common pattern of male nuptial colour divergence between haplochromine sister species: metallic grey–blue in *P. pundamilia* and bright yellow and red in *P. nyererei*. Female mating preferences for different male colours maintain the genetic and phenotypic differentiation of the two species in clear water. Previous work indicated that the red coloration of *P. nyererei* males, which is subject to directional sexual selection, may be a carotenoid-dependent signal of parasite infestation rate. In the present study, we find a parallel result for *P. pundamilia*: bright blue males are infected with fewer species of parasites. We also find that parasite infestation rates differ quantitatively between the two species in a way that is consistent with species differences in diet and microhabitat. We conclude that parasite-mediated sexual selection may have contributed to the divergence of female mating preferences between *P. pundamilia* and *P. nyererei*, and may currently strengthen reproductive isolation between these species. © 2008 The Linnean Society of London, Biological Journal of the Linnean Society, 2008, **94**, 53–60.

ADDITIONAL KEYWORDS: haplochromine cichlids – mate choice – nuptial coloration – parasite load – *Pundamilia* – speciation.

INTRODUCTION

We investigated how natural and sexual selection contribute to species divergence in East-African haplochromine cichlids. These fish are classic examples of explosive speciation, mediated at least in part by sexual selection: female mating preferences for male coloration exert sexual selection within species (Maan *et al.*, 2004; Pauers, McKinnon & Ehlinger, 2004) and maintain reproductive isolation between species (Seehausen & Van Alphen, 1998; Knight & Turner, 2004). We studied the mechanisms underlying the divergence of a sibling species pair from Lake Victoria. *Pundamilia pundamilia* and *Pundamilia nyererei* represent a common type of colour pattern variation in the Lake Victoria haplochromines (Seehausen, Van Alphen & Witte, 1999). *Pundamilia pundamilia* males are metallic grey–blue and *P. nyererei* males are yellow laterally and bright red dorsally. The two species are morphologically similar; the cryptically coloured females can be distinguished only with difficulty. Females of both species have assortative mating preferences and use male coloration as a choice criterion (Seehausen & Van Alphen, 1998).

Within *P. nyererei*, female mate choice exerts directional sexual selection on male red coloration (Maan *et al.*, 2004), possibly driven by 'good genes' selection for parasite resistance (Hamilton & Zuk, 1982): bright red males have lower macroparasite loads (Maan

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et al., 2006b). A similar mechanism may be present in *P. pundamilia*: if sexual selection on male coloration has contributed to the divergence of *P. pundamilia* and *P. nyererei*, blue-preferring females would be expected to receive some benefit from mating with bright blue males, just as red-preferring females do from mating with bright red males. In the present study, we therefore investigated the relationship between male coloration and parasite load in *P. pundamilia*.

Pundamilia pundamilia males are blue on the body and in the dorsal fin, but they also express red coloration, mainly in the caudal and anal fins. We quantified both aspects of male coloration and investigate whether they are related to parasite infestation rate in wild-caught adult males. We also analysed the carotenoid content in both components of *P. pundamilia* coloration, because carotenoids may mediate a trade-off between sexual signalling and parasite resistance (Lozano, 1994). Previous work has shown that the red coloration of *P. nyererei* is based on carotenoids (Maan *et al.*, 2006b). To determine whether similar trade-offs exist in both species, we compared the colour pigments and parasite load of *P. pundamilia* with those of *P. nyererei*.

MATERIAL AND METHODS

Fish collection and preservation

Using hook and line, we collected 26 males between December 2002 and March 2003 at Makobe Island (south-eastern Lake Victoria; western Speke Gulf). Immediately after capture, males were photographed for colour analysis. Fish were subsequently sacrificed on melting ice and measured [standard length (SL) to the nearest 0.1 mm] and weighed (to the nearest 0.1 g). Fish were preserved in 4% formalin, and the body cavity slit open ventrally to allow preservation of organs and internal parasites. After 1–4 weeks, they were transferred to 30% ethanol, at least 1 week later to 60% ethanol and, again, at least 1 week later, to the final solution of 70% ethanol. We calculated condition factor (CF) as $CF = 100 \times (\text{weight}/SL^3)$ (Sutton, Bult & Haedrich, 2000). We also scored the amount of fatty tissue (Collyer & Stockwell, 2004) on a five-point scale, with a score of 1 for individuals without any fat, and a score of 5 for those with fat tissue completely covering the intestine and liver. Gonadal maturation was also scored on a five-point scale. Sexually mature males included those scored as 4 and 5 (i.e. testes swollen to more than 80% of the maximum observed).

Colour analysis

For photography, males were placed in a perspex cuvette with water and gently squeezed against the front window of the cuvette with a grey polyvinyl chloride sheet in the background. We used a digital camera (Sony DSC-F707) and adjusted white balance in PhotoShop 6.0 (Adobe Systems Inc.) using a white patch (Kodak colour card) attached to the front of the cuvette. We quantified the amount of red coloration in the dorsal, anal, and caudal fins in SigmaScan Pro 4.0 (SPSS Inc.). We calculated the area of the fins that matched our redness criteria [a combination of hue (0–26 plus 232–255) and saturation (40–97%); identical to those used for *P. nyererei* (Maan *et al.*, 2004)]. This area was divided by the total fin area, yielding a percentage of fin coverage subsequently referred to as redscore. Ultraviolet-reflectance of *P. pundamila* male nuptial coloration is negligible (Maan *et al.*, 2006a). The blue coloration (bluescore) was also quantified: we counted the number of blue-reflecting scales on the body and added this number to twice the number of blue-reflecting dorsal fin membranes (each membrane between two spines or rays occupies approximately twice the area of one scale). The number of scales was unrelated to male size (Pearson correlations, $N = 26$, standard length: $r = 0.10$, $P = 0.62$, weight: $r = 0.10$, $P = 0.63$). To assess whether this measure corresponded to overall 'blueness' as perceived by humans, we also scored blueness by eye on a scale from 0 (no blue at all) to 5 (bright blue in both dorsal fin and on the body). The two measures were highly correlated (Spearman correlation: $r_s = 0.65$, $P < 0.001$) and we continued the analysis with the more precise bluescore.

Determination of parasite load

With a dissecting microscope, we examined the skin, fins, gills, abdominal cavity, gonads, liver, and gastrointestinal tract for parasites. Parasite identification followed Paperna (1996). In addition to counts for each parasite species, we calculated three summary variables as estimates of overall parasite infestation rate: TPL (total parasite load; the sum of all parasites infecting one fish), PS (the number of parasite species infecting one fish), and MPL (median parasite load). MPL takes the differences in abundance between parasite species into account: for each parasite species (p), we normalized the number of individuals infecting one fish (load in individual i) with the median load of that particular parasite species in the sample, and summed these relative loads: $\text{MPL}_i = \sum_p \left(load_{p,i} \Big/ medianload_p \right)$.

Pigment analysis

We followed the same procedures as described previously for the closely related *P. nyererei* (Maan *et al.*, 2006b). One adult male, laboratory F_1 offspring of

wild caught *P. pundamilia* from Makobe Island, was sacrificed on ice. We took one sample from the skin of the side of the fish (grey–blue) and another from the fins (including soft part of dorsal, anal and caudal fins; bright red). After drying for a few minutes, samples were weighed (g) and measured (mm^2). Pigments were extracted in acetone for 24 h, evaporated overnight and re-dissolved in hexane (8 mL for the red fin sample; 1 mL for the blue skin sample). Absorption spectra were determined in the range of 220–600 nm in a spectrophotometer (Unicam UV1). Carotenoid content was estimated from absorbance at λ_{max} using the absorption coefficient $A^{1\%}_{1\,cm} = 2500$ for carotenoid mixtures (Britton, Liaaen-Jensen & Pfander, 1995). To detect drosopterins (a red pteridine pigment: Fox & Vevers, 1960; Hudon, Grether & Millie, 2003), the hexane-dissolved extracts were dried and re-dissolved in 30% EtOH, acidified with HCl to pH 2, and tumbled for 24 h at room temperature. For comparison, drosopterins were extracted from 50 *Drosophila melanogaster* heads using the same procedure. Extracts were analysed using spectrophotometry and high-performance liquid chromatography (HPLC) (Waters 990 photodiode array) with an Allsphere ODS 2 column (5 μm) (15 cm × 4.6 mm) (Alltech) and a mobile phase of 70 : 20 : 10 (volume percentage) acetonitril : CH_2Cl_2 : methanol (flow rate = 1 mL min^{-1}; pressure = 500 psi).

Species comparison

We compared the parasite infestation rates and pigment composition of *P. pundamilia* to that of a sample of territorial *P. nyererei* males that were caught at Makobe Island in the same time period, often on the same day (Maan *et al.*, 2006b). For this comparison, we included only sexually mature males of both species.

Data analysis

Comparisons of groups and bivariate relationships were analysed using *t*-tests and Pearson correlations for normally distributed data, and Mann–Whitney *U*-tests and Spearman rank correlations for non-normally distributed data (SPSS 10.0). Normally distributed data are reported as means ± SEs. We investigated the relationships between parasite load and male characteristics (standard length, redscore, bluescore) for the subsample of sexually mature males because these are the males available for female mate choice. The same subsample was used for comparisons with the sibling species *P. nyererei*; all other analyses included both mature and immature males We used generalized linear models with Poisson distributions and logarithmic link functions, in R software (Ihaka & Gentleman, 1996; http://www.r-project.org). Stepwise removal of nonsignificant variables from saturated models yielded minimal adequate models; significance was determined by *F*-tests examining the change in deviance following removal of each variable. Test statistics were adjusted for over- and underdispersion (Venables & Ripley, 2002).

RESULTS

Pigment analysis

The absorption spectrum of the hexane extract from the red fin sample showed a shoulder at 418 nm, one peak at 440 nm and the highest peak at 468 nm. This is a typical carotenoid pattern and it is practically identical to the spectrum decribed previously for the red pigment in *P. nyererei* (Maan *et al.*, 2006b). Total carotenoid content in fins of *P. pundamilia* was 0.51 mg g^{-1} and 0.16 μg mm^{-2} (*P. nyererei* red skin: 0.58 mg g^{-1} and 0.24 μg mm^{-2}). The blue skin sample showed a very similar absorption spectrum: one peak at 416 nm, the highest peak at 439 nm, and a third peak at 468 nm. However, the carotenoid content was approximately 30-fold lower than in the red fin sample: 0.014 mg g^{-1} and 0.006 μg mm^{-2}. Given the areas on the fish body and fins that have red coloration in both species, and assuming that the *P. nyererei* fins contain similar amounts of carotenoids as found in *P. pundamilia*, the total amount of carotenoid deposited in visible red coloration in *P. pundamilia* can be estimated at approximately 10% of that in *P. nyererei*.

We did not detect drosopterins in the EtOH extract of the blue skin sample. The red fin extract, similar to the *P. nyererei* red skin extract and the *Drosophila* extract (Maan *et al.*, 2006b), showed a broad absorption peak at 476 nm, corresponding to the known absorption peak of drosopterins in acid solution (475 nm; Needham, 1974). The retention times of this compound in the HPLC were identical for the fish and the *Drosophila* extracts (2.06 min). For the red fin sample of *P. pundamilia*, the contribution of drosopterins to the total absorption of visible light was small: peak absorbance amounted to only 4.4% of carotenoid peak absorbance (*P. nyererei*: 3.5%; Maan *et al.*, 2006b).

Parasites

We found the same six macroparasite species as in *P. nyererei*: four ectoparasites and two endoparasites (Maan *et al.*, 2006b). In the gills, we found two species of ectoparasitic copepods [*Lamproglena monodi* (Lernaeidae) and *Ergasilus lamellifer* (Ergasilidae)] and one monogenean [*Cichlidogyrus* sp. (Dactylogyridae)]. Encapsulated larvae of an unidentified bivalve

mollusc were present in the gills of a small number of fish. In the skin and fins, we found encysted metacercariae belonging to the trematode genus *Neascus* (Digenea). Larval nematodes (*Contracaecum* sp.) were commonly found in liver and abdominal cavity.

Species comparison

There were significant differences in parasite load between the two species. *Pundamilia pundamilia* males had significantly higher loads of *Contracaecum* nematode larvae than *P. nyererei*, but *P. nyererei* had significantly higher copepod loads of both *L. monodi* and *E. lamellifer* (Table 1). In *P. pundamilia*, TPL was largely determined by nematode load and significantly higher than in *P. nyererei*. The PS per individual fish was significantly higher in *P. nyererei*. Parasite load may be related to fish body size and *P. pundamilia* males were larger than *P. nyererei* males (weight (g): $n_1 = 14$, $n_2 = 17$, mean ± SE 30.10 ± 2.25 versus 12.70 ± 0.39, $t = 7.62$, $P < 0.001$). Per gram bodyweight, *P. nyererei* had significantly higher loads of *Neascus*, *L. monodi*, and *E. lamellifer* and a significantly higher PS. *P. pundamilia* had higher numbers of *Contracaecum* per gram bodyweight than *P. nyererei*, but TPL per bodyweight did not differ significantly between the species (Table 1).

Parasite load in *P. pundamilia*

Larger *P. pundamilia* males had higher gonadal maturity scores (Spearman rank correlation, $N = 26$: $r_s = 0.82$, $P < 0.001$) and higher bluescores ($r_s = 0.54$, $P = 0.004$). Redscore did not increase with male size ($r_s = -0.03$, $P = 0.89$). Bluescore was not related to redscore ($r_s = 0.14$, $P = 0.51$). Some parasites increased in number with fish size (SL; *Cichlidogyrus*: $r_s = 0.43$, $P = 0.029$; *Neascus*: $r_s = 0.35$, $P = 0.077$); others were not related to size (mollusc, *L. monodi*, *E. lamellifer*, nematodes: $r_s < 0.3$, $P > 0.13$).

Among the sexually mature males ($N = 14$; Table 2, Fig. 1), large males tended to have lower TPL, but not MPL, nor PS. Males with high bluescore had significantly lower *Neascus* load. Bluescore was the best, significant, predictor of low MPL ($P = 0.046$) and PS ($P = 0.03$); standard length and redscore were not related to MPL or PS ($P > 0.28$). High fin redscores tended to be associated with high TPL ($P = 0.061$).

None of the parasites or summary variables was significantly related to body condition or fat score (Spearman rank correlations: $-0.35 < r_s < 0.35$, $P > 0.2$). Redscore was positively related to body condition ($r_s = 0.53$, $P = 0.049$) but not fat score ($r_s = 0.05$, $P = 0.86$); bluescore was not related to body condition ($r_s = 0.03$, $P = 0.93$), although there was a tendency for a positive relationship with fat score ($r_s = 0.51$, $P = 0.061$).

DISCUSSION

We investigated the parasite communities of the sister species *P. pundamilia* and *P. nyererei* at a site where they are ecologically differentiated and do not exchange much genes (Seehausen, Van Alphen & Witte, 1997). We found the same species of macroparasites in both species, except for intestinal trematodes, that occurred in a few *P. nyererei* individuals (Maan *et al.*, 2006b) but were absent in *P. pundamilia*.

Parasite infestation rates differed quantitatively between the species, indicating that they differ in parasite exposure and/or aspects of immune defence. Both may be related to differences in habitat and diet: at Makobe Island, *P. pundamilia* feeds primarily on benthic insect larvae whereas *P. nyererei* feeds on zooplankton (Bouton, Seehausen & van Alphen, 1997). Both species inhabit rocky shores but, in the Makobe community, *P. pundamilia* is most abundant in crevices 0.5–1.5 m in depth and *P. nyererei* is most abundant outside crevices 4–7 m in depth (Seehausen & Bouton, 1997). The higher *Contracaecum* load in *P. pundamilia* is probably related to its shallow and nearshore habitat: *Contracaecum* larvae depend on piscivorous birds as final hosts, in which they produce eggs. These eggs wash into the water with bird faeces and infect their first intermediate host (i.e. small crustaceans) that are subsequently eaten by fish (Paperna, 1996). At Makobe Island, egrets and cormorants aggregate in large numbers on the shoreline, covering the rocks with guano. As a result, the abundance of infectious *Contracaecum* stages is likely to decrease with the distance to the shore. By contrast, parasitic copepods, with higher loads in *P. nyererei*, reach their host directly via the water flow through the gills. Due to its zooplanktivorous, limnetic feeding style, *P. nyererei* may experience increased exposure to these copepods compared with the more benthic-feeding *P. pundamilia* (Knudsen, Curtis & Kristoffersen, 2004).

In *P. nyererei* males, low parasite loads are associated with high body redscores (Maan *et al.*, 2006b). Although the red coloration of *P. pundamilia* fins seems chemically similar to that in *P. nyererei* body coloration, we did not find significant associations with parasite load. We found a nonsignificant trend for high redscores to predict high nematode load in *P. pundamilia* whereas, in *P. nyererei*, there was a significant negative relationship between redscore and nematode load (Maan *et al.*, 2006b). The relationships between body condition and redscore in the

Table 1. Parasite load in sexually mature males of *Pundamilia pundamilia* and *Pundamilia nyererei*

	Parasite load: descriptive statistics				Parasite load: species difference					
	Pundamilia pundamilia (14 males)		*Pundamilia nyererei* (17 males)		Mann–Whitney *U*-test					
					Raw data			Controlled for body weight		
	%	Median (range)	%	Median (range)		Z	*P*		*Z*	*P*
Neascus sp.	100	6.5 (3–13)	100	5 (1–19)		1.25	0.21	$n > p$	2.38	**0.017**
Cichlidogyrus sp.	93	8.5 (0–16)	88.2	6 (0–12)	$p > n$	1.90	0.058	$n > p$	1.75	0.081
Lamproglena monodi	50	0.5 (0–9)	100	7 (2–15)	$n > p$	3.95	**0.000**	$n > p$	4.67	**0.000**
Ergasilus lamellifer	14	0 (0–1)	64.7	2 (0–5)	$n > p$	3.06	**0.002**	$n > p$	3.14	**0.002**
Mollusc	29	0 (0–1)	47.1	0 (0–12)		1.20	0.23	$n > p$	1.72	0.086
Contracaecum sp.	100	34 (1–94)	88.2	4 (0–20)	$p > n$	3.90	**0.000**	$p > n$	2.38	**0.017**
TPL		51 (25–110)		24 (11–52)	$p > n$	3.61	**0.000**		0.87	0.38
PS		4 (3–6)		5 (3–6)	$n > p$	2.47	**0.013**	$n > p$	4.45	**0.000**

Parasite load: descriptive statistics for both species; % denotes prevalence: the proportion of infected individuals.

In the Mann–Whitney *U*-test results for the difference between the two species, the direction of the difference is indicated for results with $P < 0.10$; $p > n$ indicates higher parasite load in *P. pundamilia*; $n > p$ indicates higher parasite load in *P. nyererei*. Significant differences are indicated in bold.
TPL, total parasite load: the sum of all parasites infecting one fish; PS, number of parasite species infecting one fish.

Table 2. Generalized linear model (GLM) results for the relationships between parasite load and male size and colour in sexually mature *Pundamilia pundamilia* males ($N = 14$)

		Standard length			Redscore			Bluescore		
	%	Effect	$F_{1,12}$	P	Effect	$F_{1,12}$	P	Effect	$F_{1,12}$	P
Neascus sp.	100	–	0.10	0.76	+	0.02	0.90	–	8.68	**0.012**
Cichlidogyrus sp.	93	+	0.26	0.62	+	0.02	0.89	+	0.86	0.37
Lamproglena monodi	50	+	0.32	0.58	–	0.24	0.63	–	1.71	0.22
Ergasilus lamellifer	14	–	11.23	**0.006**	–	0.24	0.63	–	3.96	0.070
Mollusc	29	–	2.17	0.17	+	0.11	0.75	–	3.06	0.11
Contracaecum sp.	100	–	3.81	0.075	+	3.32	0.094	+	0.47	0.51
TPL		–	4.34	0.059	+	4.28	0.061	+	0.27	0.61
MPL		–	0.84	0.38	+	0.12	0.73	–	4.94	**0.046**
PS		–	1.35	0.28	+	0.01	0.94	–	6.05	**0.03**

GLM results are reported for all parasites; the low prevalence of some species (Table 1) warrants cautious interpretation of these relationships. Significant differences are indicated in bold.

TPL, total parasite load: the sum of all parasites infecting one fish; MPL, median parasite load; PS, number of parasite species infecting one fish.

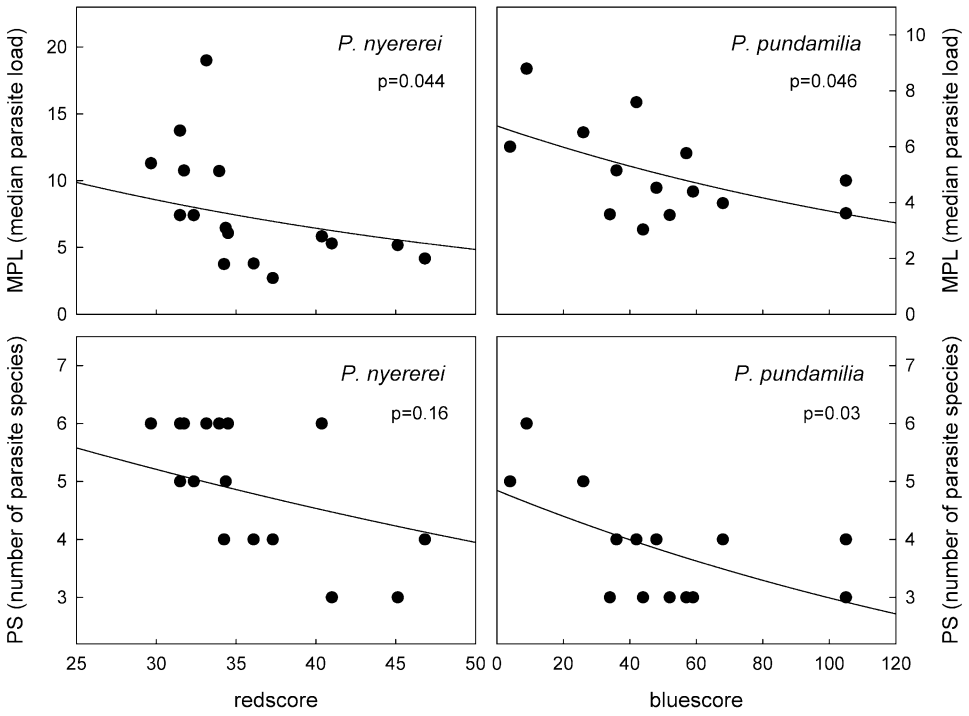

Figure 1. Colour scores and measures of parasite load in sexually mature *Pundamilia* males. Right panels: bluescore in relation to median parasite load (MPL) and number of parasite species (PS) in *Pundamilia pundamilia*. For comparison, the left panels show the relationships between *Pundamilia nyererei* redscore and MPL and PS; adapted from Maan *et al.* (2006b).

two species were inverse: significantly negative in *P. nyererei*, but significantly positive in *P. pundamilia*. Thus, the information conveyed by carotenoid-dependent colour signals appears to differ between the species. *Pundamilia nyererei* males may be more carotenoid-limited than *P. pundamilia* because they allocate approximately ten-fold more carotenoid to red coloration. Quantitative comparison, however,

requires analysis of the carotenoid content in the diet of both species.

In *P. pundamilia*, high bluescores rather than redscores may predict low parasite loads: bluescore was significantly negatively related to *Neascus* load, MPL, and PS. Blue coloration is not based on pigments, but produced by microstructures that scatter long wavelengths and reflect short wavelengths. By contrast to carotenoid-based sexual ornaments, relatively little is known about the signal value of structural colours. There is evidence for sexually selected structural coloration in birds (Sheldon *et al.*, 1999; McGraw *et al.*, 2002; Doucet & Montgomerie, 2003; Siefferman & Hill, 2003) and fish (Kodric-Brown & Johnson, 2002; Cummings, Rosenthal & Ryan, 2003; Boulcott, Walton & Braithwaite, 2005), but the underlying physiological mechanisms are not resolved.

Females of *P. pundamilia* and *P. nyererei* use male nuptial coloration in interspecific mate choice (Seehausen & Van Alphen, 1998) and, in *P. nyererei*, male red coloration is subject to intraspecific directional sexual selection through female choice (Maan *et al.*, 2004). We do not know whether female *P. pundamilia* similarly select for bright blue coloration among conspecific males, but the variation in male parasite load indicates that such mate choice could be adaptive. Although parasite infestation rates were below pathological levels in both species (Paperna, 1996) and did not correlate with lower body condition or fat score, it is possible that the documented variation in parasite load of adult males translates into more consequential variation in resistance of the offspring: cichlid fry and juveniles are often more vulnerable to the harmful effects of parasites (Paperna, 1996). Laboratory studies should address the heritability and fitness effects of parasite resistance in these species (Barber *et al.*, 2001).

Our results suggest that parasite-mediated sexual selection within each species could cause divergent selection between the species on male coloration and parasite resistance. Hence, divergent sexual-selection may not be inconsistent with 'good genes' models of sexual selection (Lorch *et al.*, 2003; Edelaar, van Doorn & Weissing, 2004; Reinhold, 2004; Skarstein, Folstad & Ronning, 2005). Whether or not parasite-mediated selection has played a primary role in the divergence of the two species could be tested by studying the relationship between parasite communities and male coloration in genetically more admixed but phenotypically variable populatons, where female mating preferences are polymorphic but reproductive isolation is incomplete (Seehausen, in press). Because blue versus red and blue versus yellow polymorphisms or sister species are common among haplochromine cichlids (Seehausen *et al.*, 1999), and given the evidence for parasite-mediated sexual selection in other cichlid species (Taylor *et al.*, 1998), this mechanism may not be unique to the species pair we studied.

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