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## **Sexual selection and speciation: mechanisms in Lake Victoria cichlid fish**

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## Chapter 3

*'The development, however, of certain structures (...) has been carried to a wonderful extreme which, as far as the general conditions of life are concerned, must be slightly injurious to the male. From this fact we learn that the advantages which favored males derive from conquering other males in battle or courtship, and thus leaving a numerous progeny, are in the long run greater than those derived from rather more perfect adaptation to their conditions of life.'*

Darwin, 1871



# Fitness correlates of male coloration in a Lake Victoria cichlid fish: evidence for parasite-mediated sexual selection on a speciation trait

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(submitted)

Sexual selection by female choice has contributed to the rapid evolution of phenotypic diversity in the cichlid fish species flocks of East Africa. Yet, very little is known about the ecological mechanisms that drive the evolution of female mating preferences. We studied fitness correlates of male nuptial coloration in a member of a diverse Lake Victoria cichlid lineage, *Pundamilia nyererei*. In this species, male red coloration is subject to intraspecific sexual selection by female mate choice. Male nuptial coloration also underlies reproductive isolation between this species and the closely related sympatric species *P. pundamilia*. Here, we show that *P. nyererei* male coloration is carotenoid based, illustrating the potential for honest signaling of individual quality. In a wild population, we found that variation in male coloration was not associated with variation in a set of strongly intercorrelated indicators of male dominance: male size, territory size and territory location. Instead, the male characters that determine female choice, territory size and red coloration, may be independent predictors of male quality: males with bright red coloration and large territories had lower parasite infestation rates. As a result, female preferences tended to select against heavily parasitized males. Consistent with parasite-mediated sexual selection, males had higher and more variable parasite loads than females.

## Introduction

The several hundred species of haplochromine cichlid fish endemic to Lakes Victoria and Malawi are textbook examples of explosive speciation (Fryer & Iles 1972; Schluter 2000; Kocher 2004). Female mate choice has played an important role in the phenotypic diversification of these species flocks: female preferences for male coloration exert sexual selection within species (Chapter 2; Pauers et al. 2004) and maintain reproductive isolation between sympatric incipient and sibling species (Seehausen et al. 1997a; Knight et al. 1998; Van Oppen et al. 1998; Seehausen 2000). Little is known however about the ecological mechanisms that determine the origin and evolution of these mating preferences: what do females gain by being choosy with regard to male coloration? Identifying the mechanisms by which female preferences evolve is fundamental for understanding mechanisms of rapid speciation.

Theoretical models that investigate the odds of speciation by sexual selection often rely on the assumption that female preferences are selectively neutral (or entail a small cost) and do not enhance offspring survival (e.g. Lande 1981; Turner & Burrows 1995; Van Doorn et al. 2004; for reviews see Panhuis et al. 2001; Turelli et al. 2001). This assumption allows evolution of preference and trait in many directions and facilitates divergence, but it is opposed by numerous studies describing sexually selected traits that reliably indicate individual quality (for reviews, see Andersson 1994; Candolin 2003; Neff & Pitcher 2005). Female preferences for males that provide genetic quality or direct benefits are subject to natural selection and may not diverge so easily (Kirkpatrick & Nuismer 2004; but see Lorch et al. 2003; Edelaar et al. 2004; Reinhold 2004). Therefore, to understand the role of sexual selection in speciation we must identify the selection pressures acting on mating preferences.

We have previously demonstrated directional sexual selection on male red coloration in the Lake Victoria cichlid *Pundamilia nyererei* (Chapter 2). Male coloration is important also in interspecific female mate choice, which is the main source of reproductive isolation between *P. nyererei* and its close relative *Pundamilia pundamilia* (Seehausen & Van Alphen 1998). Laboratory breeding of *P. nyererei* indicates that both male coloration and female preferences are heritable (Seehausen et al. 1997a; Haesler & Seehausen 2005), making a coevolutionary process feasible. Here, we study the other extreme of the ‘sexual selection continuum’ (Kokko et al. 2002), where female selectivity may increase offspring health and survival. We investigate possible sources of selection on female mating preferences within *P. nyererei*. Lake Victoria haplochromine cichlids are female mouthbrooders and males do not contribute to brood care. Direct benefits are therefore unlikely to influence female choice, but bright colours may signal heritable quality through a variety of mechanisms (Andersson 1994). We ask whether male coloration is an indicator of male quality, and thereby a predictor of offspring viability. Specifically, we study the potential for parasite-mediated sexual selection (Hamilton & Zuk 1982) in a wild *P. nyererei* population.

Red, orange and yellow ornaments are often due to carotenoid deposition (e.g. in fish: guppies [Kodric-Brown 1989], sticklebacks [Brush & Reisman 1965] and cichlids [Evans & Norris 1996]). Besides their role in colour signals, carotenoids are attributed various beneficial physiological functions, particularly in immune defence (Olson & Owens 1998; Hill 1999). Animals obtain carotenoids from food (Goodwin 1984), but they are considered a scarce resource (Grether et al. 1999) and may constitute a trade-off between sexual ornamentation and survival (Folstad & Karter 1992; Lozano 1994). Carotenoid-based sexual ornaments thus fulfil the requirements of an honest signal of quality (Zahavi 1975) and several examples of this mechanism have been found, especially in birds (Saino et al. 1999; McGraw & Ardia 2003; Saks et al. 2003) and fish (Barber et al. 2001; Grether et al. 2004).

We test whether the red and yellow colours of male *P. nyererei* are carotenoid-based. Subsequently we investigate in wild territorial males whether yellow and red coloration and/or other visible characteristics predict parasite load, and whether the latter is related to variation in mating success measured in the wild. Because females may select high-quality mates by choosing males that are dominant in male-male competition (Cox & LeBoeuf 1977; Berglund et al. 1996), we also investigate the relationships between mating success, male coloration, parasite load and indicators of male dominance such as male size, territory size and territory location.

## Methods

### *Behavioural observations and fish collection*

We studied a *P. nyererei* population between December 2000 and February 2001 (subsequently referred to as '2001') and between November 2002 and March 2003 ('2003') at Makobe Island in the western Speke Gulf, Tanzania (Seehausen & Bouton 1997). The relatively clear water at this island allows underwater observations (Secchi reading  $\text{mean} \pm \text{se} = 221 \pm 7$  cm in the study period [84 measurements]). *P. nyererei* is mainly zooplanktivorous and also feeds on insect larvae and Nostoc (Bouton et al. 1997). Mature *P. nyererei* males defend territories on the rocky bottom at four to seven meters water depth and they attract females by vigorous courtship displays (Seehausen & Van Alphen 1998). When mating occurs, the female takes the fertilised eggs in her mouth and leaves the territory. Females mouthbrood their clutches for about three weeks and after first releasing them, guard the fry for another week (Seehausen 1996). Females and non-territorial males inhabit the same depth range and are typically seen in large feeding shoals with other zooplanktivores.

### *Observations on individually marked territorial males*

Using SCUBA, we identified 28 territorial males in the year 2001, and collected between 5 and 10 observations of 10 minutes each for every male, yielding a total of 50 to 100 minutes observation time per male. We recognised males individually

by their coloration pattern and/or injuries or scars. Observations were carried out between 09:00 and 13:00 hours and all observations for any one male were completed within a period of 6 to 32 days. We recorded all interactions between these males and conspecific females. Female response was defined as the number of courted females responding positively (i.e. approaching the male) to male courtship. We used the number of male quivering bouts displayed to a female as a measure of male courtship intensity. We measured water depth at the territory (T depth; m) and territory size (T size; m<sup>2</sup>); territorial borders were deduced from the positioning of the territory owner during aggressive behaviour towards other fish. We counted and measured (diameter, cm) all rocks in each territory and determined the number of crevices between rocks that were used by the territory owner. Territories were bimodally distributed over the sampled depth range with concentrations at depths of  $4.21 \pm 0.06$  m ( $n=11$ ) and  $6.26 \pm 0.06$  m ( $n=17$ ).

In the year 2003, we found *P. nyererei* territories in a continuous depth range from 4.3 to 6.5 meters in the same area of Makobe Island. Territory size and depth distribution did not differ between years (average territory size and depth:  $t < 1.2$ ,  $p > 0.24$ ; depth distribution:  $\chi^2 = 5.67$ ,  $df=6$ ,  $p > 0.2$ ). We identified 23 territorial males and measured territories of 14 of these males. We caught 8 males holding measured territories and 9 males holding territories that were not measured. Behavioural observations were not conducted.

In both years, territorial males were caught in gillnets or by hook and line, both using SCUBA. They were slowly brought to the surface in transparent plastic bags, which minimised the occurrence of stress-induced colour changes.

### *Observations on additional males and females*

We collected an additional sample for parasite load analysis in 2001: 26 males and 19 females caught in gillnets (l:h: 30:1.5 m; stretched mesh sizes of 12.5, 16.5 and 18.5 mm) at approximately 6 meters water depth. Nets were set for a maximum of one hour to avoid colour loss in males. Female abundance at different depths was determined in 2003. We set 12.5 mm gillnets at 3.5, 4, 5 and 6 meters depth in the study area, and collected 104 females in 380 minutes. This sample emanated from experimental fishing for other species and we could not retain these fish. Therefore, these catches could only be used to determine the numbers of *P. nyererei* females at different depths, and fish were not dissected. To determine the depth distribution of females in relation to gonadal maturation stages we used a second sample, taken at 3.5, 4, 5, 6 and 6.5 meters depth (138 females).

### *Preservation*

Immediately after capture, males were photographed for colour analysis. Fish were sacrificed on melting ice and measured (standard length: SL; to the nearest 0.1 mm). Fish were preserved in 4% formalin, 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 one week later to 60% and again at least one week later to the final solution of 70%. They were then weighed [W; to the nearest

0.1 g]) and condition factor (CF) was calculated as  $CF = 100 \cdot (W/SL^3)$  (Sutton et al. 2000).

### *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 PVC sheet. For all pictures, we 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 calculated colour scores in SigmaScan Pro 4.0 (SPSS Inc.). Following Chapter 2, we used criteria delimiting the body area (excluding fins and eyes) covered by red and yellow based on a combination of hue and saturation (red: hue=0-26 plus 232-255, saturation 40-97%; yellow: hue=27-45, saturation 40-97%) and subsequently calculated the area of the fish body that matched these criteria. This yielded a percentage of body coverage, subsequently referred to as 'redscore' and 'yellowscore'. We similarly defined criteria for blackness to quantify the body coverage of the black vertical bars and ventral aspects of the body (intensity=0-75; 'blackscore').

In 2001, we used an SLR camera and two flashes on either side and subsequently digitised pictures. In 2003, we used a digital camera without flash. To enable comparison of colour scores between years, we calculated colourscores of the Kodak colour card that was photographed with both the SLR and digital camera ( $n=10$  pictures for each camera). This yielded a calibration factor of 1.16 for both red- and yellowscore. Blackscore was too different between methods to allow for calibration. When using calibrated colourscores, this is indicated in the text.

### *Determination of parasite load and sexual maturity*

With a dissecting microscope, we examined the skin, fins, gills, abdominal cavity, gonads, liver and gastro-intestinal tract and counted all parasites. Parasite identification followed Paperna (1996). We report parasite counts for each parasite species separately. We calculated additional summary variables as estimates of overall parasite infestation rate: TPL (total parasite load) is the sum of all parasites infecting one fish; PS is the total number of parasite species infecting one fish. For territorial males, we calculated a third summary variable (median parasite load, MPL) that takes the differences in abundance between parasite species into account: for each species, we normalised the number of individuals infecting one fish with the median parasite load of that species in the sample of territorial males of either year, and summed these relative loads.

Sexually mature, gravid, females were those that were ready to spawn within a few days, i.e. their ovaries were full with eggs that had a diameter of at least 80% of the maximum observed. Sexually mature males had their testes swollen to more than 80% of the maximum observed.

### *Skin pigment analysis*

One female and one male, adult F1 offspring of wild caught *P. nyererei* from Makobe, were sacrificed on ice. Of each fish, two skin samples of approximately 2 cm<sup>2</sup> were taken: one from the dorsum (red in males), the other from the side of the fish (yellow in males; both brownish in females). These were dried for a few minutes and then weighed (g) and measured (mm<sup>2</sup>). Pigments were extracted in acetone for 24 hours. Absorption spectra of the acetone extracts, measured in the range of 220-600 nm in a spectrophotometer (Unicam UV1) showed that male skin samples significantly absorbed in the 420-470 nm range, whereas female skin samples did not. Acetone extracts of the male skin samples were evaporated overnight, re-dissolved in hexane and absorption spectra determined. Carotenoid content of these extracts was estimated from absorbance at  $\lambda_{\max}$  using the absorption coefficient  $A_{1\text{cm}}^{1\%} = 2500$  for carotenoid mixtures (Britton et al. 1995). Besides carotenoids, orange and red colour patterns in fish can contain drosophpterins (Fox & Vevers 1960; Hudon et al. 2003). To detect these, hexane extracts were dried and re-dissolved in 30% EtOH, acidified with HCl to pH 2, and tumbled for 24 hours at room temperature. For comparison, drosophpterins were extracted from 50 *Drosophila melanogaster* heads using the same procedure. Extracts were analysed using spectrophotometry and HPLC (Waters 990 photodiode array) with an Allsphere ODS 2 column (5  $\mu\text{m}$ ) (15 cm  $\times$  4.6 mm) (Alltech) and a mobile phase of 70:20:10 (volume %) acetonitril:CH<sub>2</sub>Cl<sub>2</sub>:methanol (flow rate 1 ml/min, pressure 500 psi).

### *Data analysis*

Comparisons of groups and bivariate relationships were analysed using paired t-tests and Pearson correlations for normally distributed data, and Mann-Whitney-U tests, Wilcoxon signed ranks tests, Chi-square tests and Spearman correlations for non-normally distributed data (SPSS 10.0, SPSS Inc.). Means of normally distributed data are reported with standard errors. Multivariate relationships were analysed with generalised linear models (GLM) using R (Ihaka & Gentleman 1996; <http://www.r-project.org>). Models with counts as dependent variables used Poisson distributions and logarithmic link functions. The proportion of gravid females was analysed with a binomial model and a logit link function. All other models assumed normal distributions; distributions of residuals were consistent with this assumption. Stepwise removal of non-significant variables from saturated models yielded minimal adequate models; significance was determined by F-tests examining the change in deviance following removal of each variable. In Poisson models, test statistics were adjusted for over- and underdispersion (Venables & Ripley 2002).

To determine whether and how variation in parasite load was reflected in variation in characteristics of territorial males that could be assessed by females, we calculated minimal adequate GLMs with parasite load as dependent variable and the following male characteristics as independent variables: male redscore, yellowscore, blackscore, standard length, courtship intensity, and territory size and water depth. Because male redscore, courtship intensity and territory size de-

termine female choice (Chapter 2), we report GLM results for these traits separately. Courtship intensity was recorded in 2001 only. In all other models, we pooled the data from both years and included ‘year’ as a factor. Because territory size varied with water depth, depth was included as a covariate in models with territory size as independent variable. For all tests of relationships with parasites, we applied sequential Bonferroni corrections (Sokal & Rohlf 1995), using  $k$  = the number of parasite species analysed or  $k$  = the number of summary variables.

## Results

### *Yellow and red skin pigments are carotenoid-based*

The absorption spectrum of the hexane extract from the red skin sample showed a typical carotenoid pattern: a shoulder at 418 nm, one peak at 439 nm and the highest peak at 468 nm. Total carotenoid content was 0.58 mg/g and 0.24  $\mu\text{g}/\text{mm}^2$ . The yellow sample showed a very similar absorption spectrum: one peak at 416 nm, the highest peak at 439 nm and a third peak at 467 nm. Carotenoid content was 0.16 mg/g and 0.085  $\mu\text{g}/\text{mm}^2$ . Thus, the red skin sample contained approximately three times more carotenoid than the yellow skin sample. The EtOH extract of the yellow skin sample did not contain drosopterins. Both the red skin extract and the *Drosophila* extract 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 minutes). For the red fish skin sample, the contribution of drosopterins to the total absorption of visible light was small: peak absorbance amounted to only 3.5% of carotenoid peak absorbance.

### *Territorial males: male colour is not related to body size or territory size*

Male coloration did not covary with male size: none of the colour scores were related to male size in either year (Pearson correlations, standard length: all  $p > 0.17$ ; weight: all  $p > 0.29$ ). Likewise, male coloration was not related to territory size (Pearson correlations, all  $p > 0.40$ ).

Both territory size and male size varied with water depth (Figure 3.1 and Table 3.1). Male territories were larger in shallow water in both years. In 2001, males in shallow water were larger and had higher condition factors than males in deeper water. In 2003, there were non-significant relationships in the same direction. In 2001, males in deeper water had higher yellowscores ( $n_1=17$ ,  $n_2=11$ ,  $t=2.36$ ,  $p=0.029$ ) but not redscores ( $p=0.18$ ). In 2003, similar trends were present (red:  $r=0.46$ ,  $p=0.067$ ; yellow:  $r=0.43$ ,  $p=0.089$ ). Blackscores tended to decrease with depth (2001:  $t=1.91$ ,  $p=0.079$ ; 2003:  $r=-0.24$ ,  $p=0.35$ ). The relationships between male size, territory size and territory depth were not affected by male coloration (GLM; all  $p > 0.20$  in both years). Thus, males with high (or low) colour scores were not larger and did not hold larger territories in either year, but males in deep water were smaller and tended to have higher colour scores.

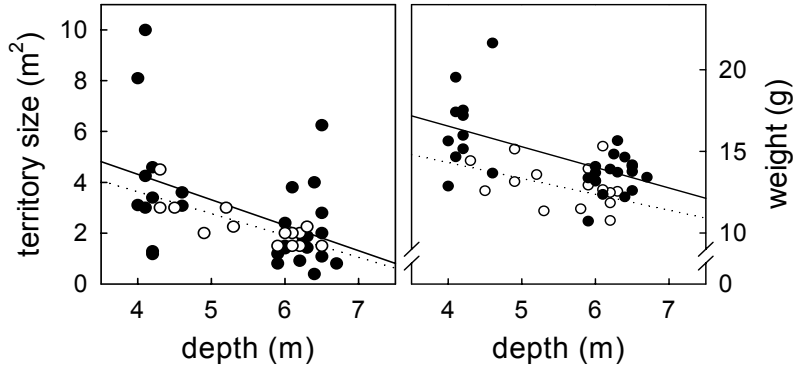


Figure 3.1 Territory size and male weight as functions of water depth in 2001 (black symbols, solid line) and 2003 (open symbols, dotted line).

Table 3.1 Means, standard errors and test results for the differences between deep and shallow territorial males in 2001 and 2003.

|                                  | 2001              |                | 2003 |        |       |        |     |
|----------------------------------|-------------------|----------------|------|--------|-------|--------|-----|
|                                  | shallow<br>$n=11$ | deep<br>$n=17$ | $t$  | $p$    | $r$   | $p$    | $n$ |
| SL (mm)                          | $82.6 \pm 0.8$    | $79.8 \pm 0.6$ | 2.95 | 0.0067 | -0.18 | 0.48   | 17  |
| weight (g)                       | $16.5 \pm 0.8$    | $13.6 \pm 0.3$ | 3.58 | 0.0035 | -0.39 | 0.12   | 17  |
| condition (g/mm <sup>3</sup> )   | $2.9 \pm 0.1$     | $2.7 \pm 0.1$  | 2.33 | 0.028  | -0.28 | 0.28   | 17  |
| territory size (m <sup>2</sup> ) | $4.1 \pm 0.8$     | $2.0 \pm 0.4$  | 2.70 | 0.012  | -0.80 | <0.001 | 14  |

Deep and shallow habitats differed in several ways. First, although the number of rocks in a territory was identical (median=23 in both deep and shallow territories), rocks in shallow water were larger (rock diameter in shallow territories:  $51 \pm 4.6$  cm [ $n=11$ ]; deep:  $34 \pm 2.8$  cm [ $n=17$ ],  $t=3.35$ ,  $p=0.0026$ ) and provided more interstitial crevices (median and range 4 [1-5] versus 2 [1-4] per territory, MWU  $Z=-2.057$ ,  $p=0.047$ ; data from 2001). Second, the total abundance (catch per effort) of adult females increased significantly with increasing depth (GLM estimate= $0.50 \pm 0.095$ ,  $F_{1,2}=28.5$ ,  $p=0.033$ ). As the proportion of gravid females (i.e. ready to spawn) was larger in shallower water however (GLM estimate= $-0.41 \pm 0.061$ ,  $F_{1,3}=46.6$ ,  $p=0.0064$ ; data from 2003), the resulting expected number of gravid females that males would encounter did not differ between depth classes ( $\chi^2=3.32$ ,  $df=3$ ,  $p=0.34$ ). In line with this, depth did not influence female choice (Chapter 2) and deep and shallow males did not differ in female response rate ( $n_1=17$ ,  $n_2=11$ , MWU  $Z=-1.03$ ,  $p=0.31$ ), nor in the total number of interactions with females ( $Z=-0.59$ ,  $p=0.58$ ) or the number of aggressive interactions with females ( $Z=-1.16$ ,  $p=0.26$ ).

Territorial males caught in 2003 were significantly smaller than those caught in 2001 ( $n_1=28$ ,  $n_2=17$ ; standard length:  $80.9 \pm 0.5$  vs.  $76.4 \pm 0.7$  mm,  $t=5.1$ ,  $p<0.001$ , weight:  $13.7 \pm 0.4$  vs.  $12.7 \pm 0.4$  g,  $t=3.12$ ,  $p=0.0032$ ) but there was no difference in condition factor ( $t=0.812$ ,  $p=0.37$ ). In 2001, male condition decreased with increasing redscore ( $n=28$ ,  $r=-0.44$ ,  $p=0.020$ ). In 2003 this relation-

ship was absent ( $n=17$ ;  $r=0.14$ ,  $p=0.60$ ). GLM analysis showed that this relationship differed significantly between years ( $F_{1,42}=4.98$ ,  $p=0.031$ ). Yellow score and black score were not related to body condition in either year (Pearson correlations, all  $p>0.27$ ). Males in 2003 had significantly higher scores for all colours ( $t>4.3$ ,  $p<0.001$ ). For black score, this difference may have been entirely due to the different cameras used. The differences in red and yellow score however remained significant after calibration (red: 2001:  $17.8\pm1.5$ , 2003:  $34.9\pm1.5$ ,  $t=7.48$ ,  $p<0.001$ ; yellow: 2001:  $1.7\pm0.4$ , 2003:  $4.9\pm0.7$ ,  $t=3.85$ ,  $p=0.001$ ).

## Parasites

### a) Parasite composition

We found seven species of macroscopic parasites: two endoparasites and five ectoparasites. In the skin and fins we found encysted metacercariae belonging to the trematode genus *Neascus* (Digenea). Three gill parasites were relatively common: two species of ectoparasitic copepods (*Lamproglana 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. Larval nematodes (*Contracaecum* sp.) were commonly found in liver and abdominal cavity; trematodes (Digenea) were found in the intestines of a few fish in 2001 only.

### b) Males have more parasites and larger variance in infestation rates than females

Males carried more parasites than females (Table 3.2 and 3.3a). However, male *P. nyererei* are larger than females and numbers of several parasites were related to fish size (GLM; results not shown). In a GLM with parasite loads as dependent variables and fish sex and size (standard length and weight) as independent vari-

Table 3.2. Parasite infestation rates of territorial males, unidentified males and unidentified females. % Denotes parasite prevalence: the proportion of infected individuals. TPL= total parasite load, PS= number of parasite species.

|                          | territorial males<br>2001 ( $n=28$ ) |               | territorial males<br>2003 ( $n=17$ ) |               | unidentified males<br>2001 ( $n=27$ ) |               | unidentified females<br>2001 ( $n=19$ ) |               |
|--------------------------|--------------------------------------|---------------|--------------------------------------|---------------|---------------------------------------|---------------|-----------------------------------------|---------------|
|                          | %                                    | median(range) | %                                    | median(range) | %                                     | median(range) | %                                       | median(range) |
| <i>Neascus</i> sp.       | 89.3                                 | 2 (0-9)       | 100                                  | 5 (1-19)      | 46.2                                  | 0 (0-20)      | 52.6                                    | 1 (0-3)       |
| <i>L. monodi</i>         | 89.3                                 | 5 (0-12)      | 100                                  | 7 (2-15)      | 84.6                                  | 2 (0-14)      | 84.2                                    | 3 (0-9)       |
| <i>E. lamellifer</i>     | 78.6                                 | 1 (0-9)       | 64.7                                 | 2 (0-5)       | 53.8                                  | 1 (0-10)      | 36.8                                    | 0 (0-2)       |
| mollusc larvae           | 14.3                                 | 0 (0-1)       | 47.1                                 | 0 (0-12)      | 7.7                                   | 0 (0-1)       | 10.5                                    | 0 (0-1)       |
| <i>Cichlidogyrus</i> sp. | 100                                  | 18.5 (4-52)   | 88.2                                 | 6 (0-12)      | 96.2                                  | 7 (0-41)      | 100                                     | 4 (1-11)      |
| trematodes               | 10.7                                 | 0 (0-5)       | 0                                    | 0             | 11.5                                  | 0 (0-3)       | 5.3                                     | 0 (0-1)       |
| nematodes                | 96.4                                 | 5.5 (0-136)   | 88.2                                 | 4 (0-20)      | 80.8                                  | 2.5 (0-112)   | 84.2                                    | 5 (0-56)      |
| TPL                      |                                      | 38 (19-166)   |                                      | 24 (11-52)    |                                       | 16 (4-124)    |                                         | 16 (6-70)     |
| PS                       |                                      | 5 (3-6)       |                                      | 5 (3-6)       |                                       | 4 (1-6)       |                                         | 4 (2-6)       |

Table 3.3. a) Differences in parasite load between males and females in 2001. b) Differences in parasite load between territorial males in 2001 and 2003. TPL= total parasite load, PS= number of parasite species. Test results that remain significant after sequential Bonferroni correction are printed in bold script.

|                          | a)                                 |                  |       | b)                                        |                  |           |
|--------------------------|------------------------------------|------------------|-------|-------------------------------------------|------------------|-----------|
|                          | Males (55) vs. females (19) (2001) |                  |       | Territorial males 2001 (28) vs. 2003 (17) |                  |           |
|                          | MWU Z                              | p                | sign  | MWU Z                                     | p                | sign      |
| <i>Neascus</i> sp.       | 1.49                               | 0.14             | ♂ > ♀ | 3.11                                      | <b>0.0019</b>    | 2001<2003 |
| <i>L. monodi</i>         | 1.77                               | 0.077            | ♂ > ♀ | 2.71                                      | <b>0.0067</b>    | 2001<2003 |
| <i>E. lamellifer</i>     | 2.90                               | <b>0.0038</b>    | ♂ > ♀ | 0.91                                      | 0.37             |           |
| mollusc larvae           | 0.43                               | 0.67             |       | 2.49                                      | 0.013            | 2001<2003 |
| <i>Cichlidogyrus</i> sp. | 5.24                               | <b>&lt;0.001</b> | ♂ > ♀ | 4.78                                      | <b>&lt;0.001</b> | 2001>2003 |
| trematodes               | 0.85                               | 0.40             |       | 1.38                                      | 0.17             |           |
| nematodes                | 1.02                               | 0.31             |       | 0.68                                      | 0.50             |           |
| TPL                      | 2.42                               | <b>0.016</b>     | ♂ > ♀ | -2.59                                     | <b>0.0096</b>    | 2001>2003 |
| PS                       | 2.24                               | <b>0.025</b>     | ♂ > ♀ | -0.47                                     | 0.64             |           |

ables, males still carried significantly more *E. lamellifer* and *Cichlidogyrus* than females ( $F_{1,72} > 5.7$ ,  $p < 0.020$ ). Because infestation rates of some parasite species were higher in territorial males than in unidentified males (Table 3.2), we also analysed a subsample including only sexually mature fish (40 males, 10 females). This yielded similar results to the previous GLM analysis, except for a now significantly higher number of parasite species (PS) in males ( $F_{1,48} = 9.28$ ,  $p = 0.0038$ ). The variance in parasite infestation rates was also larger in males (Wilcoxon signed ranks test comparing parasite load variance for each parasite species between males and females:  $Z = -2.197$ ,  $df = 6$ ,  $p = 0.028$ ; including only sexually mature fish:  $Z = -2.366$ ,  $df = 6$ ,  $p = 0.018$ ).

In 2003, total parasite load of territorial males was significantly lower than in 2001, mainly due to lower infestation rates of *Cichlidogyrus* (Table 3.3b). We found significantly more *Neascus* and *L. monodi* in 2003. Parasite load was not related to body condition in either year (males and females: all  $F < 3.97$ ,  $p > 0.07$ ). Including colourscores in the models for males did not influence these results.

### c) Redscore and territory size predict male parasite load

Of all parasite species, only the nematodes were significantly related to male characteristics that could be assessed by visiting females: high redscore and small size (SL) predicted low nematode load ( $F \geq 7.78$ ,  $p \leq 0.008$ ; Table 3.4; Figure 3.2). Large fish (SL) tended to carry fewer *E. lamellifer* ( $p = 0.019$ , critical  $p$  after Bonferroni correction = 0.010) and mollusc larvae ( $p = 0.022$ , critical  $p$  after Bonferroni correction = 0.013) in their gills. There were non-significant negative relationships between territory size and *L. monodi* infection ( $p = 0.084$ ) and between redscore and *Cichlidogyrus* ( $p = 0.082$ ). Intestinal trematodes were not analysed separately because of their low incidence, but they were included in the summary variables.

Table 3.4 Male parasite load and characteristics that can be assessed by visiting females. TPL: total parasite load, MPL: median parasite load, PS: number of parasite species. Test results that remain significant after sequential Bonferroni correction are printed in bold script. a) Minimal adequate GLM for the relationships between male parasite load and male characteristics that can be assessed by visiting females. b-d) GLM results for the relationships between male parasite load and redscore, territory size and courtship intensity. Except for the analyses involving courtship intensity, data of 2001 and 2003 are pooled and a factor ‘year’ is included in the models.

|                          |          | a) minimal adequate model     |                          |               | b) relation to redscore          |                          |               |
|--------------------------|----------|-------------------------------|--------------------------|---------------|----------------------------------|--------------------------|---------------|
|                          | variable | estimate±se                   | <i>F</i>                 | <i>p</i>      | estimate±se                      | <i>F</i> <sub>1,42</sub> | <i>p</i>      |
| <i>Neascus sp.</i>       | SL       | -0.043±0.039                  | 1.19                     | 0.28          | +0.002±0.017                     | 0.009                    | 0.93          |
| <i>L. monodi</i>         | T size   | -0.024±0.014                  | 3.17                     | 0.084         | -0.010±0.012                     | 0.63                     | 0.43          |
| <i>E. lamellifer</i>     | SL       | -0.124±0.051                  | 5.91                     | 0.019         | +0.012±0.021                     | 0.35                     | 0.56          |
| mollusc larvae           | SL       | -0.244±0.104                  | 5.68                     | 0.022         | -0.020±0.057                     | 0.12                     | 0.73          |
| <i>Cichlidogyrus sp.</i> | red      | -0.019±0.011                  | 3.17                     | 0.082         | -0.019±0.011                     | 3.17                     | 0.082         |
| nematodes                | red      | -0.094±0.027                  | 10.90                    | <b>0.0020</b> | -0.081±0.030                     | 7.78                     | <b>0.0079</b> |
|                          | SL       | +0.194±0.065                  | 8.77                     | <b>0.0051</b> |                                  |                          |               |
| TPL                      | red      | -0.024±0.011                  | 9.59                     | <b>0.0041</b> | -0.031±0.010                     | 8.83                     | <b>0.0049</b> |
|                          | T size   | -0.028±0.010                  | 8.32                     | <b>0.0070</b> |                                  |                          |               |
| MPL                      | T size   | -0.032±0.011                  | 9.22                     | <b>0.0047</b> | -0.020±0.011                     | 4.32                     | 0.044         |
|                          | red      | -0.014±0.011                  | 4.16                     | 0.050         |                                  |                          |               |
| PS                       | T size   | -0.005±0.003                  | 3.17                     | 0.084         | -0.006±0.004                     | 2.08                     | 0.16          |
|                          |          | c) relation to territory size |                          |               | d) to courtship intensity (2001) |                          |               |
|                          |          | estimate±se                   | <i>F</i> <sub>1,33</sub> | <i>p</i>      | estimate±se                      | <i>F</i> <sub>1,26</sub> | <i>p</i>      |
| <i>Neascus sp.</i>       |          | -0.017±0.020                  | 0.78                     | 0.38          | -0.183±0.258                     | 0.52                     | 0.48          |
| <i>L. monodi</i>         |          | -0.024±0.014                  | 3.17                     | 0.084         | -0.190±0.197                     | 0.96                     | 0.34          |
| <i>E. lamellifer</i>     |          | -0.037±0.023                  | 2.96                     | 0.094         | -0.464±0.336                     | 2.03                     | 0.17          |
| mollusc larvae           |          | -0.006±0.074                  | 0.007                    | 0.93          | +0.295±0.682                     | 0.17                     | 0.68          |
| <i>Cichlidogyrus sp.</i> |          | -0.017±0.010                  | 2.74                     | 0.11          | -0.172±0.177                     | 0.98                     | 0.33          |
| nematodes                |          | -0.086±0.049                  | 4.51                     | 0.041         | -0.518±0.685                     | 0.61                     | 0.44          |
| TPL                      |          | -0.034±0.012                  | 9.97                     | <b>0.0034</b> | -0.309±0.203                     | 2.43                     | 0.13          |
| MPL                      |          | -0.036±0.012                  | 11.06                    | <b>0.0022</b> | -0.400±0.175                     | 5.55                     | 0.026         |
| PS                       |          | -0.005±0.003                  | 3.17                     | 0.084         | -0.036±0.051                     | 0.51                     | 0.48          |

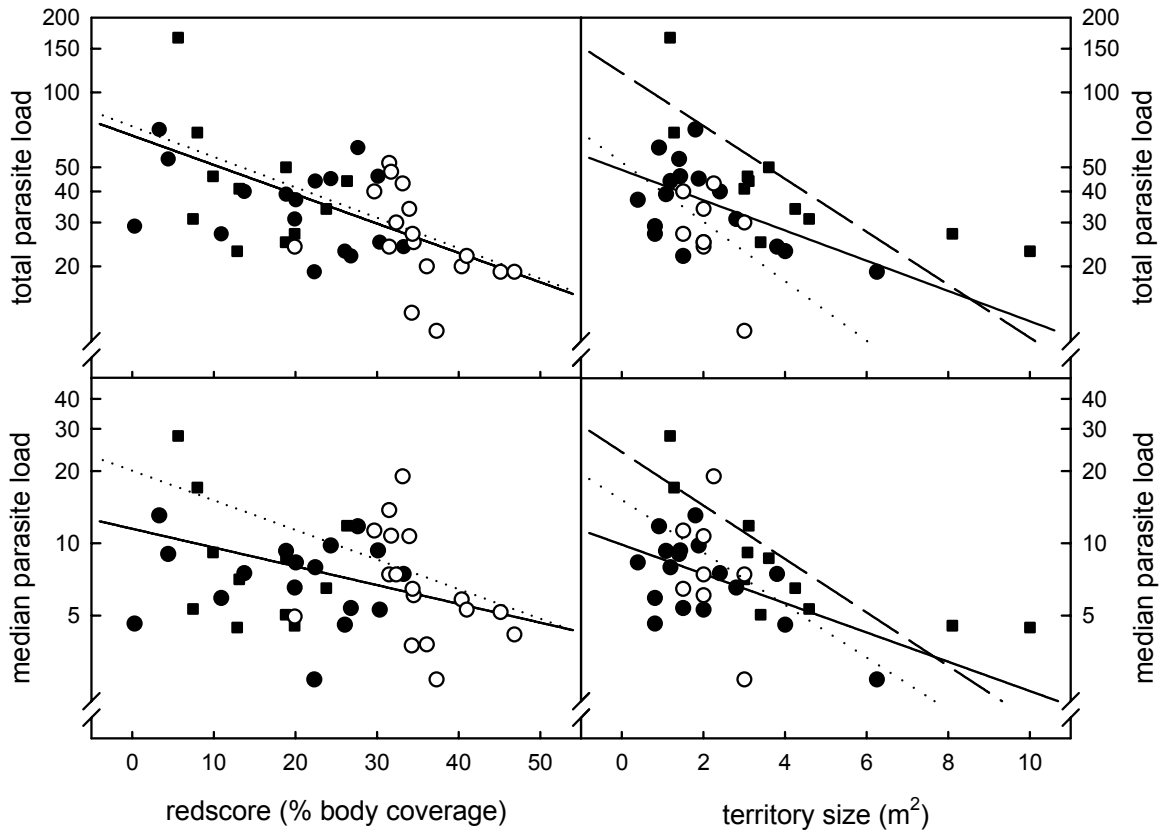


Figure 3.2. Male parasite load and characteristics that can be assessed by females. Total parasite load is the sum of all parasites infecting one fish; median parasite load is the sum of normalised parasite loads. Black symbols indicate territorial males of 2001 in deep (●) and in shallow (■) water, open symbols (○) indicate territorial males of 2003. Left panels: total and median parasite load in relation to redscore. Solid lines represent 2001 males; dotted lines represent 2003 males. For 2001, redscores are adjusted for photography method (i.e. multiplied by 1.16; see methods). The trend in the lower left panel is not significant (see Table 3.4b;  $p=0.044$ ; critical  $p$  after Bonferroni correction  $=0.025$ ). Right panels: total and median parasite load in relation to territory size. Solid lines represent males with territories in deep water (2001); dashed lines represent males with territories in shallow water (2001); dotted lines represent 2003 males.

Both male redscore and territory size were significantly negatively related to total parasite load ( $F \geq 8.32$ ,  $p \leq 0.0070$ ) and a large territory also predicted low median parasite load ( $F_{1,33}=11.06$ ,  $p=0.0022$ ). The number of parasite species infecting individual fish was not significantly related to any of the variables. Male courtship behaviour was not significantly related to parasite load ( $p > 0.026$ ; critical  $p$  value after Bonferroni correction  $p < 0.017$ ). Likewise, male yellowscore and blackscore were not related ( $p > 0.10$ ) to infestation rates with any of the parasite species.

#### d) Females tend to select against heavily parasitized males

Previous work showed that female response rate was positively related to male redscore, courtship intensity and territory size (Chapter 2). There was a tendency for these preferences to yield selection against heavily parasitized males: there

were negative relationships ( $p < 0.05$ ) between female response and male parasite load for *Neascus* and *Cichlidogyrus* infection and for total parasite load (Table 3.5). After Bonferroni correction however, these relationships were not significant.

Table 3.5 Relationships between male parasite load and female response in 2001. TPL: total parasite load, MPL: median parasite load, PS: number of parasite species.

| Male parasites and female choice (2001) |       |       |
|-----------------------------------------|-------|-------|
|                                         | $r_s$ | $p$   |
| <i>Neascus sp.</i>                      | -0.43 | 0.023 |
| <i>L. monodi</i>                        | -0.13 | 0.52  |
| <i>E. lamellifer</i>                    | -0.01 | 0.97  |
| mollusc larvae                          | 0.20  | 0.31  |
| <i>Cichlidogyrus sp.</i>                | -0.39 | 0.039 |
| trematodes                              | -0.19 | 0.32  |
| nematodes                               | -0.26 | 0.18  |
| TPL                                     | -0.39 | 0.038 |
| MPL                                     | -0.32 | 0.10  |
| PS                                      | -0.14 | 0.48  |

## Discussion

The red and yellow coloration of *Pundamilia nyererei* males is carotenoid based. Extracts from the red skin areas contained three times as much carotenoid as the yellow skin extracts. Drosopherins, present in the red skin extracts, contributed less than 4% to total absorbance. Because we used laboratory-bred individuals for the pigment analysis, we cannot rule out the possibility that the quality and quantity of the pigments encountered may differ from that in wild fish. However, the colours expressed by laboratory-bred *P. nyererei*, fed with a mixture of fresh shrimps and peas, are indistinguishable from those expressed by wild-caught individuals. Further, the differences in coloration that exist between different *P. nyererei* populations are maintained in laboratory-bred generations (Seehausen et al. 1997a).

We found no evidence that the redness of male coloration, which is the most important criterion for female mate choice (Chapter 2), is important in male dominance. It was not related to male body size or territory size. Territories in shallow water were larger, provided better shelter and were occupied by larger males. Since body size predicts fighting ability in male *Pundamilia* (Dijkstra et al. 2005), the observed distribution of males may result from competition among males with shallow water being the preferred habitat. At Makobe Island, both sexes of *P. nyererei* are most abundant around six meters depth (Seehausen & Bouton 1997; personal observation), suggesting that food conditions are not better in shallow water. Possibly, the larger rocks and greater number of crevices in shallow water provide better opportunities for predator avoidance. Moreover, territorial *P. nyererei* males compete for territories also with other haplochromine species. There is a general interspecific trend for larger species to inhabit shallower waters (Seehausen et al. 1998b). In our study, males in shallow water had frequent ag-

gressive interactions with *Neochromis omnicaeruleus* males (30% larger), whereas males in deep water had disputes predominantly with *Pundamilia* “pink anal” males, which are similar in size to *P. nyererei* (Seehausen 1996).

Most fish show indeterminate growth. Irrespective of the mechanism behind the observed distribution of territorial males, male size may thus be a reliable indicator of survival which in turn is expected to reflect genetic quality (Brooks & Kemp 2001). We did not find evidence that *P. nyererei* females preferred large males or males with territories in shallow water. Assuming that female choice is adaptive, this suggests that these traits do not reflect male genetic quality, or that other visible cues are better indicators.

Indeed, our data suggest that if female *P. nyererei* preference is aimed at discriminating against heavily parasitized males, they should pay attention to male red coloration and territory size. In two samples of territorial males that we took with an interval of two years, red coloration and territory size emerged as the best predictors of male parasite load. Most individual parasite species showed trends of being negatively correlated with both characters. This corresponds with the observation that male red coloration and territory size are important criteria for female mate choice in this species (Chapter 2). To our knowledge, this is only the second study of parasite-mediated sexual selection in cichlid fish (see also Taylor et al. 1998), and the first to implicate a carotenoid trade-off. Parasite load does not necessarily reflect the genetic quality of the immune system (Shykoff & Widmer 1996; Getty 2002): parasites may affect overall health and consequently weaken male competitive ability and colour expression. However, we did not find any relationship between parasite load and body condition. Assuming that feeding behaviour and physiological trade-offs have a genetic basis, it seems unlikely that the observed variation in parasite load is due to environmental effects alone. Therefore, our results may rather suggest that female choice for males that are bright red and possess large territories has evolved under selection for heritable fitness.

The result that redness rather than yellowness is related to both female choice and parasite load, is consistent with the idea that red is a more costly carotenoid display than yellow (Hill 1996), and is supported by the finding that the red areas of the male skin contain much higher carotenoid concentrations than the yellow areas.

The relationship between total parasite load and red coloration was similar in 2001 and 2003, but the territorial males in the 2003 sample were brighter red and carried fewer parasites, mainly due to lower infestation rates with *Cichlidogyrus* (Figure 3.2). We may speculate that female choice is driving the population temporarily towards lower parasite loads and associated increases in redscore, until another parasite species or another genotype of the same species comes to prevalence and causes a renewed increase in infestation rate. Perhaps more likely, infestation rates of *Cichlidogyrus* may have been lower in 2003 for environmental reasons, and subsequently have allowed for higher average redness. Territorial males were smaller in 2003 than in 2001, but this difference seems to be unrelated to the difference in redscore or parasite load: male size was not related to colour, and

parasites with higher infestation rates in larger fish (e.g. nematodes) were not less abundant in 2003.

Our observations that several parasite species occur in significantly larger numbers in males than in females, especially in territorial males (Table 3.2), and that infestation rates are significantly more variable in males, hint at male-specific trade-offs between immune defence and reproductive investment. These may be mediated by carotenoid-based male breeding coloration. Evidence for carotenoid limitation in males but not females has been found in guppies (Grether et al. 2004). However, the parasite species that was most strongly related to male coloration in our study, the nematode *Contracaecum*, did not differ in abundance between males and females. Conversely, the two species that were more abundant in males (*Ergasilus*, *Cichlidogyrus*) were not significantly related to male coloration. Perhaps the different parasite loads in males and females in *P. nyererei* are related to ecological differences between the sexes (Reimchen & Nosil 2001). Either way, the higher infestation rates of males make the production of carotenoid-based coloration particularly costly for males.

In contrast to selection for arbitrary traits, sexual selection for heritable fitness has been proposed to constrain rather than promote species divergence (Kirkpatrick & Nuismer 2004). This implies that our results present a challenge for the hypothesis that sexual selection has contributed to the divergence of *P. nyererei* and its close relative *P. pundamilia*. However, *P. nyererei* and *P. pundamilia* differ in diet and depth range (Seehausen 1996), which likely entails differences in parasite exposure. Consequently, directional sexual selection for extreme male phenotypes (Chapter 2; Pauers et al. 2004) may be accompanied by ‘phenotype-matching’ selection, due to preferences for locally adapted males. Theory suggests that the latter kind of selection has considerable potential to drive sympatric speciation (Arnegard & Kondrashov 2004; Edelaar et al. 2004; Reinhold 2004). Likewise, variation in female preferences could evolve by selection for MHC compatibility (Aeschlimann et al. 2003). Further work should evaluate the relative importance of these mechanisms for haplochromine speciation.

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