

Aggressive biases towards similarly coloured males in Lake Malawi cichlid fishes

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In haplochromine cichlids, female mate choice based on male nuptial coloration has played an important role in speciation. Recent studies suggest that male coloration strongly influences the distribution of these fishes based on male–male aggression; males direct more aggression towards similarly coloured opponents while tolerating differently coloured individuals. We explored the role of male nuptial colour in aggression among the mbuna of Lake Malawi, examining aggression by male *Metriaclima mbenjii*, the red top cobalt zebra, towards conspecific opponents, similarly coloured heterospecific opponents and differently coloured heterospecifics. In trials in which focal males were offered a single opponent, while the total number of aggressive behaviours did not vary among opponent species, the types of behaviours did; focal males directed more lateral displays towards conspecifics than towards the other opponent species. When focal males were offered two opponents simultaneously, *M. mbenjii* directed more aggressive behaviours and more lateral displays towards similarly coloured opponents, regardless of species. Furthermore, when offered a conspecific and a similarly coloured opponent simultaneously, there were no differences in behaviour towards either opponent. Thus, nuptial coloration is used by males to identify competitors, and it suggests that male–male aggression may have also been an important diversifying force in speciation in rock-dwelling Lake Malawi cichlids.

Keywords: haplochromine; cichlid; speciation; Lake Malawi; sexual selection; male–male aggression

1. INTRODUCTION

The explosive radiation of haplochromine cichlids in Lake Malawi has been attributed to sexual selection (Turner 1994; Barlow 2000). While female mate choice, based largely on male nuptial coloration, has played a prominent role in the rock-dwelling ‘mbuna’ (Jordan *et al.* 2003), an additional hypothesis is the possible role of male–male aggression in the

diversification of cichlid flocks. Turner (1994), citing an unpublished paper by Kornfield *et al.*, suggested this possibility in mbuna, but this idea went largely unexplored until Seehausen & Schluter (2004), who found that the distribution of Lake Victorian haplochromines was influenced by male nuptial coloration; similarly coloured congeners were rarely found in sympatry. This suggests that males direct a disproportionate amount of aggression towards similarly coloured individuals, and that differently coloured species may coexist.

Behavioural studies of Lake Victoria cichlids supported Seehausen & Schluter’s (2004) hypotheses. Dijkstra *et al.* (2005, 2006) found that these males directed more aggression towards similarly coloured opponents, though this bias disappeared when colour differences among opponents were muted experimentally. This is contrary to what Korzan & Fernald (2006) found in a Lake Tanganyika haplochromine (*Astatotilapia burtoni*), in which the males of two differently coloured sympatric morphotypes directed more aggression towards the males of the opposite morphotypes.

Few examinations of male–male aggression in the mbuna exist, though a field study by Holzberg (1978) found the pattern suggested by Seehausen & Schluter (2004); aggression in male *Metriaclima zebra* is biased towards similarly coloured conspecifics. A more recent field study found that males direct more aggression towards conspecifics, but ignore other mbuna species, though no colour-based biases were noted (Genner *et al.* 1999). Given that male nuptial coloration is similar among mbuna species, and that similarly coloured congeners rarely co-occur (Konings 2001), we hypothesize that male–male aggression based on coloration is an important diversification mechanism in the rock-dwelling Lake Malawi cichlid fauna. Owing to the competition among similarly coloured male conspecifics for territory and mates (Turner 1994; Barlow 2000), we also hypothesize that male nuptial coloration, regardless of species, is likely to be a cue by which males recognize competitors.

2. MATERIAL AND METHODS

(a) Experimental animals

We used seven wild-caught adult male *Metriaclima mbenjii* as the subjects of these experiments. This species features a red dorsal fin and a solid blue body, often called a red top (RT) colour pattern. We examined aggressive behaviour in *M. mbenjii* by presenting individuals with opponents of three species: *M. zebra* ‘Chilumba’ ($n=5$; blue dorsal fin, heavily barred blue–black body; a ‘BB’ coloration); *Labeotropheus fuelleborni* ‘Chidunga’ ($n=5$; RT coloration); or a conspecific. *Metriaclima mbenjii* co-occurs with these two species in the wild, though not with these exact populations (Konings 2001). Owing to limited numbers of fish, all *M. mbenjii* served as both focal and stimulus fish in the experiments (Dijkstra *et al.* 2006); the order in which they did was haphazard. These fish were housed in two groups, and never given tankmates as opponents.

Fish were kept and experiments were conducted at the Milwaukee County Zoo. Each group of fish was housed separately in 151 l aquaria; group housing ensured the maintenance of social behaviours. Barriers were placed between aquaria to prevent observations of behavioural interactions of neighbours and assessments of potential opponents. Subjects were fed a mixture of foods twice daily. Aquaria were filtered with box filters and water changes were performed weekly. Fish were allowed to acclimate for approximately 30 days prior to experimentation.

(b) Experimental procedures

Experiments were conducted in a 151 l aquarium in which an artificial coral provided territory for the focal male. Smaller aquaria

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Table 1. Multivariate ANOVA demonstrating the effects of opponent species on behaviour in single-opponent trials (Wilks' $\Lambda = 0.362$; $F_{8,30} = 2.483$, $p = 0.034$).

effect	SS	d.f.	MS	<i>F</i>	<i>p</i>
square root of total aggression	5.646	2	2.823	0.658	0.530
error	77.254	18	4.292		
square root of frontal displays	8.820	2	4.410	0.821	0.456
error	96.674	18	5.371		
square root of lateral displays	21.022	2	10.511	5.603	0.013
error	33.767	18	1.876		
square root of butts/bites	15.907	2	7.953	3.497	0.052
error	40.941	18	2.275		

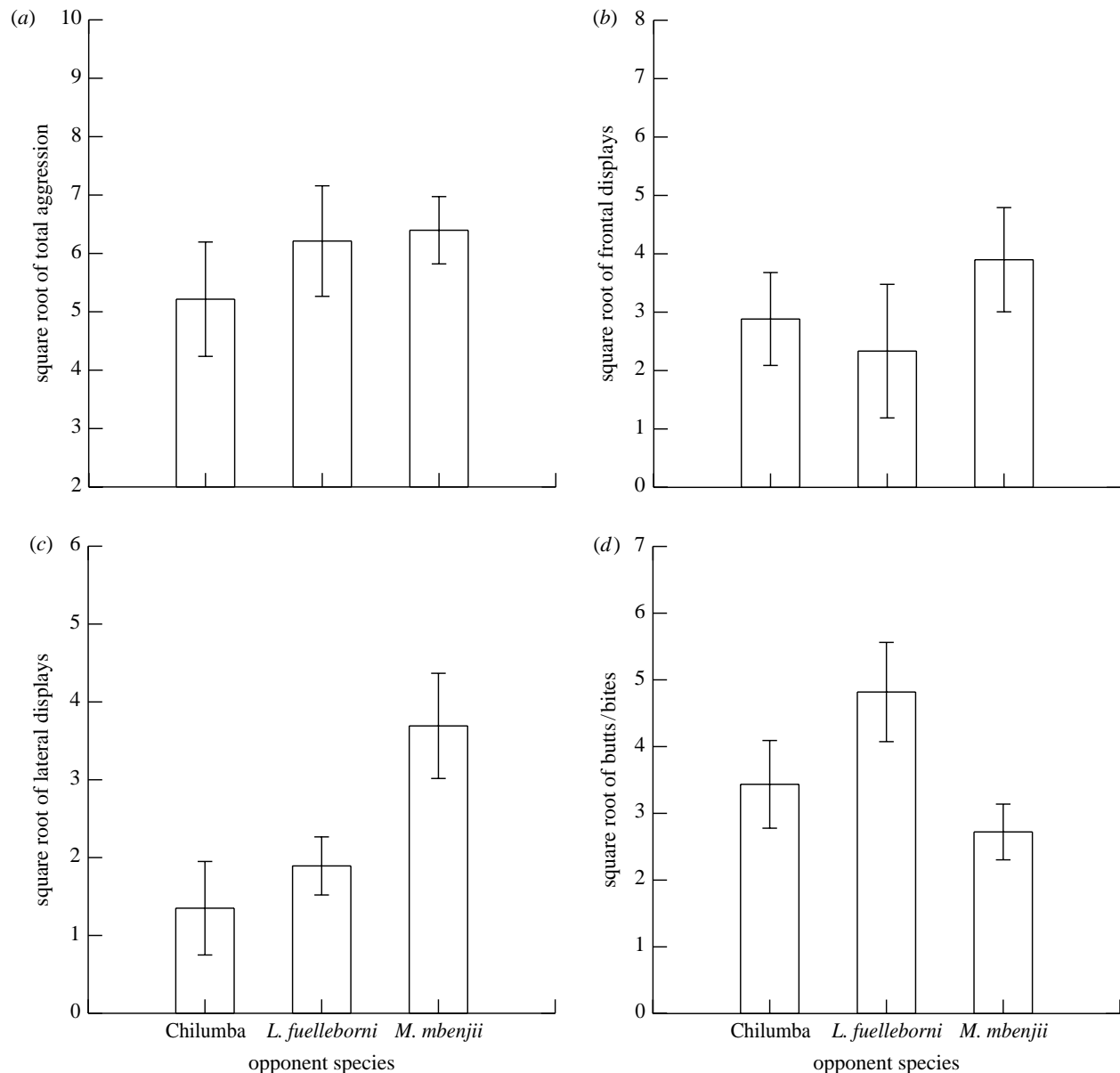


Figure 1. Differences in the numbers of behaviours directed to single opponents by the focal male *M. mbenjii*. (a) Total aggression, (b) frontal displays, (c) lateral displays and (d) butts/bites. See table 1 for statistical tests.

(approx. 6 l; one for experiment 1 and two for experiment 2) were suspended in the arena to house the opponent fish. These containers provided only visual contact between subjects and allowed the focal fish to interact with the opponent, but eliminated aggression-related injuries.

A single focal male was placed in the arena and allowed to acclimate for approximately 24–48 hours prior to observation. We haphazardly selected opponents (one for experiment 1 and two for experiment 2), placed them into the suspended aquaria and videotaped the behavioural interactions for 10 min with no humans

present. After experimentation, all subjects were returned to their respective holding tanks. Both focal and opponent males were used no more frequently than once every three experiments.

Aggressive behaviour was scored from the videotapes, and the numbers of behaviours recorded by observers averaged per experiment. We selected three types of aggressive behaviours from Baerends & Baerends-van Roon (1950), recording the number of each performed only by the focal male. These included lateral displays, frontal displays and butting or biting (see Baerends & Baerends-van Roon (1950) for further description).

In experiment 1, focal males were offered one opponent. We presented each focal male ($n=7$) with three opponent species in three separate trials, yielding a total of 21 experiments scored by three observers. In experiment 2, focal males ($n=6$) were simultaneously presented with two opponents, such that they were given all possible combinations of two opponent species (*M. mbenjii* and *L. fuelleborni*; *M. mbenjii* and *M. zebra* Chilumba; and *L. fuelleborni* and *M. zebra* Chilumba), yielding 18 experiments. Because the same focal fish were used in both the experiments, we added a fourth observer to score experiment 2, which ensured that the patterns of behaviour observed in experiment 1 were verifiable and not resulting from inter-observer influence.

(c) Statistical analyses

All data were square-root transformed for normality. For experiment 1, the numbers of individual behaviours and their sum ('total aggression') were dependent variables in a multivariate ANOVA to determine whether or not the opponent species had an effect on the behaviour of the focal fish. Any behaviours significantly impacted by opponent species, along with total aggression, were used in analysing experiment 2. In experiment 2, a series of paired *t*-tests was used to determine whether the focal males were more aggressive to one opponent species than the other. Since our sample size was small and fish reused, and owing to the total number (6) of *t*-tests performed, we used a two-tailed *t*-test and a Holm–Bonferroni correction of the *p*-values obtained from these *t*-tests.

Since focal males were offered the same opponent species in both experiments 1 and 2, we wanted to determine whether or not prior experience had an influence on focal fish behaviour in experiment 2. We used total aggression towards each opponent species in experiment 1 as the independent variable in an ANOVA; total aggression towards each opponent species in experiment 2 was the dependent variable. There was no effect of prior experience on a focal male's behaviour in experiment 2 ($F_{2,12}=0.385$; $p=0.689$).

3. RESULTS

(a) Experiment 1

In experiment 1, male *M. mbenjii* behaved differently towards different opponent species. A multivariate ANOVA revealed that while there were no differences in the responses of *M. mbenjii* among opponents in either the total number of aggressive behaviours or the number of frontal displays performed, there were differences in the number of lateral displays and butts/bites performed (table 1; figure 1*a,b*). The focal male *M. mbenjii* performed significantly more lateral displays towards the opponent male *M. mbenjii* than they did towards the other two opponent species (figure 1*c*). Opponent male *L. fuelleborni* tended to receive more butts/bites than the other two species, though this difference was not significant (figure 1*d*).

(b) Experiment 2

When offered two opponents, the behaviour of focal males depended upon the opponent pair offered. Because the only significant behaviour in experiment 1 was the number of lateral displays, we conducted our analyses on that and total aggression. When offered a pair of opponent males consisting of a *M. mbenjii* and a *M. zebra* Chilumba, the focal male directed a greater total number of aggressive behaviours (paired *t*-test: $t_5=4.077$; $p=0.010$) and a greater number of lateral displays (paired *t*-test: $t_5=4.597$; $p=0.006$) towards the opponent *M. mbenjii*. A similar trend was seen in opponent pairs consisting of a *L. fuelleborni* and a *M. zebra* Chilumba; more total aggression (paired *t*-test: $t_5=3.011$; $p=0.030$; n.s. after Holm–Bonferroni correction) and more lateral displays (paired *t*-test: $t_5=2.411$; $p=0.061$; n.s.) were directed towards the RT *L. fuelleborni* than were directed towards the *M. zebra* Chilumba. Finally, when offered an opponent

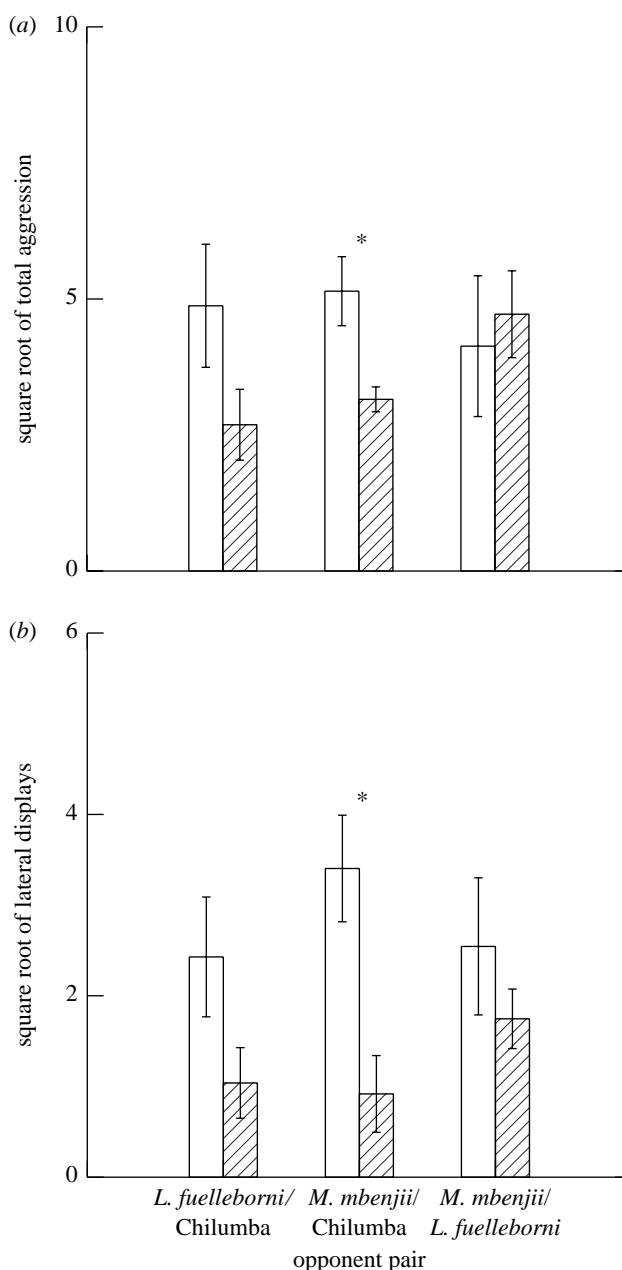


Figure 2. Differences in the numbers of behaviours directed to opponents by focal male *M. mbenjii* when two opponents (open bars, RT opponent; striped bars, other opponent) are simultaneously presented. (a) Total aggression and (b) lateral displays. For both, 'RT opponent' can refer to either *M. mbenjii* or *L. fuelleborni*, but refers to *M. mbenjii* specifically in *mbenjii/fuelleborni* opponent pairs (* $p \leq 0.01$).

pair consisting of a *L. fuelleborni* and a *M. mbenjii*, there was no difference in either total aggression (paired *t*-test: $t_5=-0.339$; $p=n.s.$) or the number of lateral displays (paired *t*-test: $t_5=1.050$; $p=n.s.$) performed to either species (figure 2).

4. DISCUSSION

When offered single opponents, male *M. mbenjii* exhibited behavioural differences depending on the opponent species: opponent *M. mbenjii* received more lateral displays and opponent *L. fuelleborni* received more butts/bites. Thus, *M. mbenjii* do not change the overall number of aggressive behaviours performed towards different species, but instead change the

types of behaviours used. When offered two opponents, the focal male *M. mbenjii* still makes a distinction, one based on opponent coloration. When the RT-coloured opponents (*M. mbenjii* or *L. fuelleborni*) were offered alongside a *M. zebra* Chilumba, these opponents received more lateral displays and more total aggression than the BB *M. zebra* Chilumba. When offered pairs of similarly coloured opponents (*M. mbenjii* and *L. fuelleborni*), the focal male *M. mbenjii* did not behave differently towards either the conspecific or heterospecific opponent.

Our findings that *M. mbenjii* males directed less intense (experiment 1) or fewer (experiment 2) aggressive behaviours towards *M. zebra* Chilumba agree with a field study by Genner *et al.* (1999), which found that males of the 'zebra' complex tolerated heterospecifics within their feeding territories. Our findings, however, differ from Genner *et al.*'s (1999) in that *M. mbenjii* directed intense and numerous aggressive behaviours towards *L. fuelleborni*; when offered *M. mbenjii* and *L. fuelleborni* concurrently in experiment 2, there was no difference in the aggression of focal males towards either species. We attribute this difference to the fact that the *L. fuelleborni* we used had the same nuptial colour pattern as our focal male *M. mbenjii*; this idea is supported by the aggressive bias displayed towards *L. fuelleborni* in opponent pairs consisting of *L. fuelleborni* and *M. zebra* Chilumba in experiment 2.

The behavioural differences in the treatment of opponents are very interesting. In aggressive encounters in cichlids, lateral displays intimidate opponents through exhibitions of size, strength and colour (Baerends & Baerends-van Roon 1950). Intimidation suggests that the individual views his opponent as a rival, and by performing these displays attempts to communicate his superiority, rather than simply doing physical harm (Barlow 2000). In experiment 1, focal male *M. mbenjii* performed significantly more lateral displays towards opponent *M. mbenjii* than they did towards the other two opponent species. This makes sense, given that conspecific males are rivals for territory and mating opportunities (Genner *et al.* 1999). The other two opponent species were typically bitten at or butted, which are attempts to chase them away (Barlow 2000). Given the treatment of the similarly coloured *L. fuelleborni* in experiment 2, opponent coloration probably plays a significant role in the decision to intimidate versus chase away opponents.

Our findings suggest a novel role for male nuptial coloration in the mbuna of Lake Malawi: the identification of opponents and the modulation of aggressive behaviour towards them. Although this has been demonstrated in Lake Victoria cichlids (Seehausen & Schluter 2004), our results are among the first, to the best of our knowledge, suggesting that

aggression among the mbuna is modulated via male coloration. This implies that male-male aggression plays a role in the distribution of mbuna species throughout Lake Malawi, and explains why similarly coloured congeners are rarely found in sympatry (Konings 2001). Our results also offer further support for Seehausen & Schluter's (2004) hypothesis that male-male aggression may act as a diversifying force in haplochromine cichlids.

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