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Male-male competition and speciation: aggression bias towards differently coloured rivals varies between stages of speciation in a Lake Victoria cichlid species complex

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sympatric speciation.

Abstract

Sympatric speciation driven by sexual selection by female mate choice on a male trait is a much debated topic. The process is problematic because of the lack of negative frequency-dependent selection that can facilitate the invasion of a novel colour phenotype and stabilize trait polymorphism. It has recently been proposed that male-male competition for mating territories can generate frequency-dependent selection on male colouration. Rare male cichlid fish would enjoy a fitness advantage if territorial defenders bias aggression towards male cichlid fish of their own colour. We used blue (ancestral type) and red phenotypes of the Lake Victoria cichlid species complex *Pundamilia*. We tested the aggression bias of wild-caught territorial blue male cichlid fish from five separate populations for blue vs. red rival male cichlid fish using simulated intruder choice tests. The different populations vary in the frequency of red male cichlid fish, and in the degree of reproductive isolation between red and blue, reflecting different stages of speciation. Blue male cichlid fish from a population that lack red phenotypes biased aggression towards blue stimulus male cichlid fish. The same was found in two populations where blue and red are reproductively isolated sister species. This aggression bias may facilitate the invasion of a novel colour phenotype and species coexistence. Blue male cichlid fish from two populations where red and blue are hybridizing incipient species biased aggression towards red stimulus male cichlid fish. Thus, after a successful invasion of red, aggression bias alone is not likely to generate frequency dependence required to stabilize the coexistence of phenotypes. The findings show that aggression bias varies between stages of speciation, but is not enough to stabilize the process of speciation.

Introduction

Empirical research on potential mechanisms of sympatric speciation has concentrated primarily on disruptive natural selection through competition for ecological resources leading to the evolution of ecological specialization and reproductive isolation (Filchak *et al.*, 2000; Schluter, 2000; Turelli *et al.*, 2001; Rundle & Nosil, 2005). This partly reflects the developments in theoretical

research: in classical theoretical scenarios of sympatric speciation, sexual selection plays a secondary role, if any (Maynard Smith, 1966; Bush, 1975; Rosenzweig, 1978; Kondrashov & Mina, 1986; for review see Coyne & Orr, 2004). Different speciation models have demonstrated the theoretical feasibility that selective mating exerting sexual selection can cause sympatric speciation (Lande & Kirkpatrick, 1986; Turner & Burrows, 1995; Payne & Krakauer, 1997; van Doorn *et al.*, 1998; Higashi *et al.*, 1999; Kawata & Yoshimura, 2000; van Doorn & Weissing, 2001). Many of these models were inspired by the explosive speciation of haplochromine cichlids in Lake Victoria and Lake Malawi (Kocher, 2004). Closely related haplochromine cichlid species typically differ markedly in

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colouration but little otherwise (Bouton *et al.*, 1997; Seehausen & Bouton, 1997; Albertson *et al.*, 1999; Genner *et al.*, 1999a; Allender *et al.*, 2003). This colour variation in male cichlid fish is associated with variation in female mate preferences (Seehausen & van Alphen, 1998; Knight & Turner, 2004).

More recent theoretical investigations indicate that sympatric speciation by sexual selection alone requires stringent conditions (Turelli *et al.*, 2001; Arnegard & Kondrashov, 2004; van Doorn *et al.*, 2004; Kirkpatrick & Nuismer, 2004). A fundamental problem is the initial hurdle for a novel male phenotype to invade a population against the predominant female preference. Also, the coexistence of incipient species, both during and after the evolution of reproductive barriers, is problematic (van Doorn *et al.*, 2004). Successful invasion requires a fitness advantage for a novel male phenotype, but maintenance of a trait polymorphism requires that this advantage is negatively frequency dependent (van Doorn *et al.*, 2004). Male–male competition for access to mating territories could generate such frequency dependence in sexual selection when exerting selection on the same traits that are involved in female mate choice (van Doorn *et al.*, 2004; Mikami *et al.*, 2004; Seehausen & Schluter, 2004). For example, if territorial defenders bias aggression towards phenotypes coloured like themselves, rare male phenotypes would receive less aggression, possibly resulting in a higher chance of rare male types to establish a high-quality territory. If female cichlid fish assess prospective mates by not only colour but also territory quality (e.g. territory size, see Maan *et al.*, 2004; P.D. Dijkstra *et al.* unpublished), they may face a conflict between species recognition and preference for mates with a high-quality territory. This effect could bestow an initial fitness advantage upon rare male cichlid fish facilitating the propagation of their genes in the population. The advantage is likely to decrease with increasing relative abundance because of more aggressive encounters with own-type individuals. This ‘rare male advantage’ would facilitate both the invasion of novel colour types, and prevent competitive exclusion of either type. It could potentially operate in the haplochromine cichlid radiations. In their lek-like mating system, male cichlid fish vigorously defend permanent territories, do not take part in parental care, and it appears that possession of a territory is a prerequisite for mating success (Maan *et al.*, 2004). Hence, competition over territory sites is intense, and may well exert sexual selection (Fryer & Iles, 1972; Seehausen *et al.*, 1998a; Seehausen & Schluter, 2004).

Intra- and interspecific aggression have mostly been studied to understand co-existence through ecological resource partitioning (e.g. Ebersole, 1985; Martin & Thibault, 1996; Genner *et al.*, 1999b; for reviews in haplochromines see McKaye, 1991). Genner *et al.* (1999b) showed that in the haplochromine cichlid *Pseudotropheus* from Lake Malawia territorial behaviour might promote coexistence of species with different

feeding strategies while enhancing competition among species with a similar specialization. The role of aggression in sexual selection and speciation has only recently been fully appreciated (van Doorn *et al.*, 2004; Mikami *et al.*, 2004; Seehausen & Schluter, 2004; Dijkstra *et al.*, 2005, 2006).

Here we tested for aggression biases in wild-caught territorial blue male phenotypes of the *Pundamilia* species complex from Lake Victoria towards either red (*Pundamilia nyererei*) or blue (*Pundamilia pundamilia*) rivals using simulated intruder choice tests. Red and blue phenotypes are anatomically similar that behave like reproductively isolated species in some locations, and like hybridizing incipient species in other locations (Seehausen, 1996, 1997). Blue phenotypes have a lake-wide distribution whereas red phenotypes have a patchy distribution and always co-occur with blue phenotypes. Among the members of the *Pundamilia* complex, the blue form has the highest record of sympatric occurrences with other members of the *Pundamilia* complex (Seehausen, 1996; Seehausen & van Alphen, 1999). It seems therefore likely that blue represents the ancestral state and that blue populations have been invaded repeatedly and independently by red phenotypes during speciation (Seehausen, 1997; Seehausen & van Alphen, 1999; Seehausen & Schluter, 2004).

In this study we have taken advantage of the fact that blue *Pundamilia* populations exist that differ in the frequency of red male cichlid fish, as well as in the degree of gene-flow between red and blue phenotypes (Seehausen, 1997). We take these population types as representing different stages of speciation, allowing us to ask whether the direction and strength of an aggression preference in blue male cichlid fish depends on speciation stage. Firstly, we measured aggression preference in wild-caught male cichlid fish from a location with a single blue *Pundamilia* species (single-species population). This population represent the pre-invasion stage. Secondly, we measured the aggression preference in wild-caught blue male cichlid fish from two populations where blue and red are clearly distinguishable incipient species or colour morphs, in which intermediate (hybrid) phenotypes are present (hybridizing-species populations). This population type represents an incipient stage of speciation after an invasion and increase in frequency of red male cichlid fish. Finally, we measured the aggression preference in wild-caught blue male cichlid fish from two populations where blue and red are completely distinct species (two-species populations). This population type represents a situation where reproductive isolation has been completed. If male–male competition is to facilitate speciation, we predict own-type biases in aggression in all population types (van Doorn *et al.*, 2004; Mikami *et al.*, 2004; Seehausen & Schluter, 2004). However, a previous study on *Pundamilia* indicates that own-type aggression biases do not always exist. In this study, we found that red male cichlid fish from different populations biased aggression

towards male cichlid fish of their own colour (Dijkstra *et al.*, 2006). In contrast, in the same study blue male cichlid fish from hybridizing-species populations showed a nonsignificant bias towards red intruders (Dijkstra *et al.*, 2006). This suggests that theory does not completely account for what actually happens in nature and that aggression biases of blue male cichlid fish may depend on speciation stage. This warrants sampling of aggression biases in more blue populations that differ in the degree of speciation, including a population consisting of a single blue species.

Method

Species and subjects

Pundamilia pundamilia (Seehausen *et al.*, 1998b) and *P. nyererei* (Witte-Maas & Witte, 1985) are endemic Lake Victoria cichlid species. Male cichlid fish were brought from the Mwanza Gulf into the Zoological Laboratory in Haren in February 2003. Some male cichlid fish from Python and Kissenda Island arrived in May 2001. We only tested wild-caught male cichlid fish. Male cichlid fish of *P. nyererei* are crimson dorsally, yellow on their flanks, and have a crimson dorsal fin. We refer to these as 'red'. Male cichlid fish of *P. pundamilia* are greyish white dorsally and on the flanks, and have a metallic blue dorsal fin. We refer to them as 'blue'. Both are confined to rocky shores and islands in Lake Victoria (Seehausen, 1996). Below we describe the five populations from which we collected blue male cichlid fish. The frequencies of colour phenotypes in the wild for each population is shown in Table 1.

The *single-species population* originated from Luanso Island. This population consists of individuals that show a continuous distribution of colours between blue and red (Seehausen, 1996, 1997). Most Luanso *Pundamilia* male cichlid fish are similar to *P. pundamilia*, hence we refer to these male cichlid fish as 'blue'. Some other male cichlid fish are intermediate between the blue and red phenotypes, but distinctly red male cichlid fish (Fig. 1, score 4 and upwards) are extremely rare, and fully red male

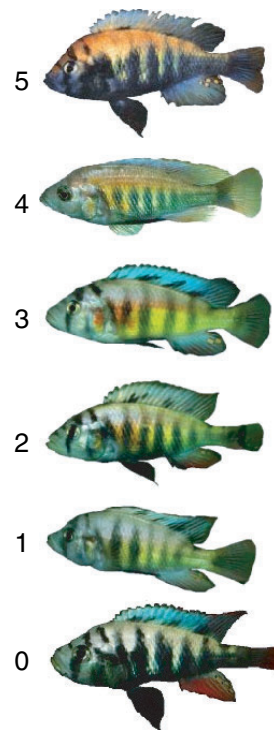


Fig. 1 *Pundamilia* hybrid colour scale for quantification of colour phenotypes, showing *Pundamilia pundamilia* (0), *Pundamilia nyererei* (5) and intermediate phenotypes (see online version for colour).

cichlid fish (score 5) have not been reported (see Table 1).

The *hybridizing-species populations* consisted of *P. nyererei*- and *P. pundamilia*-like phenotypes from Python and Kissenda Islands, in the Mwanza Gulf of Lake Victoria (Tanzania). Around these islands, red and blue occur syntopically. Differentiated red and blue *Pundamilia* are common around these islands, together with intermediate forms that are less abundant and closely resemble laboratory hybrids (Table 1). We only used distinct blue and red male phenotypes (Fig. 1, male cichlid fish with colour scores 0 and 5 respectively).

The *two-species populations* consisted of fish from Makobe Island and Senga Point, in the Speke Gulf of Lake Victoria (Tanzania). At these locations, *P. pundamilia* and *P. nyererei* are reproductively isolated sympatric sibling species as intermediate phenotypes have never been observed among hundreds of fishes (Table 1). At Senga Point they are also fully syntopic, whereas depth ranges do not overlap much at Makobe Island.

In this experiment, we tested for aggression bias in wild caught blue male cichlid fish towards either red or blue stimulus male cichlid fish. We tested 28 male cichlid fish from Luanso Island (standard length, SL, mean \pm SE = 92.8 \pm 1.3 mm), 22 male cichlid fish from Python Island (SL mean \pm SE = 96.6 \pm 2.1), seven from Kissenda Island (SL mean \pm SE = 98.4 \pm 3.4), 23

Table 1 The frequency of *Pundamilia* colour phenotypes (0–5, see hybrid colour scale Fig. 1) shown for each population.

	Phenotype score					
	0	1	2	3	4	5
Luanso Island	6	16	5	1	0	0
Python Island	19	2	0	0	1	12
Kissenda Island	8	0	1	0	4	11
Makobe Island	75	0	0	0	0	55
Senga Point	27	0	0	0	0	15

The fish were collected by hook and line and gillnets. The data of Python and Kissenda Island are from 2001, that of the other populations from 2002/2003.

P. pundamilia male cichlid fish from Makobe Island (SL mean \pm SE = 114.0 + 2.5), and 11 male cichlid fish from Senga point (SL mean \pm SE = 115.24 + 2.8).

Stimulus male cichlid fish were blue and red male cichlid fish from Kissenda or Python Island, scored either as 0 or as 5 phenotypes respectively (Fig. 1). Kissenda and Python male cichlid fish are indistinguishable. In light manipulation experiments in which we masked differences in nuptial colouration of red and blue stimulus male cichlid fish from these islands, we have shown that aggression biases are based on differences in nuptial colouration rather than other possible morph-specific differences (Dijkstra *et al.*, 2006). This makes these male cichlid fish suitable stimuli to measure colour-based aggression preferences. Male cichlid fish of all five populations were tested with stimulus male cichlid fish bred from wild-caught parents from Kissenda Island. Some male cichlid fish from Makobe Island (10 out of 23) were also tested with wild-caught stimulus male cichlid fish from Python and Kissenda Island. Specifically, we selected red stimulus male cichlid fish from a group of 58 red *Pundamilia* male cichlid fish (SL range: 68.5–103 mm, 40 laboratory-bred and two wild Kissenda male cichlid fish, six laboratory-bred and 10 wild Python male cichlid fish), and blue male cichlid fish from a group of 50 blue *Pundamilia* male cichlid fish (SL range: 66.3–105, 35 laboratory-bred Kissenda male cichlid fish, seven laboratory-bred and eight wild Python male cichlid fish). Any stimulus male cichlid fish was only used once for each test population. The size asymmetry within stimulus pairs, calculated as $(L - S)100/S$, where L is SL of the larger, S that of the smaller fish, was less than 6.2%.

Housing

The test male cichlid fish were kept in single-population aquaria with wild-caught female cichlid fish and only blue male cichlid fish. Male cichlid fish from different populations were housed in separate aquaria. The sides and the back of the aquaria were covered with black plastic sheets. All aquaria were connected to a central biological filter system and water circulated continuously. Water temperature was kept at 25 ± 2 °C and a 12:12 h light:dark cycle was maintained. All aquaria contained a gravel substrate. The fish were fed flake food (TetraMin Tropical Fish Flakes) seven times per week and a mixture of ground shrimp and peas two times per week.

Test and stimulus male cichlid fish were transferred to individual aquarium compartments at least a week prior to commencement of the simulated intruder choice tests to minimize the effects of short-term experiences. All male cichlid fish were kept in separate compartments within which a PVC tube was placed as a refuge. They had visual contact to a male cichlid fish through a transparent plastic sheet. Test male cichlid fish were visually exposed to only blue male cichlid fish, stimulus male cichlid fish to both red and blue male cichlid fish.

This pre-experimentation housing in aquarium compartments ensured that male cichlid fish became territorial and avoided unwanted effects of social isolation while preventing physical interaction.

Simulated intruder choice test

Test male cichlid fish were placed in the test aquarium 1–3 days before a trial to allow acclimatization to the aquarium. A PVC tube was provided as a refuge. A test aquarium consisted of a large experimental compartment (55 × 35 × 39 cm high) for the test male cichlid fish, and a smaller compartment (5 × 35 × 39 cm high) for a neighbouring male cichlid fish. The neighbouring male cichlid fish was a small laboratory-bred *P. pundamilia* male cichlid fish (Kissenda or Python Island), which was required to maintain territorial condition of the test male cichlid fish. The neighbouring male cichlid fish was separated from the test male cichlid fish by a transparent partition. At the start of a trial, a blue and red stimulus male cichlid fish confined in a transparent watertight tube were simultaneously placed in the experimental compartment 20 cm apart from one another. The test male cichlid fish perceived these stimulus male cichlid fish as intruders, and typically responded aggressively to both stimulus male cichlid fish, whilst the neighbouring fish was ignored. The trial was recorded on videotape for 5 min starting immediately after introduction of the stimulus male cichlid fish. Across trials we alternated the left and right positions of the stimulus male cichlid fish with respect to colour.

Analysis

From the videotapes, we scored the number of attacks and display behaviours of the test male cichlid fish towards both stimulus male cichlid fish (Baerends & Baerends-Van Roon, 1950). The display ratio of each male cichlid fish was the fraction of displays directed towards the blue stimulus calculated as the number of displays towards blue divided by the sum of displays towards blue and red. The attack ratio was generated in the same way. All ratio data were arcsine square root transformed to meet assumptions of parametric testing. We tested against 50% using one-sample *t*-tests to test whether male cichlid fish have an aggression preference for either colour type. To test for differences in aggression bias between populations, we used analysis of variance (ANOVA) and Tukey HSD *post hoc* tests. Statistical analyses were performed in SPSS 12.0.1. All quoted probabilities are for two-tailed tests of significance.

Results

Male cichlid fish directed aggressive behaviours to both types of stimulus male cichlid fish. On average, male

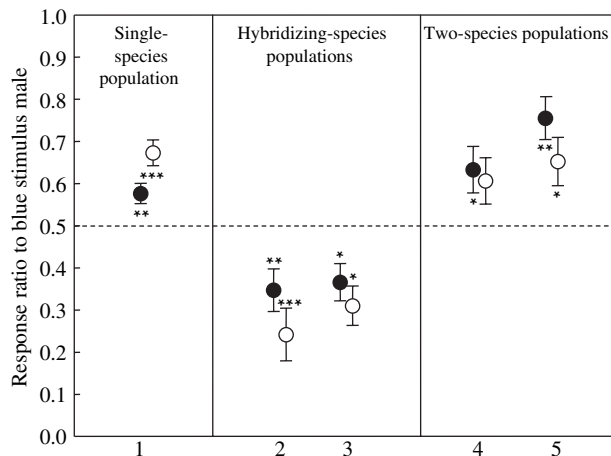


Fig. 2 The display (filled) and attack ratios (open) of *Pundamilia* male cichlid fish from five populations. The different populations of *Pundamilia* phenotypes are indicated below with numbers: the single-species population (1: *Pundamilia*, Luanso Island, $n = 28$), the hybridizing-species populations (2: *P. pundamilia*-like phenotypes, Python Island $n = 22$, 3: Kissenda Islands $n = 7$) and the two-species populations (4: *P. pundamilia* Makobe Island $n = 23$, 5: *P. pundamilia* Senga Point, $n = 11$). The response ratio is the response to the blue stimulus divided by the sum of the responses to the red and blue stimulus. A response ratio of 50% represents identical responses to the two stimuli (—). Data points indicate mean + SE values. In most instances, the response ratios deviated significantly from 50% (for statistics see text); significance of deviation from 50% is indicated by asterisks (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

cichlid fish performed 38.0 displays (\pm SE 2.7, $n = 91$) and attacks 32.8 (\pm SE 2.3) during a 5-min trial.

Figure 2 shows the response ratios of blue male cichlid fish of each population. Both the display and attack ratios differed significantly across the five populations (display: ANOVA: $F_{4,86} = 10.462$, $P < 0.001$, attack: $F_{4,86} = 12.498$, $P < 0.001$; Fig. 2). Male cichlid fish from the single-species population directed significantly more aggression to blue than to red stimulus male cichlid fish (Fig. 2; one-sample t -test: display: $t_{27} = -3.176$, $P < 0.005$, attack: $t_{27} = -4.883$, $P < 0.001$). In contrast, blue male cichlid fish from both hybridizing-species populations paid on average more attention to red than to blue stimulus male cichlid fish (Fig. 2; one-sample t -tests: Python Island, display, $t_{21} = 3.073$, $P < 0.01$, attack: $t_{21} = 3.846$, $P < 0.001$; Kissenda Island, display: $t_6 = 2.858$, $P < 0.05$, attack: $t_6 = 3.247$, $P < 0.05$). Blue male cichlid fish from the two two-species populations biased their aggression significantly towards the blue stimulus male cichlid fish (Fig. 2; one-sample t -tests: Makobe Island, display: $t_{22} = -2.333$, $P < 0.05$, attack: $t_{22} = -1.996$, $P = 0.058$; Senga Point, display: $t_{10} = -4.242$, $P < 0.005$, attack: $t_{10} = -2.593$, $P < 0.05$).

We can now test whether response ratios depend on stage of speciation. Within-population type, or speciation stage, we found no differences in response ratios between

populations (Kissenda vs. Python Island, Tukey HSD: display: $P = 1.0$, attack: $P = 0.97$; Makobe Island vs. Senga Point: display: $P = 0.48$, attack: $P = 1.0$). The response ratios in the single-species population differed significantly from those in the two hybridizing-species populations (single-species population vs. Kissenda Island: Tukey HSD: display: $P = 0.14$, attack: $P < 0.001$; single-species population vs. Python Island: display: $P < 0.01$, attack: $P < 0.001$). The response ratios in the hybridizing-species populations differed from those in the two-species populations (Kissenda vs. Makobe Island: Tukey HSD: display: $P < 0.05$, attack: $P < 0.01$; Kissenda Island vs. Senga Point: display: $P < 0.001$, attack: $P < 0.001$; Python vs. Makobe Island: display: $P < 0.001$, attack: $P < 0.001$; Python Island vs. Senga Point: display: $P < 0.001$, attack: $P < 0.01$). The response ratios in the single-species population did not differ from those in the two-species populations (single-species population vs. Makobe Island: Tukey HSD: display: $P = 0.86$, attack: $P = 0.96$; single-species population vs. Senga Point: display: $P = 0.08$, attack: $P = 1.0$).

Discussion

It has recently been proposed that male–male competition for mating territories may facilitate the invasion of novel male phenotypes and stabilize sympatric speciation and species coexistence through negative frequency-dependent selection (van Doorn *et al.*, 2004; Mikami *et al.*, 2004; Seehausen & Schluter, 2004). Such frequency dependency may arise when territory owners bias aggression towards same-coloured phenotypes (van Doorn *et al.*, 2004; Mikami *et al.*, 2004; Seehausen & Schluter, 2004). Blue *Pundamilia* male cichlid fish from the single-species population and the two two-species populations showed an aggression bias to blue stimulus male cichlid fish, consistent with the hypothesis. In contrast, blue male cichlid fish from the two hybridizing-species populations biased aggression towards red stimulus male cichlid fish. The findings are consistent with our expectation that the aggression bias depends on the stage of speciation. Indeed, the response ratios of the hybridizing-species populations were significantly different from those in both the single-species population and the two-species populations. The aggression bias towards blue in the single-species population may bestow an initial fitness advantage upon (red) colour mutant male cichlid fish, facilitating their establishment in the population. Aggression biases towards own colour may also stabilize coexistence of reproductively isolated sister species, because both species (red has consistent own-type bias, Dijkstra *et al.*, 2006) would receive more aggression with increasing relative abundance.

The data are consistent with previous findings. Around Makobe Island, we have also studied another blue reproductively isolated species, *P. 'pink anal'*, that co-occurs with *P. nyererei*. Aggression choice tests in the

field using SCUBA diving revealed an own-type aggression bias in *P. 'pink anal'* (Dijkstra *et al.*, 2006), similar to the two-species populations in the present study. Furthermore, in the same study, 10 out of 15 blue male cichlid fish of hybridizing-species populations biased aggression towards red male cichlid fish (Dijkstra *et al.*, 2006).

The aggression bias towards conspecifics in reproductively isolated species is in accordance with the findings of Seehausen & Schluter (2004). In a field survey on Lake Victoria cichlid communities, they found that closely related species that occur at the same locality tend to differ markedly in colouration. In the same study, it was found that territorial male cichlid fish tend to have territorial neighbours of species that are different in nuptial colouration from themselves (Seehausen & Schluter, 2004). These patterns can be explained when individuals among closely related species bias their aggression towards conspecifics and male cichlid fish of phenotypic similar species, exerting negative frequency-dependent selection on male nuptial colouration (Seehausen & Schluter, 2004).

Interestingly, the aggression bias towards red intruders of blue territorial defenders in the hybridizing-species populations suggests that, during the incipient stage of speciation, aggression bias alone is unlikely to stabilize the process. Other aspects of male–male competition may affect coexistence of (incipient) species, such as colour effects on behavioural dominance (Dijkstra *et al.*, 2005), and/or differences in intrinsic aggression level. There is evidence that red male cichlid fish were more aggressive than blue male cichlid fish in hybridizing-species populations (Dijkstra *et al.*, 2006; Dijkstra unpublished). Whether the combined effect of different elements of male–male competition can stabilize a trait polymorphism remains to be tested.

The marked difference in aggression biases between the hybridizing-species populations and the two-species populations can be partially understood by taking into account the degree of competition for female cichlid fish. Gene-flow between red and blue male cichlid fish at the two hybridizing-species populations suggests that male cichlid fish of the two phenotypes compete partly for the same set of female cichlid fish. This may render it less advantageous to concentrate aggression on male cichlid fish of its own colour than in a situation where red and blue are fully reproductively isolated species, as is the case in the two-species populations.

In the experiments we tested wild-caught fish. The aggression biases that we measured therefore likely mimic the situation in nature. It is possible that aggression biases not only have a heritable basis but are also shaped by experience. Learning effects are unlikely to explain the difference in aggression bias in the case of hybridizing-species and two-species populations as male cichlid fish from both population types are familiar with red male cichlid fish. Yet, in the case of aggression bias towards blue in male cichlid fish from the single-species

population, we cannot exclude the possibility that this is the result of a lack of experience with red male cichlid fish. Testing to what extent aggression biases have a heritable basis and are shaped by individual experience during ontogeny provides a fascinating avenue for future research.

The present study is the first test of a crucial theoretical assumption of models of sympatric speciation by sexual selection (van Doorn *et al.*, 2004; Seehausen & Schluter, 2004). The primary conclusion is that aggression bias varies between stages of speciation. The hypothesized negative frequency dependence arising from this bias could occur in the initial and final speciation stage but not in an incipient stage. Therefore, aggression bias alone is unlikely to stabilize the process of speciation.

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