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Can male-male competition stabilize speciation? A test in Lake Victoria haplochromine cichlid fish

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Abstract It has been suggested that sympatric speciation can be driven by sexual selection on male mating traits alone. However, a fundamental problem for this process is the lack of ecological differentiation that would stabilize the coexistence of incipient species through frequency-dependent selection. Such selection can also occur if male aggression is primarily directed towards similar rather than towards dissimilar phenotypes, so that rare male phenotypes would enjoy a negatively frequency-dependent fitness advantage. We experimentally tested such an aggression bias in two recently diverged, ecologically and anatomically

similar sympatric cichlid species pairs of the genus *Pundamilia* from Lake Victoria. Territorial males of a pair of partially reproductively isolated species with red and blue nuptial coloration, respectively, studied in the laboratory were confronted simultaneously with both colour types enclosed in transparent tubes. Red males were more aggressive to red stimuli under white light but not when colour differences were masked under green light. Blue males were equally aggressive to both stimuli in both light conditions. Males of two apparently fully reproductively isolated species, again one with red and one with blue nuptial coloration, studied in the field, both directed more aggressive behaviour towards conspecific than towards heterospecific stimulus males. The differential allocation of aggression would create an advantage for males of the less abundant phenotype or species, thereby potentially supporting stable coexistence of the phenotypes. The finding that this effect was less clear in the partially reproductively isolated species pair than in the fully isolated species pair is discussed.

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Introduction

Sympatric speciation, in which species arise without geographic isolation, is a much contested issue in evolutionary biology (Maynard Smith 1966; Felsenstein 1981; Bush 1994; Via 2001). There is theoretical and empirical evidence that sympatric speciation may occur in the wake of divergent ecological adaptation driven by competition for resources (Filchak et al. 2000; Schluter 2000; Turelli et al. 2001). In the traditional view, speciation is initiated by disruptive natural selection, although sexual selection by female mate choice on male ornaments may play a secondary role. Some models indicate that sympatric speciation can also be driven by sexual selection without

disruptive natural selection (Lande and Kirkpatrick 1986; Turner and Burrows 1995; Payne and Krakauer 1997; Higashi et al. 1999; Kawata and Yoshimura 2000; Van Doorn and Weissing 2001) or with a secondary role for the latter (Van Doorn et al. 1998). Under certain conditions, male trait and the corresponding female preference can jointly evolve by two runaway processes, creating two reproductively isolated populations. Many of these models were inspired by the extraordinary diversity of cichlid fish in Lake Victoria and Lake Malawi in East Africa (Fryer and Iles 1972; Seehausen et al. 1997; Seehausen and van Alphen 1999; Albertson et al. 1999). Sexual selection exerted by female preferences for male nuptial colour patterns has been proposed to be an important mechanism underlying explosive speciation in these cichlids (Dominey 1984; Seehausen et al. 1997; Seehausen and Van Alphen 1999; Smith and Kornfield 2002; Kocher 2004). This hypothesis was based on the observation that closely related species in the most rapidly radiated groups display striking differences in nuptial coloration, while ecological and morphological differentiation among sister species are muted (Seehausen et al. 1998b; Genner et al. 1999a; Albertson et al. 1999) or even lacking among sympatric conspecific morphs (Seehausen et al. 1999). In addition, the promiscuous mating system of these cichlids, with exclusive female parental care in the form of mouthbrooding, is conducive to strong sexual selection by female mate choice.

Although the cichlid fish in Lake Victoria show many characteristics that are consistent with sympatric speciation by sexual selection on male nuptial coloration, recent theoretical models indicate that sympatric speciation by sexual selection alone (Arnegard and Kondrashov 2004; Kirkpatrick and Nuismer 2004; Van Doorn et al. 2004) and even sympatric speciation generally requires stringent conditions (Gavrilets 2004). In the absence of ecological differentiation, it is unclear how novel phenotypes can invade. Also, the stable coexistence of incipient species is problematic, both during and after speciation. All this is due to a lack of frequency-dependent selection (Van Doorn et al. 2004). Van Doorn et al. (2004) and Lande et al. (2001) showed that frequency-dependent selection can be generated by female-female competition for mates. However, even under these conditions, sympatric speciation was only observed when additional and independent sources of disruptive selection were assumed. Van Doorn et al. (2004), Seehausen and Schluter (2004) and Mikami et al. (2004) independently proposed that male-male competition for access to breeding territories could generate such disruptive selection. Males of Lake Victoria and Malawi cichlid fish establish breeding territories to attract females and defend these territories aggressively against other males. Usually, only territorial males participate in reproduction (Parker and Kornfield 1996; Maan et al. 2004). Hence, competition over territory sites is intense and likely to exert sexual selection (Fryer and Isles 1972; Seehausen et al. 1998b). Simulation

models (Van Doorn et al. 2004) indicate that if territorial individuals direct more aggression to phenotypically similar than to dissimilar rivals, sympatric speciation by sexual selection is theoretically feasible. Such a bias in aggression to like-coloured males would result in a fitness advantage of males of a rare phenotype relative to males of the more abundant phenotype in a given local cichlid community. This advantage is negatively frequency-dependent because as the frequency of the rare phenotype increases, its advantage decreases due to more aggressive encounters with its own type. This would facilitate the invasion of both types, but this would prevent either type to drive the other extinct. In this way male-male competition can support the coexistence of several morphs and, hence, stabilize the speciation process.

Traditionally, intra- and interspecific aggression has been studied to understand coexistence through ecological resource partitioning (e.g. Ebersole 1985; Genner 1999b). Experimental evidence exists that some fish direct more aggression towards conspecifics than towards heterospecifics (Losey 1982; 1990; Kohda 1998), although other studies are ambiguous (Brockmann 1973; Thresher 1978; Genner 1999b; Leiser 2003). Genner et al. (1999b) showed that territorial male cichlids of the *Pseudotropheus* species complex in Lake Malawi never tolerated males and females of the same species complex in their territories, with a stronger aggression bias for dietary specialists than for dietary generalists. Further, Lake Malawi cichlids have non-overlapping territories at the intrageneric level, but males that belong to different genera may have overlapping territories (McKaye 1991). Fighting heterospecifics is likely to be a waste of energy and time, unless they are direct competitors for ecological resources. Between ecologically similar incipient and sibling species, the same might be true for competition for mates. However, studies that focus on differential aggression among ecologically similar species are lacking, and the possible role of intra- and interspecific aggression in sexual selection and speciation has largely been neglected (Seehausen and Schluter 2004).

In laboratory and field experiments, we tested for aggression biases in two sympatric pairs of red and blue sibling species of *Pundamilia* that are similar in ecology and anatomy. Sympatric red/blue or yellow/blue sibling species pairs are common in haplochromine cichlid communities (Seehausen and Van Alphen 1999; Seehausen and Schluter 2004). In a laboratory experiment, we tested a pair of fully sympatric partially reproductively isolated species by presenting territorial males of either colour type with stimulus males of both types. To test for the effect of nuptial coloration on biasing aggression, the experiment was carried out once under white light and once under green light, which effectively masked the colour differences between red and blue males. In a field experiment, we tested a pair of an apparently reproductively isolated sibling species in the field under natural light conditions. For both experiments,

we predicted that males of each phenotype direct more aggression towards males of their own colour type.

Materials and methods

Experiment A: partially reproductively isolated red and blue species pair

Species and subjects

The pair of occasionally hybridizing species consisted of a *Pundamilia nyererei* (Witte-Maas and Witte 1985)-like and a *Pundamilia pundamilia* (Seehausen et al. 1998a)-like phenotype, both from Python and Kissenda Islands in the Mwanza Gulf of Lake Victoria (Tanzania). Males of *P. nyererei* type are crimson dorsally, yellow on their flanks and have a crimson dorsal fin. We refer to this red phenotype as ‘red morph’. Males of *P. pundamilia* type are greyish white dorsally and on the flanks and have a metallic blue dorsal fin. We refer to this phenotype as ‘blue morph’. Both are confined to rocky shores and islands in Lake Victoria (Seehausen 1996) and occur sympatrically and syntopically around Python and Kissenda Islands. Differentiated red and blue phenotypes are common around Python and Kissenda Islands, with intermediate forms occurring at lower frequencies (Seehausen 1997, Seehausen, unpublished). Males were brought from the Mwanza Gulf into the Zoological Laboratory in Haren in May 2001. We used only wild-caught males, 26 red and 23 blue males.

Housing

Males and females were kept in stock aquaria of 100×45×38, with 13–25 individuals of the same morph per aquarium. Experimental aquaria were of the same size. The short sides and the back of the aquaria were covered with black plastic. 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. The bottoms of the aquaria were covered with gravel. Fish were fed flake food (TetraMin Tropical Fish Flakes) 6 days a week.

Prior to the experiments, males were individually housed for at least 2 months in separate compartments, within which a polyvinyl chloride (PVC) tube was placed as a refuge. Males had visual contact with one male of the same colour through a transparent plastic sheet. This arrangement ensured that males became territorial (illustrated by aggressive displays and butting behaviour towards the neighbouring male) and avoided unwanted effects of social isolation, while preventing them from engaging in physical interaction.

Aggression choice tests

An experimental tank consisted of a central compartment (60×45×38 cm) for the test fish. On both sides of this central compartment, we created two side compartments (20×45×38 cm) for dither fish using transparent partitions. Each side compartment contained three to four juvenile fish with no nuptial coloration [*P. pundamilia* from Kissenda Island, standard length (SL) 2–2.5 cm]. Visual exposure to these dither fish ensured that the test fish retained territorial condition. A test male was placed in the central compartment 1 day before a trial for acclimatization. A PVC tube was provided as a refuge. At the start of a trial, two stimulus males, one red and one blue (see below), were confined in transparent watertight tubes and placed at the right and the left ends of the central compartment. The behaviour of the test male was recorded on videotape for 10 min from behind a blind. The stimulus males were switched between the two sides halfway through the test to correct for any possible side biases. Each stimulus pair was used for one blue and one red test male to allow for pairwise comparison of blue and red male behaviour. Across trials we alternated the initial left and right position of red and blue stimulus males.

Standard length of each male was measured to the nearest 0.1 mm with callipers. Stimulus pairs were formed by matching a blue and a red male for SL. The size asymmetry, calculated as $(L-S)100/S$, where L is the SL of the larger fish, and S is the SL of the smaller fish, was less than 7%. Red and blue males did not differ in average SL within pairs [Wilcoxon signed rank tests (WSR), $Z=-1.601$, $P=0.109$, $N=15$]. Due to a limited stock of wild fish, most males acted both as test and stimulus male in different trials. The interval between reusing fish was at least 5 days.

Table 1 Experiment A: tests against 50% of response ratios (Fig. 1a,b) of red (white light, $N=14$; green light, $N=12$) and blue males (white light, $N=15$; green light, $N=12$) in a partially reproductively isolated species pair

Behaviour	Red morph				Blue morph			
	White light		Green light		White light		Green light	
	Z	P value	Z	P value	Z	P value	Z	P value
Display ratio								
Frequency	-1.789	0.074	-0.078	0.937	-0.157	0.875	-0.178	0.859
Duration	-2.605	0.009	-1.098	0.272	-0.227	0.820	-0.078	0.937
Attack ratio								
Frequency	-1.726	0.084	-0.089	0.929	-0.625	0.532	-0.445	0.657
Duration	-1.915	0.056	-1.020	0.308	-1.306	0.191	-0.471	0.638

Z values and significance are indicated for WSR tests. All tests are two-tailed

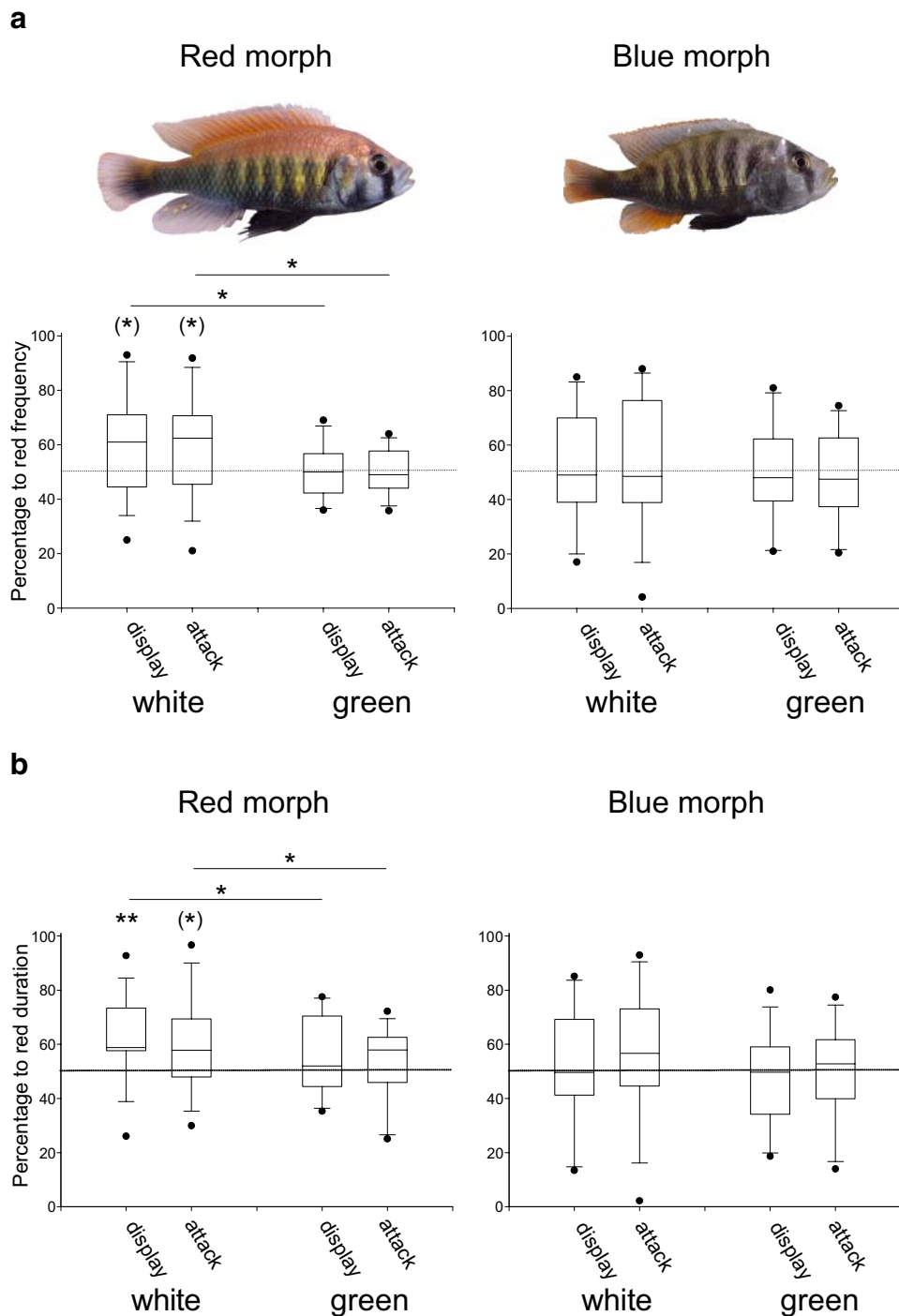


Fig. 1 a, b The display and attack ratios of red and blue males in the partially reproductively isolated species pair based on frequencies (**a**) and durations (**b**). Response ratios are presented for white light (red males $N=14$, blue males $N=15$) and green light conditions (males of both morphs $N=12$). The response ratio is the response to

the red stimulus divided by the sum of the responses to the red and blue stimuli. A response ratio of 50% represents identical responses to the two stimuli (*broken line*). Geometric means are presented with the 25th and the 75th percentiles, *error bars* show the 10th and 90th percentiles, and *dots* are outliers

We tested 15 blue and 15 red males: blue test males were on average larger than red test males [blue males, $N=15$; median, 94.8 mm; range, 85.8–118.7; red males, $N=14$ (one omitted due to courtship, see below); median, 89.1;

range, 81.4–106.8; WSR, $Z=-2.198$, $P=0.028$, $N=14$], which reflects the situation at Python and Kissenda Island, where blue males attain a larger body size than red males (Seehausen 1996).

Table 2 Experiment A: comparison of response ratios (Fig. 1a,b) between white and green light of red and blue males (males of both morphs $N=12$) in a partially reproductively isolated species pair

	Red morph		Blue morph	
	Z	P value	Z	P value
Behaviour				
Display ratio				
Frequency	-1.804	0.036	-0.157	0.875
Duration	-1.804	0.036	-0.941	0.347
Attack ratio				
Frequency	2.197	0.014	-0.628	0.530
Duration	-1.647	0.0495	-1.098	0.272

Z values and significance are indicated for WSR tests. Tests for red males are one-tailed, and tests for blue males are two-tailed

Light manipulation

To isolate effects of nuptial coloration of stimulus males from possible effects of other differences between them on aggression bias in the test male, we did each test once under white and once under green light. Illumination was provided by a 58-W fluorescent light tube (Osram L58W/21-840 cool white) in hoods suspended 80 cm above the water surface. Green light was created with a single layer of green sheets (Filter Primary Green, code 139, Lee Filters). To have white and green light of similar intensities, we wrapped two layers of neutral filters (Filter Neutral Density, code 209 0.3ND, Lee Filters) around the hoods in the white light treatment, which decreases light intensity without loss of spectrum. Light intensity was measured at eight fixed points in the experimental room. White light was 13% more intense than green light (white light $X \pm SD = 224.25 \pm 19.83$ lx, $N=8$; green light: $X \pm SD = 252.75 \pm 26.14$ lx,

$N=8$). Under both light treatments males exhibited the full range of aggressive behaviours. After five to eight trials in one light treatment, corresponding to 1 day, we replaced the filters (green light or white light, see below). The next day we repeated the test of the males of the previous day under the second light treatment with the same set of stimulus males. Sets of trials were balanced and alternated with respect to the sequence of the light treatment.

Behavioural observations

The videotapes were analysed with a video interface and time code generator using Observer Software version 3.0 (Noldus Information Technology, Wageningen, The Netherlands). The following behaviour patterns were recorded (see Baerends and Baerends-van Roon 1950): display behaviour (i.e. frontal and lateral display) and attack behaviour (i.e. biting and butting) at the walls of the tubes that contained the stimulus males. A display event was defined as lateral or frontal display, ending with a change in posture. An attack bout was defined as a series of rapid bite attempts with less than 1-s time intervals between the bites. The Observer Software yields both time budgets and frequencies of behaviours.

Experiment B: fully reproductively isolated red and blue species pair

Species and subjects

The fully reproductively isolated species pair consisted of *P. nyererei* (Witte-Maas and Witte 1985) and *Pundamilia 'pink anal'* (an as yet undescribed species, Seehausen 1996) from Makobe Island in the Speke Gulf of Lake

Table 3 Experiment A: aggression level (median, 25th and 75th quartile) expressed as the sum of frequencies and time spent attacking and displaying (in seconds) towards both stimuli for red males (white light, $N=14$; green light, $N=12$) and blue males (white light, $N=15$; green light, $N=12$) of a partially reproductively isolated species pair under both light conditions

	Red morph		Blue morph		Z	P
	Median	25th and 75th quartile	Median	25th and 75th quartile		
White light						
Behaviour						
Display						
Frequency	64.50	50.75–72.25	46.00	37.00–60.00	-0.157	0.875
Duration	180.85	142.43–189.61	116.15	96.83–152.30	-2.103	0.035
Attack						
Frequency	51.00	38.00–60.25	44.00	30.00–55.00	-0.039	0.969
Duration	231.70	157.78–293.00	190.50	145.35–300.90	-0.722	0.47
Green light						
Behaviour						
Display						
Frequency	57.00	36.75–85.50	47.50	44.00–54.75	-0.039	0.969
Duration	147.65	90.28–195.10	166.20	103.45–197.00	-0.51	0.96
Attack						
Frequency	48.50	34.50–57.25	45.00	37.25–54.25	-0.667	0.505
Duration	190.60	122.68–230.90	211.55	186.72–225.53	-0.561	0.58

Z values and significance are indicated for WSR tests. Tests are two-tailed

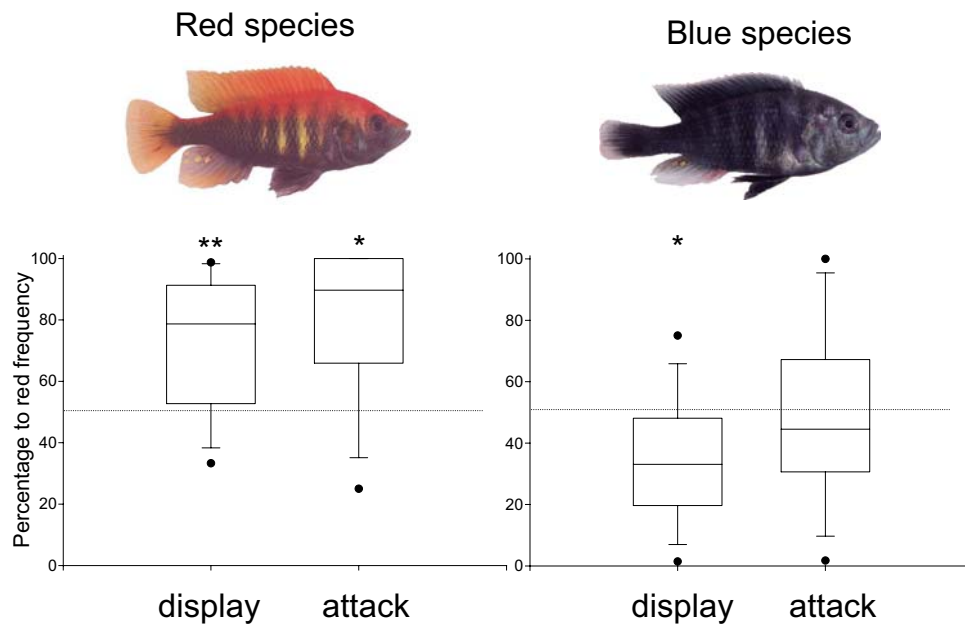


Fig. 2 The display and attack ratio of blue males ($N=14$, statistics based on $N=10$) and red males ($N=12$) of a sympatric and fully reproductively isolated species pair based on frequencies. The response ratio is the response to the red stimulus divided by the sum of the responses to the red and blue stimuli. A response ratio of 50%

represents identical responses to the two stimuli (*broken line*). Geometric means are presented with the 25th and the 75th percentiles, *error bars* show the 10th and 90th percentiles, and *dots* are outliers

Victoria (Tanzania). Hybrid phenotypes have never been seen at Makobe Island (Seehausen et al. 1998b; P.D. Dijkstra, M.E. Maan and O. Seehausen, personal observation). *P. nyererei* from Makobe Island is referred to as ‘red species’. It differs from the *P. nyererei*-like individuals from Python and Kissenda Islands in that males are brighter red in color. Males of *P. ‘pink anal’* are dark metallic blue and have a characteristic pink anal fin and pink lap-pets on the dorsal fin. We refer to them as ‘blue species’. Both species occur sympatrically and syntopically around Makobe Island (for details, see Seehausen 1996; Seehausen and Bouton 1997). Their breeding sites fully overlap, but *P. ‘pink anal’* is less abundant than *P. nyererei* on the breeding site (Seehausen et al. 1998b). The relatively clear waters make Makobe Island one of the few locations in Lake Victoria where experimental work using scuba is possible. We used 27 red and 25 blue males.

Aggression choice tests

Fieldwork was conducted by scuba diving. Territorial males of both species were located along a transect line at a depth of 6–9 m. Each male territory was marked with a coded tile placed between the rocks. A diver positioned a red and a blue stimulus male (size-matched, SL asymmetry <6%), each individually confined in a watertight transparent tube, into the centre of a territory. Stimulus males were obtained by gillnetting or angling at nearby locations around Makobe Island. The centre of a male’s territory was defined as the most frequent position of the territory owner, usually at a pile of rocks or close to a crevice. Typically, upon placing a stimulus pair, the territorial male either retreated in a crevice or fled then reappeared within a minute. If a male did not return or respond within the first 5 min, the trial was aborted. We successfully tested 14 blue and 12 red males.

Table 4 Experiment B: aggression level (median, 25th and 75th quartile) expressed as the sum of frequency of displays and attacks directed to both stimuli for red males ($N=12$) and blue males ($N=14$) of a fully reproductively isolated species pair

Behaviour	Red species		Blue species		MWU	P
	Median	25th and 75th quartile	Median	25th and 75th quartile		
Display	48.50	38.00–69.50	39.00	32.75–57.00	69.0	0.46
Attack	22.00	7.25–45.50	29.50	12.50–45.75	74.0	0.63

Significance is indicated for MWU tests. Tests are two-tailed

A diver recorded the frequencies of display events and attack bouts to each stimulus male with a pencil on a PVC sheet. Like the laboratory tests, each field trial lasted 10 min, and halfway through the trial, we switched the left and right positions of the stimulus males.

Analysis

Experiment A

In experiment A, under white light, one blue male, and under green light, two red males and three blue males performed courtship behaviour. These cases were excluded from the analysis. Since neither response ratios nor aggression level differed between males from Python and Kissenda Islands [Mann-Whitney U tests (MWU), response ratios, $P > 0.1$, aggression level, $P > 0.1$], we pooled the data. To analyse if a bias exists in aggression, we first generated response ratios based on frequencies of as well as time spent on both display and attack behaviour. The display duration ratio of each male was expressed as the time spent displaying towards a red stimulus (D_r) relative to the total time spent displaying towards both stimuli, calculated as $D_r / (D_r + D_b)$. We calculated the display frequency ratio in the same way by using frequencies of displays. The attack duration and frequency ratios were generated in an analogous way. The aggression level was defined as the sum of frequencies of and the time spent displaying and attacking to both the red and blue stimulus males.

To test if males had an aggression bias towards either their own or the other morph, the attack and display ratios were tested against 50% using Wilcoxon matched-pairs signed-rank (WSR) tests. We used WSR tests also to compare the response ratios between morphs tested with the same stimulus pair and between light treatments using the same test pairs. All tests were two-tailed, except for the light treatment comparison of red males, where we were able to make an a priori prediction about the direction of the results, namely, that masking colour differences would only lead to less discrimination and not to more discrimination of red and blue stimuli. Sample sizes varied depending on the type of comparison or test. For example, if one male performed courtship behaviour under green light, the corresponding case under white light was omitted to allow for pairwise comparison. Differences in overall aggression levels between morphs and light treatments were also compared with WSR tests.

Experiment B

Experiment B was analysed like experiment A. However, we calculated response ratios and aggression level only from frequencies of display events and attack bouts. In addition, differences between species were examined with MWU tests because stimulus pairs could not always be presented pairwise to one male of each species.

Each stimulus pair was only presented to a single male of each species except for four stimulus pairs which were each presented to two different blue males. For the analysis of aggression level we treated these data points as independent events (blue males, $N=14$). However, for the analysis of response ratios, we only took those cases with the highest aggression level to avoid pseudoreplication (blue males, $N=10$), although the analysis, including all cases, yielded the same P values.

Comparison between experiment A and B

The response frequency ratios between red and blue morph from the Mwanza Gulf and red and blue species from Makobe Island were compared with MWU tests.

Results

Experiment A: partially reproductively isolated red and blue species pair

White light

Red males paid on average slightly more attention to red than to blue stimuli; the display frequency ratio tended to deviate from 50% (Table 1 and Fig. 1a), and the display duration ratio deviated significantly from 50% (Table 1 and Fig. 1b). Both the attack frequency and duration ratios tended to deviate from 50% (Table 1 and Fig. 1a,b). In contrast, blue males did not discriminate between the two stimuli; none of the response ratios deviated from 50% (Table 1 and Fig. 1a,b). Response ratios of blue and red males did not differ significantly (WSR tests: $-1.412 < Z < -0.282$, $0.158 < P < 0.778$, $N=14$). When the ratios of blue and red males were pooled, the response ratios deviated from 50%, although this was only significant for the duration ratios (WSR tests: display frequency ratio $Z = -1.435$, $P = 0.151$; display duration ratio $Z = -2.152$, $P = 0.031$; attack frequency ratio $Z = -1.676$, $P = 0.094$; attack duration ratio $Z = -2.303$, $P < 0.021$, $N=29$). This indicates that overall, red males evoked more aggression than blue males.

Light treatment effect

Under green light, neither red nor blue males discriminated between red and blue stimuli (Table 1 and Fig. 1a,b). For red males, all response ratios differed between the two light treatments (Table 2 and Fig. 1a,b). Such a difference was not observed for blue males (Table 2 and Fig. 1a,b). These results indicate that the bias of red males towards aggressing red competitors more than blue ones was due to the differences in nuptial coloration between the stimulus males rather than other differences between the morphs.

Under white light red males spent significantly more time displaying than blue males (Table 3). Under green

light there was no difference in aggression level between morphs (Table 3). For red males, attack duration was shorter under green light than under white light, but the other measures of aggression level did not differ between light treatments (WSR tests: display frequency $Z=-0.157$, $P=0.875$; display duration $Z=-0.157$, $P=0.875$; attack frequency $Z=-0.039$, $P=0.969$, attack duration $Z=-2.197$, $P=0.028$, $N=12$). The aggression level of blue males did not differ between light treatments (WSR tests: display frequency $Z=-0.039$, $P=0.969$; display duration $Z=-1.177$, $P=0.239$; attack frequency $Z=-0.667$, $P=0.505$; attack duration $Z=-0.157$, $P=0.875$, $N=12$).

Experiment B: fully reproductively isolated red and blue species pair

Both red and blue males directed more display behaviour to males of their own species (Fig. 2, WSR tests: red $Z=-2.578$, $P=0.010$, $N=12$; blue $Z=-2.073$, $P=0.038$, $N=10$). The attack ratio deviated from 50% only for red males (WSR tests: red $Z=-2.835$, $P=0.005$; blue $Z=-0.415$, $P=0.678$). Both display and attack ratios to the red vs the blue species differed significantly between the species (display ratio $MWU=9.5$, $P<0.001$, $N_1=12$, $N_2=10$; attack ratio $MWU=21.5$, $P=0.009$). Red males exhibited a stronger bias in attack to conspecifics than blue males ($MWU=18.5$, $P=0.004$, $N_1=12$, $N_2=10$), but the strength of the display bias to conspecifics did not differ between the species ($MWU=46.5$, $P=0.381$). Overall, blue and red males directed more displays ($Z=-3.323$, $P=0.001$, $N=22$) and attacks ($Z=-2.542$, $P=0.011$, $N=22$) to their own species. Blue and red males did not differ in the absolute frequency of attacks and displays (Table 4).

Comparison between partially and fully reproductively isolated species pair

The own-species bias in aggression exhibited by the fully reproductively isolated red species tended to be stronger than the own-morph bias by the red morph of the partially reproductively isolated species, significantly so for the attack frequency ratio (display frequency ratio $MWU=55$, $P=0.145$; attack frequency ratio $MWU=33$, $P=0.008$, $N_1=12$, $N_2=14$). The display bias of the blue species towards conspecifics was significantly stronger than the bias in the blue morph (display frequency ratio $MWU=31.5$, $P=0.014$, $N_1=10$, $N_2=15$), but this difference was not observed in the attack frequency ratio ($MWU=59$, $P=0.397$).

Discussion

It has recently been proposed that a bias in aggression towards the own species in male-male competition may facilitate stable coexistence of incipient species, both during and after speciation (Mikami et al. 2004; Van Doorn et al. 2004, Seehausen and Schluter 2004). The

results in the present study are partly consistent with this hypothesis: males of reproductively isolated, but closely related red and blue species that occur sympatrically and syntopically directed significantly more agonistic behaviour towards conspecific males than towards males of the other species. In interspecies competition for territories, males of both species may, hence, experience elevated fitness when their species is locally the less abundant of the two. This mechanism may promote stable coexistence of red and blue phenotypes after speciation, even without ecological divergence.

The aggression bias for conspecifics in reproductively isolated red and blue species is consistent with the findings of Seehausen and 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 coloration. In the same study, it was found that territorial males tend to have territorial neighbours of species that are different in nuptial coloration from themselves (Seehausen and Schluter 2004). There is evidence that a similar segregation pattern among territorial males of several coexisting species also exists in cichlid communities in Lake Tanganyika (Kohda 1998). These patterns can be explained when individuals among closely related species bias their aggression towards conspecifics and males of phenotypically similar species, exerting negative frequency-dependent selection on male nuptial coloration (Seehausen and Schluter 2004).

We expected own-type biases also in males of the partially reproductively isolated species pair. Inconsistent with this hypothesis, blue males in the partially reproductively isolated species pair that we studied did not exhibit any aggression bias to males of their own colour type. Consistent with the hypothesis, however, red males in the same pair were more aggressive to other red males than to blue males. Although the analysis on the basis of behavioural frequencies did just not reach statistical significance, the analysis on the more accurate basis of time spent on aggression yielded significant results. Although these colour effects on male-male aggression will cause the fitness of red phenotypes to be maximized when red is rare, and to decrease with increasing relative abundance, they would never generate a fitness advantage of red over blue. Therefore, it appears that in the case reported here, aggression bias alone does not stabilize the polymorphism. However, results of a previous investigation (Dijkstra et al. 2005) suggest that the disadvantage that males of the red morph would suffer from the colour-based aggression bias in the partially reproductively isolated species pair may well be offset by a significant colour-based dominance advantage of red males in physical combat in the same species pair (Dijkstra et al. 2005). It is possible that the two together stabilize the polymorphism.

Importantly, the assumption that aggression bias is based on colour differences and not on other morph-specific traits is indicated by our light manipulation experiment. Males of the red and blue morph did not distinguish between red and blue stimulus males under green light, and the aggression bias of red males under white light was significantly

reduced under green light. It appears, therefore, that nuptial colour in *Pundamilia* is important not only in female mate choice (Seehausen and van Alphen 1998) but also in intra-sexual signalling.

The adaptive significance of differential aggression in promoting the establishment of polymorphisms and the coexistence of species can be understood by taking into account the degree of competition for females. Gene flow between red and blue in the partially reproductively isolated species pair suggests that males of the red and blue morph compete partly for the same set of females. This may render it less advantageous for blue males to concentrate aggression on males of its own colour than in a situation where red and blue are fully reproductively isolated species, e.g. at Makobe Island. This may explain the difference in own-type biases between the partially and the fully reproductively isolated species pair that we studied.

The own-type bias of red males was stronger than that of blue males in both the partially and the fully reproductively isolated species pair. The deviation from symmetry in frequency-dependent selection caused by male-male aggression biases suggests that blue *Pundamilia* phenotypes are likely to be favoured by male-male competition at least unless blue is clearly more abundant than red. This may explain why red *Pundamilia* populations appear to always occur sympatrically with at least one blue species or colour morph, whereas entirely blue populations are not uncommon (Seehausen and van Alphen 1999).

Taken together, our results suggest that (1) red phenotypes have a stronger own-type bias than blue phenotypes, that (2) an own-type bias in aggression alone is not likely to promote coexistence of partially reproductively isolated species, and that (3) aggression bias may stabilize syntopic coexistence of reproductively isolated sister species.

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