Behavioral Ecology
doi:10.1093/beheco/arn125
Advance Access publication 26 November 2008

Color polymorphism and intrasexual competition in assemblages of cichlid fish

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The origin and maintenance of phenotypic polymorphisms is a classical problem in evolutionary ecology. Aggressive male–male competition can be a source of negative frequency-dependent selection stabilizing phenotypic polymorphisms when aggression is biased toward the own morph. We studied experimental assemblages of red and blue color morphs of the Lake Victoria cichlid fish *Pundamilia*. Aggression was investigated in mixed-color and single-color assemblages. We found that aggression was indeed biased toward males of the same color, which could in theory reduce aggression levels in mixed-color assemblages and promote coexistence. However, previous studies showed high aggression levels in red and dominance of red over blue males in dyadic interactions, which could hinder coexistence. We found that coexistence in mixed-color assemblages reduced the level of aggression in red males but not in blue males. Red and blue males were equally dominant in mixed-color assemblages, suggesting that predictions derived from dyadic interactions may not be valid for an assemblage situation. The results are consistent with field data: the geographic range of red is nested within that of blue, suggesting that red cannot displace blue. Our study suggests that male–male competition may be a significant force for maintaining phenotypic diversity. *Key words:* cichlid fish, color polymorphism, Lake Victoria, male–male competition, sexual selection. *[Behav Ecol 20:138–144 (2009)]*

source: https://doi.org/10.7892/boris.36772 | downloaded: 12.10.2024

The maintenance of phenotypic polymorphisms within species and of closely related sympatric species are 2 related and important themes in evolutionary ecology (Ricklefs and Schluter 1993; Coyne and Orr 2004). Frequency-dependent natural selection is often thought of as a powerful force that can generate and maintain phenotypic polymorphisms (for review, see Rundle and Nosil 2005). However, several species-rich taxa exhibit conspicuous polymorphisms in sexually selected traits like nuptial coloration (e.g., Uy and Borgia 2000; Masta and Maddison 2002; Boake 2005; for review, see Gray and McKinnon 2007). A classical example is the East African haplochromine cichlid fish (Fryer and Iles 1972; Genner and Turner 1999; Seehausen 2000). The observation of these polymorphisms has lead to the hypothesis that disruptive sexual selection by female mate choice operates on male nuptial coloration (Dominey 1984; Payne and Krakauer 1997; Seehausen et al. 1997).

Although divergent sexual selection through female choice may drive the evolution of phenotypic differentiation, it was long unclear how it can generate the frequency dependence needed to stabilize phenotypic polymorphisms (Arnegard and Kondrashov 2004; Van Doorn et al. 2004). It has recently been proposed that interference competition among males for mating territories can be a source of negative frequency-dependent sexual selection (Mikami et al. 2004; Seehausen and Schluter 2004; Van Doorn et al. 2004). If males bias aggression toward phenotypically similar rival males, rare male phenotypes would receive less aggression than common male phenotypes. This could in theory confer a fitness advantage to males of a rare phenotype relative to males of the more abundant phenotype, thereby promoting invasion of new phenotypes and facilitating coexistence of different phenotypes.

Aggression has been implicated in causing character displacement of sexual traits between closely related species (Alatalo et al. 1994; Tynkkynen et al. 2004). It may also affect the evolution of color polymorphisms (e.g., Barlow 1983; Calsbeek and Sinervo 2002; Kingston et al. 2003; Pryke and Griffith 2006). For instance, in the color polymorphic Gouldian finch (*Erythrura gouldiae*), red-headed males dominated black-headed males, both of which dominated yellow-headed males in staged contests (Pryke and Griffith 2006). The authors suggest that asymmetric dominance relationships between the different morphs influence the relative fitness of each morph and hence contribute to maintenance of these 3 discrete phenotypes in the wild.

The radiations of haplochromine cichlid fishes in some African Great Lakes are characterized by exceptional color diversity among closely related species (Kocher 2004; Seehausen 2006). Haplochromines have a polygynous mating system, with highly territorial males defending individual mating territories. Territory ownership may often be a prerequisite for gaining access to spawnings (Parker and Kornfield 1996; Maan et al. 2004), and territory quality is important in mate choice (Dijkstra, van der Zee, et al. 2008). Because color is an important cue in intrasexual communication (Dijkstra et al. 2006), frequency dependence arising from male–male competition could occur in haplochromines. Field data on Lake Victoria cichlid communities were consistent with negative frequency-dependent selection on male color through male–male competition. Seehausen and Schluter (2004)

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Received 19 March 2008; revised 7 June 2008; accepted 21 June 2008.

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found 1) that closely related species that occur at the same locality differ significantly more often in male nuptial coloration than males of randomly chosen species and 2) among 2 closely related species within a site, males had more often territorial neighbors with different nuptial coloration than expected by chance.

Pundamilia is a widespread haplochromine genus in Lake Victoria (Seehausen forthcoming). In Kissenda Island (Mwanza Gulf, Tanzania), *Pundamilia* population displays a genetic color polymorphism, composed of red nuptial phenotypes resembling *Pundamilia nyererei* from other sites, blue nuptial phenotypes resembling *Pundamilia pundamilia* from other sites, and intermediate phenotypes (for phenotype distribution at Kissenda Island, see Dijkstra, Seehausen, et al. 2007). These intermediate phenotypes suggest that red and blue are hybridizing. In this study, we only used distinct red and blue phenotypes; for convenience, we refer to these males as red and blue color morphs, respectively.

Pundamilia males establish individual territories. Although males may also benefit from the food resources and shelter contained in them, it appears that territories mainly serve to attract females (Maan et al. 2004). Red males in this population have an 85% chance of winning a combat with a weight-matched blue male in staged dyadic interactions. This is largely an effect of red color itself because the advantage disappears under green light (Dijkstra et al. 2005). Another part of the behavioral dominance of red is attributable to their higher aggression level (Dijkstra et al. 2006). However, the geographical distribution of red and blue *Pundamilia* populations in Lake Victoria suggests that the red phenotype does not displace the blue phenotype. Whereas populations are often entirely composed of blue males, red *Pundamilia* populations without exception occur sympatrically with blue ones (Seehausen and van Alphen 1999). Dominance relationships measured in experimentally isolated pairs of individuals do not necessarily reflect those between the color morphs in a natural setting where multiple males interact simultaneously in an assemblage that is maintained over a longer time span. Dominance relations in the latter situation result not only from fixed traits but also through self-reinforcing effects of winning and losing conflicts among assemblage members (Chase et al. 2002, 2003; Hemelrijk and Wantia 2005). Hence, whereas dyadic interaction paradigms are useful to characterize the specific interactions between phenotypes, theoretical predictions of effects at the population level require tests using an experimental assemblage paradigm.

In this study, we investigated the effects of color on aggressive behavior in mixed-color (blue and red males together) and single-color (only red or only blue males) assemblages of *Pundamilia* fish. The experimental assemblage paradigm used here permits investigation of the combined effects of aggression bias and dominance in dyadic interactions. We tested the following predictions derived from theoretical models (Mikami et al. 2004; Van Doorn et al. 2004; Hemelrijk and Wantia 2005), from the geographic distribution of red and blue in Lake Victoria (Seehausen and Schluter 2004), and from our earlier results (Dijkstra et al. 2005; Dijkstra 2006): 1) Males of both morphs bias aggression toward their own morph in the mixed-color assemblages. 2) If so, the assemblage-wide aggression level should be reduced in mixed-color assemblages because males encounter more males with whom they share a mutually "tolerant" attitude (Mikami et al. 2004). This prediction is particularly eminent for red males due to their higher level of aggression. 3) Despite dominance of red in dyadic interaction, red males do not dominate the blue males in mixed-color assemblages because intense/exhausting aggression among red males would reduce their dominance advantage over blue males.

MATERIALS AND METHODS

Fish

Laboratory crosses revealed that red and blue nuptial coloration in *Pundamilia* are genetically determined, with hybrid crosses resulting in intermediate phenotypes (Haesler and Seehausen 2005; Van der Sluijs et al. forthcoming). Red males at Kissenda Island occupy a range of different water depths from shallow to deep, whereas blue males occur in the shallowest 3 m (Seehausen forthcoming). Hence, their habitat is fully overlapping in shallow waters, making it likely that males of each color morph compete with one another over territories. Intermediate phenotypes do not suffer from intrinsically reduced fitness in terms of fertility, growth rate, and survival (Van der Sluijs et al. 2008).

We used first-generation offspring bred from 41 fish collected in June 2001 around Kissenda Island, Lake Victoria, Tanzania. Experimental males (61 red and 60 blue) were sexually mature and at least 11 months of age.

Housing

Males were reared in sib groups. When the first fish started to mature (at ~6 months of age), they were translocated into stock aquaria, containing males and females of both color morphs. The sides and the back of all 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. All aquaria contained gravel substrate. Fish were fed with flake food (TetraMin Tropical Fish Flakes) at least once per day and a mixture of ground shrimps 2 times per week. Prior to the experiment, we housed males for at least 1 week individually in aquaria measuring 90 × 36 × 30 cm (l × w × h), divided into 8 compartments by PVC screens. To avoid social deprivation, all males were kept in compartments with another randomly chosen red or blue male visible behind a transparent PVC screen. Each compartment contained a PVC tube that the fish used as a hiding place.

Experiment

We used the following 3 experimental treatments (the first 2 are single-color treatments): 1) red ($N = 7$ assemblages): 8 red males in one aquarium; 2) blue ($N = 7$ assemblages): 8 blue males; and 3) mixed color ($N = 12$ assemblages): 4 red and 4 blue males together in one aquarium. To avoid effects of size asymmetry on dominance, fish in all 3 treatments were size matched within groups. Information about the mean male weight in each assemblage, the variation in male weight in each assemblage, and the mean weight of red and blue males in mixed-color assemblages are summarized in Table 1. None of the parameters differed significantly between treatments or morphs (independent *t*-tests, P values > 0.05). The experimental aquaria, measuring 250 × 66 × 46 cm (l × w × h), contained 3 vertical PVC tubes (diameter 15 cm and length 21 cm) placed approximately 60 cm apart from one another. These tubes mimic a crevice that *Pundamilia* males use as the focal point of their territory (Dijkstra, Hekman, et al. 2007).

To reduce the aggression level among experimental males, we used 15 *Melanotaenia lacustris* (rainbow fish) as background fish in each experimental aquarium (Dijkstra, Hekman, et al. 2007). These fish are standard background fish in our laboratory facilities.

Forty-three red males (out of a total of 61) and 44 blue males (out of a total of 60) were employed once in the single-color treatment and once in the mixed-color treatment. The interval

Table 1
The weight of test fish for each assemblage type separately

Treatment	Morph	*N*	Weight		CV	
			Mean	SE	Mean	SE
Blue	Blue	7	15.10	1.16	0.06	0.01
Red	Red	7	14.04	0.96	0.08	0.01
Mixed		12	14.84	0.79	0.06	0.01
	Blue		14.99	0.74		
	Red		14.70	0.84		

Also shown is the weight of blue and red males in mixed-color assemblages. The variation in weight is indicated by the average coefficient of variation (CV). The CV for each assemblage is calculated as mean weight/standard deviation. Shown are the mean and SE calculated from (mean) values across assemblages.

between use of the same male was at least 15 days. Half of the males first experienced the single-color and then the mixed-color treatment and the other half vice versa. The other males were used only in one treatment. Prior to a test, we weighed all males to the nearest 0.01 g. We clipped males for individual recognition on their dorsal (2 positions) and caudal fins (3 positions), using scissors. Fin clips grow back within 1–2 weeks and cause no lasting damage to the fish (Dijkstra, Hekman, et al. 2007).

Behavioral observations

We observed each assemblage on the first 3 consecutive days after release of the fish into the experimental aquarium. On each day, we observed 45 min, amounting to a total observation time of 135 min per assemblage. The males displayed the common aggressive behaviors of cichlids (Baerends and Baerends-Van Roon 1950). We recorded aggressive interactions that resulted in a displacement, with the fish being displaced defined as loser and the other one as winner. Displacements could come about via 1) attack (overt aggression) and 2) display (covert aggression). These behaviors were recorded on a tape recorder. A displacement via an attack was defined as one fish charging or chasing another fish. A displacement via a display was defined as one fish showing lateral or frontal display, followed by fleeing of his opponent, or as 2 fish displaying to each other, followed by one of them fleeing. The size of the group and the frequency of interactions were such that it was possible to record all social interactions. Background fish never attacked focal cichlid males. In 21 treatment groups, we also recorded attacks of cichlid males directed to background fish.

Data analysis

The social hierarchy was stable over the 3-day observation period, as indicated by strong correlations of dominance ranks between days 1 and 2 (relationship in rank between days was calculated using the Spearman correlation for each assemblage separately [$N = 26$]; Fisher's combination test was used to combine significance levels over several samples: $\chi^2 = 93.9021$, degrees of freedom [df] = 52, $P = 0.0003$) as well as between days 2 and 3 (Fisher's combination test, $\chi^2 = 178.7005$, df = 52, $P < 0.00001$). For all analyses, we tested for effects of day of observation; there was only an effect of day in the analysis of aggression level (see below). Therefore, apart from the analysis of aggression level, all analyses (dominance and aggression bias) are based on values averaged over the 3 daily scores.

Dominance

In order to determine the degree of dominance of males of one particular morph relative to the other, we first established the dominance hierarchy for each mixed-color assemblage. To this end, we calculated for each male his dominance index (defined as the number of times the individual won against an opponent divided by the total number of fights they engaged in) in relation to each assemblage member he interacted with (maximum 7 opponents) and then took the average of these values (Hemelrijk et al. 2005). The average dominance index allowed us to rank males in each assemblage from 1 (= most dominant) to 8. Dominance rankings were calculated separately for each day of observation. If interactions with certain assemblage members were missing, the average dominance index was obviously calculated over fewer members (Hemelrijk et al. 2005), an important correction because individuals usually did not interact with all other assemblage members (average number of interaction partners, mean ± standard error [SE] day 1: 4.16 ± 0.15; day 2: 4.80 ± 0.12; day 3: 5.05 ± 0.10; $N = 208$).

The dominance of males of one particular morph relative to the other morph in the mixed-color assemblages was inferred in 2 ways.

First, we compared the number of red "alpha" males with the number of blue alpha males. The alpha male is the most dominant male in an assemblage; it is usually also the most brightly colored male (Dijkstra, Hekman, et al. 2007) and typically defends a territory, centered around either 1 or 2 tubes. The male with the highest average dominance index was considered the alpha male. In few cases (see Results), 2 males in the same assemblage were defined as alpha males because they shared the highest average dominance index. To test for morph-specific dominance, we compared the number of red and blue alpha males using a sign test.

Second, the degree of dominance of red males over blue males in an assemblage was also estimated by the method developed by Hemelrijk and coauthors (Hemelrijk et al. 2003; 2008). It is based on the relative dominance of males of one morph over those of the other morph using a standardized Mann–Whitney *U* statistic (Siegal and Castellan 1988). For each red male, the number of blue males ranking below him was counted. The value of the Mann–Whitney *U* statistic is calculated as the sum of these counts. Note that for a mixed-color assemblage of 8 males (4 red and 4 blue), the maximum possible value for each morph is $4 \times 4 = 16$. The standardized *U* value is corrected for the maximum possible value at this sample size. Thus, it is calculated as the *U* statistic of red over blue, divided by the maximum possible value. A value of 0.5 indicates equal dominance of red and blue males, a value >0.5 dominance of red males, and <0.5 dominance of blue males. Complete dominance of red, which implies that all red males are dominant over all blue males, equals 1, and complete dominance of blue equals 0. To test for dominance of red over blue, we tested whether the standardized *U* value (averaged over the 3 daily scores) deviates from 0.5 using a 1-sample *t*-test.

Aggression bias and aggression level

To evaluate aggression biases, we calculated attack ratios, which is the number of attacks males directed to males of their own morph divided by the sum of their attacks to all males. The attack ratios were calculated for each morph and day separately using the sum of the attacks of males in an assemblage. If there is no aggression bias, the attack ratio is 3/7 in a mixed-color assemblage because a red or a blue male had the opportunity to attack 4 males of the other color, but only 3 males of its own color. We tested for aggression bias in each morph separately by testing the attack ratio (averaged over the 3 daily scores) against

3/7 using a 1-sample *t*-test. Previous studies, using simulated intruder choice tests, showed that both red and blue bias aggression toward males of their own color (Dijkstra 2006). We therefore based significance on a 1-tailed test of significance.

For the analysis of the aggression level, we focussed on both the rate of received attacks and the rate of performed attacks. These rates of attacks (per individual per 45-min observation session) were calculated for each morph and day separately using the average attacks of all males in an assemblage. In terms of costs of male–male competition, both measures of aggression level are relevant (e.g., Hsu et al. 2006). The rates of received and performed attacks for males of a given morph are by definition equal in single-color assemblages but not in mixed-color assemblages because in the latter case attacks can be received from both morphs. We tested the hypothesis that aggression is reduced in mixed-color assemblages relative to single-color assemblages. To this end, we used for each morph separately a repeated measure (RM) analysis of variance (ANOVA) with day as repeat and response variable either the rate of received attacks or the rate of performed attacks. For red males, we had a 1-tailed test of significance because both the own-color aggression bias and the high level of aggression of red males (see Results) specifically predict reduced aggression levels in mixed-color assemblages compared with single-color assemblages.

Proportions were arcsine square root transformed and levels of aggression were ln $(x + 1)$ transformed to meet assumptions of parametric testing. Statisticalanalyses were carried out with SPSS 12.0.1. All reported probabilities are for 2-tailed tests of significance, unless we have a clear preconception about the direction of an effect. When assessing the significance of the effect of day in a RM ANOVA, we checked the assumption of sphericity. If this assumption was violated, we used the Greenhouse–Geisser adjustment to reduce df and the probability of a type I error (Zar 1999). Although the aggression level was analyzed with RM ANOVAs to control for changes over the 3-day observation period, to simplify graphical presentation, we plotted the mean (±SE) based on the average of the 3 daily scores.

RESULTS

Dominance

Color of alpha male in mixed-color assemblages

To test for dominance of males of one morph over males of the other, we examined the number of red and blue alpha males in each mixed-color assemblage on every day (Table 2). Most assemblages were dominated by a single alpha male on any one day. We averaged these numbers over the 3 days and found no significant difference in the number of blue and red alpha males (sign test, $P = 1.0$), suggesting that red was not dominant over blue in mixed-color assemblages.

Average dominance of both morphs in mixed-color assemblages

The proportion of blue males ranking below red males in the dominance hierarchy of each mixed-color assemblage (standardized *U* value) averaged over the 3 days is shown in Table 2 (last column). The standardized *U* value did not deviate from 0.5 (mean ± SE of the standardized *U* value: 0.46 ± 0.05; 1-sample *t*-test: $t = -0.868$, df = 11, $P = 0.40$). This suggests that in a social group, red and blue males did not systematically differ in dominance.

Own-color bias in aggression in mixed-color assemblages

As expected, in mixed-color assemblages, males directed attacks to both morphs. Both red and blue males biased their aggression toward males of their own color (Figure 1): the attack ratios averaged over the 3 daily scores deviated significantly from 3/7 (1-sample *t*-test, red males: $t = 2.454$, df = 11, 1-tailed $P = 0.016$; blue males: $t = 2.467$, df = 11, 1-tailed $P = 0.015$).

Aggression level in single-color and mixed-color assemblages

The level of aggression, expressed as the rate of received and the rate of performed attacks per individual, is shown in Figure 2. As expected, the aggression level in red assemblages was higher than in blue assemblages (only single-color assemblages, 1 tailed; RM ANOVA: $F = 8.378$, df = 1, 12, $P = 0.007$). There was a significant day effect (RM ANOVA: $F = 14.532$, df = 2, 24, $P = 0.00007$) due to a lower aggression level on the first

Table 2

The color of alpha males for each mixed-color assemblage and day separately

Alpha male information							*U* value mean of red over blue	
Assemblage number	Day 1	Day 2	Day 3	Red	Blue	More red than blue alpha males?	Mean	SE
1	R^a	R^a	R^a R^b	1.00	0.00	Yes	0.63	0.10
2	R	B^a	B^a	0.33	0.67	No	0.48	0.10
3	R	B^a	B^a	0.33	0.67	No	0.36	0.10
4	R^a	R^a	R^a	1.00	0.00	Yes	0.50	0.04
5	B^a	B^a	B^a	0.00	1.00	No	0.19	0.03
6	R^a B	R^a	R^a	0.83	0.17	Yes	0.63	0.03
7	B^a	B^a	B^a	0.00	1.00	No	0.33	0.03
8	R^a	R^a	R^a	1.00	0.00	Yes	0.60	0.15
9	B	R^a	R^a	0.67	0.33	Yes	0.62	0.25
10	R^a	R^a R^b	B	0.67	0.33	Yes	0.63	0.24
11	R	B^a	B^a	0.33	0.67	No	0.41	0.11
12	B^a	B^a	B^a	0.00	1.00	No	0.13	0.06
			Sum	6.16	5.84			

Each letter, R (red) or B (blue), represents one alpha male: there was usually one alpha male per assemblage, except in 3 cases where 2 fish were equally dominant. Letters in superscript indicate the identity of the alpha male over the 3-day observation period. Different letters denote different males. The number of times we observed a red and a blue alpha male averaged over the 3 days is also listed; note that in case the alpha position was shared by 2 males, we divided their scores by 2. We also noted whether we counted more red than blue alpha males in an assemblage. Finally, we show the standardized *U* values averaged over the 3 days for each assemblage. The standardized *U* value (*U* value) indicates the average fraction of blue males ranking below each red male (for full explanation, see Materials and methods). A value of 0.5 indicates equal dominance for red and blue males, a value >0.5 dominance for red males and <0.5 dominance for blue males.

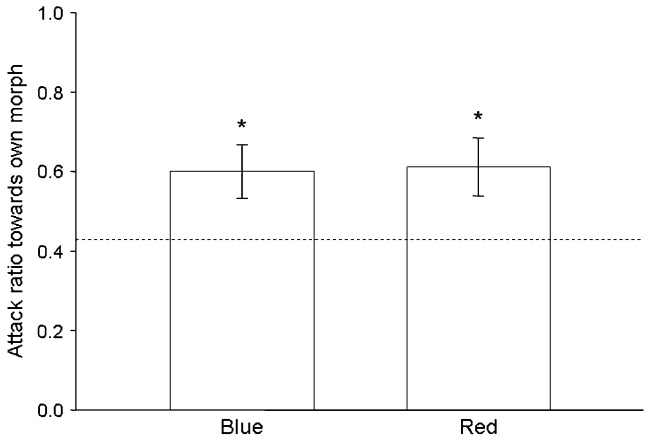

Figure 1
The attack ratio toward ones own morph, calculated as the number of attacks toward males of ones own morph divided by the total number of attacks in mixed assemblages. Shown are the means and SEs based on the average of the daily scores for red males and blue males ($N = 12$). Asterisks indicate significant differences ($P < 0.05$) from the no-preference ratio of 3/7 (dotted line).

observation day: this temporal trend was also apparent in the results reported below.

We detected no difference in aggression level in blue males between mixed-color and blue assemblages (RM ANOVAs, rate of received attacks: $F = 0.042$, df = 1, 17, $P = 0.84$; day effect: $F = 9.044$, df = 2, 34, $P = 0.0007$; rate of performed attacks: $F = 0.627$, df = 1, 17, $P = 0.44$; day effect: $F = 6.323$, df = 1.465, 24.908, $P = 0.011$). In contrast, red males experienced a lower level of aggression in mixed-color than in red assemblages (RM ANOVAs, rate of received attacks: $F = 3.145$, df = 1, 17, 1-tailed $P = 0.047$; day effect: $F = 8.945$, df = 1.395, 23.718, $P = 0.003$; rate of performed attacks: $F = 3.005$, df = 1, 17, 1-tailed $P = 0.05$).

Aggression to background fish

The behavior of cichlid males toward the background fish did not confound the results. The frequencies of attacks toward background fish averaged over the 3 days were (mean ± SE): 9.5 ± 3.6 ($n = 7$ blue assemblages); 8.6 ± 3.1 ($n = 7$ red assemblages); and 8.7 ± 3.9 ($n = 7$ mixed-color assemblages). The attack frequency did not differ between treatments (RM ANOVA: $F = 0.098$, df = 2, 18, $P = 0.91$; day effect: $F = 13.729$, df = 2, 36, $P = 0.00004$). Also, within the mixed-color treatment, red and blue males did not differ in the frequency of attacks toward the background fish (blue males: 3.6 ± 1.7; red males: 5.1 ± 2.9; RM ANOVA: $F = 0.025$, df = 1, 12, $P = 0.88$; day effect: $F = 9.271$, df = 2, 24, $P = 0.001$).

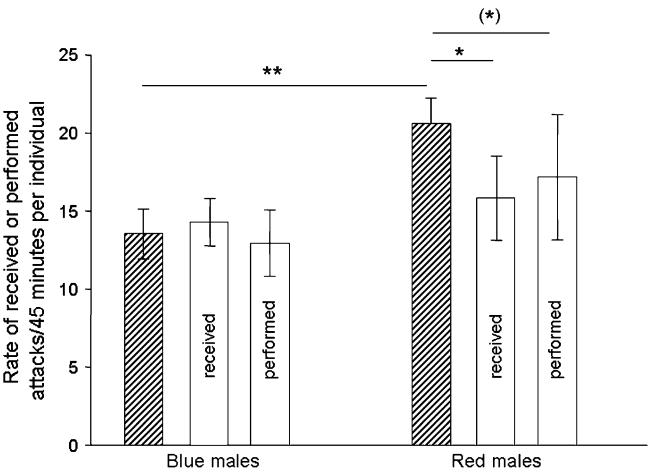

Figure 2
Aggression level, expressed as the rate of received and performed attacks per individual per 45-min observation session. Shown are the means and SEs for each morph in single-color assemblages (hatched) and mixed-color assemblages (open). Received and performed attacks are by definition equal in single-color assemblages and are hence not presented separately. Significant differences are indicated by asterisks [(*) $P = 0.05$, *$P < 0.05$, and **$P < 0.01$], for statistics see text. Sample sizes are 7 blue, 7 red, and 12 mixed-color assemblages.

DISCUSSION

We investigated aggression and social hierarchies among male Lake Victoria cichlids of the genus *Pundamilia* in assemblages composed of males of a single-color morph and in assemblages composed of males of 2 different color morphs. We found that in mixed assemblages, blue and red males did equally well in terms of social dominance, consistent with the possibility of stable coexistence. Even though in dyadic interaction with blue males red males enjoy a significant advantage (Dijkstra et al. 2005), neither the number of red alpha males relative to blue alpha males nor the dominance index score of red over blue (standardized U value) indicated dominance of males of either color in mixed-color assemblages.

The likelihood that red was dominant over blue in the assemblage situation (U values of Table 2) was much lower than that in the dyadic context (0.85; see Figure 2 in Dijkstra et al. 2005). This effect was significant (1-sample t-test: $t = -7.933$, df = 11, $P = 0.000007$). We need to note, however, that this comparison is somewhat problematic because experiments were carried out at different times. Yet, males were raised under the same standardized conditions and bred from the same stock of wild-caught fish for both experiments.

A major difference between mixed-color assemblages and dyadic interactions is that in mixed-color assemblages, red males experienced aggressive encounters not only with blue males but also with other red males in which they lack the dominance advantage of red–blue interactions. Reduced dominance of red over blue in a mixed assemblage may arise from the energetic costs of high levels of red–red aggression. It is also possible that red males experience stronger loser effects (i.e., negative effect on future dominance arising from a defeat, Hsu et al. 2006) than blue males.

A similar contrast in dominance relationship between a dyadic and an assemblage situation was found in great tits in which individuals were classified as either bold or cautious, with the latter being less aggressive than the former (Verbeek et al. 1996, 1999). Bold individuals dominated cautious individuals in dyadic interactions (Verbeek et al. 1996), but the dominance relationship became reversed in groups (Verbeek et al. 1999). Our results illustrate the importance to validate in an experimental assemblage paradigm observations on dominance relationships made in pairwise interactions (see also Chase et al. 2002, 2003). Dominance relationships often form in networks of animals rather than in isolated pairs (Oliveira et al. 1998). Theoretical work emphasizes the need to view the process of territory establishment as a fluid process where punishment and persistence, rather than "winner takes it all," shape observed patterns (Stamps and Krishnan 2001).

Males of both morphs exhibited a bias in aggression toward males of their own color. This could in theory generate negative frequency-dependent selection because rare phenotypes receive fewer attacks than the more abundant phenotype (Mikami et al. 2004; Seehausen and Schluter 2004; Van Doorn et al. 2004). We also found that there was more aggression in red assemblages than in blue assemblages. These results confirm aggression biases and differences in aggression levels

measured in an individual test situation, using simulated intruder choice tests (Dijkstra 2006; Dijkstra et al. 2006; Dijkstra, Seehausen, et al. 2007). Own-color biases in aggression predict that in a mixed-color assemblage, males behave less aggressively than in a single-color assemblage. Therefore, in terms of general expenditure on aggression and establishing territories, the mean fitness of males would be higher in a mixed-color assemblage than in a single-color assemblage at identical total densities. This prediction is particularly strong for red males because there was more aggression in red than in blue assemblages (see, e.g., in bold and cautious pigs, Hessing et al. 1994). Although not highly significant, we found indeed that coexistence of males of both morphs reduced the overall aggression level for red males, whereas there was no difference for blue males. It is likely that for blue males, the presence of the more aggressive red males outweighed the effect of reduced own-color bias–based aggression in mixed-color assemblages.

It follows from the above that being in a mixed-color assemblage is more "beneficial" for red males than for blue males: red males experience less costly male–male competition in the presence of blue males. These results are consistent with a large field data set on distributions of phenotypes in Lake Victoria: populations of red phenotypes of *Pundamilia* always occur in the presence of blue phenotypes (either as conspecific morph or as a closely related species), whereas *Pundamilia* populations of exclusively blue phenotypes are widespread (Seehausen and van Alphen 1999; Seehausen and Schluter 2004).

Male–male competition is one of several different sources of selection that contributes to the maintenance of color polymorphisms in general (Roulin 2004; Gray and McKinnon 2007). Ecological factors and mate choice can also be important sources of selection. In sympatric red and blue *Pundamilia*, other sources of divergent selection measured in the Kissenda population include divergence in habitat (Seehausen et al., forthcoming) and visual adaptation (Seehausen et al. 2008). In other places of the lake, where red and blue *Pundamilia* are well-differentiated species, additional mechanisms include divergent diets (Bouton et al. 1997) and parasites (Maan et al. forthcoming). Finally, male nuptial coloration is not only a cue in aggressive interactions (Dijkstra et al. 2005, 2006) but also a primary target of female mate choice decisions (Seehausen and van Alphen 1998).

We found that red males were more aggressive than blue males. Discrete morphs may vary in agonistic behaviors in other animal species too (e.g., Sinervo and Zamudio 2001; Kingston et al. 2003; Pryke and Griffith 2006; Korzan and Fernald 2007). For example, Pryke and Griffith (2006) studied the color polymorphic Gouldian finch (*E. gouldiae*). They found intrinsic dominance–related behavioral differences between 3 color morphs. In the pygmy swordtail fish (*Xiphophorus pygmaeus*), the gold morph dominated the blue morph (Kingston et al. 2003). Likewise, in the midas cichlid (*Amphilophus citrinellus*), the gold morph dominated the gray morph (Barlow 1983). Korzan and Fernald (2007) studied yellow and blue color morphs of the cichlid *Astatotilapia burtoni*. Like in our study, they found that the 2 color morphs differ in the nature of aggressive behavior, with yellow males being more aggressive toward neighboring territorial males than blue males and also more dominant. Interestingly, they found that both color morphs directed more aggression to rivals with the opposite color. This could favor the more abundant color morph, theoretically destabilizing the color polymorphism. However, in contrast to the *Pundamilia* system, the color polymorphisms in *A. burtoni* is not fixed because males can change colors from yellow to blue and vice versa. In these examples, like in ours, morph-specific behavioral dominance traits are likely to contribute to the selective forces maintaining the color polymorphism in the wild.

Frequency dependence arising from intrasexual competition has been hypothesized to facilitate sympatric speciation by sexual selection (Van Doorn et al. 2004). Although a "rare morph" advantage through intrasexual selection per se does not contribute to the emergence of reproductive barriers, it can pave the way for speciation (Van Doorn et al. 2004; Gray and McKinnon 2007; Dijkstra, Seehausen, et al. 2008) because it removes 2 major hurdles to sympatric speciation: First, it could facilitate the invasion of a novel phenotype against the predominant female mating preference. Second, it can stabilize the coexistence of diverging sets of genotypes, allowing time for the emergence of reproductive barriers (Seehausen and Schluter 2004).

In summary, we found that males of both color morphs biased aggression toward their own color; co-occurrence of another color morph reduced aggression levels for the more aggressive red males, but not for blue males; and dominance relationships between both morphs derived from dyadic interactions were strikingly different than those under more seminatural conditions of a mixed-color assemblage. Our study supports the notion that male–male competition may be a significant selective force in species-rich communities of closely related species with striking variation in male secondary sexual displays.

FUNDING

The Netherlands Organization for Scientific Research (grant 810.64.013, Rubicon-grant 825.07.001).

We thank Dagmar Heidinga and Ruthy Fraterman for help with the experiment. Mhoja Kayeba, Mohamed Haluna, John Mrosso, and Martine Maan are acknowledged for fish collection. Serge Daan, Niels Dingemanse, Neil Metcalfe, and 2 reviewers gave valuable comments on the manuscript. The research was carried out with an animal experiment license (DEC 2812) from Groningen University and complied with current laws in the Netherlands.

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