

Colour biases in territorial aggression in a Neotropical cichlid fish

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Received: 1 May 2013 / Accepted: 24 December 2013 / Published online: 11 January 2014
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Abstract Discrete colour morphs have provided important insights into the evolution of phenotypic diversity. One of the mechanisms that can help to explain coexistence of ecologically similar colour morphs and incipient species is (colour) biased aggression, which has the potential to promote continued existence of the morphs in a frequency-dependent manner. I addressed colour biases in territorial aggression in a field-based study on a Neotropical cichlid fish species, *Amphilophus sagittae*, which has two ecologically indistinguishable colour morphs that mate assortatively. I found that *A. sagittae*, in particular females, were more aggressive towards models of their own colour than those mimicking colours of the other morph. Such a behavioural pattern should result in a selection regime that benefits the rarer morph, and hence could help explain how novel, rare phenotypes may avoid competitive exclusion.

Keywords Aggressive behaviour · Colour polymorphism · Frequency-dependent selection · Sexual selection · Species complex

Introduction

Species and species groups with discrete colour morphs can provide important insights into mechanisms that underlie individual variation and phenotypic diversity (Roulin 2004; Bond 2007; Gray and McKinnon 2007; Puebla et al. 2007). Mechanisms that can contribute to coexistence of divergent colour morphs include, among others, niche separation, assortative mating, fluctuations in selection regimes and frequency-dependent predation pressure (Roulin 2004; Bond 2007; Gray and McKinnon 2007; Puebla et al. 2007). A frequency-dependent advantage of colouration has been demonstrated, for example, in guppies (*Poecilia reticulata*), in which individuals with a rare colour pattern were found to have a clear survival advantage compared to those with common colour patterning, due to lower predation pressure on the former (Olendorf et al. 2006).

Biases in aggressive behaviour may also have an important role in the context of evolution of colour patterns and coexistence of colour morphs. For example, in certain damselflies, males are more aggressive towards heterospecific males that have colour patterns similar to their own than towards heterospecifics with dissimilar colour patterns. As a result, colour patterns of the two species tend to be more dissimilar when they live in sympatry than in allopatric populations (Tynkkynen et al. 2004; Anderson and Grether 2010). Multiple colour morphs exhibiting different levels of aggression coexist, e.g. in Gouldian finches (*Erythrura gouldiae*): red-headed males dominate black-headed and yellow-headed males (Pryke and Griffith 2006). However, when the relative density of the aggressive red-headed morph is high, males of this morph suffer from increased stress and reduced physiological capacity, stabilising the proportions of the different morphs in a frequency-dependent manner (Pryke et al. 2007). The level of aggression may

Communicated by Aaron J Wirsing.

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also depend on the colour of both the territory holder and the territorial intruder, as is the case with vocal responses of males of the polychromatic white-throated sparrows (*Zonotrichia albicollis*) (Horton et al. 2012).

In general, aggression biases may help establishment of novel (colour) morphs and incipient species either by benefitting them directly or by resulting in a selection regime that benefits whichever phenotype has a low frequency (van Doorn et al. 2004; Mikami et al. 2004; Seehausen and Schluter 2004). In particular, one of the colour morphs may be more aggressive (Mikami et al. 2004; Dijkstra et al. 2010). This is especially interesting if the more aggressive morph has a benefit only when it is rare, e.g. due to the high costs of aggressive encounters when it is common (Pryke et al. 2007). It is also possible that less aggression is directed towards individuals of certain colours (Pryke 2009). One interesting possibility for such a situation is a difference in reproductive strategies employed by individuals of different colours. The reproductive strategies, in turn, could then be under balancing or frequency-dependent selection. Finally, aggression may be biased towards individuals that have colours similar to the aggressor (Dijkstra et al. 2007; Pauers et al. 2008). Such a pattern may result in a frequency-dependent advantage for the individuals of the rare colour type because these individuals should experience lower overall levels of aggression (given that the common morph is encountered more often by all individuals).

Increased aggression toward similar-looking individuals may be associated not only with enhanced coexistence of distinct morphs but also their divergence if the aggression bias is associated with assortative mating (Seehausen and Schluter 2004; Dijkstra and Groothuis 2011). Indeed, colour-biased aggression is also relevant in the context of speciation, with recent theoretical considerations suggesting that a major moderator of net speciation rates may be the shortness of time that most novel incipient species persist before being eliminated by competition, introgression, or other hazards faced by small populations (Rundell and Price 2009; Etienne and Rosindell 2012; Rosenblum et al. 2012). Sexual selection, in particular, is thought to produce ecologically equivalent incipient species, the coexistence of which has been regarded unlikely (M'Gonigle et al. 2012). However, it is not known how commonly colour-biased aggression functions in a frequency-dependent fashion in wild populations. This is surprising because, to understand evolutionary processes maintaining a polymorphism, or alternatively leading to divergence between morphs, one of the first steps should be to characterise the nature of morph-biased aggression.

In this study, I investigated the importance of aggression biases in *Amphilophus sagittae*, a species belonging to a rapidly diversifying lineage of Neotropical crater lake fish, the Midas cichlid species complex (Barluenga

and Meyer 2010; Elmer et al. 2010; Geiger et al. 2010), hereafter abbreviated as MCSC. Many, but not all, MCSC populations include two colour morphs, often called 'dark' and 'gold', the latter typically being orange in colour (Barlow 1983; Elmer et al. 2009, 2010). I manipulated territory intrusions in the field in order to assess the following three mechanisms, which are important in the context of evolution of colour polymorphisms, but which have typically not been considered together in any one study. Specifically, I tested whether (1) one colour morph is more aggressive than the other, (2) less aggression is directed towards individuals of a certain colour, and (3) aggression is biased towards individuals that have colours similar to the aggressor.

Materials and methods

The study was conducted in Crater Lake Xiloá, Nicaragua (12°12.8'N, 86°19.0'W), between December 2010 and January 2011 using SCUBA. In the focal species, *A. sagittae*, as with other members of the MCSC (and related cichlids in general), notable aggression in the wild is directly linked to reproduction. In particular, a pair ready to spawn claims a territory with a suitable cavity or structure for depositing eggs and later hiding small juveniles from would-be predators. The territory is then aggressively defended until the juveniles are ready to disperse, approximately a month later (McKaye 1977; Rogers 1988; Barlow 2000; Lehtonen 2008, 2011a, b, and personal observations). Aggression is therefore mostly targeted towards (1) brood predators (both conspecific and heterospecific), (2) competitors for territory space (both conspecific and heterospecific), and (3) sexual competitors (McKaye 1977; Barlow 2000; Lehtonen et al. 2010, 2011b, 2012).

Similar to a very closely related species *A. xiloensis*, which is also endemic to Lake Xiloá, *A. sagittae* has two assortatively mating colour morphs, 'dark' and 'gold'. In *A. xiloensis*, the colour morphs have genetically differentiated (and hence could be regarded as incipient species), whereas in *A. sagittae*, the genetic differentiation status of the colour morphs is currently unknown (Elmer et al. 2009). Although gold × gold pairs are much more frequent in both species than expected if mating was random (in *A. sagittae*, approximately 4 vs. 0.5 % of the pairs), 'mixed pairs' (with one individual dark and the other gold) are nevertheless not rare (5 %), due to only 7 % of individuals in the breeding population being 'gold' (Elmer et al. 2009). Lake-wide, the distribution of these 'gold'-coloured individuals seems patchy (Elmer et al. 2009), but is relatively even (notwithstanding colour-assortative mating) at the site where this study was conducted (personal observations). Territories of the different pair types included in the current study were

observed at depths that were not significantly different from each other: dark \times dark [10 ± 1.4 m (mean \pm SD), $n = 30$], gold \times gold (11 ± 2.1 m, $n = 21$) and mixed (11 ± 1.9 m, $n = 33$) (ANOVA, $F_{2,81} = 1.67$, $P = 0.20$). For each pair type, the nearest neighbouring territory holders were most often either a pair of dark *A. sagittae* or Nicaragua cichlids, *Hypsophrys nicaraguensis* (personal observations).

I simulated aggressive encounters by using models of territory intruders (hereafter referred to as dummies; see below for details). Each replicate was initiated by placing a dummy at a distance approximately 50 cm from the centre of the *A. sagittae* territory. This positioning corresponds to a typical distance territory holders swim when deterring territorial intruders (Lehtonen et al. 2011b, 2012). After a habituation period of one minute, I counted the total number of aggressive encounters by both territory owners (male and female) towards the dummy for 5 min, giving the total ‘aggression rate’. In addition to noting the rate of aggression, I also classified the mode of each act of aggression as either ‘display’ (typically flared fins and puffed out opercula without rushed movements) or ‘mobile’ (attacks and bites). Such classification of aggression is relevant for members of the MCSC, because displays in these fish represent no immediate threat of physical injury to the target and are less costly (e.g. energy expenditure and risk of injuries to the aggressor) than attacks and bites (Lehtonen et al. 2010, 2011b, 2012). I also approximated the total length of each territory holder. Calibrated underwater body size estimates have earlier been found to be accurate (Lehtonen et al. 2011a) and repeatable (Lehtonen, unpublished). I calibrated my length estimates frequently (usually daily) by comparing live fish to scales of known lengths.

I tested for the following patterns that are relevant in the context of (frequency-dependent) selection by aggression biases: (1) territory holders of one colour (morph) are more aggressive than individuals of another colour, (2) more intense—or a higher rate of—aggression is directed towards individuals of a certain colour, and (3) territorial defenders bias aggression towards fish of their own colour. Here, the total rate of aggressive responses was defined as the sum of all aggressive events (displays + mobile aggression) per 5-min observation. The counts were first square root-transformed to satisfy normality and then analysed using generalised linear mixed models. The main model was fitted by maximising the log-likelihood, with territory holder sex, territory holder colour, dummy colour and size of the dummy relative to the territory holder as explanatory fixed factors, with pair/territory ID treated as a random factor to take into account the potential interdependence between the actions of the female and male forming a pair and defending the same territory. This model was simplified by stepwise removal of non-significant interaction terms using likelihood ratio tests (G^2 with a removal

criterion of $P > 0.10$). Mechanisms 1 and 2, above, were assessed directly by their main effects in the model, whereas mechanism 3 was identified by the presence or absence of a significant interaction between territory holder colour and dummy colour. To examine the three mechanisms in more detail, and to facilitate comparisons to previous studies (see Dijkstra and Groothuis 2011), I then analysed separately (1) males and females (a linear model without the random factor for each sex), (2) dark and gold morphs, and (3) dark and ‘gold’ dummies. Finally, to investigate biases in the type of aggression (displays vs. mobile aggression), I again used generalised linear mixed models. Specifically, the square root-transformed counts of aggression were analysed with aggression type, territory holder colour, dummy colour and size of the dummy relative to the territory holder as fixed factors, and the identity of the pair/territory holder as a random factor (see above). Note here that total aggression was broken down to a response variable that consisted of both the counts of display aggression and mobile aggression (see also Lehtonen et al. 2011b). The types of aggression were analysed separately for (1) sexes, (2) the two colour morphs, and (3) dummy colours. Non-significant interaction terms (G^2 tests with $\alpha = 0.10$) were again removed in a stepwise fashion. I used R 3.0.0 software (R Development Core Team) for all analyses.

The experiment involved in total 84 biparentally defended *A. sagittae* territories. Specifically, I used all encountered gold \times gold ($n = 21$) and mixed ($n = 33$) pairs that had offspring of suitable size (already hatched but not yet longer than 2.5 cm), whereas dark \times dark pairs with similarly sized offspring ($n = 30$) were chosen haphazardly in the vicinity of gold \times gold and mixed pairs. However, I avoided sampling territories too close to each other so that the subjects would not get pre-exposed to the dummies in an uncontrolled fashion. All the sampled territories were within a ‘mixed’ habitat, which is characterised by pebbles lying on a finer substratum of sand and organic material. Occasionally, the species also breeds using cavities associated with the crater wall, i.e. habitat of pure rock (Lehtonen et al. 2012). None of these less common territories were involved here, controlling for the potential influence of habitat type on aggressive encounters (Danley 2011). Territories without swimming juveniles were sparsely encountered and were only sampled after the juveniles had emerged, in order to control for the phase of the reproductive cycle. Each dummy mimicked either a dark or orange-coloured (i.e. ‘gold’) territory intruder, either 16, 20 or 24 cm long. These three size categories corresponded to the size distribution of *A. sagittae* territory holders sampled during this study [dark females: 18 ± 1.3 cm (mean \pm SD), $n = 49$; gold females: 18 ± 1.3 cm, $n = 35$; dark males: 21 ± 1.2 cm, $n = 43$; gold males: 22 ± 1.2 cm, $n = 41$]. In total, I had 30 unique dummies with 2×3 (=6) different

colour \times size combinations. I used each dummy only once per territory type (i.e. d \times d, g \times g, mixed), except for three haphazardly chosen dummies, each of which were presented at two different ‘mixed’ pair territories (as I had prepared only 30 dummies but found 33 ‘mixed pair’ territories). None of the territories was subject to more than one dummy presentation. I chose to manipulate intruder encounters using dummies because (1) the rate of natural encounters with the more rare gold individuals is in most cases too low for logistically feasible underwater observations, and (2) natural, uncontrolled encounters are potentially confounded, for example if the differently coloured individuals have slightly different habitat preferences, or (3) differ in their aggressiveness, submissiveness, or other behavioural patterns, while (4) the field-based approach also avoids the problem that aggression biases may differ between wild and laboratory-raised individuals (Dijkstra and Groothuis 2011). Furthermore, Midas cichlids are too large to be caught and presented to territory owners in transparent jars (see Dijkstra et al. 2006), a method that could otherwise help to solve points (1), (2) and (4) above.

Instead of using uniform dark grey and orange dummies that might also have elicited aggressive responses (see Rowland 1999; Ochi and Awata 2009), I decided to make each dummy look more realistic, as well as unique (hence avoiding pseudoreplication; see, e.g., Hurlbert 1984; Kroodsmas et al. 2001), by gluing waterproof, photographic colour prints of a lateral side of a live MCSC individual (of unknown sex) onto both lateral sides of a fish-shaped floating plate (thickness = 6 mm). The dummy was then attached to a sinker with a thin, transparent fishing line, allowing it to float in a natural position approx. 25 cm above the lake bottom. As a too low number of high quality photographs of *A. sagittae* were available, due to the relative rarity of the gold morph in that species (Elmer et al. 2009), I also used photographs of *A. xiloensis* and *A. cf. labiatus* (see “Discussion” for further details). Both naturally and artificially coloured dummies have been successfully used in a wide array of studies on fish (Rowland 1999), including Midas cichlids (Barlow and Siri 1994) and other cichlid species (Ochi and Awata 2009). In the current study, dummies elicited aggressive responses in 77 of the 84 females (92 %) and 71 out of the 84 males (85 %). Three females (3.6 %) and seven males (8.3 %) fled from the vicinity of the dummy before exhibiting any aggression.

Results

Total aggression rate

With regard to the total counts of aggression (per the 5-min observation period), after simplification of the main linear

mixed model (i.e. stepwise removal of interactions with $P > 0.10$), there were significant interactions between the sex of the territory holder and the dummy size relative to territory holder size ($G^2 = 5.118$, $P = 0.024$), as well as between the territory holder colour and dummy colour ($G^2 = 5.302$, $P = 0.021$). The former result implies that relatively large dummies were subject to the highest rates of aggression especially by males (Fig. 1), while the latter result was due to territory holders directing more aggression towards dummies of their own colour than the other colour (agreeing with mechanism 3; Fig. 2). I next considered females and males in separate models. In females, the interaction between female colour and dummy colour was significant (Fig. 2a; the final, simplified model in Table 1). In males, in turn, all interactions were non-significant ($P > 0.10$) and hence dropped from the final model. The simplified model indicated that the only significant main effect was dummy size relative to male size (Table 2): territory-holding males were more aggressive towards large dummy opponents (Fig. 1). Next, I analysed the two colour morphs separately. In dark fish, after stepwise model simplification, the only significant effect was dummy colour ($t_{61} = 2.424$, $P = 0.018$): dark fish directed a higher rate of aggression towards dark than ‘gold’ dummies (Fig. 2). Regarding the gold morph, there were borderline interactions between sex and the relative dummy size ($G^2 = 2.775$, $P = 0.096$), as well as between the dummy colour and relative size ($G^2 = 2.845$, $P = 0.092$): gold males rather than females tended to react more strongly to relatively large dummies, and the own colour aggression bias (i.e. higher aggression towards ‘gold’ dummies) tended

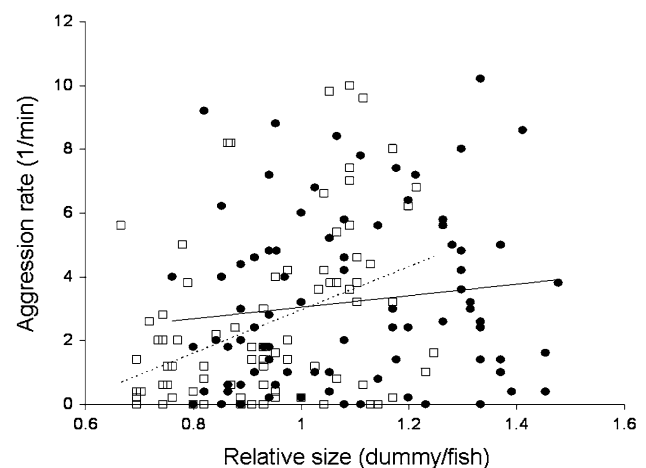


Fig. 1 The total rate aggression by *Amphilophus sagittae* in relation to the relative size of the dummy intruder. Females [aggression = $1.784(\text{size}) + 1.265$; $R^2 = 0.005$; $n = 84$] are marked with solid, round symbols and with a solid trend line, whereas males [aggression = $6.762(\text{size}) - 3.813$; $R^2 = 0.156$; $n = 84$] are indicated with square symbols and a dashed trend line

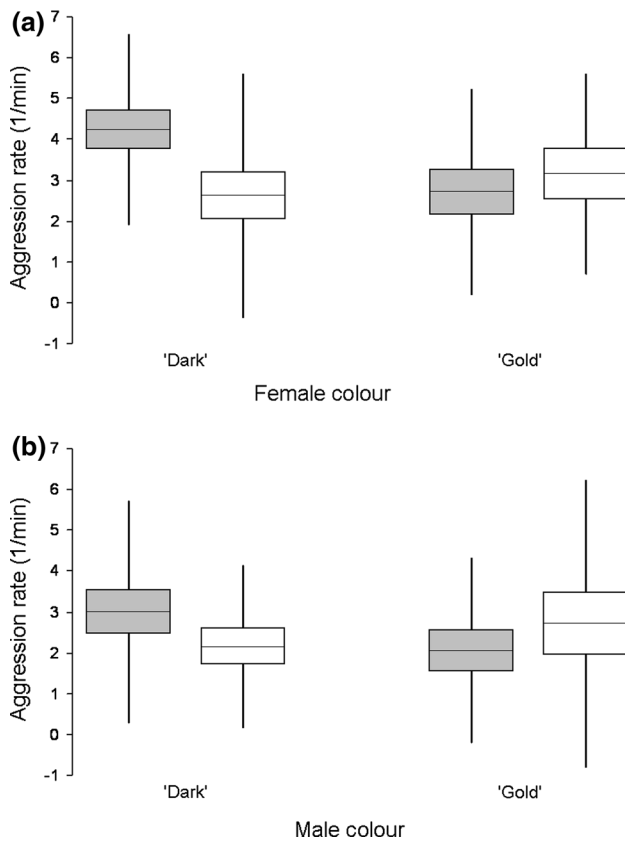


Fig. 2 The total rate of aggression towards dummy intruders by **a** females and **b** males. Shaded boxes indicate dark dummies and white boxes are for dummies with orange, ‘gold morph’ coloration. Central horizontal lines within the boxes indicate means, margins of the boxes are for standard errors, and whiskers indicate standard deviations. Sample sizes from left to right for **a** females: 23, 26, 20, 15; and **b** males: 24, 20, 19, 21

Table 1 The simplified linear model for the total rate of aggression by territorial *Amphilophus sagittae* females towards dummy intruders

Effect	<i>F</i> value	<i>df</i>	<i>P</i>
Relative size (dummy/female)	1.427	1, 79	0.24
Female colour	0.552	1, 79	0.46
Dummy colour	2.636	1, 79	0.11
Female colour × Dummy colour	4.245	1, 79	0.043

The final model includes all three main factors and a significant interaction term

to be stronger toward relatively large than small dummies. Finally, when considering these patterns from the perspective of the differently coloured dummies, dark dummies were confronted at a higher rate by dark than gold territory holders ($t_{40} = 2.482$, $P = 0.017$), whereas total attack rates towards ‘gold’ dummies depended on the interaction between sex and relative size ($G^2 = 5.871$, $P = 0.015$). In

Table 2 The simplified linear model for the total rate of aggression by territorial males towards dummy intruders

Effect	<i>F</i> value	<i>df</i>	<i>P</i>
Relative size (dummy/male)	13.35	1, 80	0.0005
Male colour	1.044	1, 80	0.31
Dummy colour	0.269	1, 80	0.61

Only the three main factors remain in the final model

particular, males—more than females—biased their aggression towards large gold-coloured dummies.

Types of aggression

The different types of aggressive behaviour (displays vs. attacks + bites) mostly matched the above patterns of total aggression rate. In particular, there was a significant three-way interaction between aggression type, female colour and dummy colour ($G^2 = 8.348$, $P = 0.0039$): a larger proportion of female aggression consisted of mobile attacks and bites (vs. immobile displays) when females faced a dummy of their own colour than the other colour (Fig. 3). Similarly, in males, the results for the use of different types of aggressive behaviour followed a pattern very similar to the total rate of male aggression (significant aggression type × dummy/male size interaction: $G^2 = 7.019$, $P = 0.0081$, whereas all other interactions were non-significant and hence removed from the model). In other words, the larger the dummy (relative to the male), the larger the proportion of male aggression was mobile aggression (attacks + bites) instead of displays, whereas no other factors had a significant effect on the use

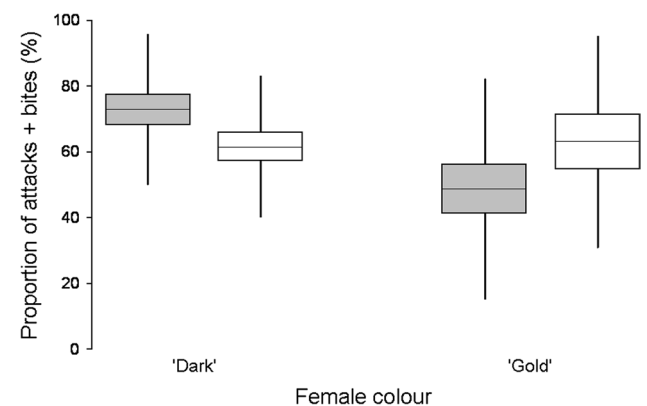


Fig. 3 The proportion of attacks and bites (i.e. mobile aggression) of all acts of female aggression towards ‘dark’ (shaded boxes) and ‘gold’ (white boxes) dummies. Central horizontal lines within the boxes indicate means, margins of the boxes are for standard errors, and whiskers indicate standard deviations. Sample sizes from left to right: 22, 22, 19, 14

of different aggression modes in males. When analysing dark-coloured fish separately, I found a borderline three-way interaction between aggression type, dummy colour and sex ($G^2 = 3.648$, $P = 0.056$): dark females—more than dark males—tended to bias the use of the more intense aggression type (i.e. mobile aggression) towards dark dummies. In the gold morph, in turn, the same pattern (three-way interaction between aggression type, dummy colour and sex) was significant ($G^2 = 6.004$, $P = 0.014$), i.e. gold females—more than males—biased intense aggression towards gold dummies. Finally, I assessed the use of the two aggression types separately for dark and ‘gold’ dummies. The pattern of aggression (types) towards dark dummies was characterised by a three-way interaction between aggression type, fish colour and sex ($G^2 = 5.470$, $P = 0.019$): the own-colour bias in aggression type was more evident in females than males. The own colour aggression bias towards ‘gold’ dummies, in turn, was more pronounced when the dummy was large relative to the territory holders than when the dummy was relatively small (aggression type \times fish colour \times relative size: $G^2 = 5.145$, $P = 0.023$).

Discussion

The main aim of this study was to test for the following patterns of biased aggression, each of which could help a rare phenotype to become established in a population: (1) the rare phenotype is more aggressive than the common phenotype, (2) more aggression is directed towards individuals of the more common phenotype, and (3) individuals bias aggression towards their own phenotype. I did not find any evidence for the first mechanism, and, while the results did not directly contradict the second pattern (Figs. 2, 3), the main model explicitly supported the third mechanism. In other words, the main model suggested that, at the population level (i.e. sexes and colour morphs combined), *A. sagittae* territory holders bias aggression towards their own morph. Because individuals—regardless of their phenotype—are more likely to encounter individuals of the common phenotype, the own colour aggression bias should result in a higher level of aggression being directed towards individuals of the common phenotype.

The evolutionary implications of this aggression bias depend on its realised fitness consequences. Given that both types of individuals mostly encounter competitors of the more common type (which seems to be the case here), the aggression bias should result in a selection regime that favours the less common morph or phenotype in a frequency-dependent manner. As a result, with everything else being equal, and in the absence of any other colour-dependent selection regimes, we would expect the rarer

morph to become more common until the fitness advantage disappears at equal morph frequencies. In *A. sagittae*, as well as in the MCSC in general, however, the gold morph is much less numerous than the dark morph (Elmer et al. 2009, 2010). This suggests that some other factors interact with colour-biased aggression, influencing the relative morph frequencies. Predation pressure has been implicated as one such factor that affects the frequency of the brightly-coloured gold individuals (e.g. McKaye 1980), although a higher predation pressure on gold individuals in the MCSC has never been convincingly demonstrated (see also Annett 1989). Despite the other, currently largely unknown selection pressures, the colour-dependent aggression bias may nevertheless provide an advantage to gold individuals when they are particularly rare, potentially resulting in a higher relative frequency than they would attain in the absence of the aggression bias. In this regard, future studies assessing the relationship between colour-biased aggression and relevant fitness measures—such as success in territory acquisition and offspring survival—would be highly interesting.

Why should *A. sagittae* bias their aggression towards individuals that are of the same colour as they are? In general, the more similar an intruder's phenotype to that of the territory holder, the higher risk of resource and sexual competition the intruder may pose; individuals with same colouration may be recognised as more serious competitors (Grether et al. 2009). Evolution of aggression biases in the context of competitor recognition has earlier been demonstrated by, for example, brook sticklebacks (*Culaea inconstans*), which are more aggressive towards competing ninespine sticklebacks (*Pungitius pungitius*) when the two have earlier lived in sympatry versus allopatry (Peiman and Robinson 2007). In the context of the current study, it is important to note that, in biparental cichlids, including *A. sagittae*, aggression mostly takes place in the context of reproduction rather than foraging (see, e.g., Barlow 2000). In this regard, it is interesting that I found evidence suggesting that colour-dependent aggression might be stronger in females than males (see also Figs. 2, 3). In particular, the own morph bias was significant for females but not males when the two were analysed separately, and there was also a significant gender effect in two out of the eight partial dataset analyses that included both sexes. One possible explanation for this pattern is that, in *A. sagittae*, as in many other species, males and females have evolved different roles in territory defence (Rogers 1988; Lehtonen et al. 2011b), with males being more prone to desert their mate and offspring, presumably to seek for additional mating opportunities (Lehtonen et al. 2011a, b). Therefore, females, more so than males, may recognise individuals of their own morph as sexual competitors (for a review on competitor recognition, see Grether et al. 2009). Similarly, males—rather than females—may regard the largest

dummies as their immediate competitors, with the largest dummy size (24 cm) being out of the normal female size range. Males may therefore be motivated to bias their aggression towards large dummies more than are females (Fig. 1). A subset of the detailed analyses also indicated that the own morph aggression bias may have been somewhat more pronounced in dark than gold individuals (see also Figs. 2, 3). At the population level, behavioural patterns of dark individuals can be expected to have a greater impact, as they are much more numerous than gold individuals (Elmer et al. 2009).

Due to logistic constraints, some photographs used for constructing the dummies were of congeneric species, resulting in dummies that—to human observers, at least—looked like *A. sagittae* individuals with respect to colour (dark or gold) and general body shape. This does not, however, guarantee that the fish perceived the models in a similar fashion. Indeed, while *A. sagittae* territory holders seemed to be sensitive at least to colour and size of the dummies, the total range of factors that influence their aggressive responses is not currently known. Fish may also see printed colours differently compared to human observers (Baldauf et al. 2008). However, even in the case when the focal individuals did consider some dark and gold dummies as closely-related heterospecific individuals, the approach was relevant. Specifically, in the habitats occupied by the MCSC, interactions among closely related species in the contexts of territory space competition and offspring defence are very common (McKaye 1977; Lehtonen et al. 2010) and play an important role in defining reproductive success (McKaye 1977; Lehtonen 2008). However, if such species recognition did take place and if the psychological mechanism that allowed *A. sagittae* to make colour-specific aggression adjustments was very fine-tuned rather than general, then some of the above considerations on the factors that contributed to evolution of the observed own-colour aggression bias (especially sexual competition) would not be valid, and some other factors could instead explain the aggression bias.

Prior to the current study, Barlow and coworkers had tested colour-biased aggression in the MCSC using a laboratory stock of Midas cichlids (*A. citrinellus*). In particular, they showed that, in a confined space and outside of the breeding context, gold individuals are socially more dominant than dark individuals (Barlow and Ballin 1976; Barlow and Wallach 1976; Barlow 1983). This dominance was thought to be due to a fear response induced by the bright coloration (Barlow and Wallach 1976; Barlow 1998), as no difference in aggression between the colour morphs was found (Barlow and Wallach 1976; Barlow and Siri 1994; Barlow 1998). Furthermore, aggression of single individuals towards dummy intruders presented outside their aquaria was found to be either biased towards

gold-coloured intruders or was not colour-biased at all, depending on the context (Barlow and Siri 1994). However, these studies were conducted either on juveniles (Barlow and Ballin 1976; Barlow and Wallach 1976) or non-paired adults outside of the reproductive context (Barlow 1983; Barlow and Siri 1994), typically in small tanks inducing escalated fights (Barlow 1983; Barlow et al. 1986), which are rarely observed in the wild (none during this study). It is therefore unclear to what extent the previous results are comparable to the current ones conducted on wild *A. sagittae*. Although the previous results did not demonstrate a frequency-dependent mechanism, they nevertheless agree with the current ones in that they demonstrated biases in social interactions that should benefit the less numerous ‘gold’ individuals.

Besides the work of Barlow and coworkers, colour morph-specific aggression biases have previously been particularly well documented by laboratory-focused studies on haplochromine cichlids (Dijkstra and Groothuis 2011; see Dijkstra et al. 2006 for a field experiment). Like the MCSC, these fish have also speciated at exceptionally high rates (Kocher 2004; Seehausen 2006), but differ from the MCSC in many important ways, such as (haplochromines always mentioned first): (1) occurrence in very large African rift lakes versus small Neotropical crater lakes, (2) female only mouth-brooding versus mostly biparental offspring care, (3) male only breeding territories versus biparental breeding territory acquisition and defence, and (4) colour polymorphism usually being evident in one sex only versus both sexes sharing the same colour polymorphism. Hence, the two lineages have very different mating systems, as well as genetic architectures of colour polymorphism (Dickman et al. 1988; Dijkstra and Groothuis 2011). Together with the earlier findings, the current results therefore suggest that colour-sensitive aggression (which has the potential to result in a frequency-dependent selection regime) can occur in systems with very different mating systems and (colour) polymorphisms.

The results of this study indicate that further assessments of the importance of frequency-dependent aggression biases would be highly interesting. For example, because aggression in the MCSC and other systems can be biased in relation to quite subtle (colour) cues (Lehtonen et al. 2010), aggression biases that can result in frequency-dependent selection may be important also within and among species that do not include spectacularly coloured individuals. It could therefore be useful to assess the topic in a larger array of different taxa, as well as to link quantitative assessments of colour perception with studies on maintenance of colour polymorphism. Furthermore, if the observed pattern of colour-biased aggression turns out to translate into fitness effects, it would provide a mechanism that could contribute to the particularly high rate of diversification within

the MCSC (Barluenga et al. 2006; Elmer et al. 2010, 2013), hence having a direct bearing on the longstanding debate about the relevance of sympatric speciation (Coyne and Orr 2004; Gray and McKinnon 2007; Sobel et al. 2010).

Acknowledgments This work benefited from discussions, comments and logistic support by Alan Hudson, Bob Wong, Eric van den Berghe, Isabel Santos Magalhaes, Karine Gagnon, Ken McKaye, Lasse Lehtonen, Lucia Paiz Medina, Luis Canda, Marta Barluenga, Olivia Roth, Pekka and Jonna Katajisto, Will Sowersby, and anonymous referees. Funding was provided by the Department of Biology at the University of Turku.

References

- Anderson CN, Grether GF (2010) Interspecific aggression and character displacement of competitor recognition in *Hetaerina* damselflies. *Proc R Soc Lond B* 277:549–555. doi:10.1098/rspb.2009.1371
- Annett C (1989) Differential predation on colour morphs of the Midas cichlid, *Cichlasoma citrinellum*. *Anim Behav* 37:935–942. doi:10.1016/0003-3472(89)90138-3
- Baldauf SA, Kullmann H, Bakker TCM (2008) Technical restrictions of computer-manipulated visual stimuli and display units for studying animal behaviour. *Ethology* 114:737–751. doi:10.1111/j.1439-0310.2008.01520.x
- Barlow GW (1983) Do gold Midas cichlid fish win fights because of their color, or because they lack normal coloration? A logistic solution. *Behav Ecol Sociobiol* 13:197–204. doi:10.1007/BF00299923
- Barlow GW (1998) Sexual-selection models for exaggerated traits are useful but constraining. *Am Zool* 38:59–69. doi:10.1093/icb/38.1.59
- Barlow GW (2000) Cichlid fishes: nature's grand experiment in evolution. Perseus, Cambridge
- Barlow GW, Ballin PJ (1976) Predicting and assessing dominance from size and coloration in the polychromatic Midas cichlid. *Anim Behav* 24:793–813. doi:10.1016/S0003-3472(76)80010-3
- Barlow GW, Siri P (1994) Polychromatic Midas cichlids respond to dummy opponents: color, contrast and context. *Behaviour* 130:77–112. doi:10.1163/156853994X00154
- Barlow GW, Wallach SJ (1976) Colour and levels of aggression in the Midas cichlid. *Anim Behav* 24:814–817. doi:10.1016/S0003-3472(76)80011-5
- Barlow GW, Rogers W, Fraley N (1986) Do Midas cichlids win through prowess or daring? It depends. *Behav Ecol Sociobiol* 19:1–8. doi:10.1007/BF00303836
- Barluenga M, Meyer A (2010) Phylogeography, colonization and population history of the Midas cichlid species complex (*Amphilophus* spp.) in the Nicaraguan crater lakes. *BMC Evol Biol* 10:326. doi:10.1186/1471-2148-10-326
- Barluenga M, Stölting KN, Salzburger W, Muschick M, Meyer A (2006) Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature* 439:719–723. doi:10.1038/nature04325
- Bond AB (2007) The evolution of color polymorphism: crypticity, searching images, and apostatic selection. *Annu Rev Ecol Evol Syst* 38:489–514. doi:10.1146/annurev.ecolsys.38.091206.095728
- Coyne JA, Orr HA (2004) Speciation. Sinauer, Sunderland
- Danley PD (2011) Aggression in closely related Malawi cichlids varies inversely with habitat complexity. *Environ Biol Fish* 92:275–284. doi:10.1007/s10641-011-9838-7
- Dickman MC, Schliwa M, Barlow GW (1988) Melanophore death and disappearance produces color metamorphosis in the polychromatic Midas cichlid (*Cichlasoma citrinellum*). *Cell Tissue Res* 253:9–14. doi:10.1016/j.tree.2011.09.002
- Dijkstra PD, Groothuis TGG (2011) Male-male competition as a force in evolutionary diversification: evidence in haplochromine cichlid fish. *Int J Evol Biol* 2011:689254. doi:10.4061/2011/689254
- Dijkstra PD, Seehausen O, Gricar BLA, Maan ME, Groothuis TGG (2006) Can male-male competition stabilize speciation? A test in Lake Victoria haplochromine cichlid fish. *Behav Ecol Sociobiol* 59:704–713. doi:10.1007/s00265-005-0100-1
- Dijkstra PD, Seehausen O, Pierotti MER, Groothuis TGG (2007) Male-male competition and speciation: aggression bias towards differently coloured rivals varies between stages of speciation in a Lake Victoria cichlid species complex. *J Evol Biol* 20:496–502. doi:10.1111/j.1420-9101.2006.01266.x
- Dijkstra PD, Lindström J, Metcalfe NB, Hemelrijk CK, Brendel M, Seehausen O, Groothuis TGG (2010) Frequency-dependent social dominance in a color polymorphic cichlid fish. *Evolution* 64:2797–2807. doi:10.1111/j.1558-5646.2010.01046.x
- Elmer KR, Lehtonen TK, Meyer A (2009) Color assortative mating contributes to sympatric divergence of neotropical cichlid fish. *Evolution* 63:2750–2757. doi:10.1111/j.1558-5646.2009.00736.x
- Elmer KR, Kusche H, Lehtonen TK, Meyer A (2010) Local variation and parallel evolution: morphological and genetic diversity across a species complex of neotropical crater lake cichlid fishes. *Philos Trans R Soc Lond B* 365:1763–1782. doi:10.1098/rstb.2009.0271
- Elmer KR, Lehtonen TK, Fan S, Meyer A (2013) Crater lake colonization by Neotropical cichlid fishes. *Evolution* 67:281–288. doi:10.1111/j.1558-5646.2012.01755.x
- Etienne RS, Rosindell J (2012) Prolonging the past counteracts the pull of the present: protracted speciation can explain observed slowdowns in diversification. *Syst Biol* 61:204–213. doi:10.1093/sysbio/syr091
- Geiger MF, McCrary JK, Schliwen UK (2010) Not a simple case: a first comprehensive phylogenetic hypothesis for the Midas cichlid complex in Nicaragua (Teleostei: Cichlidae: *Amphilophus*). *Mol Phylogenet Evol* 56:1011–1024. doi:10.1016/j.ympev.2010.05.015
- Gray SM, McKinnon JS (2007) Linking color polymorphism maintenance and speciation. *Trends Ecol Evol* 22:71–79. doi:10.1016/j.tree.2006.10.005
- Grether GF, Losin N, Anderson CN, Okamoto K (2009) The role of interspecific interference competition in character displacement and the evolution of competitor recognition. *Biol Rev* 84:617–635. doi:10.1111/j.1469-185X.2009.00089.x
- Horton BM, Hauber ME, Maney DL (2012) Morph matters: aggression bias in a polymorphic sparrow. *PLoS ONE* 7:e48705. doi:10.1371/journal.pone.0048705
- Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. *Ecol Monogr* 54:187–211. doi:10.2307/1942661
- Kocher TD (2004) Adaptive evolution and explosive speciation: the cichlid model. *Nat Rev Genet* 5:288–298. doi:10.1038/nrg1316
- Kroodsma DE, Byers BE, Goodale E, Johnson S, Liu W-C (2001) Pseudoreplication in playback experiments, revisited a decade later. *Anim Behav* 61:1029–1033. doi:10.1006/anbe.2000.1676
- Lehtonen TK (2008) Convict cichlids benefit from close proximity to another species of cichlid fish. *Biol Lett* 4:610–612. doi:10.1098/rsbl.2008.0378
- Lehtonen TK, McCrary JK, Meyer A (2010) Territorial aggression can be sensitive to the status of heterospecific intruders. *Behav Process* 84:598–601. doi:10.1016/j.beproc.2010.02.021
- Lehtonen TK, Wong BBM, Lindström K, Meyer A (2011a) Species divergence and seasonal succession in rates of mate desertion in closely related Neotropical cichlid fishes. *Behav Ecol Sociobiol* 65:607–612. doi:10.1007/s00265-010-1061-6

- Lehtonen TK, Wong BBM, Svensson PA, Meyer A (2011b) Adjustment of brood care behaviour in the absence of a mate in two species of Nicaraguan crater lake cichlids. *Behav Ecol Sociobiol* 65:613–619. doi:[10.1007/s00265-010-1062-5](https://doi.org/10.1007/s00265-010-1062-5)
- Lehtonen TK, McCrary JK, Meyer A (2012) Introduced predator elicits deficient brood defence behaviour in a crater lake fish. *PLoS ONE* 7:e30064. doi:[10.1371/journal.pone.0030064](https://doi.org/10.1371/journal.pone.0030064)
- M'Gonigle LK, Mazzucco R, Otto SP, Dieckmann U (2012) Sexual selection enables long-term coexistence despite ecological equivalence. *Nature* 484:506–509. doi:[10.1038/nature10971](https://doi.org/10.1038/nature10971)
- McKaye KR (1977) Competition for breeding sites between the cichlid fishes of Lake Jilao, Nicaragua. *Ecology* 58:291–302. doi:[10.2307/1935604](https://doi.org/10.2307/1935604)
- McKaye KR (1980) Seasonality in habitat selection by the gold color morph of *Cichlasoma citrinellum* and its relevance to sympatric speciation in the family Cichlidae. *Environ Biol Fish* 5:75–78. doi:[10.1007/BF00000953](https://doi.org/10.1007/BF00000953)
- Mikami OK, Kohda M, Kawata M (2004) A new hypothesis for species coexistence: male–male repulsion promotes coexistence of competing species. *Popul Ecol* 46:213–217. doi:[10.1007/s10144-004-0189-5](https://doi.org/10.1007/s10144-004-0189-5)
- Ochi H, Awata S (2009) Resembling the juvenile colour of host cichlid facilitates access of the guest cichlid to host territory. *Behaviour* 146:741–756. doi:[10.1163/156853909X446181](https://doi.org/10.1163/156853909X446181)
- Olendorf R, Rodd FH, Punzalan D, Houde AE, Hurt C, Reznick DN, Hughes KA (2006) Frequency-dependent survival in natural guppy populations. *Nature* 441:633–636. doi:[10.1038/nature04646](https://doi.org/10.1038/nature04646)
- Pauers MJ, Kapfer JM, Fendos CE, Berg CS (2008) Aggressive biases towards similarly coloured males in Lake Malawi cichlid fishes. *Biol Lett* 4:156–159
- Peiman KS, Robinson BW (2007) Heterospecific aggression and adaptive divergence in brook stickleback (*Culeae inconstans*). *Evolution* 61:1327–1338. doi:[10.1111/j.1558-5646.2007.00113.x](https://doi.org/10.1111/j.1558-5646.2007.00113.x)
- Pryke SR (2009) Is red an innate or learned signal of aggression and intimidation? *Anim Behav* 78:393–398. doi:[10.1016/j.anbehav.2009.05.013](https://doi.org/10.1016/j.anbehav.2009.05.013)
- Pryke SR, Griffith SC (2006) Red dominates black: agonistic signalling among head morphs in the colour polymorphic Gouldian finch. *Proc R Soc Lond B* 273:949–957. doi:[10.1098/rspb.2005.3362](https://doi.org/10.1098/rspb.2005.3362)
- Pryke SR, Astheimer LB, Buttemer WA, Griffith SC (2007) Frequency-dependent physiological trade-offs between competing colour morphs. *Biol Lett* 3:494–497. doi:[10.1098/rsbl.2007.0213](https://doi.org/10.1098/rsbl.2007.0213)
- Puebla O, Bermingham E, Guichard F, Whiteman E (2007) Colour pattern as a single trait driving speciation in *Hypoplectrus* coral reef fishes? *Proc R Soc Lond B* 274:1265–1271. doi:[10.1098/rspb.2006.0435](https://doi.org/10.1098/rspb.2006.0435)
- Rogers W (1988) Parental investment and division of labor in the Midas cichlid (*Cichlasoma citrinellum*). *Ethology* 79:126–142. doi:[10.1111/j.1439-0310.1988.tb00706.x](https://doi.org/10.1111/j.1439-0310.1988.tb00706.x)
- Rosenblum EB, Sarver BAJ, Brown JW, Des Roches S, Hardwick KM, Hether TD, Eastman JM, Pennell MW, Harmon LJ (2012) Goldilocks meets Santa Rosalia: an ephemeral speciation model explains patterns of diversification across time scales. *Evol Biol* 39:255–261. doi:[10.1007/s11692-012-9171-x](https://doi.org/10.1007/s11692-012-9171-x)
- Roulin A (2004) The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. *Biol Rev* 79:815–848
- Rowland WJ (1999) Studying visual cues in fish behavior: a review of ethological techniques. *Environ Biol Fish* 56:285–305. doi:[10.1023/A:1007517720723](https://doi.org/10.1023/A:1007517720723)
- Rundell RJ, Price TD (2009) Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends Ecol Evol* 24:394–399. doi:[10.1016/j.tree.2009.02.007](https://doi.org/10.1016/j.tree.2009.02.007)
- Seehausen O (2006) African cichlid fish: a model system in adaptive radiation research. *Proc R Soc Lond B* 273:1987–1998. doi:[10.1098/rspb.2006.3539](https://doi.org/10.1098/rspb.2006.3539)
- Seehausen O, Schluter D (2004) Male–male competition and nuptial-colour displacement as a diversifying force in Lake Victoria cichlid fishes. *Proc R Soc Lond B* 271:1345–1353. doi:[10.1098/rspb.2004.2737](https://doi.org/10.1098/rspb.2004.2737)
- Sobel JM, Chen GF, Watt LR, Schemske DW (2010) The biology of speciation. *Evolution* 64:295–315. doi:[10.1111/j.1558-5646.2009.00877.x](https://doi.org/10.1111/j.1558-5646.2009.00877.x)
- Tynkynen K, Rantala MJ, Suhonen J (2004) Interspecific aggression and character displacement in the damselfly *Calopteryx splendens*. *J Evol Biol* 17:759–767. doi:[10.1111/j.1420-9101.2004.00733.x](https://doi.org/10.1111/j.1420-9101.2004.00733.x)
- van Doorn GS, Dieckmann U, Weissing FJ (2004) Sympatric speciation by sexual selection: a critical reevaluation. *Am Nat* 163:709–725. doi:[10.1086/383619](https://doi.org/10.1086/383619)