

1 **Rare morph Lake Malawi mbuna cichlids benefit from reduced aggression from con- and hetero-specifics**

2 Running title: Reduced aggression benefits rare morph mbuna

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4 Alexandra M. Tyers\*, Gavan M. Cooke & George F. Turner

5 School of Biological Sciences, Bangor University, Deniol Road, Bangor. Gwynedd. Wales. UK. LL57 2UW

6 \* Current address: Max Planck Institute for Biology of Ageing, Joseph-Stelzmann-Straße 9B, 50931, Köln

7

8 **Corresponding author:** A.M. Tyers, [alexandra.m.tyers@gmail.com](mailto:alexandra.m.tyers@gmail.com)

9

10 **Abstract**

11 Balancing selection is important for the maintenance of polymorphism as it can prevent either fixation of one  
12 morph through directional selection or genetic drift, or speciation by disruptive selection. Polychromatism can  
13 be maintained if the fitness of alternative morphs depends on the relative frequency in a population. In  
14 aggressive species, negative frequency-dependent antagonism can prevent an increase in the frequency of rare  
15 morphs as they would only benefit from increased fitness while they are rare. Heterospecific aggression is  
16 common in nature and has the potential to contribute to rare morph advantage. Here we carry out field  
17 observations and laboratory aggression experiments with mbuna cichlids from Lake Malawi, to investigate the  
18 role of con- and heterospecific aggression in the maintenance of polychromatism and identify benefits to rare  
19 morphs which are likely to result from reduced aggression. Within species we found that males and females bias  
20 aggression towards their own morph, adding to the evidence that inherent own-morph aggression biases can  
21 contribute to balancing selection. Over-representation of rare morph territory owners may be influenced by  
22 two factors; higher tolerance of different morph individuals as neighbours, and ability of rare morphs to spend  
23 more time feeding. Reduced aggression to rare morph individuals by heterospecifics may also contribute to rare  
24 morph advantage.

25

26 **Key words:** Malawi, cichlid, blotch polymorphism, aggression, rare morph advantage

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28

## 29 Introduction

30 Permanent polymorphism, the presence of multiple genetically determined morphological or behavioural  
31 phenotypes within a population, is common in nature and indicates some type of selective balance between  
32 morphs. Balancing selection is important for the maintenance of polymorphism as it can prevent either fixation  
33 of one morph through directional selection or genetic drift, or speciation by disruptive selection (Huxley 1955;  
34 Wellenreuther *et al.* 2014; Kim *et al.* 2019). Polychromatism (colour polymorphism) can be maintained if the  
35 fitness of alternative morphs differs in time or space in heterogeneous environments, or if the fitness of a  
36 phenotype depends on its relative frequency in a population (Hughes *et al.* 2013; Pérez i de Lanuza *et al.* 2017;  
37 Surmacki *et al.* 2013; Svensson 2017; Henze *et al.* 2018).

38 In many taxa, species-recognition cues have diverged through reproductive or antagonistic character  
39 displacement to reduce hybridisation or unnecessary exertion and risk of injury among heterospecifics which  
40 are not in direct competition for mates or resources (Seehausen & Schluter 2004; Grether *et al.* 2009). Rare  
41 colour morphs can benefit from lack of recognition by receiving less mating-related harassment (Takahashi *et al.*  
42 2010) or less intrasexual aggression from conspecifics (Dijkstra *et al.* 2008; Lehtonen 2014; Pérez i de Lanuza G  
43 *et al.* 2017; Scali *et al.* 2020). In aggressive species, negative frequency-dependent antagonism, generated  
44 through either evolution of an own-morph bias (Dijkstra *et al.* 2008; Lehtonen 2014; Scali *et al.* 2020) or by a  
45 dynamic common morph bias based on experience (Bolnick *et al.* 2016), can prevent an increase in the  
46 frequency of rare morphs as they would only benefit from increased fitness (due to reduced aggression) while  
47 they are rare (Seehausen & Schluter 2004; Dijkstra *et al.* 2007; Bolnick *et al.* 2016).

48 The existence of conspecific aggression biases does not preclude heterospecific aggression completely.  
49 Indeed, recent studies suggest that heterospecific aggression as a result of resource competition and  
50 reproductive interference may be more common than previously assumed (Grether *et al.* 2009; Drury *et al.*  
51 2020). Regardless of whether heterospecific aggression is due to convergence in territorial signals among  
52 species competing for resources or due to misdirection of aggression because closely related species still share  
53 similar signals (Losin *et al.* 2016), in a variety of taxa aggression is often higher among more similar coloured  
54 than more differently coloured species (Genner *et al.* 1999; Pauers *et al.* 2008; Anderson & Grether 2010; Losin  
55 *et al.* 2016). In taxa where multiple ecologically and phenotypically similar species co-exist in the same habitat  
56 there is therefore potential for rare morphs to benefit not only from reduced conspecific aggression, but also

57 from reduced heterospecific aggression. A recent study of Midas cichlids, however, demonstrated increased  
58 aggression towards rare heterospecific morphs and suggested that this disadvantage may help to explain their  
59 lower frequency in natural populations (Lehtonen *et al.* 2015). The role of heterospecific aggression in relation  
60 to polychromatism requires further exploration to improve our understanding of how this may contribute to its  
61 evolution and maintenance.

62 The mbuna cichlids of Lake Malawi (and the closely-related ecologically-similar Mbipi of Lake Victoria)  
63 provide an excellent system for the investigation of colour polymorphism. Mbuna inhabit densely packed multi-  
64 species communities in the shallow-waters and identify conspecific mates and rivals predominantly by their  
65 species-specific colour and pattern (*e.g.* Seehausen & van Alphen 1998; Couldridge & Alexander 2002; Jordan  
66 2008; Pauers *et al.* 2008). Several species display a polychromatism characterised by the presence of rare  
67 “blotched” morph individuals, which occur at different frequencies in different species and populations (Lande  
68 *et al.* 2001; Ribbink *et al.* 1983; Konings 2007). While it is likely that predation has played some role in the  
69 evolution of this polychromatism (Seehausen *et al.* 1999; Streelman *et al.* 2003; Maan *et al.* 2008), and mate  
70 choice may have been involved in the evolution of (partial) sex-linkage (Seehausen *et al.* 1999; Lande *et al.*  
71 2001; Roberts *et al.* 2009), it is thought that intrasexual competition plays a large role in its maintenance  
72 (Dijkstra *et al.* 2008; Dijkstra *et al.* 2009b). Although in most species the frequency of rare morphs remains  
73 relatively low in all populations, in some, for example *Maylandia callainos* at Thumbi West Island in Lake  
74 Malawi, rare morphs can occur with higher frequency, which allows greater ease of observation and collection.  
75 Here we used this population to conduct field observations and laboratory behavioural experiments to test  
76 alternative hypotheses regarding aggression biases: Do both morphs preferentially direct aggression towards  
77 the common (presumably ancestral) morph, or is there an own-morph bias? An own-morph bias could be  
78 sufficient to maintain polymorphism through negative frequency-dependent selection, while a common-morph  
79 bias would suggest that an additional frequency-dependent process would be necessary to limit an increase in  
80 the number of rare morph individuals. We also test for aggression biases towards the common and rare morph  
81 from a closely related heterospecific to assess whether this may contribute to balancing selection. We aim to  
82 identify potential benefits to rare morphs, which may occur as a result of receiving less aggression, in the  
83 natural environment. Additionally, as differences in selection pressures on each sex, due to differences in the  
84 type of competition they experience (competition for mates among males and competition for non-mating

85 resources among females) can result in sex differences in the types of aggressive behaviour used during  
86 contests (Arnott & Elwood 2009), we also test for sex-differences in aggressive behaviour and aggression biases.

87

## 88 **Methods**

89

### 90 **Study system**

91 *Maylandia callainos* (= *Pseudotropheus callainos* or *Metriaclima callainos*) is a member of the 'mbuna' complex  
92 of rocky shore cichlid fishes endemic to Lake Malawi. Populations of *M. callainos* are found in shallow water,  
93 with peak population density between 3 – 10m (full range 0 – 25m). Their natural range is confined to the  
94 northern end of Lake Malawi, where they are often found in sympatry with the more widely distributed  
95 ecologically similar congeneric *M. zebra*. However, due to human mediated translocations, they are also found  
96 in some southern areas. Phenotypes of common and rare morph mbuna differ between species, but within  
97 populations, the common morph is often BB (black vertical melanin bars on a blue/dark background) or solid  
98 blue/dark body colour, while rarer morphs have a disrupted melanin pattern of many or few blotches/spots on  
99 a light (orange/pink/white) body. Blotch polychromatism is not present in all *M. callainos* and *M. zebra*  
100 populations; at some localities only the plain blue (B) and BB morph are found, whereas at others, these  
101 common morphs may occur along side rare white (W) and orange-blotch (OB) and very rare white-blotch (WB)  
102 and orange (O) morphs. In this study we focus on a well established translocated population of *M. callainos* at  
103 Thumbi West Island in the Lake Malawi National Park in the southwest arm of the lake which has both B and W  
104 morphs. The likely source population of the *M. callainos* at Thumbi West is Nkhata Bay, where they co-occur  
105 with a population of *M. zebra* comprised of BB, OB and O morph individuals (fig. 1).

106 All fish were wild caught: *M. callainos* and *M. zebra* from Thumbi West Island (TW) in July 2010, *M. zebra*  
107 from Nkhata Bay (NB) and Chiofu Bay (CB - naïve to *M. callainos* in the wild and lab) in 2009. Males and females  
108 were used in this study, partially because of the lower number of rare males, but also because both male and  
109 female aggression biases have previously been suggested to be important in colour polymorphism maintenance  
110 in cichlids (e.g. Dijkstra *et al.* 2008). Furthermore, unlike many species with blotch polychromatism, this one is  
111 less strongly female limited, as numerous white *M. callainos* males were found at the study/collection site.

112

1 a) Mbuna community at Thumbi West Island



113 **Figure 1. a)** Mbuna community at Thumbi West Island. Territorial male (bottom) and female (top) of **b)** *M.*  
114 *callainos* Blue morph, **c)** *M. callainos* White morph, **d)** *M. zebra* BB morph. Coloured squares correspond to  
115 colours used for the different morphs in results plots.

116

#### 117 **Field observations (excluding aggression)**

##### 118 Frequency of blue and white morph *M. callainos* at Thumbi West Island

119 Snorkel observations were used to estimate the ratio of B to W *M. callainos* morphs in the general population.  
120 Dominant mature adult males can be easily recognised by their behaviour and colour, but females and  
121 immature males are indistinguishable and are referred to as ‘apparent females’. Hence, the number of males  
122 and apparent females of each morph was counted along three 30m transects covering an area half a meter  
123 each side of the line (n = 74 fish). The numbers of territory-holding males of each morph were counted in nine  
124 5m<sup>2</sup> quadrats (n = 142 fish). Although ideally the comparison should be made between non-territorial males  
125 and territorial males, in practice this was not possible due to the difficulty in sexing fish without catching them.  
126 However, it is likely that in the whole population, rare morph males occur at a lower frequency than rare morph  
127 females (as found in other closely related species with blotch polychromatism, Lande *et al.* 2001; Maan & Sefc  
128 2013), which would make estimates of the ratio of rare to common morph males among non-territorial fish a  
129 conservative estimate; territorial W males would be present at a much lower frequency than predicted from the  
130 ratio of W morph in the general population.

131 Territory distances between morphs

132 Territory maps were constructed by drawing the rocky substrate, within 5x5m string quadrats (n = 9), on dive  
133 slates while snorkelling. Males frequently return to their spawning cave and focal observations allowed for  
134 accurate determination of the position of this territorial focal point for each male within the quadrats. The  
135 distance between the territory focal point of each male within the centre 3m<sup>2</sup> (n = 27 B & 25 W) and closest  
136 white and blue neighbour (including fish nearer the edge of the quadrat) was then measured.

137 Grazing differences between morphs

138 Each grazing action performed by focal individuals was recorded during ten minute observations of territorial  
139 males and non-territorial fish (n = 9 individuals of each morph for each social status).

140

141 **Field observations of aggressive interactions**

142 During focal observation lasting 10 minutes per fish (n = 9 territorial males of each morph) all aggressive  
143 behaviors directed towards the two conspecific morphs were recorded. The vast majority of all aggressive acts  
144 recorded were 'chases', lateral displays were observed but rare, counts of each type of behaviour were summed  
145 for analysis. While collecting data on conspecific aggression biases, aggression towards each focal fish from  
146 heterospecifics was also recorded.

147

148 **Laboratory aggression trials**

149 To test whether there are differences in the level aggression received by blue and white morph *M. callainos*  
150 from conspecifics and heterospecifics, three experiments were carried out using the same methods. Five  
151 minute pairwise aggression trials were conducted in two replicate tanks measuring 0.9x0.3x0.3m. Each tank  
152 contained a central brick refuge to act as a territory focal point, two transparent (perforated) plastic jars to hold  
153 the stimulus fish, an air driven box filter and an internal heater to maintain water temperature at *ca.* 22-24°C.  
154 Lights were kept on a 12:12 light:dark cycle. All fish were fed flake food once a day. Females and males were  
155 used, but stimulus fish were always the same sex as focal fish. Focal fish were allowed at least 24h to  
156 acclimatise before introduction of the stimuli and recording of focal fish behaviour began after emergence from  
157 the central refuge. Individual aggressive behaviours (frontal/lateral display, quiver, lunge/butt and bite) were  
158 recorded and combined to give an overall aggression count for each individual. To control for potential tank side

159 bias, two separate trials were carried out with each focal fish, each with a different stimulus fish pair and with  
160 morphs swapped between sides. To avoid pseudoreplication from the re-use of focal males, before analysis an  
161 average was taken of the aggressive behaviour observed in the two trials by each individual.

#### 162 Exp. 1: Interspecific aggression biases between species

163 Firstly, conspecific aggression bias was confirmed by presenting BB *M. zebra* males from CB (n = 10) with pairs  
164 of conspecific BB and heterospecific B stimulus fish.

#### 165 Exp. 2: Intraspecific aggression biases between morphs

166 For this experiment all available *M. callainos* were used as focal and stimulus fish (n = 10 B male, 6 B female, 3  
167 W male and 9 W female) to test for morph-specific aggression biases among conspecifics.

#### 168 Exp. 3: Interspecific aggression to different morphs

169 BB *M. zebra* focal fish from different populations (n = 12 male/ 12 female “TW”, 12 male/ 5 female “NB”, 12  
170 male/ 12 female “CB”) were used to test for heterospecific aggression biases to B and W *M. callainos* stimulus  
171 pairs. Stimulus pairs consisted of the same *M. callainos* used in Exp. 2.

172

#### 173 **Data analysis**

174 Statistical analysis and plotting was carried out using Rstudio (v. 1.2.5033; Rstudio Team 2019) using additional  
175 packages Rmisc, pscl, ggplot2, scales. General and generalized linear models were used depending on whether  
176 the data originated from continuous measurements (territory distance), or counts (grazing and aggressive  
177 behaviour). For the laboratory aggression experiments, trials were omitted from the analysis if the average  
178 behaviour count was less than 10. In experiments where females and males were tested, sex was included in  
179 the models to test whether this was a significant factor affecting aggression biases towards the stimulus  
180 morphs.

#### 181 Frequency of blue and white morphs at Thumbi West Island

182 A G-test was used to compare the actual number of territory holding W males observed with what would be  
183 expected given the proportions of B and W morphs in the general population.

#### 184 Territory distances between morphs

185 GLMs were used to test: 1) Whether there is a significant difference in the distance between focal fish and the  
186 nearest neighbour of the same and different morph, and 2) differences in the average distance to B and W

187 neighbours from focal fish of the two different morphs.

#### 188 Grazing differences between morphs

189 GLMs were used to test for effects of dominance status and morph on grazing frequency.

#### 190 Field observations of aggressive behavior

191 Due to the small number of aggressive behaviors and relatively high number of zero counts recorded during  
192 observations of aggressive encounters in the field, standard poisson regression GLMs were compared with  
193 models corrected for zero-inflation. In most cases the zero inflated model was not significantly better, the  
194 results reported here are therefore from the standard Poisson GLMs testing: 1) aggression directed towards  
195 territorial intruders of each morph by territorial males of each morph, 2) aggression received by territorial  
196 males of each morph from heterospecifics.

#### 197 Laboratory aggression trials

198 First, GLMs were used first to test whether focal species and sex had a significant influence on the total number  
199 of aggressive behaviours (overall aggressiveness of species and sexes) displayed to both stimulus fish. To control  
200 for the effect of overall differences in level of aggression between species/populations/sexes/individuals, counts  
201 of aggressive behaviour directed towards each of the paired stimulus fish was converted to proportion of  
202 aggression. Subsequently, the following were tested: 1) whether *M. zebra* display a conspecific aggression bias  
203 when presented with conspecific and common morph heterospecific, 2) whether among conspecifics (*M.*  
204 *callainos*) overall one morph receives more aggression than the other, and whether aggression bias differs  
205 among morphs and sexes, 3) whether *M. zebra* display an aggression bias when presented with pairs of  
206 common and rare morphs of a heterospecific, and if this aggression bias differs between allopatric populations  
207 of *M. zebra*, depending on whether they co-occur with *M. callainos* or not.

208

### 209 **Results**

210

#### 211 **Field observations (excluding aggression)**

##### 212 Frequency of the blue and white morphs at Thumbi West Island

213 There were significantly (G-test:  $G_1 = 6.91$ ,  $p = 0.009$ ) more territory holding rare white (W) morph males than  
214 would be expected given the proportion of W and blue (B) morph fish in the general population (fig. 2a).



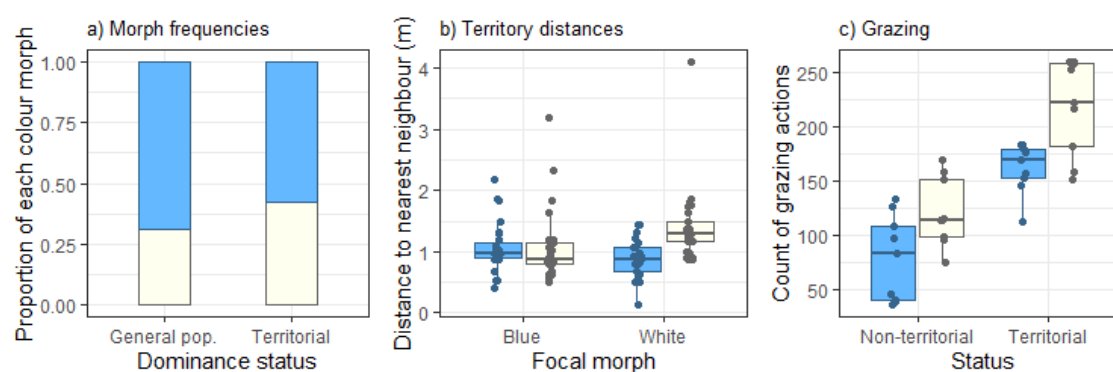
## 215 Territory distances between morphs

216 Firstly, there is a significant difference in the average distance between same and different morph territorial  
217 males (glm:  $t_{1,103} = 2.51$ ,  $p = 0.0135$ ). On average, territorial males of the same morph are found at greater  
218 distance from each other (mean 1.24m) than territorial males of different morphs (mean 0.98m). While it is not  
219 surprising to find that W and W are found furthest apart (mean 1.40m), as this would be the assumption based  
220 on the observation of a lower frequency of W morph territorial males, if distance between morphs was only  
221 based on frequency, it would also be expected that the distance between B morph males should on average be  
222 the smallest distance. This is not the case: the distance between B and nearest B is on average the same (mean  
223 1.07m) as, and not significantly different (glm:  $t_{1,53} = -0.008$ ,  $p = 0.994$ ) from, the distance between B and  
224 nearest W. We also found that the distance between W males and their nearest B neighbour is on average the  
225 smallest distance recorded between territorial males (mean 0.89m), and significantly different from the  
226 distance between W territorial males and their nearest W neighbour (glm:  $t_{1,49} = 3.73$ ,  $p = 0.0005$ ) (fig. 2b).

## 227 Grazing differences between morphs

228 Both dominance status and morph had a significant effect on grazing frequency: Compared to territorial males,  
229 non-territorial fish grazed significantly more, and regardless of social status B morph fish grazed significantly  
230 less than W morph (glm;  $z_{1,35}$  Morph = 22.07,  $p < 2e-16$ , Status = 12.18,  $p < 2e-16$ ) (fig. 2c).

231



232 **Figure 2.** Field observations at Thumbi West Island: **a)** There are significantly ( $p = 0.009$ ) more territory holding  
233 W males than would be expected given the proportion of each morph in the general population. Bars show rel-  
234 ative frequency of each morph,  $n = 216$  fish. **b)** Bar colours indicate neighbour colour. Differences in the dis-  
235 tance of territory focal point ( $n = 27$  B and 25 W males) among same and different morph males do not always

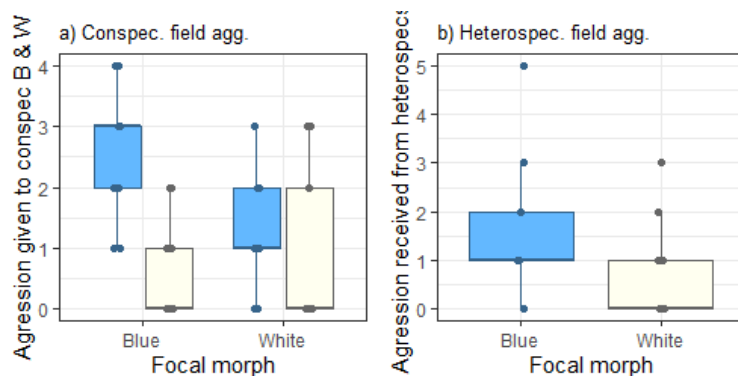
236 reflect the expectations based on the relative abundance of each in the population. Overall, same morph males  
237 hold territories further apart from each other than different morph males ( $p = 0.0135$ ). The average distance  
238 between B males and the nearest B neighbour is no different from between B and nearest W neighbour ( $p =$   
239  $0.994$ ), and the distance between W territorial males and their nearest B neighbour is on average the smallest  
240 distance recorded between territorial males, despite the lower frequency of W males. **c)** Bar colour indicate fo-  
241 cal morph,  $n = 9$  individuals of each morph and status; both territorial and non-territorial W morph fish grazed  
242 significantly more than B morph fish (Morph  $p < 2e-16$ , Status  $p < 2e-16$ ).

243

#### 244 **Field observations of aggressive interactions**

245 Overall, B morph *M. callainos* territorial intruders receive significantly more aggression than their W counter-  
246 parts (glm;  $z_{1,35} = -2.79$ ,  $p = 0.005$ ). However, aggression bias appears to differ between the morphs; B males  
247 make significantly more attacks to other B males (glm;  $z_{1,17} = -2.93$ ,  $p = 0.003$ ), whereas W morph males show no  
248 significant aggression bias (glm;  $z_{1,17} = 0.69$ ,  $p = 0.493$ ) (fig. 3a). Although there was a trend towards B fish re-  
249 ceiving more aggression from heterospecifics than W fish, this difference was not significant (mean B = 1.8, W =  
250 0.8,  $z_{1,17} = -1.82$ ,  $p = 0.068$ ) (fig. 3b).

251



252 **Figure 3.** Observation of conspecific and heterospecific aggressive interactions among territorial males in the  
253 field: **a)** Bar colour indicates intruder colour,  $n = 9$  focal males of each morph. B morph *M. callainos* territorial  
254 males show significantly more aggression towards other B males than towards W males ( $p = 0.003$ ), W males  
255 show no significant aggression bias ( $p = 0.493$ ). Overall, B morph males receive significantly more aggression ( $p$

256 = 0.005). **b)** Bar colour indicates focal fish colour. There is a non-significant trend towards B morph males also  
257 receiving more aggression from heterospecifics ( $p = 0.068$ ).

258

## 259 Laboratory aggression trials

### 260 Differences in focal fish behaviour between species/(experiment) and sex

261 Within species, both sexes show similar levels of aggression. *M. callainos* overall were more aggressive (glm;

262 Focal.spec  $z = -10.36$ ,  $p < 2e-16$ , Sex  $z = 0.08$ ,  $p = 0.939$ , fig. 4a). To test whether this difference in

263 aggressiveness was a real difference between species, or due to *M. callainos* being presented more often with

264 the possibility of being aggressive towards conspecific fish in these experiments, a subset of *M. zebra* CB and

265 *M. callainos* males from the three experiments was compared. *M. zebra* displayed a significantly higher level of

266 aggression in the experiment where the stimulus pair consisted of one conspecific and one heterospecific (Exp.

267 1) compared to the experiment where they were presented with two heterospecific stimulus fish (Exp. 3). There

268 was no significant difference, however, in the level of aggression between the species in the experiments in

269 which the stimulus pairs contained one conspecific (Exp. 1: *M. zebra* focal fish) or two conspecifics (Exp. 2: *M.*

270 *callainos* focal fish) stimulus fish (fig. 4b). This suggests that the presence or absence of a conspecific stimulus

271 fish contributed to the overall difference in the level of aggression observed between the species in these

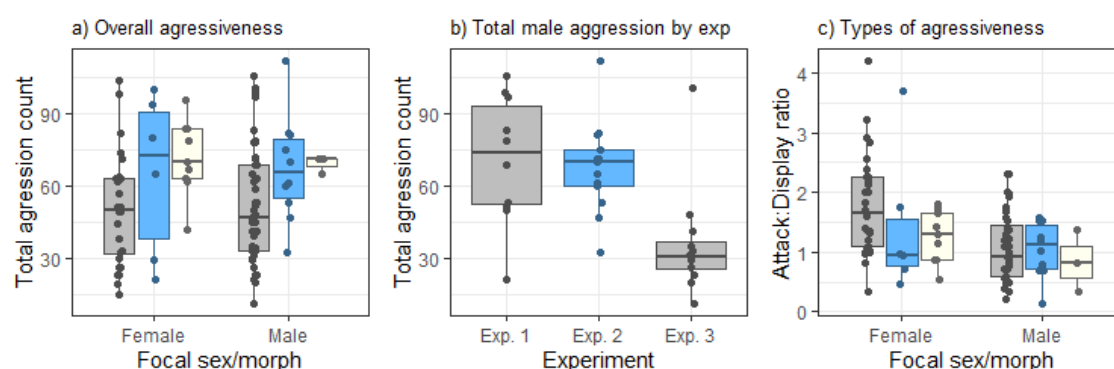
272 experiments. There was therefore no evidence of species differences in intrinsic level of aggression, rather that

273 aggression among heterospecifics is lower than among conspecifics. The attack:display ratio differed between

274 sexes, but not species: Females attack more frequently and males display more (glm; Focal.spec  $z = 1.72$ ,  $p =$

275 0.089, Sex  $z = -4.09$ ,  $p = 9.45e-05$ , fig. 4c).

276



278 **Figure 4.** Differences in levels of aggression (total aggression count) and types of aggressive behaviour (ratio of  
279 attacks:displays) used among species(/experiment) and sexes. N = 29 female/ 46 male BB *M. zebra* (grey bars);  
280 15 female/ 13 male *M. callainos* (blue and white bars). **a)** On average *M. callainos* were significantly more  
281 aggressive ( $p < 2e-16$ ), and there is no difference in the total amount of aggressive behaviour from females and  
282 males ( $p = 0.939$ ). **b)** *M. zebra* are only significantly less aggressive in the absence of a conspecific stimulus fish  
283 (Exp. 3,  $p < 2e-16$ ). N = Exp. 1, 12 *M. zebra*; Exp. 2, 13 *M. callainos*; Exp. 3 12. *M. zebra*. **c)** The attack:display  
284 ratio does not differ significantly between species ( $p = 0.089$ ), but males display more frequently than females  
285 which use a higher proportion of attacks ( $p = 9.45e-05$ ).

286

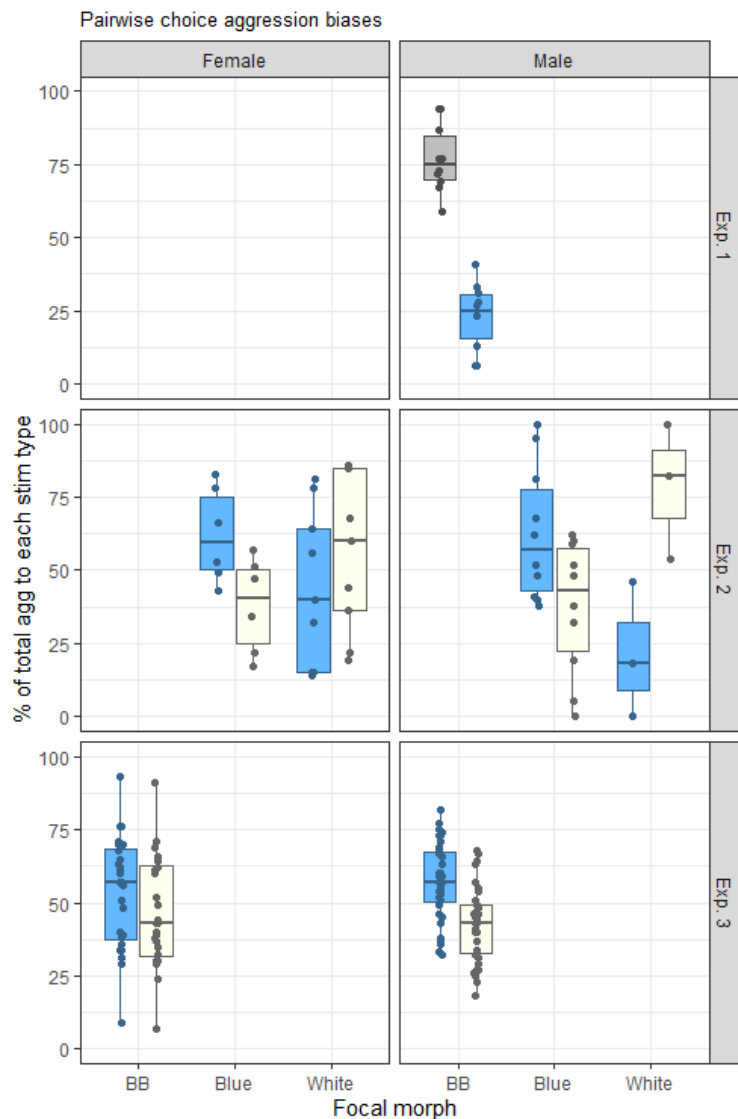
#### 287 Aggression biases in pairwise intruder choice tests

288 As expected, *M. zebra* males display significantly more aggressive behaviour towards conspecifics when given  
289 the choice of BB conspecific and B heterospecific stimulus males (glm;  $z_{1,19} = -16.03$ ,  $p < 2e-16$ , fig. 5, Exp 1).

290 Within *M. callainos*, the interaction between colour morph of focal and stimulus fish significantly affects  
291 proportion of aggression received, while sex has no effect ( $z_{1,55}$  Interaction = 12.48,  $p < 2e-16$ , Sex = 0.00,  $p =$   
292 1.00, fig. 5, Exp 2). To further clarify whether the difference in the proportion of aggression directed to stimulus  
293 fish of each colour morph is due to an own morph aggression bias (*i.e.* each colour morph is more aggressive to  
294 other males of the same colour) or an overall common morph aggression bias (*i.e.* males of both colour morph  
295 direct more aggression towards males of the common colour morph), stimulus type was recoded from  
296 Blue/White to either Other/Own or Common/Rare: Own-morph stimulus fish received significantly greater  
297 proportion of aggression than the other-morph stimulus fish, common morph stimulus fish did not receive a  
298 greater proportion of aggression overall in this experiment ( $z_{1,55}$  Other/Own  $z = 12.48$ ,  $p < 2e-16$ , Common/Rare  
299  $z = -0.30$ ,  $p = 0.768$ ).

300

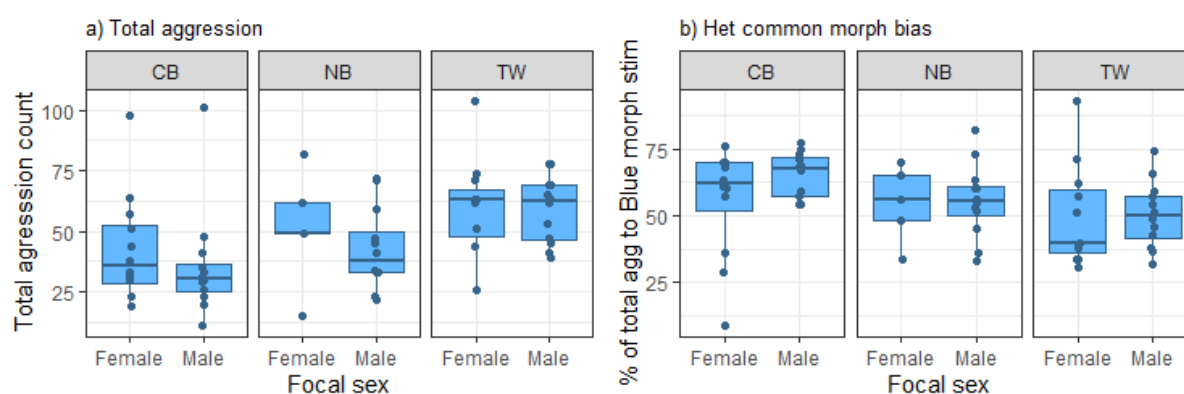
301



302 **Figure 5.** In laboratory based aggression trials: Exp.1) As expected, *M. zebra* males bias aggression towards  
 303 conspecifics ( $n = 10$ ,  $p < 2e-16$ ). Exp. 2) Both *M. callainos* morphs showed a significant tendency to bias  
 304 aggression towards intruders of the same morph as themselves ( $p < 2e-16$ ). Exp. 3) Overall the *M. callainos* B  
 305 morph stimulus fish received significantly more aggression from heterospecific *M. zebra* than the W morph ( $p <$   
 306  $2e-16$ ).  
 307  
 308 When *M. zebra* were presented with the choice of *M. callainos* B and W stimulus pairs, overall both females  
 309 and males preferentially attacked the common B morph ( $z_{1,127}$  Stim morph = -8.79,  $p < 2e-16$ , Sex = 0.01,  $p =$   
 310 0.991, fig. 5, Exp 3). However, the level of aggression (total aggression count to both morphs) and the strength  
 311 of aggression bias (proportion of aggression to B morph) differs between populations and sexes: *M. zebra* from

312 Nkhata Bay (NB) and Thumbi West (TW) were significantly more aggressive to the heterospecific stimulus fish  
313 overall than those from Chiofu Bay (CB), and females overall were more aggressive than males (glm;  $z_{2,63}$  NB =  
314 3.36,  $p = 0.0008$ , TW = 9.68,  $p < 2e-16$ , Sex = -2.62,  $p = 0.009$ , fig. 6a). In regards to strength of heterospecific  
315 common morph aggression bias, we found that NB and TW focal fish displayed a significantly lower proportion  
316 of aggression towards the blue morph than those from CB (*i.e.* a weaker bias), and that although females were  
317 more aggressive overall, they also showed a weaker common morph aggression bias than males (glm;  $z_{2,63}$  NB =  
318 -2.50,  $p = 0.012$ , TW = -4.82,  $p = 1.47e-06$ , Sex = 2.05,  $p = 0.041$ , fig. 6b).

319



320 **Figure 6.** Differences in levels of aggression and aggression biases depending on *M. zebra* source population  
321 and sex (n females/males = CB 12/12, TW 5/12, NB 12/12). **a)** *M. zebra* from CB were significantly less  
322 aggressive in these experiments (compared to NB  $p = 0.0008$ , TW  $p < 2e-16$ ), and females more aggressive  
323 overall than males ( $p = 0.009$ ). **b)** *M. zebra* from CB showed a significantly stronger common (B) morph  
324 aggression bias than those from NB ( $p = 0.012$ ) and TW ( $p = 1.47e-06$ ), and females also displayed a weaker  
325 aggression bias than males ( $p = 0.041$ ).

326

## 327 Discussion

328 Our field observations of *Maylandia callainos*, a polychromatic mbuna cichlid from Lake Malawi, indicated that  
329 common (blue) morph territorial intruders received more aggression than rare (white) morph intruders.  
330 Pairwise intruder choice tests in a controlled laboratory setting demonstrated that males and females of each  
331 morph bias aggression towards their own morph. These results add to the evidence that inherent own-morph  
332 aggression biases, which result in negative frequency dependent selection on rare colour morphs, can

333 contribute to balancing selection and thereby promote the maintenance of polychromatism (Dijkstra *et al.*  
334 2008; Lehtonen 2014; Scali *et al.* 2020).

335 While this and previous studies (*e.g.* Dijkstra *et al.* 2008; Dijkstra *et al.* 2009a; Seehausen & Schluter 2004;  
336 Lehtonen 2014; Scali *et al.* 2020) have shown that aggression biases can be involved in stabilising  
337 polychromatism, to our knowledge this is the first study to identify benefits to rare morph cichlids which may  
338 result from receiving less aggression in the natural environment. We found there to be significantly more  
339 territory holding rare morph males than would be expected given the proportions of the two colours in the  
340 general population. Our observations suggest that the over-representation of rare morph territory owners may  
341 be influenced by two factors. Firstly, blue and white males appear to have higher tolerance of each other as  
342 neighbours, being found on average significantly closer to each other than blue morph individuals. Secondly,  
343 both territorial and non-territorial white morph individuals spend more time feeding, which suggests that the  
344 rare colour morph may benefit from lack of recognition during competition for non-mating related resources  
345 (Dijkstra *et al.* 2008; Lehtonen 2014; Pérez i de Lanuza G *et al.* 2017; Scali *et al.* 2020).

346 Further to showing that rare morph individuals can benefit from reduced intraspecific aggression, we found  
347 that a closely related ecologically similar heterospecific (*Maylandia zebra*) also biases aggression towards the  
348 *M. callainos* blue morph. While these results are in conflict with those from another cichlid fish system, which  
349 suggest that rare morphs may be disadvantaged by greater heterospecific aggression (Lehtonen *et al.* 2015),  
350 given that aggression among heterospecifics is often higher among more similar coloured than more differently  
351 coloured species (Genner *et al.* 1999; Pauers *et al.* 2008; Anderson & Grether 2010; Losin *et al.* 2016), it is not  
352 surprising to find that in some cases rare morph individuals may receives less aggression from a heterospecific  
353 which is more similar in colour to the common morph. We also found, however, that although heterospecific  
354 females were more aggressive overall, they also showed a weaker blue morph aggression bias than males. We  
355 speculate that a lower level of discrimination among morphs by heterospecific females, and the greater use of  
356 direct attacks compared to display behaviours (this study and Arnott & Elwood 2009), may be due to the  
357 difference in competition among females and males (*i.e.* greater heterospecific competition among females for  
358 access to shelters among the rocks during incubation of offspring).

359 In cichlids and other taxa, laboratory studies have shown that in species which differ in colour among  
360 allopatric populations, males tend to bias aggression towards males from their own population (Tyers & Turner

361 2013; Bolnick *et al.* 2016; Cooke & Turner 2018; Yang *et al.* 2018). In this study, we found that heterospecific  
362 aggression also varies depending on whether a pair of species occurs in sympatry or allopatry. The level of  
363 aggression (total aggression count to both *M. callainos* morphs) differs between *M. zebra* populations: *M. zebra*  
364 from Nkhata Bay (NB) and Thumbi West (TW), which co-occur with *M. callainos*, were significantly more  
365 aggressive to *M. callainos* than those from Chiofu Bay (CB), which are naïve to *M. callainos*. These findings  
366 support the hypothesis that aggression among heterospecifics may often not simply be due to misdirected  
367 aggression among species (Peiman & Robinson 2010), which would be indicated by higher levels of aggression  
368 from the allopatric *M. zebra* population (CB). The persistence of heterospecific aggression at NB support the  
369 idea that it has an adaptive function in long-term co-existing multi-species communities (Peiman & Robinson  
370 2010; Losin *et al.* 2016). Although there are no *M. callainos* at Chiofu Bay, this location is home to another  
371 closely-related species (*M. esterae*) which has blue males, and brown, orange and orange blotch females. *M.*  
372 *zebra* at Chiofu Bay therefore do co-occur with a similar blue morph fish, but no white morph fish and we found  
373 that the *M. zebra* from this location has a stronger blue-morph aggression bias than the other *M. zebra*  
374 populations which co-occur with blue and white *M. callainos*. A previous study of a polymorphic frog species  
375 found stronger aggression biases among morphs when they occur in allopatry compared to when they are  
376 found in sympatry (Yang *et al.* 2018). Our results show a similar pattern in heterospecific aggression; a weaker  
377 blue morph aggression bias in *M. zebra* populations which coexist with both colour morphs.

378 Our results indicate that a rare colour morph may benefit from lack of recognition as a resource competitor,  
379 by both conspecifics and heterospecifics. This results in rare morph individuals receiving less aggression and  
380 gaining improved access to territories and food. This can benefit rare morph individuals while they are rare, but  
381 then what prevents them from increasing in frequency until fixation? Firstly, we found that rare (white) morph  
382 individuals were more aggressive towards their own morph than they were to the common (blue) morph, which  
383 would result in white morph individuals experiencing increasing levels of aggression as they became more  
384 common. Secondly, the lower level of heterospecific aggression bias towards the common morph, in  
385 populations with blue and white morphs, suggests that heterospecifics learn or evolve the ability to recognise  
386 rare morph individuals as competitors. The ability to recognise rare morph individuals may increase as they  
387 become more common: TW has the highest frequency of white morph individuals and the weakest common  
388 morph aggression bias by heterospecifics. Finally, female preference for common-morph males may result in a



389 disadvantage to rare morph males (Roberts *et al.* 2009). The genes responsible for the expression of the  
390 melanin-disrupted (“blotched”) morphs are almost always closely linked to a dominant female determiner, and  
391 so are generally much more common in females. This suggests that these colour phenotypes are  
392 disadvantageous to males, although they may be advantageous to females by providing increased crypsis or  
393 reduced aggression from conspecifics and/or heterospecifics.

394

### 395 **Conclusions**

396 Our results support previous studies indicating that negative frequency-dependent antagonism can be  
397 generated by own-morph aggression biases among conspecifics in cichlids which display polychromatism. We  
398 find that heterospecifics show reduced aggression to rare morph individuals, suggesting that heterospecific  
399 aggression may also facilitate invasion of rare colour morphs into a population. We identify potential  
400 advantages to rare morph individuals in the field, in terms of territory and foraging.

401

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409

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