HIGH-RESOLUTION CHARACTERIZATION REEVALUATES SEX LINKAGE OF ORNAMENTATION IN GUPPIES

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Abstract

Colouration plays a key role in the ecology of many species, influencing how an organism interacts with its environment, other species and conspecifics. Guppies are sexually dimorphic, with males displaying sexually selected colouration resulting from female preference. Previous work has suggested that much of guppy colour pattern variation is Y-linked. However, it remains unclear how many individual colour patterns are Y-linked in natural populations as much of the previous work has focused on phenotypes either not found in the wild, or that are aggregate measures such as total colour area. Moreover, ornaments have traditionally been identified and delineated by hand, and computational methods now make it possible to extract pixels and identify ornaments automatically, eliminating the potential for human bias. Here we developed a pipeline for automated ornament identification and high-resolution image analysis of male guppy colour patterns. Our results show that loci controlling the presence or absence of individual male ornaments in our population are not predominantly Y-linked. However, we find that ornaments of similar colour are not independent of each other, and modifier loci that affect whole animal colouration do appear to be at least in part Y-linked. Considering these results, Y-linkage of individual ornaments may not be important in driving colour changes in natural populations of guppies, or in expansions of the non-recombining Y region, while Y-linked modifier loci that affect aggregate traits may well play an important role.

Introduction

Colouration is an important morphological character in many species. It has the potential to greatly influence how an organism interacts with its environment via thermoregulation, UV protection [1–3],with other species via aposematism, mimicry and crypsis [4–7] and with conspecifics via intra- and intersexual selection [8,9]. Moreover, some colour phenotypes under sexual selection can incur significant metabolic production costs [10,11] or increased predation risks due to increased conspicuousness [12,13]. In these cases, the costs affect both sexes, but only one sex gains a mating advantage, resulting in sexual conflict [14,15]. In these cases, colour traits are likely to be dimorphic when the genetic architecture can be separated between females and males [15].

One important way to establish separate male and female genetic architecture, and thereby resolve sexual conflict, is Y-linkage. Moreover, sexual conflict is thought to accelerate the evolution of the Y chromosome and its divergence from the X [16] when a sexually antagonistic allele is in close linkage with the sex-determining (Y) locus. In males, this will lead selection against recombinants to maintain linkage, ultimately resulting in a non-recombining Y chromosome region [17–19]. Theoretical models [16,17] predict an accumulation of sexually antagonistic genes on Y chromosomes, particularly in nascent sex chromosome systems before large-scale gene loss has occurred.

Male guppies (*Poecilia reticulata*) are brightly coloured and highly variable in their patterning [20]. These patterns are composed of spots and bars that can be categorized into three types of ornament, orange and red carotenoid and pterin ornaments, melanic black ornaments, and green and blue ornaments due to structural colours [21,22]. In contrast females lack any

of these ornaments and are instead a uniform colour. Although colouration leads to increased predation [23], male guppy colouration is the result of sexual selection, with females showing a preference for more colourful, brightly patterned males [20,21,24–26]. Guppies face fewer predators in the upper reaches of the streams and in these more recently colonised upstream populations, guppies have evolved more conspicuous colouration due to increased female preference [27,28]. Male guppy colouration is therefore a sexually antagonistic trait, being selectively disadvantageous via predation if it were found in females, but being sexually advantageous for males.

Previous work has suggested that a sizable proportion of guppy male colouration is Y-linked, and a summary by Lindholm and Breden (2002) identified 16 male ornament characters as fully Y-linked, 24 that recombine between the X and Y and two that are X linked. Indeed, Y-linkage of male colour traits has been suggested as a main factor in the evolution of recombination suppression between the sex chromosomes in this system [29]. However, as Lindholm and Breden (2002) point out, much of the evidence for this Y-linkage has come from studies on phenotypes not typically found in the wild [30,31], or from studies that do not or cannot account for potential for variation in female sensitivity to hormone treatments [31–33], and/or from studies using aggregate measures of total colour area rather than specific ornaments [21,33,34]. Moreover, ornaments have traditionally been identified and delineated by hand, leading to the potential for human bias if samples are not analysed blind to parentage or place of origin [35] , even though computational methods now make it possible to extract pixels automatically, without the potential for human bias.

Here we developed a pipeline for high resolution image analysis of male guppy colour patterns. We use this pipeline along with two datasets from our outbred stock population derived from the Quare river system to; i) identify and characterize male colour ornaments agnostically with computational clustering methods, ii) determine how these ornaments relate to each other, and iii) use pedigreed families to compare and contrast inheritance patterns and possible Y-linkage of both individual ornament phenotypes and aggregate pattern phenotypes of total colour areas .

Methods

Our stock population of guppies originates from the Quare River in Trinidad, originally collected in 1998 and subsequently maintained in large numbers (>500) in benign freely-mating conditions [36]. We established pedigrees from this population by pairing four males and virgin females randomly in the first generation. We then paired offspring in subsequent generations from different families to maximise the number of grandparents in each brood, to minimize inbreeding which can make it difficult to differentiate different modes of inheritance, and to help increase the diversity of different phenotypes segregating in different families. This led to a minimum of six great-grandparents per family in generation three (see Supplementary Fig. 1). In all, our pedigree stretched across three generations and 19 crosses, and produced 214 male offspring across 15 generations two and three (average per family = 14). We maintained both the stock population and pedigree families on a 12:12 light cycle at 26C and fed a standard hobby guppy diet and artemia daily.

Imaging

We photographed males from the controlled pedigrees, described above, as well as 171 males taken from the main stock population and not used in pedigrees as a population baseline for ornamentation. For all males, fish were photographed as soon as possible after sexual maturation in a specially designed tank (photarium) with internal dimensions of Length 100mm x Width 100mm x Depth 20mm. Photos were taken with a tripod-mounted Canon EOS 100D and an 18-55m lens, using ISO 400, shutter speed 1/640 and F4.5. Lighting was standardized using a Polaroid PLLED312 312 LED light set to 5600K on full power. The standardized photographic conditions limited the need for pre-processing normalisation, however, we used the R function histMatch() from the RStoolbox package [37] to correct the small amount of variation we did observe in brightness . In addition, we also used the R function focal() from the Raster package [38] to slightly blur images for downstream colour block extraction, thereby eliminating any potential problems with glare or reflectance.

Following pre-processing, we cropped each photo to a 1801x931 (XY) pixel image using custom functions. We then employed the *k*-means clustering algorithm used by the R base function kmeans(), which partitions the image based on the RGB values of pixels into *k* clusters, so that the sum of squares from pixels to the assigned cluster RGB centres is minimized. Each image was partitioned using both *k*=20 and *k*=40, so that the image was split into 20 or 40 clusters of different pixels. With images now split into *k* clusters of different colours, we were able to separate and extract pixels from orange and black ornaments.

130 For each of the two types of ornaments we extracted ornaments from the *k*-image that gave
131 the most distinct and defined ornaments. Although we used *k*-means images to reduce the
132 complexity of the colour on the fish to ease pixel selection, we did however extract the
133 original RGB values from the non *k*-means image.

134 Following the extraction of the ornament pixels for each fish, we placed 99 equally spaced
135 semi-landmarks around the body outlines (ignoring both caudal, dorsal and other fins) in
136 the original photos using the TPS suite [39,40]. We used the gpagen() function from the
137 Geomorph package [41] to calculate the mean shape of the 171 male fish from the main
138 population, and used this as the reference shape against which the images of extracted
139 orange ornaments from all pedigree males were aligned. This alignment was done using
140 Thin Plate Spline warping as implemented by the computeTransform() and
141 applyTransform() functions from the package r/Morpho [42]. See supplementary fig. 1 for
142 pipeline overview.

143 *Defining ornaments and ornament inheritance*

144 After aligning the orange and black extracted images of all 171 males from the stock
145 population, we had RGB scores for each pixel in each fish. We then used these data to define
146 and delimit the male ornaments in our stock population. Pixels were marked as ornamental
147 (being part of an ornament) based on the proportion of presence across fish. For a pixel to be
148 marked as ornamental it had to be found in black> 11% of all assayed fish for orange pixels
149 and > 10% of all assayed fish for black pixels. This slightly higher threshold for orange
150 pigmentation was needed in order to separate orange ornaments CII and CIII which were
151 otherwise joined by a few black pixels (Fig 1a; 1b). We then used the function hclust() to

152 cluster pixels into a predefined number of distinct groups, defined as ornaments. For orange
153 ornaments we used a predefined number of 4 ornaments, as this was very clearly the
154 number of distinct black regions found in fish. For black ornaments we tested clustering
155 using 6,7 and 8 ornaments, with one of the black regions being the eye. From these results, it
156 was clear that there were five main patches of black pigmentation, plus the eye and one very
157 small region that we did not consider in downstream analyses. From this we were able to
158 measure the minimum and maximum XY coordinates or four orange and five black
159 ornaments, and determine the size, hue and saturation of each of these ornaments in each
160 individual.

161 To better understand the correlational relationships among ornaments which we term
162 modulation, we calculated Pearson's correlations between ornaments for size (where
163 ornaments with 10 pixels or less were marked as NA) and saturation in R using the rcorr()
164 function from the package *Hmisc* [43]. We also calculated Spearman's correlations for
165 presence/absence (with ornaments with 10 pixels or less marked as absent).

166 With the ornaments we defined from our stock populations, we used functions from the R
167 packages pedigreemm, sommer, and MCMCglmm [44–47] to estimate heritability for size
168 (the number of pixels) and presence/absence for individual ornaments from our pedigrees.
169 For size, heritability was calculated using the raw data for each ornament (in the number of
170 pixels) for all fish that had that ornament, and with fish missing that ornament marked as
171 NA. A matrix with the relatedness between all individuals in the pedigree was calculated
172 using the functions pedigree() and getA(). We then used the mmer() function to fit a
173 univariate mixed effect model to estimate heritability, with a random effect - 'animal', which

174 is linked to the relatedness matrix included in the model formula (ornament.size ~
175 (1|animal)). For binary presence/absence data, we used a threshold of 10 pixels for presence
176 with all sizes smaller than this marked as absent. We used the function *MCMCglmm*() to
177 estimate heritability as implemented for binary traits [48]. We used *MCMCglmm*() for binary
178 traits as it has been found to be more reliable for binary data than other methods available
179 [49]. We used the priors (R = list(V = 1, fix = 1), G = list(G1 = list(V = 1, nu = 1000, alpha.mu =
180 0, alpha.V = 1) with the residual variance fixed to 1 as recommended for binary data [48],
181 and used a burnin of 100000 and the number of iterations set to 2500000.

182 *Strength of Y-effects on ornamentation*

183 We examined the effects of any possible Y-linked loci on total orange area and total black
184 area, by looking at the relationship between the phenotypes of our generation three
185 offspring and the phenotypes of their paternal and maternal grandfathers. Because the Y
186 chromosome is passed down the patriline, grandsons will have the Y of their paternal
187 grandfathers but not their maternal grandfathers. Therefore, we can hypothesise that if there
188 are Y linked loci effecting total black or black area, then the slope of the paternal grandfather
189 total black or black area, should be greater than the slope of maternal grandfather phenotype
190 on male total black or black area.

191 Unfortunately, as we were unable to photograph and phenotype the grandfathers of our
192 generation two offspring with our phenotype analysis pipeline. For this analysis we
193 therefore have a smaller sample of 73 generation three offspring from seven families. We
194 carried out analyses of both maternal and paternal effects for both total orange area and total
195 black area, using mmer(). As per our heritability analysis we used a random effect - 'animal',

196 which is linked to a relatedness matrix, and which controls for relatedness between
197 individuals. We also included a fixed effect, which was either the maternal or paternal
198 grandfathers phenotype. From the coefficients of this model we then have the slope of these
199 grandfather phenotypes (paternal and maternal) on both total orange and black area in our
200 generation three offspring. By comparing these slopes we can then see whether there is
201 therefore evidence of any Y effect loci. The formula of this analysis was: (total orange/black
202 area ~ Pat/Mat-GF phenotype + (1|animal)).

203 **Results**

204 Following Thin Plate Spline alignment of orange and melanin extracted images, we
205 identified four orange (CI = CIV) and five black ornaments (MI – MV, Fig. 1) found in our
206 stock population. Although all orange ornaments overlapped with at least one black
207 ornament, the overlap regions were small with 59% of potential ornament pixels in Fig 1.
208 Orange (blackblack), 35% black (black), and only 6% both (grey).

209 We determined correlations among ornaments in terms of size and saturation to understand
210 the genetic relationship among different modules in our stock population. We observed
211 positive correlations for both size and presence/absence among many of the orange
212 ornaments (Fig. 2A, Table 1 below the diagonal, supplementary table 1). This result suggests
213 there may be modifier loci that globally affect the size of orange ornaments found in males,
214 and thus also the total amount of orange pigmentation. Many previous studies that have
215 thus used the total orange (or 'black') area as a trait would therefore have been at least in
216 part measuring the heritability or inheritance of these loci rather than any loci related to the
217 presence or absence of any individual ornament. In contrast, correlations in size between

218 adjacent orange and black ornaments were found to generally be negative (Fig 2B, Table 1,
219 Supplementary table 2). This perhaps indicates epistatic interactions between loci controlling
220 black ornaments, with loci or alleles that increase size in one ornament, seemingly reducing
221 the size of others that are adjacent. Alternatively, it may reflect that where orange and black
222 ornaments overlap, only the black area is visible. Correlations in size between orange and
223 black ornaments (average = -0.019) are far lower than between ornaments of the same colour
224 (orange average = 0.21, black average = 0.091, Table 1).

225 We also determined the correlation in saturation among ornaments (Table 1 above the
226 diagonal, Supplementary table 3). For both black and orange pigmentation, we observed
227 generally positive correlations in saturation between adjacent ornaments of the same
228 pigmentation type. Thus again, ornaments in the same pathway do not appear to be entirely
229 independent of each other, suggesting that modifier loci perhaps control saturation levels
230 affecting multiple ornaments simultaneously. As with size, the correlation in saturation
231 between orange and black ornaments was on average much lower (average = 0.175) than
232 between ornaments of the same colour (orange average = 0.47, black average = 0.305, Table
233 1), suggesting that modifier loci do not transcend biochemical pathways to affect both
234 ornament types.

235 We used our pedigreed males to investigate the pattern of inheritance from father to son
236 (Table 2). Where an ornaments presence or absence is exclusively under the control of the Y-
237 chromosome all male offspring of a male will share their father's phenotype. However, in
238 cases where the presence or absence of an ornament is genetically partially or fully under
239 the control of a locus or loci of autosomal, X or pseudoautsomal origin, sons will frequently

have different phenotypes than their fathers. Our results show that no ornament displays the inheritance pattern expected for a purely male specific Y-linked trait. For two ornaments (OII and BV), we observe a majority of male offspring have the opposite phenotype to their father. Overall these results suggest that individual orange and black ornaments are not generally linked to the Y chromosome in our population.

For presence/absence, mean heritability was roughly similar for orange ornaments (0.539) and black ornaments (0.410), and most ornaments show high heritability except for ornaments OIII and BI (Table 3). In contrast, heritability for total area differed markedly between orange (0.678) and black (0.038) ornaments.

Our analysis of paternal and maternal grandfather effects identified a strong relationship between offspring total orange area and paternal grandfather total orange area (Coeff. = 0.807). However, the relationship between offspring total black area and maternal grandfather total black area was much weaker (Coeff. = 0.232). This indicates that there may indeed be Y-linked modifier loci that affect total orange area in guppies. This is consistent with our previous analyses which show positive correlations in size among orange ornaments. We also found a strong positive relationship between offspring total black area and paternal grandfather total black area (Coeff. = 0.452). In contrast there is a negative relationship between offspring total black area and maternal grandfather total black area (Coeff. = -0.214). Again, this result suggests that Y-linked modifier loci do indeed affect the total black area in guppies, just as they appear to affect total orange area too.

Discussion

Our study uses high resolution image analysis to define individual ornaments without human bias in order to examine inheritance patterns across a dataset of 214 offspring from 19 families. By computationally clustering pixels automatically to define individual ornaments without bias [35], we provide a comprehensive and agnostic diagnostic method for colouration patterning in a semi-natural outbred population. Using this method, we identified four orange and five black ornaments (Fig. 1) in our population that we then used to study modulation, heritability and mode of inheritance. We find, somewhat surprisingly, that loci controlling the presence and absence of these ornaments are not Y-linked, despite previous studies finding evidence of Y-linkage. However, in contrast to these presence/absence loci, we do find evidence of Y-linked modifier loci that affect aggregate phenotypes like total orange and total black area.

Modulation of ornamentation

The colourful ornaments found in animals are often treated as independent phenotypes, with the relationships among different ornaments less well considered [50]. However, in many cases the functions of these ornaments are not independent, but as whole patterns in conjunction [51]. Furthermore, some ornaments, particularly those that share the same biochemical basis, may have shared aspects of genetic architecture through the pathways that control them, and so treating them as individual units may not always be appropriate.

The colour patterns of guppies are used as a sexual signal. It has been proposed that such signals might be made more conspicuous, and therefore attractive to females, by greater differences between adjacent patches of colour [50]. If this is the case for guppies, we might

expect selection for linkage between adjacent black and orange patches. We tested this by examining correlations in presence and absence between different male ornaments (Table 1). Although we found some strong positive correlations for size within ornaments of the same colour, there was little correlation among adjacent ornaments of different colours (supplementary table 2). Our results therefore do not provide evidence of linkage between adjacent and contrasting male ornaments in our population of guppies.

Our analysis does though provide several lines of evidence that guppy ornaments of the same colour are not entirely independent of each other. Interestingly, orange and black ornaments displayed different genetic characteristics, with high heritability for size (Table 3) and high correlations in size (Table 1, Fig 2) between many of the orange ornaments, but not black ornaments. However, for both black and orange pigmentation, we observed generally positive correlations in saturation between adjacent ornaments of the same pigmentation type (Table 1). In addition, for both orange and black, we find evidence of Y-linked modifier loci that globally affect total amount of orange and black pigmentation. Altogether, this provides support for the idea that ornaments in the same biochemical pathway are not entirely independent of each other, with global modifier loci affecting saturation levels and total size for multiple ornaments simultaneously, while other independent loci encode for the presence or absence of individual ornaments.

Sex linkage

Many guppy colour traits have been shown to be Y-linked, reviewed by Lindholm and Breden [52] and Kottler and Schartl [53]. However, much of this work has either been done

306 on ornaments in domestic guppy breeds and therefore unlikely to be found in the wild
307 [30,31], or on composite traits, such as aggregate colour area [21,32,33]. We assessed the
308 potential for Y-linkage in individual ornaments from our semi-natural populations under
309 the expectation that any Y-linked ornament displayed by a father would be also present in
310 all his sons. However, we did not observe Y inheritance patterns for presence or absence of
311 any single one of our nine ornaments. This suggests that individual male colour patterns in
312 guppies from our population are not Y-linked. This is also reflected in the heritability for
313 presence/absence of these ornaments, with more than half of ornaments with heritability
314 <0.5. Even for the ornaments inherited with the highest heritability (CIV), 9.7% of offspring
315 males display a different phenotype to their father.

316 Recent studies have suggested that the guppy Y chromosome is divided into a relatively
317 small region where recombination between the X and Y never occurs [54–57], and a larger
318 adjacent region where recombination is rare, occurring in between 1% and 0.1% of males
319 [55,58,59]. However, because the vast majority of recombination in males is confined to the
320 telomeric ends of the sex chromosome, nearly the entire chromosome is passed on as a Y
321 chromosome in >99% of male offspring. Therefore, rare X-Y recombination events cannot
322 explain the difference between paternal and filial phenotypes we observe in our ornaments.

323 These results are at first perplexing in light of previous work, and there are several potential
324 explanations. First, it is possible that our population has lost Y-linkage of ornaments in the
325 >20 years since it was collected from the wild. However, we think this is not terribly likely
326 given the large size of the founding colony and the fact that it has been maintained as an
327 outbred, freely mating population ever since. Both of these strategies have maintained

genetic diversity and avoided potential bottlenecks. Moreover, Y-linkage has been observed in other lab and inbred populations [21,60], and it is not clear how Y-linkage would erode.

Second, considerable variation in perceived Y-linkage exists among wild Trinidadian populations [61], with notable differences between high predation and low predation populations in particular [32,33,55,62]. It is therefore possible that difference between our results and others may simply be due to differences between populations. Although we also think this is unlikely given that the Quare population upon which our stock population is founded has been studied using aggregate methods, described below [33], it is however is important for future studies to quantify Y-linkage of individual ornaments in multiple different populations.

However, our results do perhaps make more sense when taken with our analysis of Y-linkage of aggregate phenotypes total orange and total black area. These analyses did find evidence of Y-linked modifier loci that globally affect the total amount of orange and black pigmentation. Thus perhaps the most likely explanation for our perplexing results is that although the presence or absence of individual ornaments are not often Y-linked in natural populations, the Y chromosome does carry one or more modifier loci that affect many ornaments in aggregate. Our results are therefore consistent with studies that have characterized aggregate ornamental area, which often identify significant Y contributions. For example, Postma *et al* [34] and Brooks and Endler [21] both identified significant levels of Y-linked genetic variation when assessing total ornamental areas. Therefore when studies use the total orange (or 'black') area as a trait they are in large part measuring the mode of

349 inheritance of these loci rather than any loci related to the presence or absence of any
350 individual ornament [21,33].

351 It may be that these Y-linked modifier loci works through a simple mechanism, such as
352 testosterone level or testosterone sensitivity, which has been shown to affect total ornament
353 area [31–33]. Indeed, the extensive variation exhibited in total ornament area among natural
354 [33] and introduced [32] populations hints at a simple genetic architecture, as the alternative
355 of rapid and repeated movement of many underlying genes to the relatively small Y
356 chromosome, is somewhat improbable. Given the importance of testosterone level and
357 sensitivity in fish growth rates, maturation and colouration [63–65], and the variation
358 observed in natural guppy populations in these traits [66], it is likely that testosterone and/or
359 testosterone sensitivity vary across populations, and this may interact with colouration. The
360 nature of these interactions, and the identification of the loci responsible for these Y-linked
361 effects remain interesting areas of future research.

362 *Conclusion*

363 Surprisingly, we did not observe inheritance patterns for presence and absence for any of
364 our nine ornaments that were consistent with Y-linkage. However, we did find evidence of
365 Y-linked modifier loci that affect the aggregate phenotypes of total orange and black area.
366 Given the results of our study, the Y-linkage of individual male colour traits may therefore
367 not be as important in the rapid evolution of colour changes in natural populations of
368 guppies [32] or in the expansions of the non-recombining Y region [67] as previously
369 thought. However, Y-linked modifier loci that affect aggregate traits may well play a role.

Alternatively other mechanisms to resolve sexual conflict, such as changes in gene regulation [68] may alone be enough to explain colour dimorphism in this species.

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Bibliography

1. Williams P. 2007 The distribution of bumblebee colour patterns worldwide: Possible significance for thermoregulation, crypsis, and warning mimicry. *Biol. J. Linn. Soc.* **92**, 97–118.

2. Cuthill IC *et al.* 2017 The biology of color. *Science (80-.).* **357**.

3. Roulin A. 2014 Melanin-based colour polymorphism responding to climate change. *Glob. Chang. Biol.* **20**, 3344–3350.

4. Merrill RM *et al.* 2015 The diversification of *Heliconius* butterflies: What have we learned in 150 years? *J. Evol. Biol.* **28**, 1417–1438.

5. Langham GM. 2005 Rufous-tailed jacamars and aposematic butterflies: do older birds attack novel prey? *Behav. Ecol.* **17**, 285–290.

6. Summers K, Clough ME. 2001 The evolution of coloration and toxicity in the poison frog family (Dendrobatidae). *Proc. Natl. Acad. Sci. U. S. A.* **98**, 6227–6232.

7. van't Hof AE, Edmonds N, Dalikova M, Marec F, Saccheri IJ. 2011 Industrial melanism in british peppered moths has a singular and recent mutational origin. *Science (80-.).* **332**, 958–960.

8. Steffen JE, Guyer CC. 2014 Display behaviour and dewlap colour as predictors of contest success in brown anoles. *Biol. J. Linn. Soc.* **111**, 646–655.

9. Hughes KA, Houde AE, Price AC, Rodd FH. 2013 Mating advantage for rare males in wild guppy populations. *Nature* **503**, 108–110.

10. Garratt M, Brooks RC. 2012 Oxidative stress and condition-dependent sexual signals: More than just seeing red. *Proc. R. Soc. B Biol. Sci.* **279**, 3121–3130.

11. Svensson PA, Wong BBM. 2011 Carotenoid-based signals in behavioural ecology: A review. *Behaviour* **148**, 131–189.

12. Ruell EW, Handelsman CA, Hawkins CL, Sofaer HR, Ghalambor CK, Angeloni L. 2013 Fear, food and sexual ornamentation: Plasticity of colour development in Trinidadian guppies. *Proc. R. Soc. B Biol. Sci.* **280**.

13. Morehouse NI, Rutowski RL. 2010 In the eyes of the beholders: Female choice and avian predation risk associated with an exaggerated male butterfly color. *Am. Nat.* **176**, 768–784.

14. Rowe L, Chenoweth SF, Agrawal AF. 2018 The genomics of sexual conflict. *Am. Nat.* **192**, 274–286.

15. Cox RM, Calsbeek R. 2009 Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. *Am. Nat.* **173**, 176–187.

16. Rice WR. 1987 The accumulation of sexually antagonistic genes as a selective agent promoting the evolution of reduced recombination between primitive sex chromosomes. *Evolution* **41**, 911–914.

17. Rice WR. 1984 Sex chromosomes and the evolution of sexual dimorphism. *Evolution* **38**, 735–742.

18. Charlesworth B, Jordan CY, Charlesworth D. 2014 The evolutionary dynamics of sexually antagonistic mutations in pseudoautosomal regions of sex chromosomes.

418 *Evolution* **68**, 1339–1350.

419 19. Furman BLS, Metzger DCH, Darolti I, Wright AE, Sandkam BA, Almeida P, Shu JJ,
420 Mank JE. 2020 Sex chromosome evolution: so many exceptions to the rules. *Genome*
421 *Biol. Evol.* , 1–32.

422 20. Endler JA. 1983 Natural and sexual selection on color patterns in poeciliid fishes.
423 *Environ. Biol. Fishes* **9**, 173–190.

424 21. Brooks R, Endler JA. 2001 Direct and Indirect Sexual Selection and Quantitative
425 Genetics of Male Traits in Guppies (*Poecilia Reticulata*). *Evolution.* **55**, 1002.

426 22. Pitcher TE, Rodd FH, Rowe L. 2007 Sexual colouration and sperm traits in guppies. *J.*
427 *Fish Biol.* **70**, 165–177.

428 23. Endler JA. 1980 Natural Selection on Color Patterns in Poecilia reticulata. *Evolution* **34**,
429 76.

430 24. Houde AE. 1992 Sex-linked heritability of a sexually selected character in a natural
431 population of Poecilia reticulata (Pisces: Poeciliidae). *Heredity* **69**, 229–235.

432 25. Lindholm A, Breden F. 2002 Sex Chromosomes and Sexual Selection in Poeciliid
433 Fishes. *Am. Nat.* **160**, S214–S224.

434 26. Houde AE. 1987 Mate choice based upon naturally occurring color-pattern variation
435 in a guppy population. *Evolution (N. Y).* **41**, 1–10.

436 27. Reznick D, Endler JA. 1982 The impact of predation on life history evolution in
437 Trinidadian guppies (*Poecilia reticulata*). *Soc. Study Evol.* **36**, 160–177.

438 28. Breden F, Stoner G. 1987 Male predation risk determines female preference in the
439 Trinidad guppy. *Nature* **329**, 831–833.

440 29. Fisher RA. 1931 The evolution of dominance. *Biol. Rev.* **6**, 345–368.

441 30. Winge Ö. 1927 The location of eighteen genes inLebistes reticulatus. *J. Genet.* **18**, 1–43.

31. Haskins CP, Young P, Hewitt RE, Haskins EF. 1970 Stabilised heterozygosis of supergenes mediating certain y-linked colour patterns in populations. *Heredity* **25**, 575–589.

32. Gordon SP, López-Sepulcre A, Rumbo D, Reznick DN. 2017 Rapid changes in the sex linkage of male coloration in introduced guppy populations. *Am. Nat.* **189**, 000–000.

33. Gordon SP, López-Sepulcre A, Reznick DN. 2012 Predation associated differences in sex linkage in wild guppy coloration. *Evolution* **66**, 912–918.

34. Postma E, Spyrou N, Rollins LA, Brooks RC. 2011 Sex-dependent selection differentially shapes genetic variation on and off the guppy Y chromosome. *Evolution* **65**, 2145–2156.

35. Holman L, Head ML, Lanfear R, Jennions MD. 2015 Evidence of experimental bias in the life sciences: why we need blind data recording. *PLoS Biol.* **13**, 1–12.

36. Kotrschal A *et al.* 2013 Artificial selection on relative brain size in the guppy reveals costs and benefits of evolving a larger brain. *Curr. Biol.* **23**, 168–171.

37. Leutner B, Horning N, Schwalb-Willmann J. In press. RStoolbox: Tools for remote sensing data analysis. R package version 0.2.6.

38. Hijmans RJ, van Etten J. 2012 raster: Geographic analysis and modeling with raster data. R package version 2.0-12.

39. Rohlf JF. 2013 tpsDig, digitize landmarks and outlines.

40. Rohlf JF. 2013 tpsUtil, file utility program.

41. Adams DC, Otárola-Castillo E. 2013 geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods Ecol. Evol.* **4**, 393–399.

42. Schlager S. 2017 Morpho and Rvcg - Shape analysis in R: R-packages for geometric morphometrics, shape analysis and surface manipulations.

466 43. Harrell Jr FE, with contributions from Charles Dupont and many others. 2019 Hmisc:
467 Harrell Miscellaneous.

468 44. Vazquez AI, Bates DM, Rosa GJM, Gianola D, Weigel KA. 2010 Technical note: An R
469 package for fitting generalized linear mixed models in animal breeding. *J. Anim. Sci.*
470 **88**, 497–504.

471 45. Covarrubias-Pazaran G. 2016 Genome-Assisted prediction of quantitative traits using
472 the r package sommer. *PLoS One* **11**, 1–15. (doi:10.1371/journal.pone.0156744)

473 46. Covarrubias-Pazaran G. 2018 Software update: Moving the R package sommer to
474 multivariate mixed models for genome-assisted prediction. *bioRxiv* , 354639.

475 47. Hadfield JD. 2010 MCMC Methods for Multi-Response GLMMs in R. *J. Stat. Softw.* **33**,
476 1–22.

477 48. de Villemereuil P. 2012 Estimation of a biological trait heritability using the animal
478 model.

479 49. De Villemereuil P, Gimenez O, Doligez B. 2013 Comparing parent-offspring
480 regression with frequentist and Bayesian animal models to estimate heritability in
481 wild populations: a simulation study for Gaussian and binary traits. *Methods Ecol.*
482 *Evol.* **4**, 260–275.

483 50. Endler JA. 2012 A framework for analysing colour pattern geometry: adjacent colours.
484 *Biol. J. Linn. Soc.* **107**, 233–253.

485 51. Endler JA, Cole GL, Kranz AM. 2018 Boundary strength analysis: Combining colour
486 pattern geometry and coloured patch visual properties for use in predicting
487 behaviour and fitness. *Methods Ecol. Evol.* **9**, 2334–2348.

488 52. Lindholm A, Breden F. 2002 Sex chromosomes and sexual selection in poeciliid fishes.
489 *Am. Nat.* **160**, S214–S224.

490 53. Kottler VA, Schartl M. 2018 The colorful sex chromosomes of teleost fish. *Genes* **9**, 233

491 54. Yamamoto T. 1975 The medaka, *Oryzias latipes*, and the guppy, *Lebistes*. In *Handbook of*
492 *Genetics* (ed RC King), pp. 133–149. Boston, MA: Spinger.

493 55. Haskins CP, Haskins EF, Mclaughlin JJA, Hewitt RE. 1961 Polymorphism and
494 population structure in *Lebistes reticulatus*, an ecological study. In *Vertebrate Speciation*
495 (ed WF Blair), pp. 320–395. Austin: University of Texas Press.

496 56. Tripathi N, Hoffmann M, Willing E-M, Lanz C, Weigel D, Dreyer C. 2009 Genetic
497 linkage map of the guppy, *Poecilia reticulata*, and quantitative trait loci analysis of
498 male size and colour variation. *Proc. Biol. Sci.* **276**, 2195–208.

499 57. Darolti I, Wright AE, Mank JE. 2020 Guppy Y chromosome integrity maintained by
500 incomplete recombination suppression. *Genome Biol. Evol.* , 1–30.

501 58. Lisachov AP, Zadesenets KS, Rubtsov NB, Borodin PM. 2015 Sex chromosome
502 synapsis and recombination in male guppies. *Zebrafish* **00**, 1–7.

503 59. Bergero R, Gardner J, Bader B, Yong L, Charlesworth D. 2019 Exaggerated
504 heterochiasmy in a fish with sex-linked male coloration polymorphisms. *Proc. Natl.*
505 *Acad. Sci. U. S. A.* **116**, 6924–6931.

506 60. Khoo G, Lim TM, Chan W, Phang VPE, Khoo G, Lim TM, Chan W, Phang VPE. 1999
507 Linkage analysis and mapping of three sex-linked color pattern genes in the Guppy,
508 *Poecilia reticulata*. *Zoolog. Sci.* **16**, 893–903.

509 61. Fraser BA, Künstner A, Reznick DN, Dreyer C, Weigel D. 2015 Population genomics
510 of natural and experimental populations of guppies (*Poecilia reticulata*). *Mol. Ecol.* **24**,
511 389–408.

512 62. Hughes KA, Rodd FH, Reznick DN. 2005 Genetic and environmental effects on
513 secondary sex traits in guppies (*Poecilia reticulata*). *J. Evol. Biol.* **18**, 35–45.

514 63. Yue M, Zhao J, Tang S, Zhao Y. 2018 Effects of estradiol and testosterone on the
515 expression of growth-related genes in female and male nile tilapia, *Oreochromis*
516 *niloticus*. *J. World Aquac. Soc.* **49**, 216–228.

64. Bayley M, Junge M, Baatrup E. 2002 Exposure of juvenile guppies to three antiandrogens causes demasculinization and a reduced sperm count in adult males. *Aquat. Toxicol.* **56**, 227–239.

65. Matty AJ, Lone KP. 2009 The effect of androgenic steroids as dietary additives on the growth of carp (*Cyprinus carpio*). *Proc. World Maric. Soc.* **10**, 735–745.

66. Zandonà E *et al.* 2011 Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. *Funct. Ecol.* **25**, 964–973.

67. Wright AE *et al.* 2017 Convergent recombination suppression suggests role of sexual selection in guppy sex chromosome formation. *Nat. Commun.* **8**, 14251.

68. Mank JE. 2017 The transcriptional architecture of phenotypic dimorphism. *Nat. Ecol. Evol.* **1**, 0006.

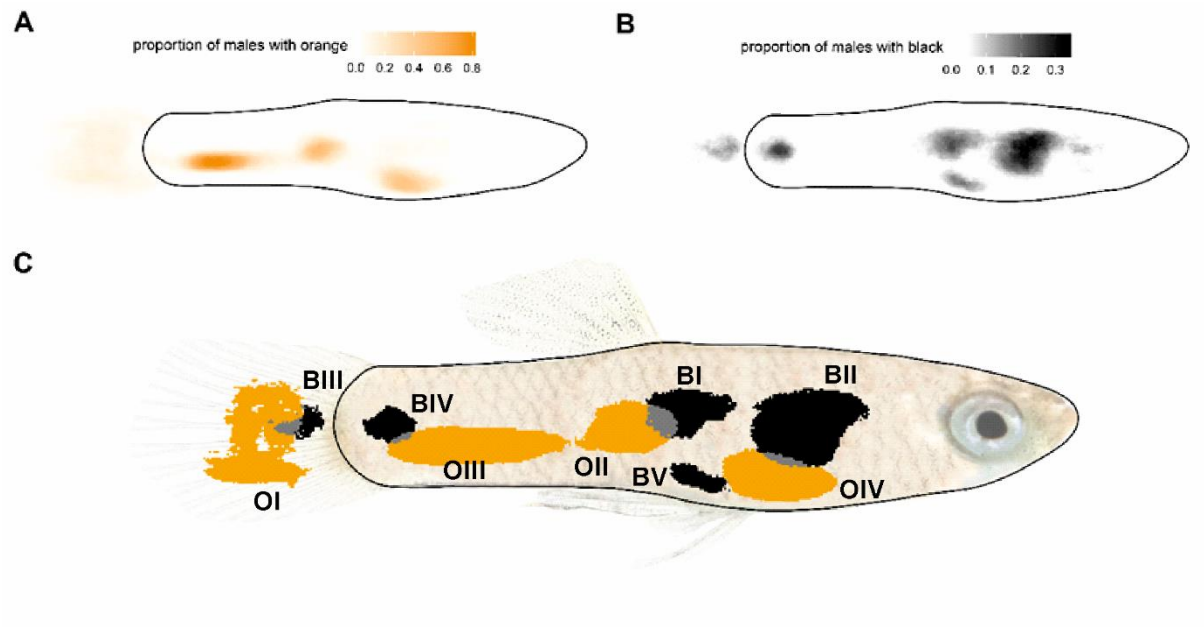

Fig 1. Male ornamentation. A) Heatmap showing the proportion of individuals from the full stock population with orange pigmentation at each pixel, B) Heatmap showing the proportion of individuals from the full stock population with black pigmentation at each pixel, with the eye masked, C) Mosaic plot showing the four orange and five black ornaments defined from the stock population.

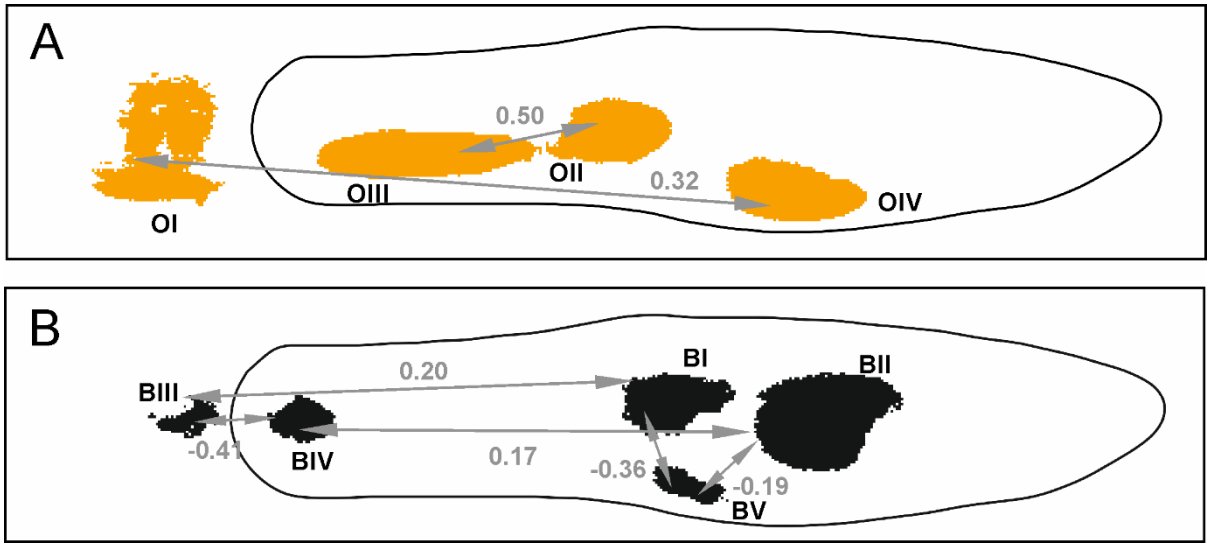

Fig 2. Significant correlations for presence and absence among orange (A) and black (B) ornaments. Correlations are significant to P< 0.05.

562 **Table 1. Strength of correlations for ornament size (below the diagonal) and saturation**
563 **(above the diagonal) among orange and black ornaments. P-value <0.05 in bold**

Ornament	OI	OII	OIII	OIV	BI	BII	BIII	BIV	BV
OI	-	**0.53**	**0.250**	-0.05	-0.05	0.07	-0.28	0.04	-0.24
OII	-0.21	**-**	**0.720**	**0.40**	0.01	-0.03	0.20	0.21	0.21
OIII	0.08	**0.19**	-	**0.41**	-0.01	0.03	0.21	0.17	0.02
OIV	-0.25	**0.28**	**0.23**	-	0.27	-0.10	0.13	-0.01	0.13
BI	-0.27	**-0.29**	-0.15	**0.34**	-	**0.35**	**0.37**	**0.41**	0.33
BII	0.13	0.12	0.01	-0.14	**-0.47**	-	0.21	**0.54**	**0.48**
BIII	-0.05	0.03	0.22	0.10	0.17	-0.24	-	**0.52**	**0.62**
BIV	0.05	**-0.44**	-0.14	-0.07	-0.14	0.02	-0.25	-	**0.57**
BV	-0.10	-0.24	-0.14	0.17	0.52	-0.03	0.12	0.24	-

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575 **Table 2. Presence/absence and heritability across both fathers and sons for each ornament.**

Ornament	**OI**	**OII**	**OIII**	**OIV**	**BI**	**BII**	**BIII**	**BIV**	**BV**	**Total Or.**	**Total Bl.**
Present	57%	89%	96%	57%	24%	98%	27%	64%	36%		
Same: different to father										-	-
Same as father	49%	44%	73%	90%	60%	87%	73%	52%	48%	**-**	**-**
h^2 (pres./abs)	0.669	0.456	0.255	0.776	0.121	0.414	0.519	0.581	0.414	-	-
h^2 (size)	0.508	0.461	0.459	0.484	0.000	0.000	0.000	0.186	0.000	0.678	0.038

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