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# The effect of purging on sexually selected traits through antagonistic pleiotropy with survival

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## Abstract

Sexually selected traits are expected to evolve to a point where their positive effect on reproductive success is counterbalanced by their negative effect on survival. At the genetic level, such a trade-off implies antagonistic pleiotropy between survival and the expression of sexually selected traits. Yet, the consequences of such a genetic architecture have been largely overlooked in studies examining how inbreeding influences sexually selected traits. These studies have solely interpreted their results as an effect of increased homozygosity. An alternative, however, is that purging of recessive alleles deleterious for survival when inbreeding increases can negatively affect the expression of sexually selected traits through antagonistic pleiotropy. We tested this hypothesis by analyzing the effects of inbreeding on several male ornaments and life-history traits across 20 captive populations of guppies (*Poecilia reticulata*) with varying levels of inbreeding. Only one ornament, orange area, decreased in its expression with an increasing level of inbreeding. This was most likely due to purging because we found no within-population relationship between orange area and the inbreeding coefficient. We further tested this hypothesis by crossing unrelated individuals from the four most inbred populations, creating a group of individuals with purged genomes but restored heterozygosity. Restoration of heterozygosity only slightly increased orange area, confirming that the decrease in orange area in the inbred populations most likely resulted from purging. These results support previous studies suggesting the existence of antagonistic pleiotropy between ornament expression and survival.

## Introduction

Strong directional sexual selection is expected to erode the genetic variation in sexually selected traits (Charlesworth 1987; Falconer 1989), leaving little potential for genetic benefits of female choice. Contrary to this, Pomiankowski and Møller (1995) found relatively high additive genetic variance in sexually selected traits compared to nonsexually selected traits. To explain this paradox, Rowe and Houle (1996) suggested that the genetic architecture of sexually selected traits includes many genes pleiotropically linked to condition. The genetic variation in sexually selected traits is, thereby, maintained through the maintenance of genetic variation in condition. Because many traits affect condition, sexually selected traits are expected to "capture" much of this genetic variation. This has been called the genic-capture hypothesis. Such genetic architecture was suggested to evolve by selection for high allocation of resources to build or maintain secondary sexual traits, which in turn leads to a strong dependence of secondary sexual traits on the resource pool (condition) of the individual.

Several studies on inbreeding (i.e., mating among relatives) have tested the genic-capture hypothesis (e.g., Drayton et al. 2007, 2010; Bolund et al. 2010; Prokop et al. 2010). These studies hypothesized that sexually selected traits should be sensitive to inbreeding because the increased homozygosity following inbreeding should reveal the genetic load of condition, and thereby, negatively influence condition-dependent

traits. This is consistent with the hypothesis that traits closely related to fitness are strongly influenced by increased homozygosity, a hypothesis empirically supported by a stronger effect of inbreeding on life-history than on morphological traits (Lynch and Walsh 1998; Roff 1998; DeRose and Roff 1999).

Negative responses to inbreeding have been generally interpreted as a direct effect of increased homozygosity, therefore assuming a change in genotype frequencies without change in allele frequencies (Lynch and Walsh 1998; Kelly 2005). This assumption does not hold, however, if the investigated traits are affected by purging. Purging is the process by which recessive deleterious alleles are removed by selection when homozygosity increases (Lande and Schemske 1985; Hedrick 1994; Lynch et al. 1995). Effects of purging have been overlooked in inbreeding studies on sexually selected traits despite the fact that purging can also explain the decrease in the expression of these traits in inbred individuals. This may seem counterintuitive because purging removes deleterious alleles that are commonly thought to decrease the expression of sexually selected traits. However, because of tradeoffs between fitness-related traits, pleiotropic alleles can be deleterious for some traits and beneficial for others (i.e., antagonistic pleiotropy). If such alleles are partly recessive, the balance between positive and negative selection acting on them will change with increasing inbreeding. The potential trade-off between sexually selected traits and survival has long been recognized in the literature (e.g., Fisher 1930; Lande 1980, 1981; Kirkpatrick 1982; Pomiankowski et al. 1991; Andersson 1994; Kokko 2001; Badyaev and Qvarnström 2002), and a negative genetic correlation between survival and attractiveness has been shown in the guppy (Brooks 2000). If the underlying genetic architecture of this tradeoff involves antagonistic pleiotropy, and alleles that allocate resources to the expression of sexually selected traits are partly recessive, we expect purging to decrease the frequency of these alleles, and consequently lower the expression of sexually selected traits in inbred populations.

The efficiency of purging depends on the genetic architecture of the traits (reviewed by Charlesworth and Willis 2009). Theoretical studies suggest that purging mainly acts on recessive alleles that are lethal or strongly deleterious (Fu et al. 1998; Wang et al. 1999). However, recessive deleterious alleles of smaller effects can also be purged if they reinforce the effect of each other in a process termed synergistic epistasis (Fu 1999). Synergistic epistasis is also necessary for purging of deleterious alleles on the Y chromsome, else selection against these alleles would not increase with increasing homozygosity.

We investigated the response to inbreeding in several ornamental and life-history traits in 20 experimental populations of guppies that accumulated varying levels of inbreeding during eight generations. Because inbreeding accumulated slowly over a relatively long period, we expected purging to occur in the most inbred populations. This was confirmed in a study by Larsen et al. (2011) on the same populations, who found strong signs of purging affecting clutch size and offspring survival in the four most inbred populations. Accordingly, at the eighth generation, we did not observe inbreeding depression in any of the life-history traits. In contrast, we observed a strong decrease of the orange-spot area with inbreeding. To test whether this response was due to an increased homozygosity or an effect of purging, as expected under the antagonistic-pleiotropy hypothesis, we analyzed the effects of inbreeding on the different traits within and among populations using contextual analyses (Blalock 1984; Raudenbush and Bryk 2002). We further estimated how much of the decline in orange area was due to increased homozygosity versus purging, by restoring heterozygosity among purged populations via interpopulation crosses. We then compared the orange-spot area between purged inbred, purged outbred, and nonpurged weakly inbred individuals.

## Methods

This study consists of two parts. In the first part, we investigated the relationship between inbreeding and the expression of morphological and life-history traits within and among populations that accumulated varying level of inbreeding during eight generations. In the second part, we wanted to distinguish between the effect of inbreeding and purging on the expression of orange coloration, the only trait affected by inbreeding at generation 8. We therefore conducted an additional experiment at generation 11 using descendants from the same populations.

### Study species

The guppy, *Poecilia reticulata* Peters (1859; Fig. 1) is an ovoviviparous freshwater fish from Trinidad and northeastern South America. Male guppies display a variety of ornaments

**Figure 1.** The study species, *Poecilia reticulata*, showing one male (top) and two females (bottom). In guppies, the male is smaller than the female and is ornamented with color spots and an enlarged caudal fin. (Photo by Per Harald Olsen.)

composed of carotenoid-based orange, melanin black, and various iridescent (structural) color spots, as well as an enlarged caudal fin (Houde 1997). Several of the genes coding for the male ornaments lie outside the recombining region of the Y chromosome (Lindholm and Breden 2002; Brooks and Postma 2011). In addition, quantitative-genetics analyses indicate a Y-linked component in the expression of orange and black color spots, and caudal-fin size (Brooks and Endler 2001a; Brooks and Postma 2011; Postma et al. 2011). However, there is also a substantial amount of autosomal variation in these traits (Brooks and Postma 2011; Postma et al. 2011). There is strong evidence for female preference for the orange color area, but the strength of this preference differs between populations (Houde 1987, 1997; Endler and Houde 1995) and between individuals (Brooks and Endler 2001b). The black spots, the body size, and the caudal-fin size also seem to affect female mate choice (Endler and Houde 1995; Houde 1997). The orange coloration is linked to condition, as demonstrated by a positive correlation between swimming performance and both the orange area (Nicoletto 1993) and the density of carotenoid pigments (Nicoletto 1991). However, the amount of food does not affect the area of orange coloration (Hughes et al. 2005), suggesting that factors other than food availability affect the condition dependence of orange area.

## Experimental populations and rearing condition

We collected approximately 500 guppies from a locality with high predation and high food availability in the lower Quare River (10°39′N, 61°12′W) of Trinidad in 1998. The founding population was large, being sampled from a river with several tributaries. Offspring of the collected guppies were used as founders of 30 experimental populations. We reared these fish under different breeding regimes and population sizes for eight discrete generations to produce three groups of 10 populations with low, intermediate, and high levels of inbreeding (referred to as the low-, intermediate-, and high-inbreeding treatment).

Each population was started by assembling pairs (one female and one male) from a random sample of the founding population, avoiding sib matings. Each mated pair produced one family. The populations in the low-inbreeding treatment had a fixed population size of 20 individuals (number of reproductive individuals at the start of each generation) with a 1:1 sex ratio, and therefore consisted of 10 families. The subsequent generations were formed with one male and one female offspring sampled from each family from the previous generation, thereby equalizing the family size and the sex ratio. Inbreeding was maintained at the lowest possible level by mating the least related females and males, based on their pedigree. Cross-mating between families was also avoided, that is, a sibling pair was never mated to another sibling pair. Note that all matings were controlled allowing us to calculate pedigree-based inbreeding coefficients ($F$) for all individuals.

The populations in the intermediate-inbreeding treatment had the same mating scheme as the populations in the low-inbreeding treatment, but a population size of 10 individuals (five males, five females). Finally, for the populations in the high-inbreeding treatment, each generation started with five males and five females selected randomly among the families (i.e., independent of family size) from the previous generation. This led to unequal family contribution to the next generation and occasionally full-sib mating.

In all populations, the fish were reared in 38-L aquaria divided into five compartments, with one family in each compartment, and water circulation among compartments (two aquaria per population in the low-inbreeding treatment, one per population in the intermediate- and high-inbreeding treatment). We maintained the fish on a 12:12 h light:dark cycle at 24 (±2)°C and fed them ad libitum once a day, alternating between dry flakes and newly hatched brine shrimp (*Artemia salina*). At the start of each generation, we placed each female together with her designated male in a compartment for 21 days. After this period, we removed the male and left the female to give birth. The female was removed after she had given birth to at least five offspring (full sibs). Male offspring were separated from their sisters at the onset of sexual maturation, when their gonopodium started to develop (Houde 1997). From this stage onwards, males were housed separately from their female siblings.

During the first six generations, we lost one population in the low-inbreeding treatment, three in the intermediate-inbreeding treatment, and six in the high-inbreeding treatment due to low recruitment in these populations. Hence, only 20 populations remained after generation six. During this period, the populations in the high-inbreeding treatment experienced purging of deleterious alleles affecting clutch size and offspring survival (Larsen et al. 2011). For these two traits, Larsen et al. (2011) observed an increase in inbreeding depression during the first four to five generations in the high-inbreeding treatment, followed by a decrease in inbreeding depression in the subsequent generations. Changes in the relationship between the inbreeding coefficient $F$ and trait expressions confirmed that the change in inbreeding depression resulted from purging of deleterious alleles.

## Inbreeding and traits expressions at generation 8

To examine the effects of inbreeding on ornamental and life-history traits at generation 8, we recorded the areas of orange and black color spots, the area of the caudal fin, the mean redness of orange spots (CIE $a^*$, where CIE is Commission International de l'Eclairage), the body length at birth, at

 

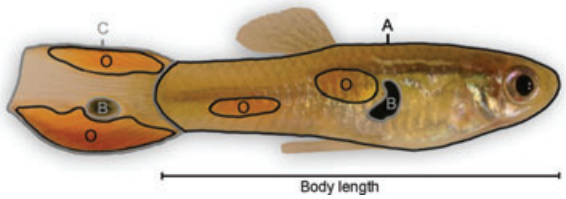


**Figure 2.** Morphological measurements taken on male guppies: body length, body area (A), caudal-fin area (C), orange-spot areas (O), and black-spot areas (B).

maturation, and 60 days after maturation, and the growth rate from birth to maturation (juvenile growth rate) and from maturation to 60 days thereafter (adult growth rate).

At generation 7, we isolated the first two offspring males that reached sexual maturity in each family and raised them individually in 1-L aquaria assigned randomly to a shelf location in a single room. We photographed all the siblings the day after birth, and the males at maturation and 60 days after maturation. All photographs were taken using a standardized assembly, including a digital camera (Canon E 300D) with remote control, two mounted lights on each side, and a moistened white plastic background. Before each photography session, we photographed a standard scale (Ted Pella, Inc. micrometer scale, 10 mm) and color plate as reference for subsequent calibration. The fish were immobilized in cold water (8–10°C) and placed on the moistened white plastic sheet before shooting. Anesthetic was not used because it alters the color patterns (Reynolds et al. 1993).

To account for the effects of clutch size on the different variables, we recorded the size of the clutch one day after birth from which the two sibling males derived. When two sibling brothers derived from different clutches, we used the average size of the two clutches in the statistical analysis. We used Image-J version 1.32 software (Abramoff et al. 2004) to measure standard body length (snout to base of the caudal fin) at birth, at maturation, and 60 days after maturation to estimate juvenile and adult growth rate (mm day$^{-1}$). From the picture at 60 days after maturation, we measured body area, caudal-fin area, and black-spot and orange-spot areas on the right side of each male (Fig. 2).

To correct for allometry, residuals from log–log regressions of the caudal-fin area and the areas of the color spots on body area and total area, respectively, were calculated. The allometric relationships (intercept ± SE, slope ± SE) were –0.47 ± 0.16 ln mm$^2$, 0.91 ± 0.04 ($R^2 = 0.697$) for caudal-fin area, –2.44 ± 0.97 ln mm$^2$, 0.92 ± 0.23 ($R^2 = 0.074$) for orange area, and –2.76 ± 1.25 ln mm$^2$, 0.68 ± 0.29 ($R^2 = 0.024$) for black area. We added the mean of each trait (on log scale) to the residuals to give the trait values a biological meaning (i.e., trait values that vary around the grand mean with the effect of size removed).

To test for qualitative variation in the orange coloration, we measured the mean redness of the orange spots using Adobe Photoshop 7.0 (Adobe Systems Inc.). First, we selected the area of the spots, then converted the pictures to CIE $L^*a^*b^*$ color space and used mean CIE $a^*$, which is the balance between red (magenta) and green, as a measure of redness. This approach was developed by P. A. Svensson (Svensson et al. 2005, 2006; Svensson 2006) to measure carotenoid-based belly coloration in two-spotted gobies (*Gobiusculus flavenscens*) for which it accurately described carotenoid concentration (Svensson et al. 2006). However, the use of this method for the guppy study system has not undergone any rigorous testing.

Repeatabilities, the ratio of among-individual variance on total variance (Falconer 1989), of the different measurements were estimated using two photographs taken on two consecutive days on 30 males. The repeatabilities were 0.98 for orange area, 0.99 for redness (CIE $a^*$), 0.99 for black area, 0.99 for body length, 0.99 for body area, and 0.97 for caudal-fin area.

The total sample size was 214 fish from 125 families. Of this, 123 fish from 76 families came from the low-inbred populations, 57 fish from 31 families came from the intermediate-inbred populations, and 34 fish from 18 families came from the high-inbred populations. (For CIE $a^*$, the sample size was 211 fish from 125 families.)

## Effect of homozygosity versus purging on orange area (generation 11)

After generation 8, we maintained the populations in the different treatments for few more generations. At generation 10, in order to further understand how inbreeding affected the orange coloration, we crossed the fish between the four most inbred populations to produce outbred individuals, taking care to perform crosses between all combinations of populations. Fish resulting from these among-population crosses had a substantial increase in heterozygosity relative to the inbred treatment as their inbreeding coefficient was zero (see Fig. 3 for a graphical representation of the different treatments). By comparing the area of orange spots between these newly outbred individuals, the four high-inbred populations and the nine low-inbred populations, we could test whether the decrease in orange area with inbreeding at generation 8 was due to increased homozygosity or was generated by selection against recessive deleterious alleles. If the decline in orange area with inbreeding was solely due to increased homozygosity, we expected a rebound in the orange area following outbreeding to a level equal to or higher than that of the low-inbred populations. If, however, the decline in orange area was mostly due to purging, we expected no difference in orange area between the newly outbred fish and the most inbred ones, because the alleles coding for large ornaments should have been removed.

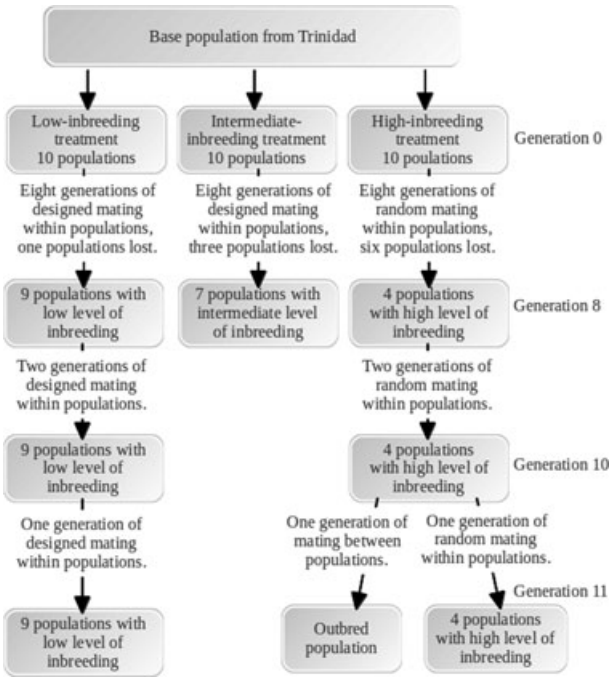


**Figure 3.** Experimental design of populations experiencing low, intermediate, and high levels of inbreeding and subsequent interpopulation crossing of the four inbred populations to create an outbred population at generation 11 (see Methods for details).

Recording of the traits at generation 11 differed slightly from the methods used at generation 8 in that we placed each matured male in a 5-L aquarium together with no more than three full-sib brothers. These aquaria were randomly placed on shelves in the same room. We recorded body area and area of orange spots on adult males by taking measurements from photographs of the left side of each fish. Photographs were taken on average 137.5 ± 2.1 days (range: 91–206 days) after maturation. Apart from this, the photographic procedure and estimation of the size-corrected orange area followed the same procedure as in generation 8 (allometric relationship, intercept ± SE, slope ± SE: –8.18 ± 0.86 ln mm$^2$, 2.22 ± 0.19; $R^2 = 0.32$).

The sample size at generation 11 was 276 fish; 172 fish from 74 families in the low-inbred populations, 60 fish from 24 families in the newly outbred population, and 44 fish from 15 families in the high-inbred populations.

## Statistical analysis

We estimated the trait means and 95% credible intervals in the different treatments at generation 8, using linear mixed-effects models without a global intercept and with population as a random factor. Family means for the traits were used as the response variable. Statistical inference was based on the 95% credible intervals.

Size of the mother, density in the growing environment (measured as clutch size), and age at maturation were correlated with several of the focal traits (Appendix A1). To control for these effects, we included these variables as mean-centered covariates (i.e., mean of zero) in the mixed-effects models when the correlation was above 0.10 (absolute value). The statistical controls for each model are given in Table 1.

If the negative response of a trait with an increasing level of inbreeding was due to variation in homozygosity, we expected the within-population relationship to explain the among-population relationship (i.e., the among- and within-population relationships should be similar). Alternatively, if a decrease in trait expression with an increasing level of inbreeding was due to purging, we expected a weak within-population relationship between the trait expression and the level of inbreeding (i.e., no true inbreeding depression), and a strong among-population relationship. Therefore, we estimated the effect of the inbreeding coefficient ($F$) on the traits among and within populations using contextual analyses (Blalock 1984; Raudenbush and Bryk 2002; and see Heisler and Damuth 1987; van de Pol and Wright 2009; Bolstad et al. 2010; Egset et al. 2011 for some biological applications). These models are multiple regressions that include predictor variables at several levels (e.g., individuals, populations, species). The interpretation of the parameters in these models depends on the zero point of the predictor variables at the various levels (Kreft et al. 1995; Enders and Tofighi 2007). When the individual measurements are centered on the population means (i.e., subtracting each population mean from the corresponding individual measurements), within-population and among-population slopes are estimated by the model. When individual measurements are not centered on the population means, the contrast between the among- and within-population slopes are estimated instead of the among-population slope (Kreft et al. 1995; Enders and Tofighi 2007). In our analysis, the among-population predictor variable was the mean inbreeding coefficient of each population, while the within-population predictor variable was the inbreeding coefficient of the family. (Full siblings have the same inbreeding coefficient.)

To estimate the mean relative orange areas of the low-inbred, high-inbred, and newly outbred populations at generation 11, we used a mixed-effects model. The fish in the newly outbred treatment had a complicated dependency, which is not trivial to control for, as they were offspring of crosses between the four high-inbred populations. By using family means as the response variable, we removed the dependence due to shared parents and common environment. To further control for dependency and avoid pseudoreplication when estimating the uncertainty of the means, we included the identity of the grandmother and grandfather as random factors. We also included the oldest male ancestor as a random

 

**Table 1.** Means of the different treatments at generation 8 with 95% credible intervals. Statistical controls with parameter estimates are given in the last column. Unit for length of mother is mm, days for age, and counts for clutch size.

| Trait | Low inbreeding | Intermediate inbreeding | High inbreeding | Statistical controls (slope ± SE) |
|---|---|---|---|---|
| Length at birth (mm) | 7.073 (6.979,7.158) | 7.059 (6.933,7.19) | 6.974 (6.818,7.142) | Length of mother (0.030 ± 0.014), clutch size (–0.040 ± 0.009) |
| Juvenile growth rate (mm day$^{-1}$) | 0.105 (0.099,0.11) | 0.104 (0.095,0.112) | 0.109 (0.097,0.12) | Clutch size (–0.0027 ± 0.0007) |
| Length at maturation (mm) | 14.237 (13.999,14.469) | 13.969 (13.651,14.305) | 14.317 (13.887,14.735) | Clutch size (–0.070 ± 0.023), age (0.024 ± 0.003) |
| Adult growth rate (mm day$^{-1}$) | 0.032 (0.03,0.033) | 0.031 (0.028,0.033) | 0.032 (0.029,0.035) | |
| Adult body length (mm) | 16.143 (15.889,16.392) | 15.806 (15.449,16.122) | 16.236 (15.789,16.669) | Clutch size (–0.062 ± 0.022), age (0.024 ± 0.003) |
| Relative orange area (ln mm$^2$) | 1.577 (1.481,1.677) | 1.585 (1.436,1.723) | 1.244 (1.059,1.431) | Age (–0.0037 ± 0.0017) |
| Relative caudal-fin area (ln mm$^2$) | 3.109 (3.092,3.124) | 3.116 (3.091,3.139) | 3.073 (3.042,3.106) | Clutch size (–0.0024 ± 0.0018) |
| Relative black area (ln mm$^2$) | 0.141 (–0.005,0.295) | 0.199 (0.003,0.393) | 0.072 (–0.188,0.329) | |
| CIE $a^*$ for orange spots (CIE $a^*$) | 150.892 (149.873,151.931) | 150.283 (148.822,151.643) | 150.952 (149.173,152.91) | |

factor to account for the dependency due to shared paternal lineage (i.e., Y chromosome).

We did not include any covariates in the model, because there was no relationship between orange area and age (neither age at photography, age at maturation, nor number of days between maturation and photography) once we accounted for the allometric relationship between orange area and total area. In addition, we did not find any effect of mother length, density before maturation (clutch size), or density after maturation (number of siblings in the same aquarium) on orange area.

All analyses were performed in R 2.13.0 (R Development Core Team 2011) using the package "lme4" (Bates et al. 2011). For all models, Markov chain Monte Carlo sampling (10,000 iterations) was used to obtain 95% credible intervals of the estimated parameters (highest posterior density interval).

# Results

## Inbreeding and trait expression at generation 8

At generation 8, the mean inbreeding coefficient was 0.08 in the low-inbred populations (range: 0.03–0.15), 0.20 in the intermediate-inbred populations (range: 0.14–0.24), and 0.33 in the high-inbred populations (range: 0.20–0.53). Population means for the inbreeding coefficient and the recorded traits are reported in Appendix A2.

For all life-history traits, we found no difference among treatments (Table 1), and there was no effect of inbreeding at the population level (Table 2; Fig. 4). Within population, inbreeding negatively affected length at birth and positively affected adult growth rate, though neither of these effects were statistically significant (Table 2; Fig. 4). In contrast, the populations with high levels of inbreeding had a 1.40 mm$^2$ (28.8% given by $1 - e^{1.24-1.58}$) smaller orange area, on average, than the low-inbred populations (Table 1). This could not be explained by the effect of inbreeding within populations and was solely due to among-population differences (Table 2; Fig. 4). Two other ornamental traits, caudal-fin area and black area, showed similar patterns with smaller trait values in the most inbred populations, but these effects were much weaker (3.4% and 6.7%, respectively) than observed for orange area, and not statistically significant.

## Effect of homozygosity versus purging on orange area (generation 11)

At generation 11, the mean inbreeding coefficient was 0.15 in the low-inbred populations (range: 0.10–0.23), 0.44 in high-inbred populations (range: 0.35–0.64), and 0 in the newly outbred population (for all individuals).

The mean size corrected orange area (95% credible interval) at generation 11 was 1.83 (1.75, 1.91) ln mm$^2$ for the

**Table 2.** Contextual analysis for the effect of the inbreeding coefficient (*F*) on the different traits both within and among populations at generation 8. Credible intervals (95%) in parentheses. The statistical controls given in Table 1 were used.

| Traits | Intercept | Within populations | Among populations |
|---|---|---|---|
| Length at birth | 7.10 (6.97,7.22) mm | −1.042 (–2.650,0.664) mm $F^{-1}$ | −0.341 (–1.036,0.367) mm $F^{-1}$ |
| Juvenile growth rate | 0.104 (0.097,0.112) mm $day^{-1}$ | −0.043 (–0.167,0.077) mm $day^{-1}$ $F^{-1}$ | 0.009 (–0.038,0.051) mm $day^{-1}$ $F^{-1}$ |
| Length at maturation | 14.25 (13.92,14.58) mm | −0.116 (–4.033,3.950) mm $F^{-1}$ | −0.528 (–2.328,1.246) mm $F^{-1}$ |
| Adult growth rate | 0.032 (0.029,0.034) mm $day^{-1}$ | 0.026 (–0.005,0.057) mm $day^{-1}$ $F^{-1}$ | −0.002 (–0.015,0.011) mm $day^{-1}$ $F^{-1}$ |
| Adult body length | 16.161 (15.809,16.518) mm | 1.469 (–2.08,5.3) mm $F^{-1}$ | −0.669 (–2.577,1.218) mm $F^{-1}$ |
| Relative orange area | 1.691 (1.549,1.836) ln $mm^2$ | 0.145 (–1.723,2.07) ln $mm^2$ $F^{-1}$ | −1.085 (–1.86,–0.281) ln $mm^2$ $F^{-1}$ |
| Relative caudal-fin area | 3.121 (3.098,3.143) ln $mm^2$ | −0.047 (–0.377,0.275) ln $mm^2$ $F^{-1}$ | −0.105 (–0.233,0.021) ln $mm^2$ $F^{-1}$ |
| Relative black area | 0.205 (0.016,0.408) ln $mm^2$ | −0.548 (–2.738,1.784) ln $mm^2$ $F^{-1}$ | −0.359 (–1.428,0.689) ln $mm^2$ $F^{-1}$ |
| CIE $a^*$ for orange spots | 150.447 (149.219,151.956) CIE $a^*$ | −2.547 (–18.773,15.647) CIE $a^*$ $F^{-1}$ | 1.751 (–6.072,8.919) CIE $a^*$ $F^{-1}$ |

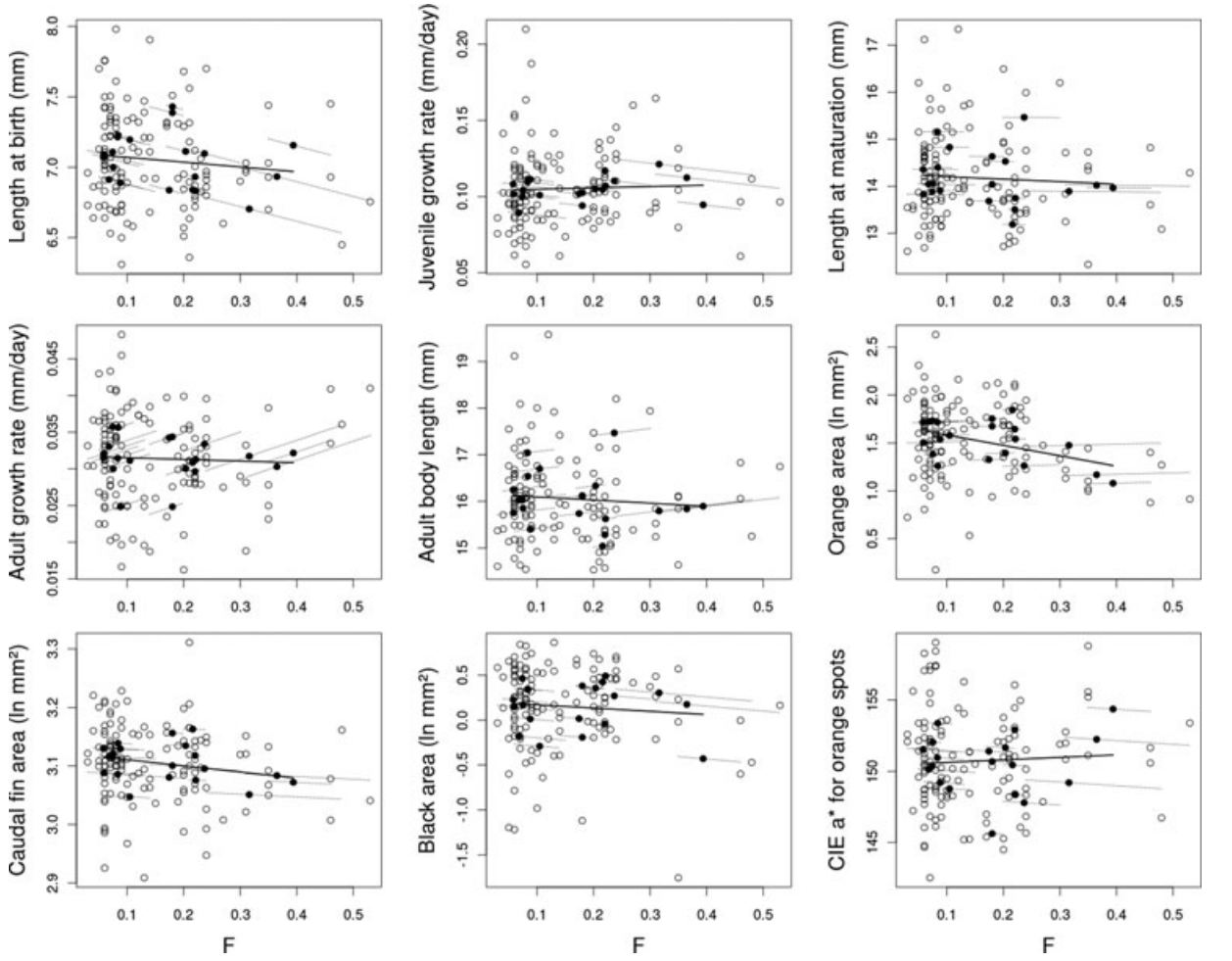


**Figure 4.** Contextual analysis of the effect of the inbreeding coefficient (*F*) on trait values both among (black lines) and within populations (dotted lines) at generation 8. Black dots are population means and open circles are family means. Parameter estimates for the regression lines are given in Table 2.

low-inbred populations, 1.43 (1.25, 1.63) ln $mm^2$ for the high-inbred populations, and 1.52 (1.36, 1.69) ln $mm^2$ for the newly outbred population. Hence, the outbred population had only slightly larger mean orange area than the high-inbred populations, but much smaller than the low-inbred populations (Fig. 5).

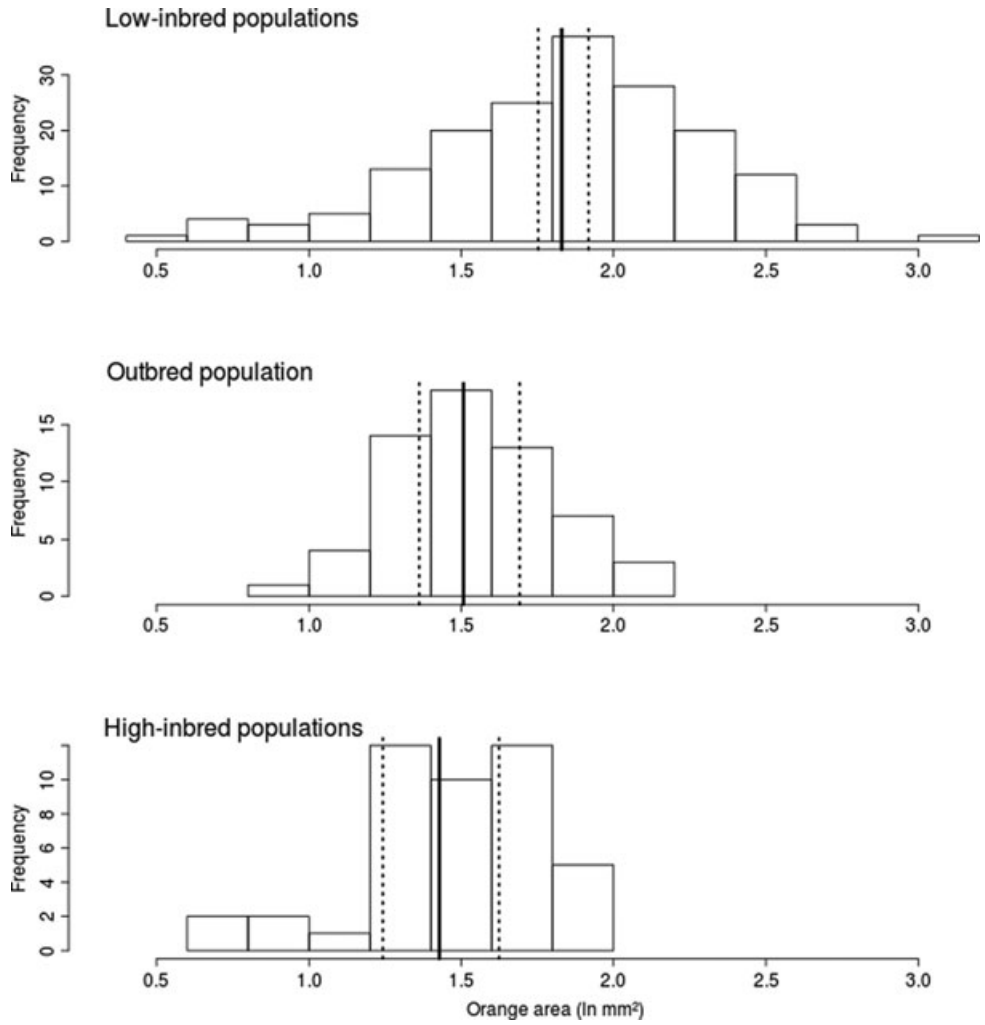


**Figure 5.** Distribution of area of orange spots in the different treatments at generation 11. Frequency gives number of males. Means with 95% credible interval are given with solid and dotted vertical lines, respectively.

## Discussion

We investigated the effects of inbreeding on several life-history and ornamental traits in 20 captive guppy populations. Despite high levels of inbreeding in some populations, we found no evidence of inbreeding depression, either in the life-history traits or in most of the ornamental traits. Lack of inbreeding depression in the life-history traits was expected as previous evidence showed purging in the high-inbred populations (Larsen et al. 2011). In contrast to the other traits, orange area was strongly reduced in the most inbred populations. This effect of inbreeding on orange area could either be due to increased homozygosity or purging. The contextual analysis suggested that homozygosity could not explain this, because the effect of inbreeding on orange area was only observed among populations. This result was supported by the absence of an increase in orange area in fish with restored heterozygosity. Taken together, our results indicate that the lowered level of orange area in the most inbred populations was due to selection (purging) and not increased homozygosity.

Five alternative hypotheses could help explain the differences in mean orange area across treatments in our second experiment. First, the mothers of the males used to create the newly outbred population were highly inbred and maternal effects could have influenced the expression of the orange area. This is unlikely, however, because the mothers in the high-inbreeding treatment at generation 6 and onwards were as fit as those in the low-inbreeding treatment for all the fitness traits examined by Larsen et al. (2011). Moreover, the males in the high-inbreeding treatment at generation 8 showed no sign of inbreeding depression in life-history

 

traits (Table 1). Second, adaptation to laboratory conditions (i.e., other than the selection against deleterious alleles) can be an important confounding factor in purging experiments (Willis 1999; Crnokrak and Barrett 2002). In our experiment, the effects of such selection were controlled for by the use of contemporary low-inbred populations that had been in the same environment for the same number of generations as the high-inbred populations. We also tried to avoid selection by sampling individuals at random among surviving siblings, allowing females plenty of time to give birth, and ensuring all fish had sufficient time to mature. (The generation interval was approximately one year; see Larsen et al. 2011.) Third, genetic drift may have increased the frequency of alleles coding for small orange area in the high-inbred populations. For example, the males within each of these four populations were fixed for the same Y chromosome type (i.e., the same paternal lineage), and by chance it is possible that these Y chromosomes may all code for small orange area. We do not deny this possibility, but consider it unlikely that drift caused an increase in the frequency of alleles coding for small orange area in all four high-inbred populations. Last, the mean inbreeding coefficient in the low-inbreeding treatment was 0.15. If recessive alleles affect orange area positively, the slight increase in homozygosity in these populations could have generated an increase in orange area. However, in the absence of purging this would predict larger orange area in the high-inbred populations than in the low-inbred populations, which is the opposite of what we observed. Therefore, purging remains the most likely explanation.

Previous studies have also found an effect of inbreeding on male ornaments in the guppy (Sheridan and Pomiankowski 1997; van Oosterhout et al. 2003; Zajitschek and Brooks 2010). These studies showed that the effect of inbreeding differs among populations, and that its effect on guppy ornamentation can be severe. However, these studies did not control for purging, although van Oosterhout et al. (2003) did so partially by controlling for line loss. Contrary to our study, these studies also found an effect of inbreeding on black area, and caudal-fin area, but the effect on black area varied across populations (Sheridan and Pomiankowski 1997). The lack of effect of inbreeding on black area, caudal-fin area, and the redness of orange spots in our study can be explained by limited dominance variation in these traits. Interestingly, our results suggest that the orange quality (as measured by CIE $a^*$) is genetically decoupled from the quantity (area of the orange spots), as quality showed no correlated response with size of orange spots.

Because sexual selection was absent in our experiment (all mating were controlled) and offspring survival was the main source of selection, we expected that purging would be mediated by the effect of deleterious alleles on survival in the most inbred populations (Larsen et al. 2011). This implies that the selection for reduced orange area was caused by a negative genetic correlation with survival. Furthermore, it suggests that the purging affecting orange occurred within populations because the effect of purging on offspring survival only occurred within population and was unaffected by the extinction of populations (Larsen et al. 2011). Even without this, the genetic interpretation of our results remains valid if the decline in orange area was due to removal of recessive deleterious alleles via among-population selection.

A negative genetic correlation between ornamentation and offspring survival has previously been observed in the guppy (Brooks 2000), and is in line with the hypothesis of an allocation trade-off between ornaments and survival (e.g., Fisher 1930; Lande 1980, 1981; Kirkpatrick 1982; Pomiankowski et al. 1991; Andersson 1994; Kokko 2001; Badyaev and Qvarnström 2002). In fact, such allocation trade-offs may be particularly strong for carotenoid-based traits, such as the orange color spots of the guppy, because carotenoids are important for health (von Schantz et al. 1999; Alonso-Alvarez et al. 2008), leading to a negative genetic correlation between trait expression and survival.

Houle's (1991) model of functional architecture of genetic correlations is informative for understanding the connection between allocation trade-offs and genetic correlations. In this model, the phenotype is determined by acquisition of resources and allocation of these resources to various traits. Genetic variation in acquisition leads to positive genetic correlations between traits, while genetic variation in allocation leads to negative genetic correlations between traits. Interpreting our results in the light of this model suggests that partly recessive alleles in a homozygous state lead to an allocation of so many resources to the expression of orange spots that it negatively affects survival. These alleles need to be partly recessive (at least for the expression of orange spots) because they are expressed in the low-inbreeding treatment, which would not occur if they were completely recessive.

Negative genetic correlations can be due to either linkage disequilibrium or antagonistic pleiotropy. For correlations due to fitness trade-offs the former is unlikely under normal levels of recombination, because they should not be favored by selection. In guppies, however, several coding genes for male ornamentation are not under normal levels of recombination as they are linked to the nonrecombining region of the Y chromosome, and are inherited like a supergene (reviewed in Lindholm and Breden 2002; Brooks and Postma 2011). A negative genetic correlation between an ornament and survival could, therefore, arise by genetic "hitch-hiking" of deleterious mutations in the nonrecombining region of the Y chromosome (see also discussion in Brooks 2000). As already mentioned, selection against deleterious mutations on the nonrecombining region of the Y chromosome should not increase under inbreeding unless these deleterious mutations are involved in synergistic epistasis with autosomal recessive alleles. Such synergistic epistasis is a plausible explanation for

 

the patterns observed in this study, but not necessary because orange area also has an autosomal component (Brooks and Postma 2011; Postma et al. 2011). Therefore, selection only on the autosomal alleles could be sufficient to explain the observed decline in orange area.

Sexual selection has been hypothesized to select against deleterious alleles, because deleterious alleles are expected to negatively affect a males' mating success (Manning 1984; Kodric-Brown and Brown 1987; Agrawal 2001; Siller 2001; Lorch et al. 2003; Whitlock and Agrawal 2009). In contrast, our study suggests that sexual selection for the orange area in guppies may indirectly select for deleterious alleles (i.e., alleles affecting survival negatively). This is in line with Fisher's prediction, that sexually selected traits will evolve to the point where their positive effect on reproductive success is counterbalanced by their negative effect on viability (Fisher 1930, p. 173). However, as long as the negative effect on viability does not completely counterbalance the positive effect of sexual selection, sexually selected traits remain indicators of "good genes" (Kokko 2001).

Although purging can have some positive effects, conservationists are advised to reduce inbreeding as much as possible in captive breeding programs (e.g., Hedrick and Kalinowski 2000). The results of this study add another reason to minimize inbreeding in such programs, because purging may lead to selection for less-attractive individuals with low reproductive potential when released in the wild for supportive breeding purposes.

This work is the first, to our knowledge, to study the effects of purging on sexually selected traits, and to demonstrate that purging probably had a negative effect on the expression of a sexually selected trait (but see Willis 1999 on corolla size). Because ignoring purging in studies of inbreeding may lead to spurious biological interpretation, we strongly recommend that future studies use designs that can separate the effect of homozygosity from the effects of purging.

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**Appendix A1.** Pearson correlations among traits at generation 8 with 95% confidence interval: density (D), age (A), length at birth (LB), juvenile growth rate (JG), length at maturation (LM), adult growth rate (AG), body length (BL), orange area (O), caudal-fin area (C), black area (B), mean CIE $a^*$ for orange spots. Estimates are based on family means.

| | D | A | LB | JG | LM | AG | BL | O | C | B | CIE $a^*$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Length of mother | 0.31 (0.14, 0.46) | 0.07 (–0.1, 0.24) | 0.17 (–0.01, 0.33) | −0.03 (–0.2, 0.15) | 0.09 (–0.09, 0.26) | −0.03 (–0.2, 0.15) | 0.08 (–0.1, 0.25) | 0.00 (–0.18, 0.17) | 0.01 (–0.17, 0.19) | −0.06 (–0.24, 0.11) | 0.02 (–0.15, 0.2) |
| Density (clutch size) | | 0.27 (0.1, 0.43) | −0.27 (–0.43, –0.1) | −0.34 (–0.49, –0.17) | −0.12 (–0.29, 0.06) | 0.08 (–0.1, 0.25) | −0.09 (–0.26, 0.09) | −0.03 (–0.21, 0.14) | −0.12 (–0.29, 0.06) | −0.04 (–0.21, 0.14) | −0.06 (–0.23, 0.12) |
| Age | | | −0.07 (–0.24, 0.11) | −0.81 (–0.86, –0.74) | 0.49 (0.34, 0.61) | −0.02 (–0.2, 0.15) | 0.49 (0.34, 0.61) | −0.18 (–0.34, 0) | 0.05 (–0.12, 0.23) | 0.07 (–0.11, 0.24) | −0.04 (–0.21, 0.14) |
| Length at birth | | | | 0.08 (–0.1, 0.25) | 0.32 (0.15, 0.47) | 0.02 (–0.16, 0.19) | 0.33 (0.16, 0.48) | 0.07 (–0.11, 0.24) | 0.06 (–0.12, 0.23) | 0.00 (–0.18, 0.18) | 0.00 (–0.18, 0.18) |
| Juvenile growth rate | | | | | −0.04 (–0.22, 0.13) | −0.09 (–0.26, 0.09) | −0.08 (–0.25, 0.1) | 0.09 (–0.08, 0.27) | −0.09 (–0.26, 0.08) | −0.06 (–0.23, 0.12) | 0.09 (–0.09, 0.26) |
| Length at maturation | | | | | | −0.23 (–0.39, –0.06) | 0.92 (0.89, 0.94) | 0.02 (–0.16, 0.19) | −0.04 (–0.21, 0.14) | 0.05 (–0.13, 0.22) | −0.04 (–0.22, 0.13) |
| Adult growth rate | | | | | | | 0.17 (–0.01, 0.33) | −0.04 (–0.21, 0.14) | 0.01 (–0.16, 0.19) | 0.01 (–0.17, 0.18) | 0.00 (–0.18, 0.17) |
| Body length | | | | | | | | 0.00 (–0.17, 0.18) | −0.03 (–0.21, 0.14) | 0.05 (–0.13, 0.22) | −0.05 (–0.22, 0.13) |
| Orange area | | | | | | | | | 0.04 (–0.14, 0.21) | 0.00 (–0.18, 0.17) | −0.18 (–0.35, –0.01) |
| Caudal-fin area | | | | | | | | | | −0.02 (–0.2, 0.16) | 0.06 (–0.12, 0.23) |
| Black area | | | | | | | | | | | −0.15 (–0.32, 0.02) |

**Appendix A2.** Descriptive statistics of the populations at generation 8: Treatment (L, low inbreeding; I, intermediate inbreeding; H, high inbreeding), number of fish measured (*N*), number of families (*N* fam), inbreeding coefficient (*F*), clutch size (CS, counts), length at birth (LB, mm), juvenile growth rate (JG, mm day$^{-1}$), length at maturation (LM, mm), adult growth rate (AG, mm day$^{-1}$), adult body length (BL, mm), orange area (O, ln mm$^2$), caudal-fin size (CF, ln mm$^2$), black area (B, ln mm$^2$), and mean CIE $a^*$ for orange spots.

| Treatment | *N* | *N* fam | *F* ± SE | CS ± SE | LB ± SE | JG ± SE | LM ± SE | AG ± SE | BL ± SE | O ± SE | C ± SE | B ± SE | CIE $a^*$ ± SE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| L | 16 | 9 | 0.06 ± 0.01 | 6.50 ± 0.87 | 7.09 ± 0.11 | 0.10 ± 0.01 | 13.8 ± 0.3 | 0.032 ± 0.001 | 15.8 ± 0.2 | 1.50 ± 0.21 | 3.09 ± 0.02 | 0.15 ± 0.20 | 151.5 ± 1.5 |
| L | 9 | 6 | 0.06 ± 0.01 | 5.67 ± 1.74 | 7.07 ± 0.15 | 0.11 ± 0.01 | 14.4 ± 0.4 | 0.032 ± 0.002 | 16.3 ± 0.3 | 1.71 ± 0.16 | 3.13 ± 0.03 | 0.23 ± 0.15 | 151.5 ± 1.4 |
| L | 15 | 9 | 0.07 ± 0.00 | 6.72 ± 0.74 | 7.11 ± 0.12 | 0.10 ± 0.01 | 13.9 ± 0.2 | 0.036 ± 0.002 | 16.0 ± 0.2 | 1.73 ± 0.19 | 3.11 ± 0.02 | 0.46 ± 0.05 | 150.4 ± 1.1 |
| L | 13 | 8 | 0.07 ± 0.01 | 9.25 ± 1.37 | 6.91 ± 0.06 | 0.09 ± 0.01 | 14.0 ± 0.3 | 0.033 ± 0.002 | 16.0 ± 0.2 | 1.72 ± 0.11 | 3.12 ± 0.03 | −0.18 ± 0.23 | 150.1 ± 0.9 |
| L | 14 | 9 | 0.08 ± 0.00 | 5.94 ± 1.31 | 7.00 ± 0.10 | 0.10 ± 0.01 | 14.1 ± 0.2 | 0.030 ± 0.003 | 15.9 ± 0.2 | 1.38 ± 0.07 | 3.12 ± 0.01 | 0.17 ± 0.09 | 152.0 ± 1.6 |
| L | 16 | 9 | 0.08 ± 0.00 | 5.39 ± 0.92 | 7.23 ± 0.07 | 0.11 ± 0.01 | 14.4 ± 0.3 | 0.036 ± 0.002 | 16.5 ± 0.3 | 1.26 ± 0.08 | 3.14 ± 0.01 | 0.34 ± 0.13 | 153.4 ± 0.8 |
| L | 13 | 10 | 0.09 ± 0.01 | 3.90 ± 1.06 | 7.21 ± 0.11 | 0.11 ± 0.01 | 15.2 ± 0.4 | 0.031 ± 0.002 | 17.0 ± 0.5 | 1.72 ± 0.07 | 3.09 ± 0.02 | 0.35 ± 0.09 | 151.0 ± 1.4 |
| L | 14 | 9 | 0.09 ± 0.01 | 3.72 ± 0.61 | 6.89 ± 0.12 | 0.11 ± 0.00 | 13.9 ± 0.2 | 0.025 ± 0.002 | 15.4 ± 0.2 | 1.54 ± 0.08 | 3.13 ± 0.02 | 0.01 ± 0.13 | 149.2 ± 0.6 |
| L | 13 | 7 | 0.10 ± 0.01 | 7.21 ± 0.91 | 7.20 ± 0.15 | 0.10 ± 0.01 | 14.8 ± 0.3 | 0.031 ± 0.003 | 16.7 ± 0.3 | 1.58 ± 0.12 | 3.05 ± 0.02 | −0.29 ± 0.19 | 148.8 ± 1.0 |
| I | 7 | 4 | 0.17 ± 0.02 | 5.00 ± 1.58 | 6.84 ± 0.15 | 0.10 ± 0.01 | 13.7 ± 0.0 | 0.034 ± 0.002 | 15.7 ± 0.1 | 1.33 ± 0.28 | 3.08 ± 0.01 | 0.02 ± 0.07 | 151.4 ± 1.1 |
| I | 6 | 3 | 0.18 ± 0.01 | 8.33 ± 2.40 | 7.43 ± 0.13 | 0.10 ± 0.00 | 14.0 ± 0.2 | 0.034 ± 0.005 | 16.1 ± 0.4 | 1.75 ± 0.20 | 3.16 ± 0.01 | 0.38 ± 0.15 | 145.6 ± 0.7 |
| I | 10 | 5 | 0.18 ± 0.01 | 7.30 ± 1.62 | 7.39 ± 0.16 | 0.09 ± 0.01 | 14.6 ± 0.6 | 0.025 ± 0.003 | 16.1 ± 0.5 | 1.68 ± 0.20 | 3.10 ± 0.02 | −0.19 ± 0.24 | 150.7 ± 0.6 |
| I | 6 | 4 | 0.20 ± 0.01 | 2.75 ± 1.11 | 7.11 ± 0.20 | 0.11 ± 0.01 | 14.5 ± 0.4 | 0.030 ± 0.003 | 16.3 ± 0.4 | 1.39 ± 0.10 | 3.13 ± 0.07 | 0.36 ± 0.19 | 151.7 ± 2.0 |
| I | 9 | 5 | 0.22 ± 0.00 | 4.30 ± 0.97 | 6.93 ± 0.11 | 0.12 ± 0.01 | 13.5 ± 0.2 | 0.030 ± 0.001 | 15.3 ± 0.3 | 1.64 ± 0.24 | 3.12 ± 0.02 | −0.04 ± 0.06 | 152.9 ± 1.0 |
| I | 10 | 5 | 0.22 ± 0.01 | 4.50 ± 0.50 | 6.84 ± 0.16 | 0.10 ± 0.01 | 13.2 ± 0.3 | 0.031 ± 0.001 | 15.0 ± 0.2 | 1.85 ± 0.08 | 3.16 ± 0.02 | 0.42 ± 0.11 | 150.4 ± 0.3 |
| I | 9 | 5 | 0.22 ± 0.01 | 6.20 ± 1.10 | 6.83 ± 0.07 | 0.11 ± 0.01 | 13.7 ± 0.3 | 0.031 ± 0.002 | 15.6 ± 0.2 | 1.54 ± 0.09 | 3.08 ± 0.04 | 0.49 ± 0.08 | 148.4 ± 1.1 |
| H | 6 | 4 | 0.24 ± 0.02 | 3.12 ± 1.09 | 7.10 ± 0.20 | 0.11 ± 0.01 | 15.5 ± 0.4 | 0.033 ± 0.003 | 17.5 ± 0.4 | 1.26 ± 0.12 | 3.1 ± 0.020 | 0.27 ± 0.16 | 147.8 ± 1.5 |
| H | 10 | 5 | 0.32 ± 0.05 | 7.00 ± 1.22 | 6.70 ± 0.08 | 0.12 ± 0.01 | 13.9 ± 0.2 | 0.032 ± 0.002 | 15.8 ± 0.2 | 1.48 ± 0.08 | 3.05 ± 0.03 | 0.30 ± 0.20 | 149.2 ± 0.9 |
| H | 8 | 4 | 0.36 ± 0.06 | 5.12 ± 0.43 | 6.93 ± 0.06 | 0.11 ± 0.02 | 14.0 ± 0.3 | 0.030 ± 0.005 | 15.8 ± 0.3 | 1.17 ± 0.10 | 3.08 ± 0.03 | 0.17 ± 0.15 | 152.2 ± 0.6 |
| H | 10 | 5 | 0.39 ± 0.03 | 6.00 ± 1.48 | 7.16 ± 0.12 | 0.09 ± 0.01 | 14.0 ± 0.5 | 0.032 ± 0.004 | 15.9 ± 0.4 | 1.08 ± 0.09 | 3.07 ± 0.02 | −0.43 ± 0.36 | 154.4 ± 1.5 |