

Artificial selection on allometry: change in elevation but not slope

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Keywords:

artificial selection;
constraints;
guppy;
morphology;
realized heritability;
scaling;
static allometry.

Abstract

To what extent within-species (static) allometries constitute a constraint on evolution is the subject of a long-standing debate in evolutionary biology. A prerequisite for the constraint hypothesis is that static allometries are hard to change. Several studies have attempted to test this hypothesis with artificial-selection experiments, but their results remain inconclusive due to various methodological issues. Here, we present results from an experiment in which we selected independently on the slope and the elevation of the allometric relationship between caudal-fin size and body size in male guppies (*Poecilia reticulata*). After three episodes of selection, the allometric elevation (i.e. intercept at constant slope) had diverged markedly between the lines selected to increase or decrease it, and showed a realized heritability of 50%. In contrast, the allometric slope remained unaffected by selection. These results suggest that the allometric elevation is more evolvable than the allometric slope, this latter representing a potential constraint on adaptive trait evolution. To our knowledge, this study is the first artificial-selection experiment that directly tests the evolvability of static allometric slopes.

Introduction

Static allometry refers to the scaling relationship between trait size and body size among individuals from the same population measured at similar developmental stages. This relationship is described by a power function $y = \alpha x^\beta$, where y and x are the trait size and body size, respectively, α is the allometric constant, and β is the allometric coefficient (Huxley, 1932; Cock, 1966; Gould, 1966). On a logarithmic scale, this relationship becomes linear: $\log(y) = \log(\alpha) + \beta \times \log(x)$ where $\log(\alpha)$ is the intercept and β the slope. If the allometric slope is unity ($\beta = 1$), the shape of the organism does not change with its size, a condition referred to as isometry (Huxley & Tessier, 1936; Gould, 1966; Mosimann, 1970). Allometries with slope different from unity describe situations in which relative increases in trait size are either smaller ($\beta < 1$) or larger ($\beta > 1$) than relative increases in body size. In both

cases, the shape of the organism is altered when its overall size changes (Huxley & Tessier, 1936).

The hypothesis that static allometries remain more or less fixed and thus constitute constraints on adaptive evolution has been of long-standing interest in evolutionary biology (Huxley, 1932; Gould, 1977; Lande, 1979, 1985; Maynard-Smith *et al.*, 1985). Although the distinction between the evolution of the allometric slope and the evolution of the allometric intercept has not always been clear, the general consensus seems to be that the allometric intercept is more variable and therefore more evolvable than the allometric slope (Maynard-Smith *et al.*, 1985; Bonduriansky, 2007). This was originally suggested by Huxley (1924, 1932) based on the idea that static allometry was a consequence of proportional growth and thus under strong developmental (internal) constraints with little adaptive significance. In support of this, several studies have reported little variation in the slope compared with the intercept when comparing static allometry among populations and species (e.g. Kurtén, 1955; Gould, 1971; Toju & Sota, 2006; Bonduriansky, 2007). Huxley's hypothesis was, however, challenged by Newell (1949) arguing that natural selection should be able to change the allometric slope

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just like any other trait. This view is reflected in more recent hypotheses predicting different static allometries in traits under different patterns of selection (e.g. stabilizing vs. directional). Many researchers have suggested that sexually selected traits, supposedly under directional selection, should display positive ($\beta > 1$) allometry (e.g. Petrie, 1988, 1992; Green, 1992), whereas traits under stabilizing selection should display negative ($\beta < 1$) allometry (e.g. Eberhard *et al.*, 1998; Eberhard, 2009; see also Armbruster *et al.*, 1999; Ushimaru & Nakata, 2001 for examples in flowering plants). These hypotheses are based on verbal models, and their theoretical support remains unclear (Bonduriansky & Day, 2003; Bonduriansky, 2007).

There is more evidence for evolution of the static allometric intercept, and early on Newell (1949) suggested that the intercept could change as a consequence of natural selection because differences in intercept (at constant slope) reflect differences in relative trait size compared with body size at the population mean (see also White & Gould, 1965; Cock, 1966; Gould, 1966, 1971). In the following, we will use the term *intercept* for the statistical parameter defined by the predicted value of the allometric regression on the y axis when $x = 0$ on logarithmic scale. We will use the term *elevation* to refer to the relative trait size at the population mean. It should be noted that differences in elevation across lines or populations equal differences in intercept when the allometric slope is constant across samples.

Over the last decades, the potential for confusion has increased due to the spread of a broader definition of allometry as any change in shape with size (Mosimann, 1970; Frankino *et al.*, 2010). As outlined in the study by Houle *et al.* (2011), this broad definition has underpinned many modern selection studies on allometry and has in most cases lead to experimental designs that study shape changes on arithmetic as opposed to logarithmic scales. This apply to the studies of Weber (1990, 1992), Wilkinson (1993) and Frankino *et al.* (2005, 2007). None of these studies assessed the changes in narrow-sense allometric slopes (i.e. slope estimated on logarithmic scale), and they did not apply selection that could be expected to change such slopes. Although valuable as studies about shape evolution in correlation with size (broad-sense allometry), they do not directly test Huxley's constraint hypothesis. Some other artificial-selection studies have used logarithmic scales, but have estimated slopes with structural-equation models that confound changes in slope with changes in the variances of the traits (Hansen & Bartoszek, 2012; see Materials and methods). The end result is that we are still lacking direct evidence on the response of narrow-sense static allometries to selection.

Here, we present the results of an experiment in which we applied two selection regimes to independently change the narrow-sense allometric slope and elevation between caudal-fin area and body area in male guppies (*Poecilia reticulata*). As far as we know, this is the first

artificial-selection study that directly tests Huxley's hypothesis of low evolvability of static allometric slopes.

Materials and methods

Study species

The guppy (*P. reticulata*) is a small (2–4 cm) neotropical freshwater fish native to rivers and streams in Trinidad and the north-eastern part of South America. Guppies are sexually dimorphic. Males are smaller and more ornamented than females. In addition to various colour patterns, males present an enlarged caudal fin sometimes prolonged by a 'sword-like' structure (Houde, 1997). Similar to other male ornaments and several life-history traits, the size of the caudal fin in guppy seems to be under conflicting selection by female preference and predation (Endler, 1995). Female guppies show, at least in some populations, mating preferences for males with large caudal fins (Endler & Houde, 1995). Furthermore, caudal-fin length, but not shape, seems to affect swimming performances in males (Nicoletto, 1991; Karino *et al.*, 2006). Although differences in the relative size of the caudal fin were observed between drainages in Trinidad, predation intensity did not explain these differences. Furthermore, the slope of the static allometry between caudal-fin length and standard length appeared invariant across predation levels and drainages (Egset *et al.*, 2011).

Study population and rearing conditions

The guppies used in this experiment were descendants from 500 individuals captured in 1998 in the Quare River, Trinidad (10°39'N, 61°12'W). This is a high-predation site where guppies are exposed to the efficient predator pike cichlid (*Crenicichla alta*). Fish were transported to the laboratory (NTNU, Trondheim, Norway) and kept in a stock population with minimal interference under a 12:12-h light/dark cycle at a water temperature of 24 °C (± 2 °C). The stock tank contained Java mosses (*Vesicularia dubyana*) giving newborn offspring shelter against potential cannibalism from adults. Fish were fed every day, alternating dried flakes and newly-hatched brine shrimps (*Artemia nauplii*). The population size always exceeded 400 individuals.

Measurements

We measured caudal-fin area and body area from digital photographs taken 60 (range 57–63) days after sexual maturation (sexual maturation was estimated by the development of the anal fin to a gonopodium in males; Houde, 1997). We standardized the time at which the caudal fin was measured with sexual maturation instead of the age of the fish because this represents a more reliable reference point regarding the development of secondary sexual characters.

Fish were immobilized in cold water (8–10 °C) and placed on a moist petri dish with white background and photographed using a digital camera (Canon E 300D; Canon, Tokyo, Japan) with two mounted lights on each side. We ensured that the petri dish contained enough water to allow the full deployment of the caudal fin. Before each photography session, a ruler was photographed for calibration. After being photographed, fish were placed at normal water temperature (ca. 24 °C) and they rapidly recovered. Body area and caudal-fin area (Fig. 1) were measured from the digital pictures using Adobe Photoshop CS3 Extended, version 10.0.1 (Adobe Systems Inc., San Jose, CA, USA). The fish were measured by a single person (CKE). A rough assessment of measurement variance was obtained from repeated measures of ten individuals. This revealed a repeatability, ratio between among-individual and total variance of 0.99 for body area and 0.89 for caudal-fin area.

Selection procedures

Outbred male offspring from 94 full-sib families were divided into two groups, with not more than one male from each family in each group to form two parental populations P1 and P2 of 94 and 93 fish, respectively. We measured the caudal-fin area and body area on all males and selected males of the first population (P1) to either increase or decrease the allometric slope. Males from the second population (P2) were selected to either increase or decrease the allometric intercept without changing the slope. Additionally, a control line was generated with males from the second population (P2). The females used for the parental generation were virgin females from the stock population (juvenile females isolated before sexual maturation).

Selection was applied for three generations. Unfortunately, the selection regime was reversed by mistake at the second episode of selection in the line selected to decrease the allometric slope. We accounted for the reverse episode of selection in our estimation of the realized heritability (see below).

Selection on the allometric slope

Selecting at the individual level for a change in the allometric slope is challenging because the slope is a



Fig. 1 Morphological measurements taken on male guppies 60 days after sexual maturation: body area and caudal-fin area.

population parameter. If Huxley's model is correct, however, the allometric slope reflects an underlying proportionality in the growth of the two characters. Our main goal was to test whether this proportionality can be changed. Hence, we assumed that individual variation in unobserved allometric parameters a and b produces an allometric relationship between two traits, Y and X , at the population level. In this model, $\log(Y)$ is linearly related to $\log(X)$ as $\log(Y) = a + b \log(X)$, where the underlying parameters a , b and $\log(X)$ are assumed to be uncorrelated random variables with a genetic basis. In this model, the individuals with the largest slope b tend to be in the upper-right and lower-left corners of the bivariate distribution of $\log(X)$ and $\log(Y)$ (light grey dots in Fig. 2a). We used this to construct a selection index to select individuals to increase or decrease the allometric slope, b . Let y and x refer to the log-transformed value of caudal-fin area and body area, respectively, ε_i is the individual residual deviation calculated as $\varepsilon_i = y_i - (\alpha + \beta x_i)$, where α and β are the intercept and slope of the observed static allometry, and let δ_i be the difference between the individual body area and the mean body area on log-transformed data (i.e. $\delta_i = x_i - \bar{x}$). At each generation, the selection index I_1 was calculated for each individual i within each selection line as $I_{1i} = \frac{\delta_i}{\sigma_\delta} \times \frac{\varepsilon_i}{\sigma_\varepsilon}$. The two components δ_i and ε_i were divided by their standard deviations in order to equalize their contribution to the selection index. This selection index attributes positive values to individuals with a body area larger than the population mean and a positive residual value of the caudal-fin area or with a body area smaller than the population mean and a negative residual value of the caudal-fin area (light grey dots Fig. 1a). Conversely, individuals with a body area larger than the population mean but a negative residual value of the caudal-fin area or a body area smaller than the population mean but a positive residual value of caudal-fin area will have a negative selection index (dark grey dots Fig. 2a).

Selection on the allometric elevation

A second selection index, I_2 , designed to change the allometric elevation equalled the residual value from the allometric regression between caudal-fin area and body area (Fig. 2b): $I_{2i} = \varepsilon_i$.

From the P1 population, we established two lines to change the allometric slope, by selecting the 25 males with the highest (to increase the slope) or lowest (to decrease the slope) values for I_1 . From the P2 population, we established two lines to change the allometric elevation by selecting the 25 males with the highest (to increase the elevation) or lowest (to decrease the elevation) values for I_2 . To prevent changes in the allometric slope due to the indirect selection (e.g. if the individual residual value was genetically correlated with the allometric slope), we always calculated I_2 using the slope estimated on the parental generation (P2). At each generation, the 25 males with the most extreme values

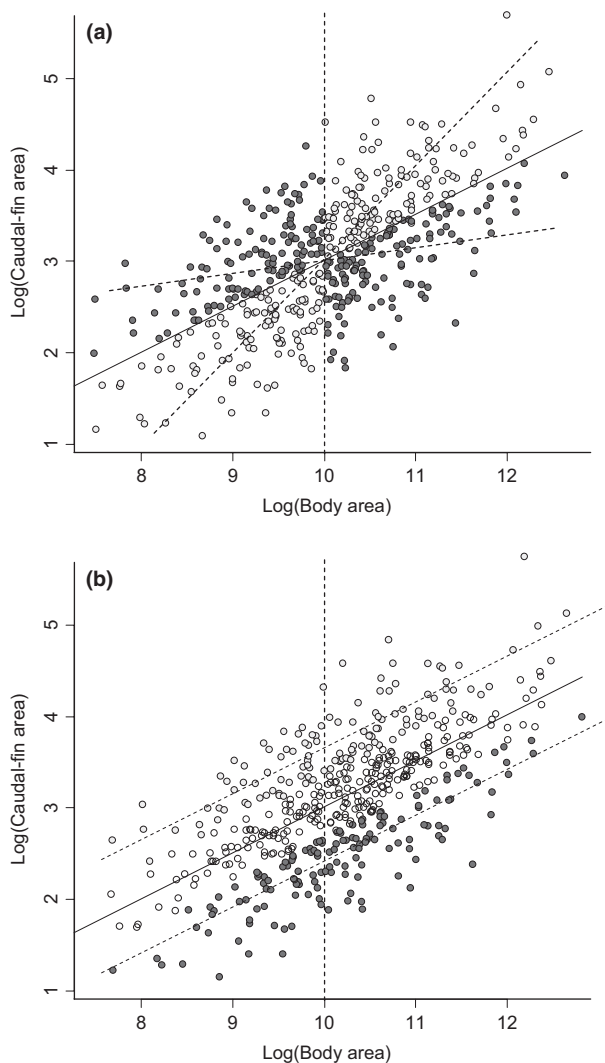


Fig. 2 Schematic representation of the selection procedure to change (a) the allometric slope and (b) the allometric intercept. In the upper graph, selection on dark grey points aims at decreasing the allometric slope, whereas selection on the light grey points aims at increasing the slope. In the lower graph, selection on the dark grey points aims at decreasing the elevation, whereas selection on the light grey points aims at increasing it. In both graphs, solid lines represent the static allometry of the original population, whereas the dotted lines represent the static allometry of the selected individuals (units on both axis are arbitrary).

for the selection index were selected in each experimental line. We also established a control line with 25 randomly selected males from the P2 population. This line was maintained under the same conditions as the selected lines.

Breeding conditions

In all selection lines and the control line, each male was mated with two females in succession. The females were

taken from families within the same line, avoiding mating between full sibs or half sibs. Each male was placed for a period of 3 weeks with a first female in a 3-L aquarium. After this period, each male was placed for another 3 weeks with a second female. Each breeding pair was visually isolated from other breeding pairs to avoid potential competition that would affect female fertility (Borg *et al.*, 2006). After 3 weeks, the males were removed and the females were left alone in their 3-L aquarium to give birth. We aimed at obtaining eight offspring from each female to ensure at least two male and two female offspring. We initially intended to measure 100 males per line at each generation (two male offspring per females). However, due to sterility, the number of males measured at each generation in each line ranged from 57 to 83. Newborn offspring were stored with a maximum density of four offspring per 3-L aquarium to minimize the competition during rearing and favour rapid growth and early maturation (Larsen *et al.*, 2011). The offspring were checked twice a week for signs of sexual maturation, and sexually mature males were removed to avoid mating with their sisters and placed alone in a 1-L aquarium where they were maintained until measurements and selection 60 days later. With the exception of the control line at the first generation, all lines were established and maintained simultaneously to ensure similar environment. Aquariums were located randomly on shelves to eliminate any differences in environmental influences between lines. In the first episode of selection, the control line was established 6 weeks after the other lines because some of the males from the P2 population included in the control line also contributed to one of the two selection lines and were therefore already mated with females during two periods of 3 weeks. Consequently, the females from the control line gave birth slightly later than the females from the selected lines.

Statistical analysis

All analyses were conducted on log-transformed traits. To test the effect of the selection treatments on the allometric slope and intercept, we conducted analyses of covariance (ANCOVA) for each selection line separately with caudal-fin area as response variable, body area as predictor variable and generation as factor. Changes in allometric slope and intercept were estimated as contrasts between the different generations and the parental generation.

We estimated selection differentials (S) as the differences in the mean selection index (\bar{I}_1 or \bar{I}_2) before and after selection for each line at each generation. Response to selection was calculated for each episode of selection using control-corrected selection indices to account for possible environmental variation in the static allometry. For selection on the slope, this was done by calculating the selection index I_1 in the offspring generation of the

selected lines using the allometric relationship of the control line as reference. This was achieved by substituting the residual deviation ε_i in the calculation of I_1 with $\varepsilon_{ic} = y_i - \bar{y}_c - \beta_c(x_i - \bar{x}_c)$, where \bar{y}_c and \bar{x}_c are the mean log caudal-fin area and mean log body area of the control line, respectively, and β_c is the allometric slope of the control line. For the selection on the allometric intercept, the control-corrected selection index I_2 was calculated by simply substituting ε_i with ε_{ic} . We then estimated the realized heritability of the two selection indices by regressing the cumulative response to selection on the cumulative selection differential. Because selection was only applied on males, the realized heritability was estimated as twice the regression slope.

Due to strong statistical correlations between genetic and phenotypic variance components, heritability is a poor measure of additive genetic variance and cannot be used to compare the evolutionary potential across traits, populations or species (Houle, 1992; Hansen *et al.*, 2011). Accordingly, Hansen *et al.* (2003, 2011) proposed to calculate evolvability as mean-scaled additive genetic variance (see also Houle, 1992), which has a direct interpretation as the expected per cent response to a unit selection gradient (the strength of selection on fitness; see also Hereford *et al.*, 2004). The standardization of the two components of I_1 by their standard deviation prevented us from calculating the evolvability of the selection index to change the allometric slope. We were, however, able to estimate the (mean-scaled) evolvability of the intercept as the product of the realized heritability with the phenotypic variance of the residuals ε_i (on log-scale), which approximates the component of evolvability of the relative caudal-fin area on the original scale due to the additive genetic variance in the intercept (Hansen *et al.*, 2011).

The type of regression used to estimate allometric relationship has been the subject of debate. Many have used major-axis or reduced major-axis regression in place of ordinary least-squares regression to estimate allometric parameters in the mistaken belief that this solves the problem of observation error in the predictor variables. Unfortunately, it can be shown that neither of these alternative regression models will give sensible estimates of allometric regression slopes when there is biological 'error' (i.e. biological deviations from the allometric line) in the model (Kelly & Price, 2004; Hansen & Bartoszek, 2012). This can be understood in a nontechnical manner for reduced major-axis regression, which estimates the allometric slope as the ratio between the standard deviations of the two traits. With this model, there is no distinction between biological and observational error, and because the covariance between the traits does not enter the estimator, there is no specific link between the parameters provided by the reduced major-axis method and our parameter of interest, namely Huxley's allometric exponent. With reduced major-axis method, any change in the variance of either of the traits

may produce changes in the estimated slope regardless of whether these changes are due to the changes in the allometric exponent or to changes in any other biological or observational variance component. Therefore, an observed change in the slope using reduced major-axis method is not valid evidence for a change in Huxley's allometric exponent. Furthermore, although allometric slopes estimated with least squares are biased with a factor equal to the repeatability of the predictor variable (see Hansen & Bartoszek, 2012 for details), with our repeatability of 99% for body area, this gives an expected 1% bias in slope, and this small bias is not likely to be much different in the different generations and treatments we compare. Therefore, we based our analysis on ordinary least-squares linear regression. All the statistical analyses have been performed in **R**, version 2.10.0 (R Development Core Team, 2010).

Results

Selection to change allometric slope

The slope of the allometric relationship between caudal-fin area and body area in the parental population was 0.82 ± 0.07 ($R^2 = 0.57$). Although small differences in the slope were observed across lines and generations (Fig. 3a, Table 1), these remained weak and erratic with regard to the selection applied.

Despite strong selection to increase or decrease the selection index, I_1 , this index remained practically unchanged and its realized heritability was not different from zero ($h^2 = 0.06 \pm 0.06$; Table 3, Fig. 3b), suggesting that I_1 harboured little selectable genetic variance.

The allometric intercept remained unchanged in the lines selected to increase or decrease the allometric slope (Tables 1 and 2), indicating that the relative caudal-fin area did not change.

Selection to change allometric elevation

The average caudal fin in the parental population was 22.42 ± 1.12 mm². As a result of selection on the allometric elevation, the caudal-fin area increased by 6% in the line selected to increase allometric elevation and decreased by 3% in the line selected to decrease the elevation (Table 1; Fig. 4a). Surprisingly, in contrast to the smooth changes in elevation in response to selection, there were erratic changes in the control line across generations (Tables 1 and 4, Fig. 4a).

The realized heritability obtained from the regression between the cumulative selection differential and the control-corrected cumulative response to selection for the allometric elevation was $h^2 = 0.50 \pm 0.14$ (Table 5; Fig. 4b). It should be noted that disregarding the control line in the calculation of the response to selection generated a smoother pattern in the regression to estimate the realized heritability but did not affect the

results (Fig. 5). The estimated mean-scaled evolvability of the allometric elevation was $e_{\mu} = 0.22\%$.

The allometric slope did not change in the lines selected for a change in elevation (Tables 1 and 4).

Discussion

Allometry has raised interest among evolutionary biologists as a potential constraint on evolutionary change (e.g. Gould, 1977; Lande, 1979, 1985; Cheverud, 1982). Huxley (1924, 1932) first raised this possibility by showing that allometric relations arise from common growth control (pp. 5–7 in Huxley, 1932). More generally, Savageau (1979) showed that allometric relationships between two or more variables connected in a dynamical system will arise whenever the whole system is controlled by a one-dimensional variable that comes to

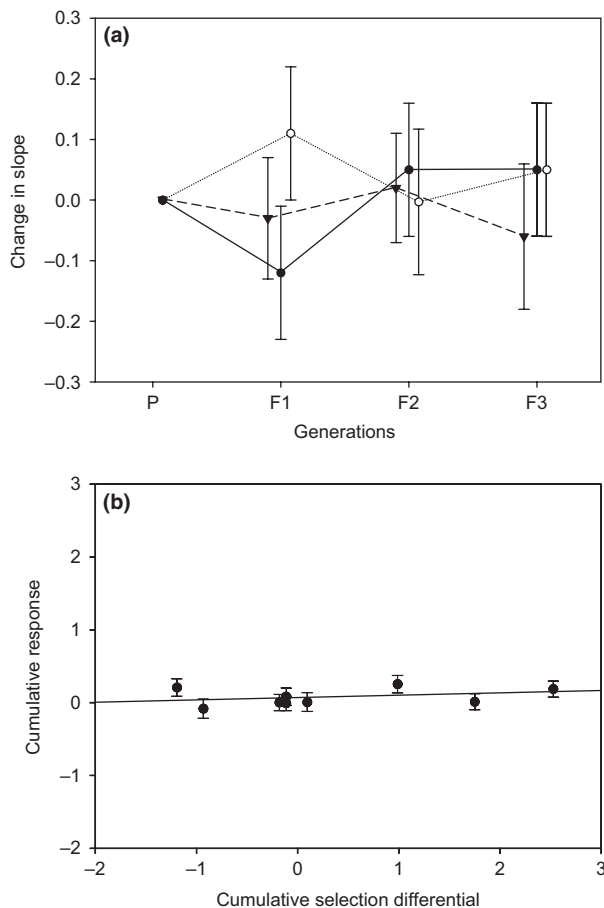


Fig. 3 (a) Changes in allometric slope (\pm SE) across generations in the lines selected for an increase (open circles), a decrease (black circles) or no change (black triangles) in the slope of the allometry between caudal-fin area and body area in male guppies. Note that the three lines started from the same parental population (P1 + P2). (b) Regression between the cumulative selection differential and the cumulative response to selection (\pm SE) in the lines selected to change the allometric slope (slope \pm SE: 0.03 ± 0.04 , $R^2 = 0.10$).

equilibrium more slowly than the rest (the equivalent of the general growth condition in Huxley's model). In such situations, deviations from the allometric relation will be diminished, and evolutionary changes may be constrained to follow a genetic line of least resistance along the allometry. Gould (1977) suggested that many cross-species (evolutionary) allometries may be the result of such constraints rather than adaptive responses to optimal trait relationships (e.g. Schimdt-Nielsen, 1984). A hybrid view is also possible if common developmental control and thus allometric relations themselves have evolved as adaptations to ensure optimal trait relations (Maynard-Smith *et al.*, 1985; Nijhout & Emlen, 1998). In either case, we can test the allometric-constraint hypothesis by asking three questions on how variation is structured: (i) How much variation and evolutionary change is independent of the allometric relation? (ii) How much variation and evolutionary change can be explained by changes in the allometric elevation? Variation in elevation could generate deviations from evolutionary allometries, but changes would still be somewhat constrained by the allometric slope. (iii) How much variation and evolutionary change are there in the allometric slope? This will tell us whether different static allometries can evolve, and is also a direct test of the developmental-control hypothesis. In this study, we have focused on the last two questions by testing whether the two parameters defining static allometry, namely the slope and the elevation, could respond to artificial selection. The elevation of the static allometry between caudal-fin area and body area responded to selection, whereas the slope remained unaffected. The realized heritability of the selection index we constructed to change the allometric slope was practically zero, suggesting that the slope of the allometric relationship is void of additive genetic variation and may act as an evolutionary constraint, whereas the allometric elevation appears evolvable.

Evolvability of the slope and elevation

Several hypotheses can explain the absence of response to selection of the allometric slope. First, one can argue that the limited number of generations of selection and the inconsistent direction of the selection during one episode in one of the two selected lines limited the power of our experiment to reveal genetic variation. The response to selection tends to be strongest during the first generations, however (e.g. Bell, 2008). Indeed, an accelerating selection response has practically never been observed from an outbred base population (Johnson & Barton, 2005).

Second, one can question the extent to which our selection index captures individuals with steep (or shallow) allometric slopes. We based our selection index on the assumption that static allometry is caused by proportional growth, as in Huxley's model, and that individuals placed on the upper right and lower left of the bivariate distribution (Fig. 2a) had steep individual allometric

Table 1 Descriptive statistics for log body area and log caudal-fin area and parameter estimates for the allometric relations between caudal-fin area and body area in the different selection lines at each generation. We also report the coefficient of determination (R^2) and the sample size (n) for each line.

Line	Generation	Body area log (mm ²) Mean (SD)	Caudal-fin area log (mm ²) Mean (SD)	Slope $\beta \pm SE$	Intercept log (mm ²) $\alpha \pm SE$	R^2	n
Parent 1	P1	3.91 (0.10)	3.11 (0.11)	0.82 ± 0.08	-0.11 ± 0.29	0.57	94
Decreasing slope	F1	4.03 (0.11)	3.20 (0.10)	0.71 ± 0.08	0.35 ± 0.30	0.56	70
	F2*	3.93 (0.10)	3.13 (0.11)	0.87 ± 0.08	-0.29 ± 0.31	0.59	83
	F3	3.97 (0.11)	3.18 (0.12)	0.87 ± 0.08	-0.27 ± 0.31	0.68	60
Increasing slope	F1	4.02 (0.11)	3.21 (0.13)	0.93 ± 0.07	-0.54 ± 0.29	0.68	78
	F2	3.93 (0.09)	3.13 (0.11)	0.82 ± 0.10	-0.09 ± 0.39	0.50	72
	F3	3.96 (0.11)	3.16 (0.12)	0.88 ± 0.08	-0.30 ± 0.32	0.62	73
Parent 2	P2	3.91 (0.11)	3.11 (0.11)	0.79 ± 0.06	0.01 ± 0.25	0.63	93
Decreasing elevation	F1	4.00 (0.10)	3.17 (0.11)	0.88 ± 0.07	-3.45 ± 0.29	0.65	76
	F2	3.89 (0.10)	3.08 (0.11)	0.86 ± 0.09	-2.78 ± 0.35	0.57	70
	F3	3.97 (0.10)	3.13 (0.11)	0.83 ± 0.07	-0.15 ± 0.29	0.64	72
Increasing elevation	F1	4.02 (0.11)	3.23 (0.11)	0.72 ± 0.07	0.34 ± 0.28	0.59	73
	F2	3.91 (0.11)	3.18 (0.12)	0.94 ± 0.07	-0.49 ± 0.26	0.72	76
	F3	3.96 (0.10)	3.22 (0.11)	0.85 ± 0.09	-0.16 ± 0.34	0.61	64
Control	F1	4.02 (0.10)	3.24 (0.10)	0.76 ± 0.07	0.17 ± 0.28	0.68	57
	F2	3.94 (0.11)	3.14 (0.11)	0.81 ± 0.06	-0.06 ± 0.24	0.70	76
	F3	3.98 (0.08)	3.16 (0.10)	0.74 ± 0.10	0.22 ± 0.45	0.41	59

*Selected in the wrong direction.

Table 2 Analyses of covariance of the allometric relationship between caudal-fin area and body area in the lines selected to change the allometric slope. Body area is on log-scale. The R^2 is 0.64 for both models. See Table 1 for parameter estimates.

Source of variation	d.f.	Mean square	F	P -value
Decreasing slope				
Body area	1	2.7126	534.15	-
Generation	3	0.0047	0.92	0.432
Body area × generation	3	0.0049	0.97	0.409
Residual	303	0.0051		
Increasing slope				
Body area	1	2.9880	542.67	-
Generation	3	0.0103	1.87	0.134
Body area × generation	3	0.0023	0.42	0.741
Residual	313	0.0055		

slopes. We further assumed that selecting these individuals would shift the slope of the static allometry upwards. We do not deny the fact that other models for static allometry could predict different relationship between individual phenotype and the steepness of the static allometry. Nevertheless, we notice that our selection index I_1 generates a fitness landscape that would favour steeper (or shallower) allometric slopes. Therefore, independently on the underlying models that could generate the allometric relations, our selection procedure should reflect selection on the slope of static allometry.

A third possibility is that selection on the slope is less efficient than selection on the elevation because it does not correspond to directional selection on the underlying traits (i.e. body area and caudal-fin area). We inferred the low evolvability of the allometric slope from the low

Table 3 Selection differentials, cumulative selection differentials and cumulative response to selection for the selection index I_1 in the lines selected for a change in allometric slope. The cumulative response to selection was calculated on a control-corrected index.

Treatments	Generation	Selection differential	Cumulative selection differential	Response
Decreasing slope	P1	-0.933	-0.933	-
	F1	0.819	-0.113	-0.083
	F2	-1.080	-1.194	0.0817
Increasing slope	F3			0.206
	P1	0.988	0.988	-
	F1	0.763	1.751	0.253
Control	F2	0.776	2.527	0.012
	F3			0.188
	P1	0.095	0.095	-
	F1	-0.209	-0.114	0.008
	F2	-0.067	-0.181	-0.0095
	F3			0.0000

realized heritability of the selection index. This apparent absence of genetic variation in the selection index may appear surprising, because this index combines two traits that harbour genetic variation. As demonstrated by the response to selection of the allometric elevation, variation in the relative caudal-fin area has a strong genetic component. Additionally, body size has been shown to have substantial additive genetic variance in the guppy (Brooks & Endler, 2001). However, the selection to increase the slope did not generate directional selection, but instead generated stabilizing selection on body area and disruptive selection on caudal-fin area, whereas

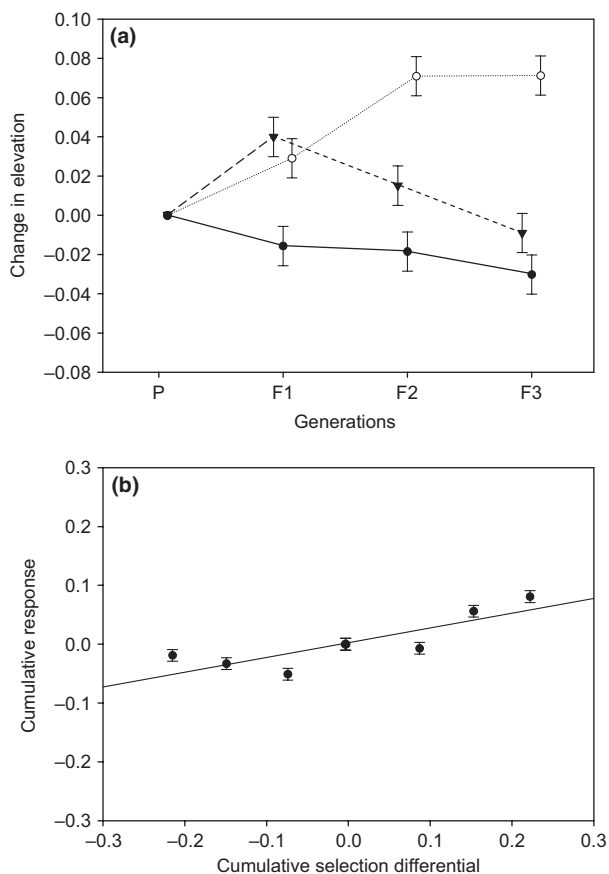


Fig. 4 (a) Changes in allometric elevation (\pm SE) in $\log(\text{mm}^2)$ across generations in the lines selected for an increase (open circles), a decrease (black circles) or no change (black triangles) in the elevation of the allometry between caudal-fin area and body area in male guppies. (b) Regression between the cumulative selection differential and the control-corrected cumulative response to selection (\pm SE) in the allometric elevation (slope \pm SE = 0.25 ± 0.07 , $R^2 = 0.71$).

selection to decrease the allometric slope generated stabilizing selection on caudal-fin area and disruptive selection on body area (see the distribution of the dark grey and light grey dots on the X and Y axes in Fig. 2a). This latter procedure parallels the hypothesis that traits under stabilizing selection should display negative allometry (Eberhard *et al.*, 1998; Eberhard, 2009). The evolutionary response of variation to selection is, however, strongly dependent on the genetic architecture (Hansen, 2006; Pélabon *et al.*, 2010), and changes in variances and covariances may be slow for highly polygenic additive genetic architectures (Bulmer, 1980; Lande, 1980). An alternative explanation for the constancy of the allometric slope is thus that the genetic variances and covariances of caudal-fin area and body area remained stable over the short duration of our experiment. Overall, we can conclude that if Huxley's model was true, then the evolvability of the growth proportionality would be low.

Table 4 Analyses of covariance of the allometric relationship between caudal-fin area and body area in the lines selected to change the allometric elevation. Note that in all cases, the allometric slope was similar among generations (interaction effect down-selected line: $F_{3,307} = 0.3071$, $P = 0.82$; up-selected line: $F_{3,302} = 1.776$, $P = 0.15$; control line: $F_{3,281} = 0.170$, $P = 0.92$), and we removed the interaction in the analysis presented here. The R^2 are 0.66, 0.70 and 0.68 for the models for decreasing elevation, increasing elevation and control, respectively. See Table 1 for model estimates.

Source of variation	d.f.	Mean square	F -value	P -value
Decreasing elevation				
Log body area	1	2.6645	593.28	–
Generation	3	0.0125	2.79	0.04
Residuals	310	0.0045		
Increasing elevation				
Log body area	1	2.9220	642.82	–
Generation	3	0.0987	21.71	< 0.001
Residuals	305	0.0046		
Control				
Log body area	1	2.4096	590.88	–
Generation	3	0.0274	6.71	< 0.001
Residuals	284	0.0041		

Table 5 Selection differentials, cumulative selection differentials and cumulative response of the selection index I_2 in the lines selected for a change in allometric elevation. The cumulative response to selection was calculated on control-corrected residuals.

Treatments	Generation	Selection differential log (mm ²)	Cumulative selection differential log (mm ²)	Response log (mm ²)
Decreasing elevation	P	-0.074	-0.074	–
	F1	-0.075	-0.149	-0.051
	F2	-0.066	-0.215	-0.033
	F3			-0.019
Increasing elevation	P	0.087	0.087	–
	F1	0.067	0.154	-0.007
	F2	0.074	0.228	0.056
	F3			0.081
Control	P	-0.003	-0.003	–
	F1	-0.000	-0.003	0.000
	F2	-0.001	-0.004	0.000
	F3			0.000

The allometric elevation appears evolvable, as illustrated by the rapid change in the relative area of the caudal fin under directional selection, and an evolvability ($e_{\mu} = 0.22\%$), which is about as expected for a morphological trait measured as area (Hansen *et al.*, 2011). The response to selection was smooth in both directions, although erratic change of the allometric elevation in the control line at the first generation disturbed this pattern. It remains difficult to understand what generated the change in the allometric elevation of the control line, and whether this change affected all selection lines or only

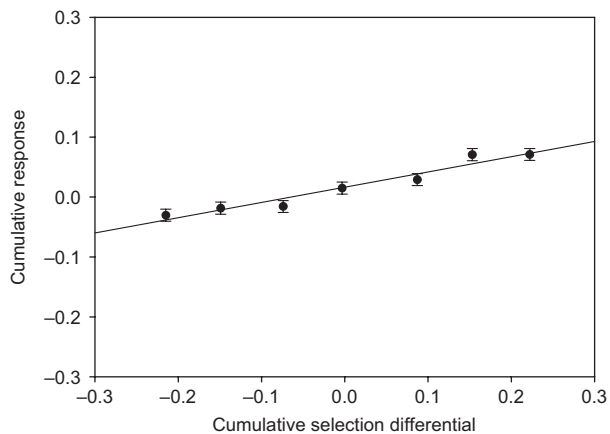


Fig. 5 Regression between the cumulative selection differential and the cumulative response to selection (not corrected for the variation in the control line) in the allometric elevation (slope \pm SE = 0.25 ± 0.05 , $R^2 = 0.85$).

the control line. A possible explanation for a change in the allometric elevation of the caudal-fin area is a difference in age at measurement between lines. Because the caudal fin in male guppies continues to grow even after the growth of the body has stopped at sexual maturation, fish measured at older age will display larger caudal fin relative to the body size. Because we standardized the measurements with the time of sexual maturation and not the age of the fish, we believe that this explanation is unlikely. We also note that the allometric elevation did not change in the two lines selected for change in the allometric slope. We therefore believe that the change in allometric elevation in the control line at the first generation only affected this line.

The response to selection on elevation was larger in the up-selected line than in the down-selected line. Considering the benign environment in which fish were raised in this experiment (individual isolated in aquarium with *ad libitum* food), this asymmetry in the response suggests some developmental constraints affecting the relative size of the caudal fin.

Artificial selection on allometry

To our knowledge, this experiment is the first that performed independent selection on the allometric slope and elevation on an appropriate scale. Previous selection experiments either performed directional selection on one trait before analysing the effects on some allometric relationships (Okada & Miyatake, 2009) or performed selection on a ratio between traits calculated on the original (i.e. arithmetic) scale (Robertson, 1962; Wilkinson, 1993; Emlen, 1996; Monteiro *et al.*, 1997; Frankino *et al.*, 2005, 2007). The choice of the scale on which selection is performed and data analysed is, however, not a matter of statistical convenience (Houle *et al.*,

2011). By performing selection and analysing the relationship between traits on the arithmetic scale, one implicitly assumes that the relationship between traits is isometric ($\beta = 1$) for the whole range of data explored. If the allometric coefficient β is different from 1, changes in the mean trait on the x axis will change the slope of the relationship on the arithmetic scale without changing the allometric relationship. Alternatively, the absence of a change in the slope on arithmetic scale does not guarantee that the slope remained unchanged on logarithmic scale. This is possibly what happened in the two selection experiments by Frankino *et al.* (2005, 2007), in which changes in the elevation of the allometric slope measured on the arithmetic scale may have resulted from changes in both allometric elevation and allometric slope (i.e. on logarithmic scale). A related problem arises in the experiment of Tobler & Nijhout (2010) who assessed the changes in the reduced major axis of wing size on body size following the selection on body size. Because the reduced major axis is the ratio between the standard deviations of the traits, the changes they observed are as likely to involve changes in residual variance as changes in an underlying allometric slope.

It is also important to keep in mind that the slope is estimated with error. In our experiment, the allometric slope varies from 0.71 to 0.94 across populations. In a recent selection experiment (Cayetano *et al.*, 2011), allometry was only measured at the start and at the end of the experiment. Although significant differences in the allometric slope were reported, intergenerational sources of variation make it problematic to interpret these differences, particularly because allometry was not the target of selection and the fit of the allometric relation was very poor (low R^2). We therefore advocate that selection experiments testing the evolution of the allometric slope should analyse and report the change in allometry across generations and not only at the first and last generation.

Allometry and sexual selection

Sexually selected traits have been suggested to display positive ($\beta > 1$) allometry (Petrie, 1988, 1992; Green, 1992; but see Bonduriansky & Day, 2003; Bonduriansky, 2007). In the guppy, males display larger and more elaborated caudal fins than females (Nicoletto, 1991; Houde, 1997; Karino & Matsunaga, 2002), suggesting that this trait is under sexual selection. This hypothesis is further supported by studies demonstrating female mating preference for males with enlarged caudal fins in some populations (Bischoff *et al.*, 1985; Endler & Houde, 1995). Contrary to the predictions, we observed a negative allometry between caudal-fin area and body area, implying that large males displayed relatively smaller caudal fin compared with small ones. This result is confirmed by a survey on 21 wild guppy populations in

Trinidad, in which a negative allometry between caudal-fin length and body length was observed (average slope \pm SE = 0.79 ± 0.05 ; Egset *et al.*, 2011). Although one can hypothesize that this negative allometry reflects higher costs (e.g. in terms of predation) than benefits (in terms of mating success) of enlarged caudal-fin size for larger males, a similar allometric slope observed among females from three guppy populations (including the one used in this experiment; C. Pélabon, C.K. Egset, G.H. Bolstad & G. Rosenqvist, submitted) suggests that the static allometry is under similar selection/constraints in both sexes. We note, however, that in some guppy populations, the growth of the male caudal fin continues far beyond sexual maturation producing in some cases an elongated 'sword'. In the populations where this occurs, the presence of sword generates different static allometries in males and females (C.K. Egset, G. Rosenqvist, C. Pélabon, unpublished).

Our artificial-selection experiment showed that it was easier to change the allometric elevation than the allometric slope. These results are consistent with the comparative analysis on wild guppy populations (Egset *et al.*, 2011) and also support the more general hypothesis that the allometric slope is less variable than the allometric elevation (Bonduriansky, 2007). The reasons for the low evolvability of the allometric slope remain unclear, however, and artificial-selection experiments specifically designed to change the allometric slope, where selection is performed over a large number of generations, seem strongly needed to better understand this question.

Acknowledgments

The authors thank Tonje Aronsen and Henriette Vaagland for data collection and fish care and Ronny Höglund, Anne-Lise Olsen, Jan Sand, Åsa A. Borg and Line-Kristin Larsen for fish care. Aline M. Lee, Anders G. Finstad, Fred Nijhout, Trine Bilde and two anonymous reviewers provided fruitful comments on earlier versions of this manuscript. The Research council of Norway (NFR project 166869/V40 and 196434 40) funded the project. The study was conducted with consent from the Norwegian Animal Research Authority.

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Data deposited at Dryad: doi: 10.5061/dryad.20bq7ns5

Received 11 November 2011; revised 30 January 2012; accepted 5 February 2012