Parker's sneak-guard model revisited: why do reproductively parasitic males heavily invest in testes?

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ABSTRACT

Alternative reproductive tactics are widespread in males and may cause intraspecific differences in testes investment. Parker’s sneak-guard model predicts that sneaker males, who mate under sperm competition risk, invest in testes relatively more than bourgeois conspecifics that have lower risk. Given that sneakers are much smaller than bourgeois males, sneakers may increase testes investment to overcome their limited sperm productivity because of their small body sizes. In this study, we examined the mechanism that mediates differential testes investment across tactics in the Lake Tanganyika cichlid fish *Lamprologus callipterus*. In the Rumonge population of Burundi, bourgeois males are small compared to those in other populations and have a body size close to sneaky dwarf males. Therefore, if differences in relative testis investment depend on sperm competition, the rank order of relative testis investment should be dwarf males > bourgeois males in Rumonge = bourgeois males in the other populations. If differences in relative testis investment depend on body size, the rank order of relative testes investment should be dwarf males > bourgeois males in Rumonge > bourgeois males in the other populations. Comparisons of relative testis investment among the three male groups supported the role of sperm competition, as predicted by the sneak-guard model. Nevertheless, the effects of absolute body size on

testes investment should be considered to understand the mechanisms underlying intraspecific variation in testes investment caused by alternative reproductive tactics.

INTRODUCTION

Sperm competition is now widely recognized as a powerful force in the evolution of male traits that contribute to fertilization success (Birkhead and Møller 1998; Simmons 2001). A fundamental mechanism of sperm competition is the raffle process (Parker 1990a), whereby a male’s fertilization success is proportional to his relative contribution to all sperm competing for a female’s ova. In this situation, increased probability of sperm competition occurring (i.e. sperm competition risk) is predicted to cause the evolution of increased male expenditure on the ejaculate (Parker 1998). Comparative studies across numerous taxa support this prediction (Byrne et al. 2002; Pitcher et al. 2005; Ramm et al. 2005; Simmons et al. 2007; Rowe and Pruett-Jones 2011, for a review see Parker et al. 1997).

Sperm competition risks could also vary within species. A typical example comes from species with alternative reproductive tactics (ARTs) in which risk of sperm competition depends on the tactics expressed by different the groups. Parker’s (1990) evolutionarily stable strategy model of sperm competition, the so-called sneak-guard (SG) model, predicts that males, that ejaculate constantly in roles with a higher risk of sperm competition (i.e. reproductively parasitic males), are selected to invest more in testes than conspecific males, that ejaculate usually in roles with a lower risk (i.e.

bourgeois males, see Taborsky 1997, for terminology). This prediction is also supported across a wide variety of taxa (Supplementary table 1, see also Taborsky 1994, 2008, for review). Studies using intraspecific variation in relative testes investment are the powerful and instructive to test the effects of sperm competition on testes investment, because intraspecific comparisons do not carry the phylogenetic problems (Harvey & Pagel 1991). Thus, ARTs play an important role in our understanding of the effects of sperm competition on ejaculate investment strategies. The SG model is now widely accepted, but its prediction may not be definitive because alternative hypotheses are seldom tested. The prediction to date has been tested by comparing testes investment between tactics or between bourgeois males to whom sperm competitors are experimentally provided or not. However, parasitic males are often considerably smaller than bourgeois males (Supplementary table 1, see also Taborsky 1998), thus potentially confounding the effects of sperm competition and body size on relative testes investment. If the parasitic males just have relative testes investment equal to that of bourgeois males, their investment will be in absolute lower because of their small body size, thus accounting for an absolute deficiency in their sperm counts. As such, small parasitic males are expected to increase their testes investment to overcome a limited capacity for sperm storage in testes in order to raise their fertility. Despite the wide prevalence of ARTs among animal species (Oliveira et al. 2008), this “body size effect” hypothesis alternative to the sperm competition effect is seldom examined (Immler et al. 2004).

One way to consider both effects at the same time would be to control the sperm competition risk in small males. However, this would be difficult to achieve because of the lack of small bourgeois males that are size-matched to sneakers, in species with ARTs. The Lake Tanganyika shell-breeding cichlid fish *Lamprologus callipterus* may provide a unique opportunity to address this issue. In this fish, bourgeois ‘nest males’ are generally much larger than parasitic ‘dwarf males’ (Sato et al. 2004; Ota et al. 2010a). In the Rumonge population of Burundi, however, nest males are small and of a size close to dwarf males of other populations (Fig. 1). Furthermore, nest males experience a lower risk of sperm competition because they effectively guard their nests, whereas dwarf males have higher sperm competition risks and invest more in testes than nest males (Sato et al. 2004). In this study, we examined which mechanism accounts for differences in testes investment by *L. callipterus* across different reproductive tactics by interpopulation comparison:

(1) the sperm competition effect; this hypothesis predicts that differences in

relative testes investment are shaped by tactic-specific sperm competition risks (i.e. SG model, Parker 1990) and the rank order of testis investment should be dwarf males > nest males in Rumonge = nest males in other populations, or

(2) the body size effect; this hypothesis predicts that body size determines relative testes investment and the rank order of testis investment should be dwarf males > nest males in Rumonge > nest males in other populations.

MATERIALS AND METHODS

Study species

L. callipterus is an obligate shell brooder using empty gastropod shells as breeding substrate (Sato 1994; Sato and Gashagaza 1997; Ota et al. 2010b). Reproductive behaviours differ considerably between the sexes, resulting in an extreme male-biased sexual size dimorphism (Schütz and Taborsky 2005; Schütz et al.2006; Ota et al. 2010b). Nest males grow large so that they can collect shells. However, they remain small in the Rumonge population because there they can use aggregations of shells formed by the digging activities of other cichlid species without having to carry shells by themselves (Sato and Gashagaza 1997; Ota et al. 2010b). This difference in nesting behaviour leads to remarkable size variations among different populations (Schütz and Taborsky 2005; Ota et al. 2010b). Among males in the other populations, nest males are larger in the populations where larger shells are available (Ota et al. 2010b), because larger males can carry larger shells (Schütz and Taborsky 2005), which explains the inter-population variation in their body size (Fig. 1).

Gravid females come from outside of the nests to spawn. Females visit the nests for spawning. If a female chooses a shell in a nest, she enters it completely and deposits all eggs by sticking them onto its inner wall. A spawning event by a female lasts for nine hours on average during which she lays eggs one by one, so that each egg requires a separate ejaculation (Schütz et al. 2010). She exclusively occupies the shell for 12–14 days to care for the broods inside (Sato 1994). This breeding ecology limits female growth (Schütz and Taborsky 2005) and consequently they grow larger in populations where larger shells are available, which causes inter-population variation (Ota et al. 2010b). Although spawning may periodically occur on a lunar cycle, the synchronicity is considerably weak and the brood-caring females can be always found in the nests irrespective of the age of the moon (i.e., days since new moon) but less frequently around new moon (Nakai et al. 1990).

Life-histories of male *L. callipterus* are fixed for life (Taborsky 2001). Nest males grow rapidly and employ bourgeois tactics in which they guard and mate with multiple females in their nests (Sato 1994; Taborsky 2001; Sato et al. 2004). On the other hand, dwarf males remain very small throughout their life and employ sneak tactics in which they covertly fertilize eggs by entering the shells, passing by the females and ejaculating at the innermost whorl of the shells until the spawning is over (Taborsky 2001; Sato et al. 2004). Dwarf males, as well as nest males, can continue to ejaculate while in close proximity to female spawners once entering the shells (Sato et al. 2004). Consequently, they can sometimes sire more offspring than bourgeois males (Meidl 1999).

Field surveys

We conducted field studies at eight populations in Lake Tanganyika using SCUBA diving: Kalundu (3°49′S, 29°14′E) and Muzimo (4°05′S, 29°24′E) from August 1987 to January 1988 by TS; Rumonge (3°58′S, 29°03′E) in January 1993 by TS; Isanga (8°39′S, 31°11′E), Kasakalawe (8°47′S, 31°04′E), Nkumbula Island (8°45′S, 31°05′E) and Wonzye Point (8°43′S, 31°08′E) from October to December 2005, 2006 and 2007 by KO; Mtondwe Island (8°42′S, 31°07′E) in November 2010 by KO, in each of which we set a study area (150-3400 m^2) at a depth of 5-30 m (see Ota et al. 2010b). During the study periods, we captured nest males and dwarf males using gill nets in and around the study area (see Ota et al. 2010a, b for detailed methods). We brought them to the laboratory and measured their standard length (SL; nearest to 0.1 mm) and body mass (BM; nearest to 0.001 g). We gently wiped their body surface dry with disposable paper towels (KimTowel, Crecia, Japan) before measuring BM. They were sacrificed after chilling them on crushed ice or anaesthetizing them with eugenol. Immediately after sacrifice, their gonads were carefully removed from their abdomen and their testes mass (TM) was weighed (nearest to 0.001 g). Mature testes mainly consist of sperm in fish (Billard 1986), and thus TM will be a good measurement for sperm production in fish species including Lake Tanganyika cichlids (Uglem et al. 2001; Awata et al. 2008, but see Leach and Montgomerie 2000). Indeed, nest male *L. callipterus* have high percentage of sperm cells in milts (mean spermatocrit = 86 %, $n = 7$ sampled at Wonzye in 2007; Ota et al., unpublished data). We used the samples from 4th to 25th moon age (i.e. samples except around new moon) in the following analyses to avoid possible effect of maturity of testes according to lunar spawning cycle. A total of 132 nest males (Isanga: n = 22; Kalundu: n = 10; Kasakalawe: n = 8; Mtondwe: n = 24; Muzimo: n = 22; Nkumbula: n = 9; Rumonge: n = 15; Wonzye: n = 22) and 94 dwarf males (Isanga: n

= 13; Kalundu: n = 8; Kasakalawe: n = 12; Mtondwe: n = 33; Muzimo: n = 3; Nkumbula: n = 8; Rumonge: n = 1; Wonzye: n = 16) were analyzed. Dwarf males were found in all populations, but we captured only one dwarf male in Rumonge. This is because the site location (deep and far from the shore) prevented us from taking enough time to search and capture dwarf males, but this will not mean that dwarf males are rare in Rumonge.

The critical determinant of hypothesis is whether the relative testis investment of Rumonge nest males is similar to that of nest males in the other populations or the investment of dwarf males. We therefore compared relative testis investment among male groups (i.e., dwarf males, Rumonge nest males and nest males in the other populations). Gonado-somatic index or GSI (i.e. TM×100/BM) had been long used as an estimate of testes investment when comparing between tactics with different size classes. However, GSI is flawed when it disproportionately changes with body size because of allometric growth. To account for testes allometry, we followed the method of Tomkins and Simmons (2002): a linear mixed model was constructed with $\log_{10}$ TM (response variable), $\log_{10}$ soma mass (SM) (= BM−TM, covariate), the three male groups (fixed factor), and moon age and populations (random factors). We included the interaction (male groups × $\log_{10}$ SM) in the full model and refined it using a backward elimination. For simplicity, we assumed no interaction between fixed and random factors. After the refinement, we compared the intercepts among the three groups after adjusting the critical α level at $0.05/3 = 0.017$ according to the Bonferroni method. Analyses using GSI were also conducted, but the results were consistent with testes allometry analysis (Supplementary figure 1).

Other factors that may affect testes investment should also be considered. We examined the potential effects of the number of mates (degree of polygyny) and female fecundity (i.e. female body size, Ota et al. 2010b) on relative testis investment. We considered these effects because more sperm are required to fertilize the eggs of more females and of larger, more fecund females (Shapiro et al. 1994). Because a female *L. callipterus* spawns in a nest, brood-caring females found in a nest can be considered as mates of the nest owner. Therefore, the degree of polygyny was examined by counting the number of brood-caring females in the shells (but see Maan & Taborsky 2008). We counted the number of them in a total of 77 nests (Isanga: n = 6 nests; Kalundu: n = 7; Kasakalawe: n = 8; Mtondwe: n = 9; Muzimo: n = 8; Nkumbula: n = 9; Rumonge: n = 8; Wonzye: n = 22) in the study area throughout the study periods except around new moon (see above). We were able to easily identify the nests because these were clumps of shells. Brood-caring females were also readily identifiable since their caudal fins were visible from the shell entrance (Ota et al. 2010b). The degree of polygyny was compared among the eight populations using a linear mixed model with populations (fixed factor) and two random factors (moon age and populations). For simplicity, we assumed no interaction between fixed and random factors. For female size, we used data that have been published elsewhere (Ota et al. 2010b) but newly examined in Mtondwe ($n = 34$) using the same methods. To examine the effects of the degree of polygyny and female fecundity on testes investment, we performed Pearson correlations between these parameters and testes investment. For these analyses, relative testis investment was quantified by the residuals of TM on SM using the common slope of the refined and final linear mixed model.

All analyses were performed using S-Plus v. 8.0 (Insightful, Seattle, WA, USA).

RESULTS

The interaction (male groups × $\log_{10}$ SM) was not significant in the full model (linear mixed model, $F_{2,164} = 2.05$, $P = 0.13$), indicating that TM-SM allometric slopes are homogeneous among the male groups. The reduced final model showed that the intercepts were different across the male groups ($F_{2,166} = 686.63$, $P < 0.001$) and TM correlated positively with SM ($F_{1,166} = 115.52$, $P < 0.001$) (Fig. 2). A post-hoc Bonferroni test revealed that the intercepts of nest males in Rumonge and the other populations were not different ($P = 0.54$) but smaller the intercept of dwarfs (Rumonge nest males vs dwarfs: $P < 0.001$, nest males in the other populations vs dwarfs: $P = 0.002$, both of which were smaller than the adjusted significant level). This indicates that the rank order of relative testis investment is dwarf males > Rumonge nest males = nest males in the other populations.

The degree of polygyny did not differ among the populations (Isanga: mean ± SD = 5.2 ± 3.1 females per nest; Kalundu: 3.9 ± 2.0; Kasakalawe: 4.1 ± 4.4; Mtondwe: 3.8 ± 3.3; Muzimo: 4.8 ± 0.7; Nkumbula Is.: 5.1 ± 4.6; Rumonge: 2.2 ± 1.6; Wonzye: 6.5 ± 3.7, mixed model, $F_{7,53} = 1.36$, $P = 0.26$). Relative testis investment was neither accounted for by the degree of polygyny (Pearson correlation, nest male: $r = 0.38$, $P = 0.35$; dwarf male: $r = -0.36$, $P = 0.38$; $n = 8$) nor female size (nest male: $r = 0.41$, $P = 0.32$; dwarf male: $r = 0.07$, $P = 0.88$; $n = 8$).

DISCUSSION

In the present study, we examined the mechanism underlying variations of relative investment in testes across reproductive tactics in *L. callipterus*. In our sample, Rumonge nest males had smaller body sizes than nest males in seven other populations and their body sizes were rather close to parasitic dwarf males. Nevertheless, they had a greatly reduced relative testis investment that is equal to nest males in the other populations. This suggests that relative testis investment is not simply a function of body size effect, but tactic-specific, supporting the Parker's SG model.

However, the results could also occur if nest males in Rumonge face other selection pressures shaping decreased relative testis investment. First, their testes investment may result from the decreased degree of polygyny and female fecundity at this population. In coral reef fish and insects, ejaculate size increases with the number of eggs available (Shapiro et al. 1994; Gage and Barnard 1996; Gage 1998). Thus, as the degree of polygyny and female fecundity increases, larger sperm storage is needed. However, we found no significant correlations between testes investment and the degree of polygyny or female fecundity. These alternative explanatory factors therefore may play less important roles in the testes investment of *L. callipterus*, although the sample sizes, and therefore presumably the statistical power of these tests, were small and thus further studies are needed. Second, their decreased testes investment may result from a lower relative abundance of dwarf males to nest males compared to other populations. Theoretically, when parasites are abundant and thus bourgeois males face a high risk of sperm competition that is equal to parasites, they invest in testes as much as parasitic males (Parker 1990, see also Simmons et al. 1999 2007 for empirical examples). Although we cannot completely rule out the possibility, this does not appear to be the case in *L. callipterus*. The spaces in the shells where the females were spawning were large enough for the dwarfs to enter the shells and obtain fertilization opportunities (Sato et al. 2004) in all populations, including Rumonge (Ota et al. 2010a, Ota and Sato unpubl. data). We believe that there is little or insufficient differences in the relative proportion of dwarfs among these populations to shape the difference in testes investment. Overall, the observed variations in relative testes investment by male *L. callipterus* would be primarily accounted for by sperm competition.

The effects of small body size of parasitic males on their investment in testes may be negligible when the fertilization opportunities of them are limited (Thomaz et al. 1997; Avise et al. 2002; Rios-Cardenas and Webster 2008) and thus their sperm may seldom be in short supply. In several species with ARTs, the fertilization opportunity of a parasitic male is considerably variable and sometimes exceeds that of bourgeois males. A sneaker of the European bitterling, for example, can sire an average of 40% eggs (range = 0–83%, Reichard et al. 2004). Such high variation in the contribution to fertilization by parasitic males is also found in Sockeye salmon (mean = 42%, range =

3–93%, Foote et al. 1997). These indicate that greater fertilization opportunities could be available even for reproductively parasitic males. This is the case in *L. callipterus*; dwarf males can usually sire more than half of the eggs (range = 50–81%, Meidl 1999). In this case, relative testes investment which is as large as that of territorial males will be often too small to enjoy the greater fertilization opportunities, possibly resulting in an absolute deficiency in their sperm counts. Therefore, we think that larger relative investment in testes will be favoured for parasitic males, regardless of sperm competition, but their larger relative testis investment seemed to be justified by sperm competition, rather than by small body size. Therefore, their testes investment strategies may not be highly influenced by their small body sizes. This conclusion might be supported by the study of Immler et al. (2004) who showed that sneaker-sized males of black goby *Gobius niger* kept and pair-spawned with females in the aquarium had small investment in testes (calculated as GSI) compared to sneakers sampled from the field, but similar investment to sneakers kept alone in the aquarium and bourgeois males sampled from the field.

There is often a difference in relative testes investment between tactics in species with ARTs, but we claimed whether this difference really results from sperm competition its prediction is still controversial, particularly in the view of the possible inevitable effects that are inherent in ARTs, i.e. the effects of the absolutely small body size. The model should be carefully tested, thereby developing our understanding of the mechanisms that underlie differences in relative testis investment across different reproductive tactics. In this study, we reconsidered the selection pressures underlying the testes investment strategies of small parasitic males while accounting for testes allometry and both the effects of body size and sperm competition. Nevertheless, their increased testes investment seems to result from their responses to sperm competition, rather than body size, suggesting that the prediction of the SG model is robust.

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Agriculture, Food and Fisheries, and complies with the laws of each country and the guidelines of the Animal Care and Use Committee of Osaka City University, Kyoto University, and the Japan Ethological Society. Funding was provided from a Grant-in-Aid for Research Fellowship from JSPS for Young Scientists and an Overseas Scientific Research grant (MEXT).

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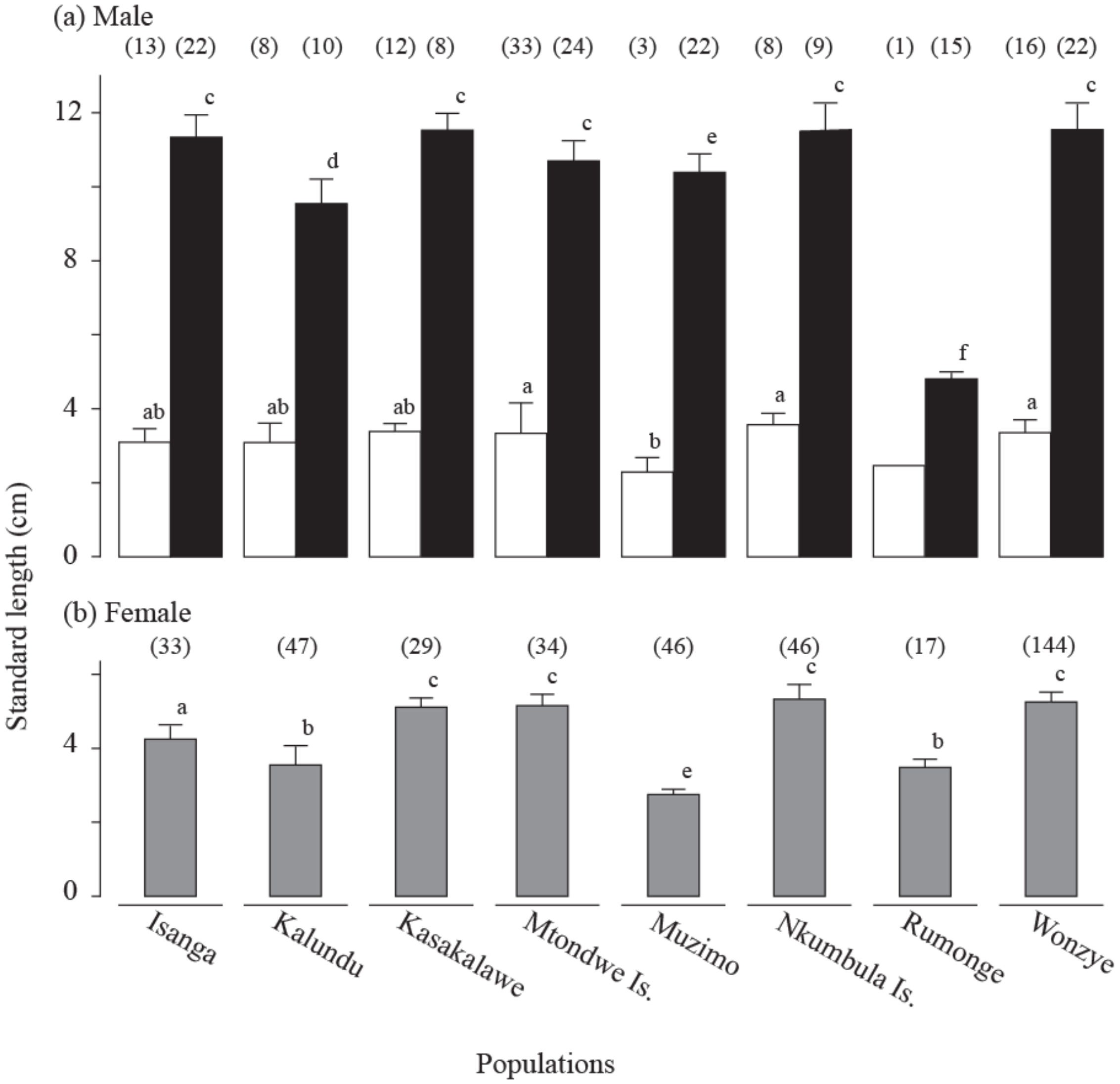
FIGURE LEGENDS

Fig. 1 Differences in (a) male and (b) female body size among eight populations (dwarf males, blank bar; bourgeois males, filled bar , two-factor ANOVA, tactic*population, $F_{7,210}$=24.3, $P < 0.001$, tactic: $F_{1,217}$=6894.7, $P < 0.001$, population: $F_{7,210}$=176.9, $P < 0.001$). Error bars represent SD. Different letters over the error bars indicate statistical significances determined using Bonferroni. Dwarf males in Rumonge were not included in the post-hoc test. Sample sizes are in parentheses above the bars.

Fig. 2 The relationships between soma mass and testes mass in both dwarf (squares) and nest (circles) males across populations (blue, Isanga; white, Kalundu; red, Kasakalawe; purple, Mtondwe; orange, Muzimo; yellow, Nkumubla; grey, Rumonge; green, Wonzye). There was only one sample of dwarf male in Rumonge. Solid lines indicate the slopes of the regressions fit for each tactic.


(a) Male
(13) (22) (8) (10) (12) (8) (33) (24) (3) (22) (8) (9) (1) (15) (16) (22)
12
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Standard length (cm)
(b) Female
(33) (47) (29) (34) (46) (46) (17) (144)
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4
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Isanga
Kalundu
Kasakalawe
Mtondwe Is.
Muzimo
Nkumbula Is.
Rumonge
Wonzye
Populations

Fig. 1

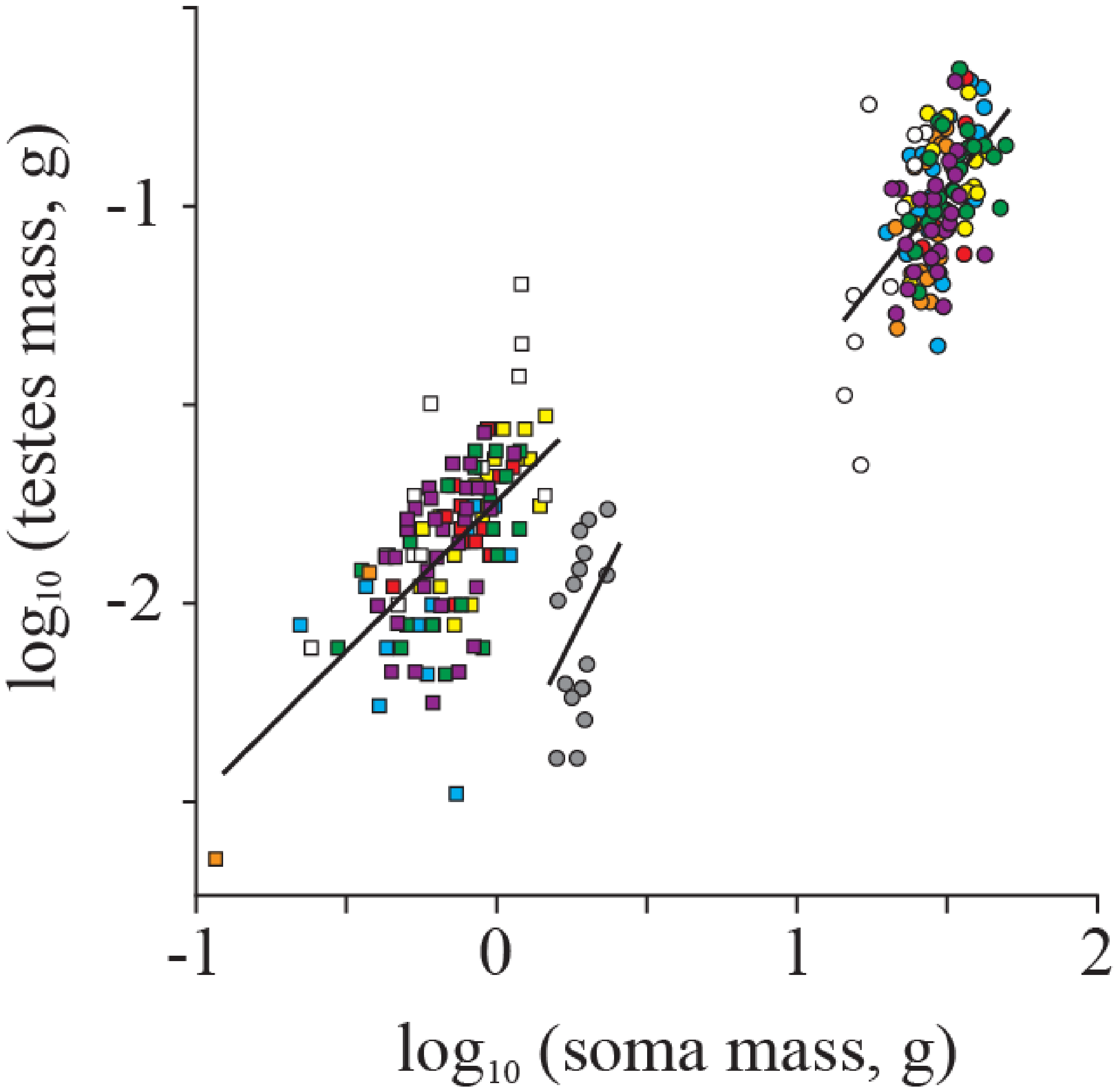

Fig. 2