

Condition-dependence and primary and secondary sexual trait allometry in the ornate dragon lizard

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ABSTRACT

The maintenance of honesty and genetic variation in secondary sexual traits remains a largely unresolved issue in evolutionary biology. Condition-dependence of secondary sexual traits may maintain genetic variance. Previous fieldwork has established that the melanised chest patch of male ornate dragon lizards is positively related to residual mass and that males with larger chest patches have access to larger territories and more females. Correlations between condition and secondary sexual traits may arise however through their mutual dependence on a third variable, such as immune function or primary sex traits such as testes. We examined the condition-dependence of the melanised chest patch of male ornate dragon lizards by investigating the relationships between chest patch area, testis mass and phenotypic condition. Path analysis revealed that the observed phenotypic condition-dependence of chest patch size is actually a consequence of the common dependence of 'condition' (residual mass) and chest patch size on testis mass. We also show that testis mass has extreme positive allometry. Our results raise the possibility of an interdependence of the signalling function of positive allometry in the chest patch and the unusual positive allometry of the testes.

Keywords: condition-dependence, sexual selection, lizard, testosterone, allometry, testis.

INTRODUCTION

Interest in evolution through sexual selection has persisted largely because of the related evolutionary problems of the maintenance of honesty and the maintenance of genetic variation in secondary sexual traits (Andersson, 1994). These evolutionary problems can be summarised as the need to understand the basis and genetics of condition-dependence (e.g. (Rowe and Houle, 1996). Phenotypic condition-dependence of secondary sexual traits can be understood as a positive relationship between secondary sexual trait expression and some measure of male phenotypic quality (Williams, 1966; Andersson, 1986). This concept of phenotypic (and recently genotypic (Rowe and Houle, 1996; Houle and Kondrashov, 2002) condition-dependence has become central to indicator models of sexual selection (Andersson, 1986; Grafen, 1990; Rowe and Houle, 1996; Houle and Kondrashov, 2002). Despite the centrality of this phenomenon to our understanding of evolution through sexual selection, and the observation of condition-dependent traits in many taxa (Andersson, 1994), relatively little is known about the trade-offs that cause condition-dependence (Kotiaho, 2000).

Phenotypic condition is frequently thought of as the size independent mass of an individual, commonly derived as the residual mass from a regression of mass on a linear measure of size (Kotiaho, *et al.*, 2001). This measure of condition is intuitively appealing because individuals that are heavy for their size are likely to have more resources at their disposal to divert towards the competing demands of their life history. However it is important to establish what the ‘currency’ of condition is, for example some subordinate birds store more fat as a bet-hedge against the unpredictability that low status brings to their foraging attempts (Hake, 1996; Cuthill, *et al.*, 1997). Hence, whether fat or muscle largely determines residual mass and which is more important to an individual’s fitness or trait expression is likely to vary between taxa. Correlations between condition and secondary

sexual traits may also arise through the mutual dependence on a third variable, such as immune function or primary sex traits such as testes. If the condition-dependent expression of sexually selected traits arises as a by-product of a third variable the questions of the maintenance of honesty in sexual signals and their genetic variance shifts dramatically (e.g. the role of the immune system (Hamilton and Zuk, 1982; Folstad and Karter, 1992). One characteristic common to condition-dependent traits that act as signals or are involved directly in competition may be positive allometry (Petrie, 1988; Petrie, 1992; Simmons and Tomkins, 1996). Positive allometry indicates that larger individuals produce relatively larger traits than small individuals (Gould, 1966; Gould, 1973) and hence that the trait has a cost that larger individuals are better able to bear: a key feature of condition-dependent signalling (see (Kotiaho, 2001) for a review of the costs of secondary sexual traits).

Here we report an investigation of the condition-dependence of the melanised chest patch in male ornate dragon lizard *Ctenophorus ornatus*. *C. ornatus* is a territorial agamid endemic to Western Australia, where it inhabits isolated granite outcrops surrounded by the unsuitable habitat of the 'wheat-belt'. Fieldwork on *C. ornatus* has shown that the chest patch is positively related to residual mass and males with larger chest patches have access to larger territories and more females (LeBas, 2001). Badges of status in other lizards have been shown to increase in response to testosterone implants (Cooper, *et al.*, 1987; Cooper and Greenberg, 1992; Salvador, *et al.*, 1996), but see (Olsson, *et al.*, 2000). Similarly, the dominant territorial colour morph of the side-blotched lizard has higher testosterone levels than the other less aggressive morphs (Sinervo, *et al.*, 2000). Hence we hypothesised that the chest patch of *C. ornatus* would also be linked to testosterone. We investigated whether the phenotypic condition dependence of the chest patch was a consequence of residual mass (i.e. residual resources) or the common dependence of both factors on testis.

METHODS

We studied specimens of *Ctenophorous ornatus* held at the Western Australian Museum. Jars containing lizards were searched for mature individuals (larger than 65 mm snout vent length) that had been collected in the central wheat-belt area of Western Australia between 29° and 33° south. This range of latitudes encompassed the centre of the lizards range, whilst excluding the extremes of the range such as the islands of the Recherché Archipelago and an isolated northern population near the town of Cue. The date and location where each individual was caught was recorded. Lizards had been originally fixed in formaldehyde in the museum, and had subsequently been transferred to jars of ethanol. The samples we studied were collected between 1957 and 1978 (median and mode = 1964), however all specimens had been transferred from formaldehyde to ethanol at the same time. In accordance, there was no evidence of any year effects on the specimens (i.e. regression of fat-body on year, SVL, soma mass, latitude and longitude, showed no significant year effect, $\beta = 0.540 \pm 3.122$, $t = 0.17$, $P = 0.86$).

After selection we kept the lizards under ethanol until they were measured and dissected. The linear morphological measurements of each lizard were recorded using a pair of Mitutoyo digital callipers. Snout-vent length (SVL) was recorded with the lizard's back straight, as the distance from the tip of the snout to the vent. Head depth was measured at the tympanum. In males the area of the melanised chest patch was measured under a binocular microscope with the *Optimas* image analysis package. The chest was flattened under a piece of glass to reduce the error arising from the unevenness of the skin. The total area of the chest and abdomen was not measured in the original sample, but was measured using *Optimas* from photographs of the ventral surface of a sample of field caught male lizards. Log chest and abdomen area was tightly related to the log of inter-limb distance squared ($F_{(1,14)} = 226.27$, P

= 0.001, $r^2 = 0.94$) with an allometric exponent close to one ($\beta = 0.916 \pm 0.061$). This exponent was used to calculate chest and abdomen area for the lizards measured from the museum, so that the allometry of chest patch area could be estimated from the linear measures taken on the museum specimens. 81 males were dissected.

After measurement the total mass of each lizard was recorded to 0.01 g on a *Sartorius* balance. The lizard's abdomen was then cut open and the fat body and testes were dissected. The lizard's organs were weighed to 0.01 mg on the same balance. The organs were placed in labelled vials containing ethanol and returned with the lizards to the Western Australian Museum. In the analysis we refer to 'body mass' as the mass of the intact lizard, we refer to 'residual body mass' as the residual of body mass regressed on SVL and we refer to 'soma mass' as body mass minus spleen mass, fat-body mass and testis mass. Residual soma mass is the residuals of soma mass regressed on SVL. To examine the seasonal variation in testis mass, date of collection was standardised to be a day between 0 and 365. Day 0 was designated as the first of February as this represents the approximate end of the breeding season, and therefore the beginning of the next cycle of reproduction.

We have used path analysis to test between competing hypotheses for the condition-dependence of chest patch area. We used the *EQS for windows* statistical package with a maximum likelihood solution to estimate the path coefficients. There are a number of parameters that can be calculated in order to estimate the goodness of fit for alternative path analysis models; we have used a Comparative Akaike's Information Criterion (CAIC) and the Root Mean Square Error of Approximation (RMSEA). The CAIC is a relative measure for comparing two or more models of the same data set, the best fitting model having the lower value. RMSEA calculates the overall fit χ^2 per degree of freedom in the model (Browne and Cudeck, 1992; Crnokrak and Roff, 2000) and hence can be used to estimate the fit of a single

model without testing alternatives. RMSEA values of zero indicate the $\chi^2 < \text{d.f.}$ and a perfect fit, values below 0.05 a good fit and those above 0.01 a poor fit.

RESULTS

Seasonal variation in testis mass

There was a significant quadratic relationship between testis mass and date of collection ($F_{(2,68)} = 15.35, P < 0.001$) with testis mass increasing from the 1st of February ($\beta = 0.907 \pm 0.177, P < 0.001$) and then decreasing from the start of the breeding season in September (day 212, $\beta = -0.002 \pm 0.000, P < 0.001$; Fig. 1). To control for this seasonal variation in testis size in later analyses, residual testis mass from this quadratic regression was used.

To examine the nature of the allometric change in testis mass between animals in the breeding season and those outside it, we performed an analysis of covariance of log testis mass on log soma mass (Tomkins and Simmons, 2002). The relationship between these variables was significantly different for males in the breeding season compared to males collected prior to the breeding season (ANCOVA, dependent variable = log testis mass, covariate = log soma mass, factor = season; season* total body mass interaction $F_{1,61} = 6.063, P = 0.017$). However this interaction was due solely to a small male (SVL 66 mm) and although this male was sexually mature and had a chest patch, it drove the interaction. The exclusion of the small male reduced the interaction to non-significance ($F_{1,59} = 0.053, P = 0.82$). Hence a conservative interpretation is of no significant difference in the slope of testis mass on soma mass relationship between males inside and outside the breeding season. The log soma mass term in the model was significant ($F_{1,60} = 21.9, P < 0.001$) indicating as expected, a significant increase in testis mass with body mass. What was unexpected was that

the testis allometry of the combined classes of males was significantly greater than one (Least squares regression (LSR), $\beta = 3.71 \pm 0.66$, $t = 4.1$, $P < 0.001$), Reduced major axis (RMA) $v_{Y \cdot X} = 6.35 \pm 0.661$, $t = 8.09$, d.f. = 61, $P < 0.001$). The main effect of season was also significant in the model ($F_{1,60} = 12.63$, $P = 0.001$), indicating that despite the same allometric slope the males in the breeding season had increased the elevation of the relationship between testis mass and soma mass, and so have larger testes for their mass (Fig. 2).

Condition-dependent determination of chest patch area

A multiple regression of male chest patch area against male SVL and male body mass found that male mass explained a significant proportion of the variation in chest patch size, independent of SVL (whole model, $F_{(2,70)} = 15.47$, $P < 0.001$; SVL $\beta = 1.746^{-3} \pm 0.03$, $t = 0.586$, $P = 0.56$; body mass, $\beta = 0.118 \pm 0.05$, $t = 2.409$, $P = 0.019$). Chest patch size was not significantly related to the day of collection in a quadratic (or linear) fashion ($F_{(2,77)} = 0.80$, $P = 0.451$). The allometry of chest patch area was estimated from the regression of log chest patch area on $(\log \text{inter-limb}^2)^{0.196}$ (see methods). Chest patch area was positively allometric (LSR $F_{(1,78)} = 21.87$, $P < 0.001$, $\beta = 1.315 \pm 0.281$) with a reduced major axis slope significantly greater than 1 ($v_{Y \cdot X} = 2.74 \pm 0.281$, $t = 6.17$, d.f. = 78, $P < 0.001$).

We tested two alternative hypotheses concerning the condition-dependent expression of the chest patch using path analysis. The first hypothesis proposes that chest patch expression increased with increasing residual mass: increased residual mass was hypothesised to increase testis mass and testis mass was itself also predicted to increase chest patch area. The partial correlations shown in Table 1 were used to determine whether a variable was entered into the path model. All variables showed some partial correlations of significance and hence all variables were included. Figure 5a shows the ‘residual mass model’, using the *EQS for*

windows statistical package with a maximum likelihood solution. The residual mass model yielded a CAIC of -31.32 and a RMSEA = 0.06 , indicating that the fit of the model is borderline. Removal of the ns paths in the model further reduced model fit.

The second hypothesis we tested was that testes were the key organ involved in chest patch expression and that androgen hormones produced by the testes increase residual mass through anabolic effects on muscle bulk (Fig. 5b). The ‘testis mass hypothesis’ is shown in Fig. 5b, the CAIC was lower than for the competing model = -34.7 and the RMSEA = 0.00 indicating a perfect fit. It should be noted that the only difference in the construction of the path models is the direction of the arrow between testis and residual mass. The direction of this arrow changes the path coefficients relating snout-vent length to both testis mass and chest patch area. However, two important features of both models are consistent: residual mass has a small and non-significant effect on chest patch area while testis mass has a moderately large and significant positive effect on chest patch area. Hence residual mass does not influence chest patch area itself, rather the correlation observed in the partial correlations above is one mediated through testis mass, and is not itself causal.

DISCUSSION

Understanding the phenotypic and genotypic basis of condition-dependence is central to understanding the evolution of traits involved in sexual signalling (Andersson, 1986; Grafen, 1990; Iwasa, *et al.*, 1991; Rowe and Houle, 1996; Houle and Kondrashov, 2002). Male ornate dragon lizards have a black chest patch, which has previously been shown to be related to male phenotypic condition (residual body mass) and territorial success in the field (LeBas, 2001). Here we have dissected museum specimens of *C. ornatus* in order to establish the

causal relationships between traits involved in the expression of the male chest patch. We have shown that the observed phenotypic condition-dependence of the chest patch is caused by a common dependence of condition (residual soma mass) and chest patch area on testis mass, rather than a causal relation between 'condition' *per se* and chest patch area. This represents a significant advance in our understanding of the phenotypic basis of condition-dependent trait expression. Furthermore our analysis raises the possibility of an interdependence of the signalling function of positive allometry in the chest patch and the unusual positive allometry of the testes.

Seasonal variation in testis mass is a pattern common to many other seasonally breeding vertebrates (Blottner, *et al.*, 1996; Blottner, *et al.*, 2000; Clesson, *et al.*, 2002). There was no evidence of a plateau in testicular investment during the breeding season to suggest that males maintained their testis investment throughout the reproductive period. Rather testis mass declined significantly through the breeding season. This decline in the mass of the testes over the breeding season is likely due to the reduction in spermatogenesis following the onset of courtship and mating. This is a pattern known to occur in the adder (*Vipera berus*) in which a discrete period of spermatogenesis occurs prior to the breeding season (Olsson, *et al.*, 1997). Spermatogenesis in the adder was found to reduce body mass as much as the efforts of courtship and mating (Olsson, *et al.*, 1997). In *C. ornatus* there is also likely to be a benefit to completing costly spermatogenesis prior to the energetically demanding period of territory defence.

Our data show that in *C. ornatus* testes increase with soma mass with an allometric exponent of 6.35 and highly significantly greater than one. Such positive allometry is extraordinary for an internal organ that in general scale with an allometry an order of magnitude smaller (Reiss, 1989). Positive allometry is generally associated with secondary sexually selected traits involved in signalling competitive ability (Gould, 1973; Petrie, 1988;

Petrie, 1992; Simmons and Tomkins, 1996) and arises because larger individuals derive greater benefits or suffer relatively smaller costs of trait expression. In this sense positive allometry reflects a key feature of the honest signalling hypothesis: high quality individuals suffer a relatively lower cost of trait expression (Grafen, 1990; Rowe and Houle, 1996). Three non-mutually exclusive hypotheses present themselves for the extreme allometry of testis mass in *C. ornatus*.

First, positive allometry may reflect the cost and timing of spermatogenesis. Parker's sperm competition models e.g. (Parker, 1990) require that investment in ejaculates carry a cost that trades ejaculate investment against other reproductive activities such as obtaining mates. The magnitude of this cost has usually been assumed to be relatively low. However there is growing empirical evidence for a taxonomically widespread and considerable cost of spermatogenesis (Wedell, *et al.*, 2002). This may apply particularly to species that perform the bulk of their seasonal sperm production in one bout (e.g. Olsson, *et al.*, 1997) rather than having a continuous production of sperm through the season. Hence the positive allometry of the testes in *C. ornatus* may arise as a consequence of a cost to spermatogenesis that is borne more easily by larger individuals. The second hypothesis is that simple optimisation of testis mass (which requires a cost) could cause large males, that are likely to achieve more matings, to invest proportionally more in testis mass. Even though multiple paternity occurs in *C. ornatus* (LeBas, 2001) there is no evidence for size-dependent tactics in this species that would select small males to increase their testis investment.

Our final hypothesis for the extreme testis allometry in this species is that positive testis allometry may reflect the role of testosterone production in signalling competitive ability or vigour. In a field study of *C. ornatus* male chest patch size was associated with male territory size and the number of females within the males territory (LeBas, 2001). Hence the chest patch, which is revealed during the regular 'push-up' displays of males, appears to have a

significant role in sexual signalling. The area of the chest patch was also found to correlate to the phenotypic condition of the male measured as residual body mass (LeBas and Marshall, 2001). In this study we have confirmed that whilst chest patch area is related to residual soma mass and can be considered condition-dependent in that sense, we have further shown that this condition-dependence arises from the mutual dependence of residual soma mass and chest patch area on testis mass (evidenced in both path analysis models). Therefore it is testis mass, not '*condition*' that drives the observed phenotypic condition-dependence of chest patch area. Our data shows that like testis mass, the area of the chest patch increases with an allometric exponent greater than one. Consistent with the premise of the immunocompetence handicap hypothesis (Folstad and Karter, 1992), if testosterone imposes evolutionarily significant costs on lizards, then chest patch area, expressed via testis mass, may be a reliable indicator of the male's ability to overcome these costs. Alternatively testosterone may be costly in the absence of immune suppression. The administration of testosterone implants increases territorial and aggressive activity in male lizards (Marler and Moore, 1988; Marler and Moore, 1989; Salvador, et al., 1996; Olsson, et al., 2000; Sinervo, et al., 2000) to the extent that in mountain spiny lizards (*Sceloporus jarrovi*) it is the debt in the energy budget of the individual that increases mortality (Marler and Moore, 1989; Marler and Moore, 1991). In other vertebrates where immune suppression is likely to have reduced significance (domestic dogs and humans), testosterone continues to have a negative effect on life span (Waters, *et al.*, 2000). Hence the positive allometry of the chest patch may indicate the ability of the male to withstand the costs associated with the relatively larger testes that are associated with a large chest patch. Although the association of females with males with large chest patches has been observed in the field, lab trials have found no evidence for female choice of males in this species (LeBas and Marshall, 2001).

Social costs associated with levels of testosterone (Marler and Moore, 1988; Salvador, et al., 1996) and mediated through chest patch area could also influence testis allometry. In lizards aggression towards subordinate males by more dominant rivals is known to suppress the secondary and primary sexual maturation of subordinates (Alberts, *et al.*, 1994). This suggests that selection on the display traits of lizards can have feedback onto the primary sexual trait responsible for its expression: the testes. For example there are likely to be large costs to small, low status individuals of overstating their competitive ability (or simply signalling 'maleness') and thereby entering into escalated contests with superior rivals. In contrast large males can avoid aggressive interactions by advertising their competitive ability (Petrie, 1988). Positive testis allometry may therefore reflect the costs and benefits associated with signalling among males.

By dissecting museum specimens of *C. ornatus* we have been able to investigate the relationship between condition and chest patch size. Our analysis revealed that although the phenotypic correlations between chest patch area and residual soma mass were significant, there was no correlation between the two when testis mass was considered in a path analysis model. Hence the observed correlation is a consequence of the co-dependence of residual mass and chest patch area on testis mass. Body mass is largely attributable to muscle bulk in the male lizards and the path analysis is therefore consistent with the intuitive notion that the androgens released by the testis increase muscle bulk as well as stimulate increased coloration of the chest patch. A relationship between androgens and muscle mass is supported by evidence for increased muscle mass with testosterone supplementation in other taxa (Fennell and Scanes, 1992; Young, *et al.*, 1993). The extraordinary positive allometry of the testes may be due to the costs of seasonal bouts of spermatogenesis or due to their mediation in the expression of the positively allometric chest patch area. Regardless of the relative importance of these factors, the positive allometry is testament to a cost that larger individuals are better

able to bear, and provides a new insight into our understanding of the relationship between primary and secondary sex traits.

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Table 1. Partial correlations between traits hypothesised to be involved in determining the size and condition-dependence of testes mass and chest patch area in male *C. ornatus*. N = 64, df = 57. SVL = snout vent length, CPA = chest patch area.

Variable	Latitude	Longitude	SVL	CPA	Fat	Testes
Longitude	-0.012	•				
SVL	0.246*	0.228*	•			
CPA	0.175	0.266*	-0.000	•		
Fat	-0.089	-0.056	0.350***	0.180	•	
Testes	0.427***	-0.443***	-0.072	0.219*	0.113	•
Soma mass	-0.424***	-0.057	0.810***	0.195	-0.281**	0.305**

* $P < 0.1$, ** $P < 0.05$, *** $P < 0.01$.

Figure Legends

Fig. 1. Curvilinear relationship between mean testes mass and day of the year on which the lizard was caught and killed. February 1st was designated as day zero as this is the end of the breeding season and represents the beginning of the cycle of testes maturation. Males in breeding season (●) and out of season (○).

Fig. 2. Testes allometry for males in the breeding season (●, —) and males outside the breeding season (○, ---), the elevation of the allometry is significantly higher in males in-season.

Fig. 3. Path diagrams showing alternative models for the condition-dependence of testes mass and chest patch area. Model (a) shows the ‘residual mass hypothesis’ and model (b) the ‘testes hypothesis’. Latitude, longitude and SVL are included in the model to control for these effects on the other variables. Both models reveal that chest patch area is dependent on residual testes mass rather than residual soma mass. The fit of model (b) is superior to model (a) (see text). Shaded boxes are independent variables, SVL = snout-vent length, testes mass is controlled for the day of the year, soma mass = body mass – organ mass, CPA is chest patch area. Standardised path coefficients are presented next to the arrow that indicates the direction of the path (arrow thickness reflects magnitude of the coefficient, dashed arrows represent paths that were not significantly different from zero). Path coefficients were estimated using the maximum likelihood method of (*EQS*). U_1 , - U_5 are coefficients of non-determination.

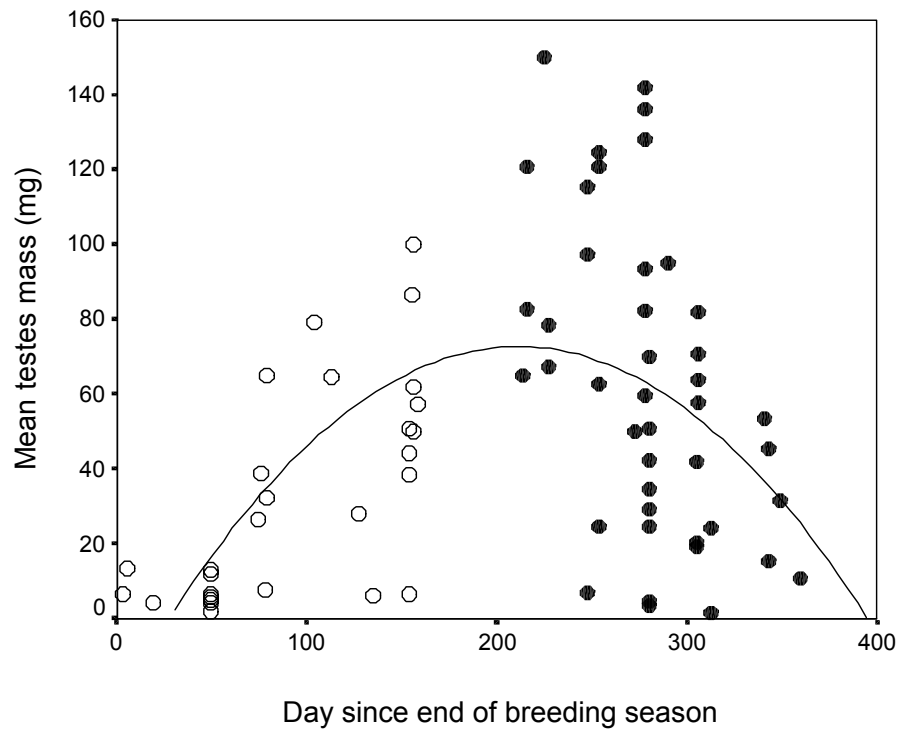


Figure 1

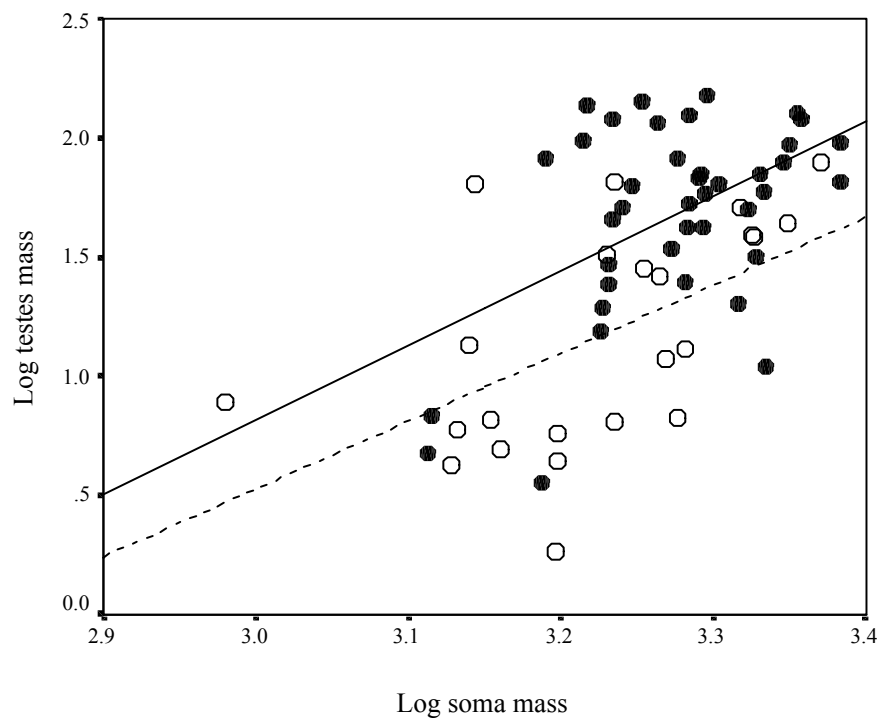


Figure 2

Figure 3a

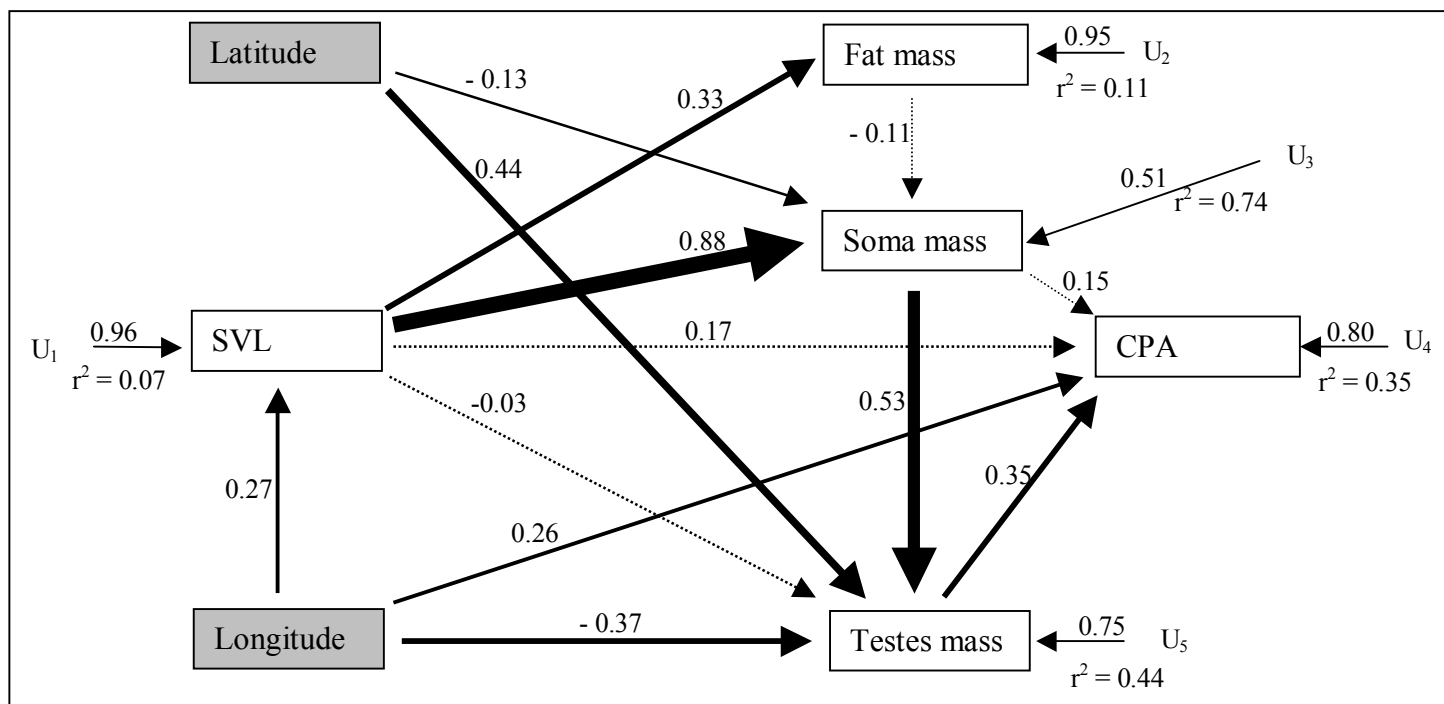


Figure 3b

