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RESEARCH ARTICLE

Neither male gonadal androgens nor female reproductive costs drive development of sexual size dimorphism in lizards

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SUMMARY

Sexual size dimorphism (SSD) is an extensively studied phenomenon in animals, including reptiles, but the proximate mechanism of its development is poorly understood. The most pervasive candidates are: (1) androgen-mediated control of growth, i.e. a positive effect of gonadal androgens (testosterone) on male growth in male-larger species, and a negative effect in female-larger species; and (2) sex-specific differences in energy allocation to growth, e.g. sex with larger reproductive costs should result in smaller body size. We tested these hypotheses in adults of the male-larger lizard *Paroedura picta* by conducting castrations with and without testosterone implants in males and manipulating reproductive status in females. Castration or testosterone replacement had no significant effect on final body length in males. High investment to reproduction had no significant effect on final body length in intact females. Interestingly, ovariectomized females and females with testosterone implants grew to larger body size than intact females. We did not find support for either of the above hypotheses and suggest that previously reported effects of gonadal androgens on growth in male lizards could be a consequence of altered behaviour or social status in manipulated individuals. Exogenous testosterone in females led to decreased size of ovaries; its effect on body size may be caused by interference with normal ovarian function. We suggest that ovarian factors, perhaps estrogens, not reproductive costs, can modify growth in female lizards and may thus contribute to the development of SSD. This hypothesis is largely supported by published results on the effect of testosterone treatment or ovariectomy on body size in female squamates.

Key words: gecko, growth, life history, phenotypic plasticity, reproductive investment, trade-off.

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INTRODUCTION

Sexual size dimorphism (SSD) is defined as a difference in body size between sexes. Since the time of Charles Darwin (Darwin, 1871), SSD has been widely regarded as a consequence of a particular sex's adaptation to its specific reproductive or ecological roles (reviewed in Anderson, 1994; Fairbairn et al., 2007). Nevertheless, proximate mechanisms leading to differences in size between the sexes are still only partially known, even in vertebrates (Badyaev, 2002). In squamate reptiles, SSD is phenotypically plastic (Madsen and Shine, 1993; Cox et al., 2006; Starostová et al., 2010), which might be mirrored by the considerable evolutionary plasticity of SSD within this group (Starostová et al., 2010). Several monophyletic lineages of squamates exhibit mixed SSD, where some closely related species possess male-biased and others female-biased SSD (e.g. Kratochvíl and Frynta, 2002; Cox and John-Alder, 2005; Starostová et al., 2010). Therefore, if a proximate mechanism controlling body size in each sex, and hence SSD, is shared by different squamate lineages, it must allow both phenotypic plasticity and rapid evolutionary changes to produce different magnitudes and even directions of SSD.

Recently, Cox and John-Alder (Cox and John-Alder, 2005) suggested that male gonadal androgens (testosterone) can represent such a mechanism. Based on results of hormonal manipulations in species from the iguanian genus *Sceloporus* with opposite SSD patterns, they proposed that testosterone can have positive effects on male growth in male-larger species but negative effects on male growth in female-larger species. Cox and colleagues (Cox et al., 2009) expanded the test of this hypothesis by conducting experimental work in another iguanian species (*Anolis sagrei*) and performing phylogenetically informed analysis of previously published results of hormonal manipulations in squamate reptiles. In their review, they reported that androgens enhance male growth in male-larger species, inhibit it in female-larger species, but have ambiguous or no obvious effect in species that are monomorphic in body size. They speculated that this bipotential effect of testosterone on growth could involve direct effects on the endocrine growth axis (e.g. growth hormone, insulin-like growth factor-1), or indirect effects such as energy trade-offs between growth and activity mediated by testosterone-induced changes in physiology and behaviour.

Aside from the hypothesis on bipotential growth regulation by gonadal androgens, the other popular alternative views SSD as a consequence of sex-specific differences in energy allocation to growth. This hypothesis is based on an assumption that in organisms with indeterminate growth, such as squamate reptiles, the amount of available resources devoted to body enlargement is directly proportional to growth (e.g. West et al., 2001). The degree of SSD, then, should largely reflect sexual differences in energy acquisition [or potentially also energy assimilation (Stahlschmidt et al., 2011)] or energy allocation to reproduction (e.g. Cox, 2006). This

hypothesis predicts that female-biased SSD should occur in species where males are limited in energy acquisition or are forced to expend energy in demanding activities such as territory defence in order to obtain mating opportunities (Cox and John-Alder, 2005). In contrast, male-biased SSD should be present in species where females acquire energy less efficiently or allocate substantially more energy to reproduction than do males, and hence it is impossible for them to sustain male-typical growth. Energy acquisition in females can be limited due to decreased mobility caused by the reproductive burden (e.g. Cooper et al., 1990; Johnson et al., 2010), or by anorexia during vitellogenesis or gravidity (e.g. Weeks, 1996). Trade-off between reproductive allocation and growth in females is intuitively appealing and represents one of the key assumptions of life-history theory (e.g. Kozłowski, 1992; Stearns, 1992). Nevertheless, empirical support for this energetic trade-off is equivocal [see e.g. Cox et al. (Cox et al., 2006) and references therein] and its consequences for the development of SSD are not yet fully explored. The hypothesis that high costs of reproduction retard growth in females was recently supported by an experimental study on the male-larger brown anole (*A. sagrei*), where preventing reproduction by ovariectomy led to substantial body size enlargement (Cox and Calsbeek, 2010). However, Cox has documented that female reproductive investment does not largely account for differences in body size between the sexes in the male-larger *Sceloporus jarrovii* (Cox, 2006).

Here, we simultaneously tested the two hypotheses regarding proximate mechanisms of SSD (i.e. androgenic control of growth in males *versus* the trade-off between growth and reproduction in females) in a complex experimental setup using the Madagascar ground gecko, *Paroedura picta* (Peters 1854). In this male-larger species, we manipulated testosterone levels and female reproductive output in reproductively mature but not yet fully grown individuals and followed their growth until reaching final (i.e. close to asymptotic) body size. According to the 'gonadal androgen' hypothesis, we predicted that individuals (both males and females) with levels of testosterone elevated within the male-typical physiological range should reach a large final body size comparable to that of intact males, while castrated males should be relatively small. In contrast, the 'reproductive cost' hypothesis predicts that females with high energy allocation to reproduction should grow to be smaller in size than both females with experimentally reduced reproductive costs and males. We prevented female reproduction in two ways: *via* ovariectomy and by maintaining intact females in social isolation to prevent mating and egg laying. This setup allowed us to test whether the effects of ovariectomy on growth can be attributed exclusively to the elimination of reproductive costs.

MATERIALS AND METHODS

Experimental animals and conditions

The Madagascar ground gecko is a species well suited for studying the proximate control of SSD development. These lizards are easily bred and maintained in the laboratory, where they grow rapidly and mature at an early age (~3 months). We have detailed information on the ontogeny of SSD from our previous growth experiment where females were mated soon after sexual maturation and reproduced regularly. Under these conditions, growth was asymptotic in both sexes, but males decelerated growth upon reaching a body size larger than that of females (Starostová et al., 2010). Males and females do not greatly differ in body shape, as both sexes follow more or less the same allometric growth of limb, head and tail dimensions relative to the trunk (Jirků, 2007). Estimation of SSD is thus not confounded by differences in body proportions between sexes [see Kratochvíl et al. (Kratochvíl et al., 2003) for an example of the opposite situation in a lizard]. Among reptiles, the Madagascar ground gecko is also known for its extremely short intervals between clutches of one or two relatively large eggs, and it can breed continuously in suitable environmental conditions (Kubička and Kratochvíl, 2009; Kubička et al., 2012; Starostová et al., 2012; Weiser et al., 2012). Female allocation to reproduction is hence substantial in this species. Females of *P. picta* continue feeding at all stages of their reproductive cycle (Weiser et al., 2012).

Environmental temperature affects final body size in a sex-specific manner (Starostová et al., 2010), frequency of reproduction (Kubička et al., 2012) and rate of energy allocation to reproduction (Starostová et al., 2012) in the Madagascar ground gecko. Therefore, we kept all experimental animals at a constant temperature of 27°C, which leads to the largest degree of SSD in this species among three constant temperatures tested (24, 27 and 30°C) (Starostová et al., 2010). To maintain constant temperature even at early embryonic stages, we housed 26 mated females in a climatic chamber set to this temperature with a 12 h:12 h light:dark light cycle. Each female was individually housed in a standardized plastic box with sand substrate, a shelter and a water dish. Females were checked twice weekly for the presence of laid eggs. When eggs were found, they were weighed, individually marked and returned to the same climatic chamber for incubation. We collected 118 eggs in a period of 5 weeks. Four times a week we checked for the presence of hatchlings, and their snout–vent length (SVL) and body mass were measured immediately. Hatchlings were individually housed and raised under the same conditions as their mothers. Crickets (*Gryllus assimilis*) of appropriate size were dusted with vitamins (Roboran, Univit, Olomouc-Klášterní Hradisko, Czech Republic) and provided as food twice a week *ad libitum*. Water enriched by calcium (Vitacalcin, Zentiva, Prague, Czech Republic) was always provided but was replaced for 1 day every 2 weeks by water supplemented with vitamins A, D_3 and E (Combinal A+D_3 and Combinal E; IVAX Pharmaceuticals, Opava, Czech Republic). Body mass and SVL of geckos were measured every month. When it was possible to determine the sex of each individual according to external morphology (enlarged hemipenal sacs in males), we assigned 40 males to three groups (intact, testosterone and castrated) and 40 females to four groups (intact, mated, testosterone and ovariectomized). Individuals were assigned to treatment groups so that each group was balanced with respect to age, body mass and SVL within each sex.

The experiment was conducted with the approval, and under the supervision, of the Ethical Committee of the Faculty of Science, Charles University, Prague (permit no. 34711/2010-30).

Treatment groups and data collection

Surgery was conducted on sexually mature but not fully grown animals between the ages of 12 to 16 weeks. Animals were anaesthetized by combining intramuscular injection of ketamine (Narkamon 5%, Spofa, Prague, Czech Republic; 130 μg g^{-1} of body mass) and hypothermia. The gonads were exposed *via* a medial ventral incision. Bilateral gonadectomy was performed on castrated males, testosterone males and ovariectomized females by ligating each gonad with surgical silk, then ablating and removing it. For the remaining groups, 'sham' surgeries were performed, during which gonads were exposed and manipulated, but left intact. Implants filled with 300 μg of crystalline testosterone were inserted into the body cavities of testosterone males and testosterone

females. Castrated males, intact males, intact females, mated females and ovariectomized females received empty implants. The incision was closed using Prolene surgical suture (Ethicon, Somerville, NJ, USA) and covered with Glubran 2 surgical glue (GEM, Viareggio, Italy). Tonic-release testosterone implants were constructed as described in Cox et al. (Cox et al., 2005) and Golinski et al. (Golinski et al., 2011). Implants consisted of Silastic tubing (Dow Corning, Midland, MI, USA; 0.058 inch i.d., 0.077 inch o.d.) with total length of 4 mm and lumen of ~1.5 mm. After recovery from the surgery (after ca. 1 month), each female in the mated female group was allowed to mate with an adult male from our breeding colony. The males were present in female cages for 1 day per month. All remaining groups were kept in isolation until circulating testosterone levels were measured in all experimental animals.

To determine final body size, we followed the growth curve of each individual. When the growth of all animals slowed considerably, ~28 weeks after surgery, we collected blood plasma from each individual to determine levels of circulating testosterone and validate success of the surgical manipulations. The influence of treatment on animal behaviour was tested for six consecutive weeks (these behavioural results will be published elsewhere). The experimental animals were then killed and measurements of final body mass, final SVL and mass of internal organs were recorded. For comparisons of final body condition and final body mass in females, we subtracted the mass of the ovaries and oviducts from the total body mass, which allowed us to control for differences in reproductive stages among individuals.

Hormone treatments were verified by assaying plasma testosterone levels at the Institute of Endocrinology (Prague, Czech Republic) from plasma samples using the method published by Hampl (Hampl, 1994). The method involves extracting steroid hormones from plasma with diethyl-ether followed by radioimmunoassay using rabbit polyclonal antiserum to testosterone-3-(carboxymethyloxime) bovine serum albumin conjugate, with homologous [^{125}I]tyrosine methyl ester derivative as a tracer. Intra- and inter-assay coefficients of variation for the analyses are typically 8.2 and 10.7%, respectively.

Estimation of asymptotic size and body condition

To describe growth, we used expression of the Gompertz function with lag phase following Perni et al. (Perni et al., 2005):

$$y = D\exp\left(-\exp(\mu_{max}e/D(\lambda - t) + 1)\right), \quad (1)$$

where y is ln(actual SVL/SVL at the time of hatching), D is the limit of y, μ_{max} is the relative maximum growth rate (day^{-1}), e is the Euler number and λ represents the lag phase (days). The asymptotic SVL for each individual was then computed as asymptotic SVL=(SVL at the time of hatching)exp$D$.

For comparison of body condition we used Fulton's index (body mass/body length^3), which was recently recommended by Peig and Green (Peig and Green, 2010). In their study, this index performed better than other alternatives such as residuals or analysis of covariance. Fulton's index is based on an idealized theoretical assumption that body mass increases with the third power of linear body dimension. The coefficient 3 is also supported empirically in our case. According to linear regression, ln(final body mass)=−11.9116 + 3.347ln(final SVL) among all experimental geckos. The scaling coefficient 3.347±0.251 (mean ± s.e.m.) is not significantly different from 3.

All data analyses were performed in STATISTICA 10.0 (StatSoft, Tulsa, OK, USA).

RESULTS

Validation of manipulations with hormone levels, reproductive and growth status

In the overwhelming majority of experimental animals, levels of plasma testosterone measured 28 weeks after manipulation met our expectations and validated our experimental procedures. Intact males exhibited large variation in circulating levels of testosterone, with values ranging from 3.30 to 144.22 ng ml^{-1} blood plasma (mean 39.63 ng ml^{-1}, N=13; Fig. 1). Implantation with exogenous testosterone elevated plasma androgens in 11 out of 14 testosterone males and in 10 testosterone females to levels comparable to those of intact males (Fig. 1). However, three testosterone males showed very low levels of testosterone, comparable to those of castrated males. These three unsuccessfully manipulated individuals were excluded from all analyses, but their inclusion does not change any result. Surgical castration reduced plasma testosterone in the castrated males (N=13) to low levels comparable to those of the intact females (N=9), mated females (N=10) and ovariectomized females (N=9). One intact female and one ovariectomized female died during the experiment, and were excluded from all analyses.

All 10 mated females in our experiment started reproducing after their first mating and produced a total of 333 eggs (range 27–41 eggs per female) until termination of the experiment. Total wet mass of eggs laid during the experiment ranged from 27.24 to 43.80 g per individual female. We did not observe the intact females kept in isolation lay unfertilized eggs during the experiment.

At the time of surgery, SVL was similar between sexes (ANOVA, $F_{1,73}$=1.922, P=0.170; males: N=37, mean ± s.e.m.=63.85±0.45 mm; females: N=38, 62.88±0.50 mm), but body mass differed between sexes (ANOVA, $F_{1,73}$=10.278, P=0.002; males: 6.47±0.18 g; females: 5.78±0.13 g). All male groups became larger than all female groups by approximately 8 weeks after the surgeries (Fig. 2). The asymptotic SVL computed from the Gompertz function and the final SVL are highly correlated (R^2=0.916, P<<0.001). From the relationship asymptotic SVL=−0.517 + 0.996(final SVL), the intercept (−0.517±3.204) estimated by linear regression is not significantly different from 0, the slope is very close to 1.0 and its 95% confidence interval (0.926–1.067) includes 1.0, which indicates an isometric relationship between asymptotic SVL and final SVL and proves that our experimental animals were fully grown at the

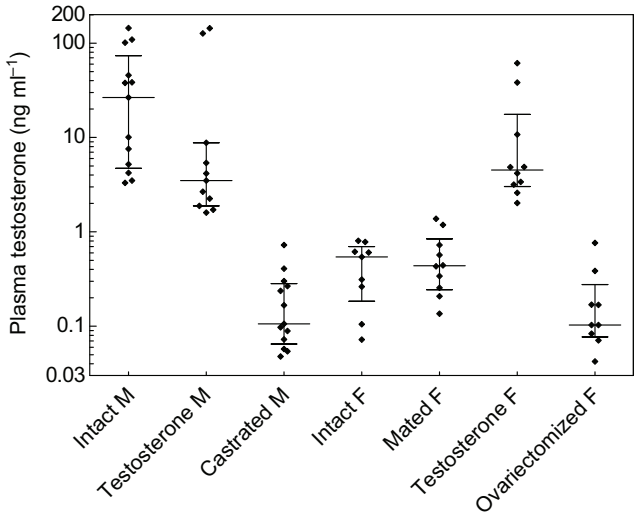

Fig. 1. Concentration of plasma testosterone levels in experimental animals (*Paroedura picta*) 28 weeks after surgery. Median and inner quartiles are shown. M, males; F, females.

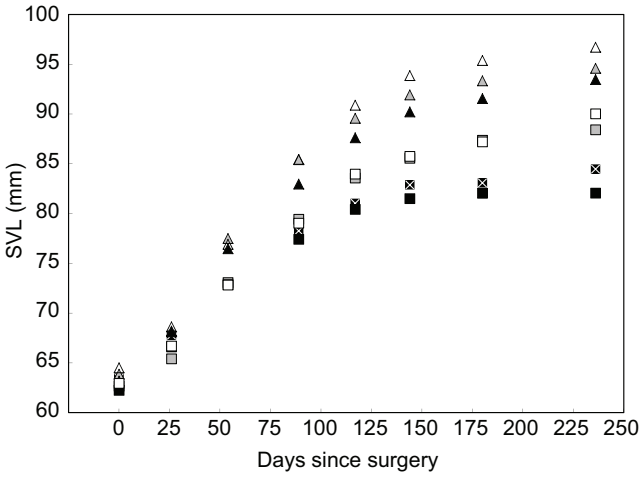

Fig. 2. Growth of experimental animals after manipulation. Means per treatment group at a given time are depicted. Open triangles represent intact males, grey triangles testosterone males, black triangles castrated males, open squares ovariectomized females, grey squares testosterone females, black squares with white cross intact females, and black squares mated females. SVL, snout–vent length.

time of experiment termination. Furthermore, the final SVL of intact males in the present study is comparable to the final SVL of males raised at the same constant temperature (27°C) in our previous growth experiment [*t*-test, N=27, t=0.530, P=0.60 (Starostová et al., 2010)]. Similarly, final SVL does not significantly differ between mated females in the present study and reproductively active females kept at the same temperature in our previous experiment (*t*-test, N=26, t=1.750, P=0.09).

Treatment effects on body size and condition in males

All male treatment groups (intact, castrated and testosterone) followed a similar growth trajectory (Fig. 2) and did not differ significantly in final SVL or final body mass (ANOVA, SVL: $F_{2,34}$=1.430, P=0.25, Fig. 3A; mass: $F_{2,34}$=0.352, P=0.71). According to Fulton's index, castrated males tended to be fatter than the other two male groups. That difference was only marginally insignificant (ANOVA, $F_{2,34}$=3.139, P=0.056; Fig. 3B).

Treatment effects on body size, condition and gonad function in females

In females, treatment significantly affected final SVL (ANOVA, $F_{3,34}$=7.118, P=0.001, Fig. 4A). Mated and intact females did not differ in final SVL [*post hoc* Fisher's least significant difference (LSD), P=0.224] but reached a smaller final SVL than did testosterone and ovariectomized females, which formed a second homogenous group (*post hoc* Fisher's LSD, P=0.411 for the difference between testosterone and ovariectomized females). Differences in modified final body mass were also significant among female groups (ANOVA, $F_{3,34}$=16.869, P<<0.001). Mated females were the lightest and differed significantly from all other groups (*post hoc* Fisher's LSD, P<0.025 in all comparisons). Intact and testosterone females reached comparable intermediate final body mass (*post hoc* Fisher's LSD, P=0.175 for comparison between these two groups), while ovariectomized females were the heaviest and differed from all the other female groups (*post hoc* Fisher's LSD, P<0.003 in all cases). The pattern did not change when female body mass including mass of reproductive organs (ovaries and oviducts)

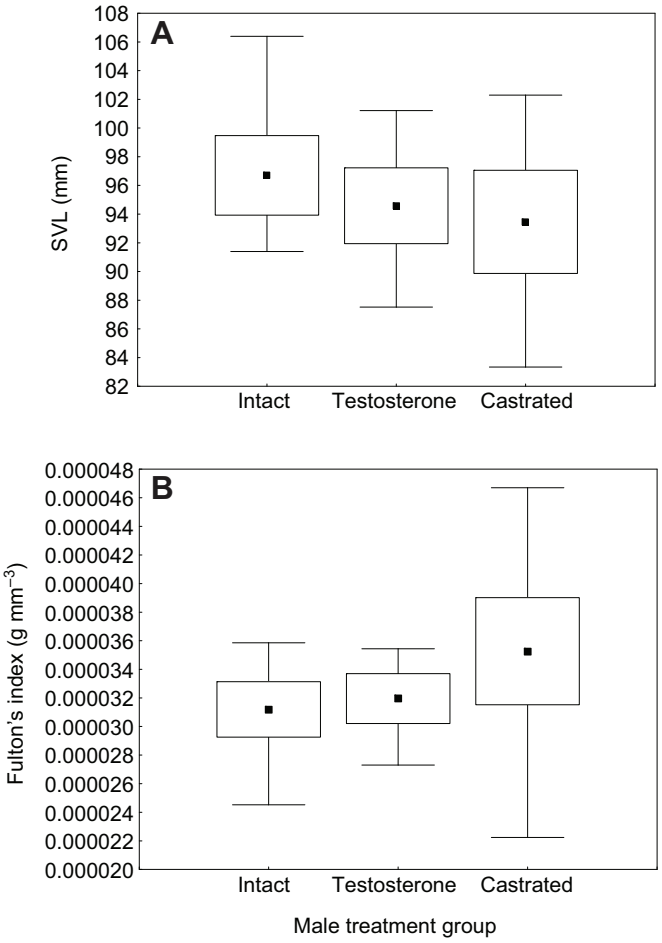

Fig. 3. Final structural body size (SVL; A) and body condition (B) in experimental males. Means, 95% confidence intervals and minimum–maximum value ranges are given.

was analyzed. Significant differences were found also in body condition ($F_{3,34}$=11.413, P<<0.001; Fig. 4B). Again, mated females showed the lowest Fulton's index (*post hoc* Fisher's LSD, P<0.022 in all cases). Intact females did not differ from testosterone females (*post hoc* Fisher's LSD, P=0.984). Ovariectomized females possessed the highest body condition (*post hoc* Fisher's LSD, P<0.002 in all cases).

Although testosterone and ovariectomized females reached unusually large body size for females of this species, their final SVL was still much smaller than that of intact males (ANOVA, $F_{2,29}$=11.772, P<0.001).

The mass of ovaries at the time of experiment termination differed significantly among intact, mated and testosterone females (ANOVA, $F_{2,26}$=45.224, P<<0.001; *post hoc* Fisher's LSD, P<0.003 in all cases; Fig. 5). Ovaries in the testosterone females were small, with no enlarged follicles. By contrast, ovaries of the intact females usually contained fully developed follicles. In the absence of males, their breeding cycles obviously stopped after vitellogenesis and did not proceed with ovulation. The intermediate mean size of the ovaries in the mated females reflects the different stages of the ovarian cycle in these regularly breeding individuals; some contained fully developed large follicles immediately preceding ovulation, while post-ovulatory females contained smaller follicles at varying stages of vitellogenesis.

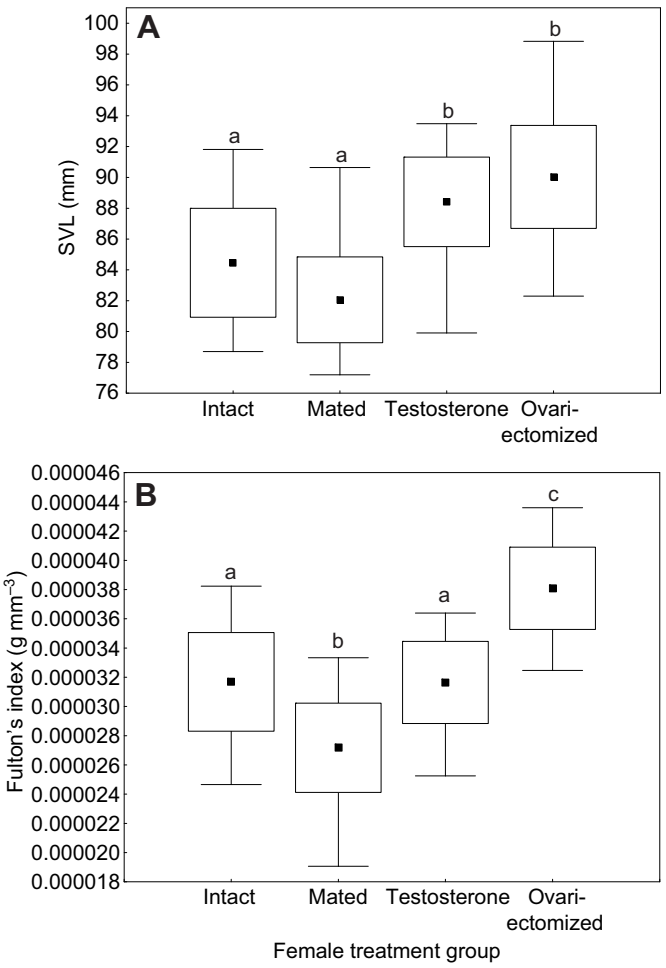

Fig. 4. Final structural body size (SVL; A) and body condition (B) in experimental females. Means, 95% confidence intervals and minimum–maximum value ranges are given. Letters denote statistically homogeneous groups.

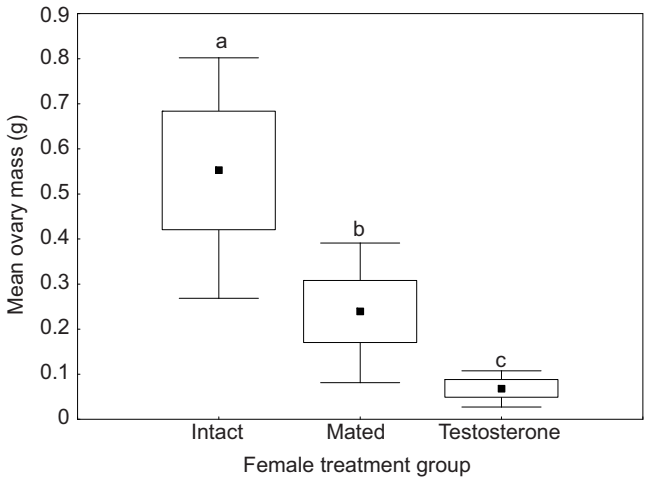

Fig. 5. Ovary mass of females at the time of experiment termination. Means, 95% confidence intervals and minimum–maximum value ranges are given. Letters denote statistically homogeneous groups.

DISCUSSION

Results of the experimental manipulations in *P. picta* do not support either the 'gonadal androgen' hypothesis or the 'reproductive cost' hypothesis. Our results instead suggest that ovarian factors – perhaps ovarian hormones – can retard growth in lizards and thus may contribute to the development of SSD. We found no effect of gonad removal and androgen replacement on final body length in males (Fig. 3A), although exogenous testosterone led to larger final SVL in females (Fig. 4A). While removal of the energetic investment into reproduction in females by ovariectomy resulted in larger body size, as predicted by the reproductive cost hypothesis, social isolation of gonadally intact females, which also prevented allocation to eggs, did not alter final body size in comparison to reproductively active females (Fig. 4A).

In their review, Cox and colleagues (their table 2) concluded that regardless of phylogenetic distribution, androgens enhance male growth in male-larger species, inhibit it in female-larger species, but have ambiguous or no obvious effect in species that are monomorphic in body size (Cox et al., 2009). They speculated that this bipotential effect of testosterone on growth could involve direct effects on the endocrine growth axis. In light of our results in *P. picta* and after critical re-evaluation of the studies mentioned in Cox et al. (Cox et al., 2009) and other resources not included in their study (Civantos, 2002; Duncan, 2011; Kubička et al., 2013), we conclude that the support for the hypothesis that gonadal androgens directly influence the endocrine growth axis in squamate reptiles is not very strong. We suggest that previously reported effects of gonadal androgens on growth in male lizards could be attributed either to unnatural levels of testosterone, or to indirect consequences of altered behaviour or social status in manipulated individuals (see also Cox and John-Alder, 2005; Cox et al., 2006).

In seven studies, the effect of testosterone on growth and body size was tested by measuring differences between gonadally intact control males and those supplemented with exogenous testosterone. Treatment with testosterone reduced structural growth (SVL) or growth rate (mm day^{-1}) in most of these studies: in hatchlings of the iguanians *Urosaurus ornatus* and *Sceloporus virgatus* (Hews et al., 1994; Abell, 1998); in adults of the iguanian *Sceloporus undulatus*, although the effect was not significant (P=0.06) (Klukowski et al., 1998); and in juveniles of the lacertid *Psammodromus algirus* (Civantos, 2002). Another study on *Psammodromus algirus* reported that individuals treated with testosterone tended to grow less than control individuals, but found no significant effect, probably due to the small sample size (Salvador and Veiga, 2000). In the snake *Thamnophis sirtalis*, testosterone also did not have significant effects on structural growth (SVL) in a short-term study with adult males (Crews et al., 1985), while addition of testosterone proved to be lethal in neonatal males of the same species (Lerner and Mason, 2001). Overall, it seems that testosterone supplementation in gonadally intact males can lead to detrimentally high, supra-physiological doses. These potentially pharmacological doses of androgens may lead to high levels of estrogens, *via* aromatization, with a negative effect on growth in manipulated individuals (see Hews and Moore, 1995). Because all these tests were performed exclusively in female-larger or monomorphic species, they seem to support the 'gonadal androgen' hypothesis (which predicts a negative effect of testosterone on growth here), but they may simply reflect general negative effects of supra-physiological levels of testosterone.

Other studies have tested effects of castration on male structural body size or growth and whether the effect is reversed by replacement of exogenous testosterone in castrated males (reviewed

in Table 1). The replacement of exogenous testosterone in castrated males is used to test whether the observed effects of castration are caused by eliminating the primary source of testosterone *versus* other functions of the testes. In two of the reviewed studies (Cox et al., 2009; Kubička et al., 2013), castration alone did not have any significant effect on male growth in comparison to control males, but administration of exogenous testosterone significantly influenced growth of castrated males. These results suggest that artificial hormone delivery, *via* implanted Silastic tubules, might fail to faithfully replicate natural *in vivo* patterns of gonadal secretion. Therefore, we recommend interpreting the effects of replacement of exogenous testosterone in castrated males with caution and we suggest that analysis of the effects of castration alone on male structural body size is a more reliable test of the control of growth by male gonads. A significant effect of castration on male structural growth was found in five out of six field or laboratory studies that lacked control over the social environment (i.e. where castrated males were in a common environment with males with elevated androgen levels). In three other studies where the social environment was regulated (i.e. where experimental individuals were kept in social isolation), castration had no effect on male growth (Table 1). In support of the hypothesis that social environment influences male growth, manipulating androgen levels only had a significant effect on male growth in the male-larger *S. jarrovii* in the field but not in the subsequent laboratory study, where individuals were kept in social isolation; however, many other environmental aspects differed between the field and the laboratory as well (Cox et al., 2006). We suggest that male gonadal androgens may act indirectly to affect lizard growth, probably *via* downstream mechanisms sensitive to social environment [see also discussion in Cox et al. (Cox et al., 2009)]. Castration causes behavioural changes in male lizards (e.g. Moore, 1988; Rhen and Crews, 2000), which can result in social stress or nutritional stress due to the inability to defend feeding territories (e.g. Cox et al., 2006). Hormonal treatment can also influence other aspects of lizard behaviour or physiology yielding indirect effects on male growth or body size. For example, Klukowski et al. (Klukowski et al., 1998) documented changes in activity pattern among males of the iguanian lizard *Sceloporus undulatus hyacinthinus* induced by increased testosterone levels, and thermoregulatory behaviour strongly influences growth rates in species of that same genus (Sinervo and Adolph, 1994). Interactions between social and thermoregulatory or feeding behaviour are also likely (e.g. aggressive males could deter less aggressive individuals from basking places). In this respect, it is important to stress that lizards in our experiments were maintained not only in social isolation (with the exception that mated females had an adult male in their cages for one day per month) but also in climate-controlled chambers, which prevents their ability to thermoregulate. In our previous study, we documented that SSD is greatly sensitive to thermal environment in *P. picta* (Starostová et al., 2010). Future laboratory studies will be needed to disentangle the effects of social, thermoregulatory and nutritional environment and their interactions with hormonal status on growth in male lizards.

Limitation of energy availability for growth due to high reproductive costs in females also does not explain the larger body length of males in *P. picta*. Previously, we had estimated that the rate of energy allocation to reproduction in continuously breeding females of *P. picta* at 27°C is approximately 12 mW (Starostová et al., 2012), i.e. approximately 1 kJ per day. As the mean energy content of eggs produced by females of *P. picta* at 27°C is 5.4 kJ (1291 cal) (Starostová et al., 2012), we estimate that the mated females in this experiment allocated an average of approximately 180 kJ toward egg production. Nevertheless, they reached a final body size comparable to that of the intact females kept in isolation to prevent reproduction. At the end of the experiment, the mated females had lower body condition than the isolated intact females, which had deposited energy to fat reserves mainly in the liver, tail and abdominal fat bodies. Starostová et al. (Starostová et al., 2012) estimated that reproductive females of *P. picta* increase resting metabolic rate by dozens of percentage points in comparison to non-reproducing individuals. We did not measure dietary consumption in this experiment, but it is likely that the mated females consumed more food to sustain their enormous energy allocation to reproduction. The 'reproductive costs' hypothesis was not supported by our previous experiment that manipulated food availability in reproductive females. Females provided low nutrition (food-restricted) invested much less into reproduction and fat storage in comparison with well-fed females, but both groups attained approximately the same final SVL (Kubička and Kratochvíl, 2009). Another experimental study in a lacertid lizard similarly documented

Table 1. Summary of the effects of castration (C) and testosterone replacement in castrated males (C + T) on male structural growth in squamate reptiles

Species	Sexual size dimorphism	Effect of C	Effect of C + T	Prevention of direct social contact	Field/Lab	Reference
Thamnophis sirtalis	F	+	0[a]	No	Lab	Crews et al., 1985
Anolis sagrei	M	0	+	No	Lab	Cox et al., 2009
Urosaurus ornatus	0/M	–	Not known	No	Lab	Hews et al., 1994
Sceloporus virgatus	F	+[b]	–	No	Field	Cox and John-Alder, 2005
Sceloporus undulatus	F	0/+[c]	–	No	Field	Cox et al., 2005
		Not known[d]	–	Yes	Lab	Duncan, 2011
Sceloporus jarrovii	M	–	+	No	Field	Cox and John-Alder, 2005
		0	0	Yes	Lab	Cox et al., 2006
Aeluroscalabotes felinus	F	0	–	Yes	Lab	Kubička et al., 2013
Paroedura picta	M	0	0	Yes	Lab	Present study

Note: effect of C is reported relative to control group (intact or sham-operated males); effect of C + T is reported relative to castrated males.

[a]Castrated juvenile males treated with testosterone grew to the same body size as castrated males, but both groups attained much larger size than sham-operated juvenile males.

[b]The interaction between growth rate and initial snout–vent length (SVL) was significant. Castration increased growth rate only in males with larger SVL at the beginning of the experiment.

[c]The positive effect of castration on growth was evident only in long-term observation.

[d]Only castrated males with and without testosterone implant were compared; no control group was included in the experiment.

a higher priority for energy allocation towards growth and maintenance rather than to reproduction (Luo et al., 2010). Additionally, the described results in *P. picta* males do not support the hypothesis that final structural size in reptiles is determined by energy availability for growth. The castrated males tended to accumulate more fat storage than the testosterone and intact males, but males in all treatment groups grew to similar final SVL (Fig. 3). Moreover, intact males and mated females followed the same growth trajectory for an extended period even after the dramatic increase in allocation to reproduction in the latter group had begun. That, too, is contrary to predictions based on the 'energy allocation' hypothesis (see also Starostová et al., 2010).

We prevented female allocation to reproduction in *P. picta* in two ways: by maintaining one treatment group in social isolation (intact females) and by ovariectomizing the other. While we predicted that both would reach similar body size, the ovariectomized females became much fatter and grew to significantly larger final SVL, which is atypical for females of this species (Fig. 4). Ovariectomy had been used in previous studies in lizards to test the effects of reproductive costs on growth and survival (Cox, 2006; Cox and Calsbeek, 2010; Cox et al., 2010). Comparisons between intact and ovariectomized females suggest that removal of female gonads not only prevents reproduction but also has dramatic effects on female growth and other aspects of physiology. Interestingly, although castration and testosterone replacement had no effect on final body size in males of *P. picta*, testosterone implants significantly increased final SVL in females in a manner similar to the effect of ovariectomy (Fig. 4A). We suggest that this effect on growth in females could be attributed to elevated testosterone levels interfering with normal function of the ovaries. The ovaries in all testosterone females were small and with no enlarged vitellogenic follicles. In contrast, the mass of ovaries varied in the mated females, reflecting the stage of their reproductive cycles at the time of termination. The intact females had large, fully vitellogenic follicles in their ovaries (Fig. 5). During social isolation without access to sperm, *P. picta* females hence stop the reproductive cycle before ovulation and remain in this stage for a long time. This strategy might be adaptive, as it can effectively prevent wasting resources to produce unfertilized eggs and at the same time allows rapid ovulation and hence onset of reproduction after access to sperm.

Effects on growth of female gonadal hormones have rarely been tested in squamate reptiles (but see Lerner and Mason, 2001) [for evidence in other vertebrates, see Malison et al. (Malison et al., 1988) or Govoni et al. (Govoni et al., 2008)], which precludes evaluating whether estrogens or progestins may affect female growth in the opposite direction in female- *versus* male-larger lizard species [see Cutler (Cutler, 1997) for support for the role of low levels of estrogens in regulation of growth in humans]. Testing this hypothesis will require further experimental work focused specifically on hormonal manipulations in females of male-larger and female-larger species. The genus *Paroedura* includes closely related species with SSD in opposing directions (Starostová et al., 2010) and its members are thus promising candidates for this future comparative work. Nevertheless, the hypothesis that ovarian hormones affect growth is indirectly supported by the results of already existing manipulative studies (Table 2). We are aware of 10 studies, including nine in squamate species, that manipulated hormonal levels in females either by ovariectomy or by addition of exogenous testosterone. Notably, such manipulations had positive effects on female body size in all male-larger species, while these effects were negative in all female-larger species (Table 2). Crews et al. (Crews et al., 1985) reported no significant effect of testosterone treatment on growth in females or castrated males (see our note in Table 1), which might be caused by non-functional testosterone implants (plasma testosterone levels were not measured in the manipulated individuals). The contrasting results from similar treatments in different species of geckos and iguanids suggest that the pattern is not driven by phylogenetic position, but that it might indeed reflect differences of growth regulation in male-larger *versus* female-larger lineages. However, one potentially confounding factor exists among the studies summarized in Table 2: ovariectomy was only performed in male-larger species, while, with a single exception (*P. picta*; present study), testosterone was only elevated in females of female-larger species. The results from our study suggest that both ovariectomy and elevation of testosterone level mediate changes in female body size by interfering with normal ovarian function. In *P. picta*, we found no differences in final structural body size between the ovariectomized females and the testosterone females (Fig. 4A), which had notably regressed ovaries (Fig. 5). Manipulations with testosterone levels had no effect on final structural body size in males of this species (Fig. 3A), thus suggesting that the observed effect of elevated testosterone on growth in *P. picta* females may not be attributed to androgenic masculinization. Based on these observations, we tend to ascribe the effect of exogenous testosterone on growth in females (as seen also in other species mentioned in Table 2) to interference with normal ovarian function that causes defeminization. Nevertheless, masculinization of female growth by increased testosterone levels cannot be ruled

Table 2. Summary of effects of ovariectomy (OVX) or addition of exogenous testosterone (T) on female structural growth in squamate reptiles

Species	Sexual size dimorphism	Manipulation	Effect of manipulation	Reference
Anolis sagrei	M	OVX	+	Cox and Calsbeek, 2009
Sceloporus jarrovii	M	OVX	+	Cox, 2006
Eublepharis macularius	M	OVX	+[a]	Tousignant and Crews, 1995
Paroedura picta	M	OVX	+	Present study
		T	+	Present study
Aeluroscalabotes felinus	F	T	–	Kubička et al., 2013
Sceloporus virgatus	F	T	–	Abell, 1998
Sceloporus undulatus	F	T	–	Duncan, 2011
Thamnophis sirtalis	F	T	–	Lerner and Mason, 2001
			0	Crews et al., 1985
Urosaurus ornatus	0/M	T	–[b]	Hews and Moore, 1995

Note: positive or negative effects of manipulation are given relative to controls.
[a]The effect of OVX was found only in females from 26°C, not from 32.5°C. The sample size at the latter temperature was very low (*N*=3 OVX females).
[b]The authors pointed to potential pharmacological doses of T.

out until it is tested in future studies by examining growth in ovariectomized females with and without testosterone implants.

We emphasize that the final structural body sizes of both testosterone females and ovariectomized females in *P. picta* in this experiment were still significantly smaller than that of intact males. This finding suggests that part of SSD in final body size can be attributed to developmental processes during embryonic development or postembryonic ontogeny before the age of surgical manipulation in this study. Note that experimental females had similar SVL, but smaller body mass than males at the time of surgery. A non-exclusive alternative is that final body size may be linked to sexual differences in genotype (sex chromosomes). Genotypic sex determination has been reported in *P. picta* based on observations that equal sex ratios hatched at several constant temperatures (Blumberg et al., 2002; Kratochvíl et al., 2008), and thus the presence of sex chromosomes can be expected in this species.

In summary, we conclude that, at least within certain limits of the conditions in our experimental study, structural body size of female lizards and hence SSD seem to be controlled by endogenous factors rather than by resource availability for growth in *P. picta*. A recent study (Duncan, 2011) found that testosterone affects levels of an important member (insulin-like growth factor 1) of the endocrine growth axis in lizards. We suggest, however, that these observed effects in lizards may be indirect and sensitive to environmental settings of particular experiments. We also suggest that female – not male – gonadal function seems to be a promising candidate for an endocrine mechanism directly affecting the growth axis in squamate reptiles and thus largely controlling SSD in structural body size. In *P. picta*, and potentially other species, we offer indirect support for the function of female gonadal hormones in the evolution of SSD.

LIST OF SYMBOLS AND ABBREVIATIONS

D	limit of y
SSD	sexual size dimorphism
SVL	snout–vent length
y	ln(actual SVL/SVL at the time of hatching)
λ	length of the lag phase of growth in days
μ_{max}	relative maximum growth rate (day^{-1})

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All authors participated in the design of the study and execution of the experiments. Data were analyzed and the first draft of the manuscript was prepared by L.K., L.K. and Z.S. All authors edited and approved the final version of the manuscript.

COMPETING INTERESTS

No competing interests declared.

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