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. positive effect of gonadal androgens (testosterone) on male growth in male-larger species, while negative in female-larger species; and (2) sex-specific differences in energy allocation to growth, e.g. sex with larger reproductive costs should reach 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 found support for neither 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 effect of testosterone treatment or ovariectomy on body size in female squamates.
Introduction

Sexual size dimorphism (SSD) is defined as a difference in body size between the sexes. Since the time of Charles Darwin (1871), SSD has been widely regarded as a consequence of a particular sex’s adaptation to its specific reproductive or ecological roles (reviewed, e.g., 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 (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 (T) can have positive effects on male growth in male-larger species but negative effects on male growth in female-larger species. Cox and colleagues (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 T 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 T-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 in energy assimilation; Stahlschmidt et al. 2010) or in 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). On the other hand, 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. 2006 and references there) 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). On the other hand, Cox (2006) has documented that female reproductive investment does not largely account for differences in body size between the sexes in the male-larger Sceloporus jarrovii.

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 T 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 T elevated within the male-typical physiological range should reach a large final body size comparable to intact males, while castrated males should be relatively small. On the other hand, 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.

Material and methods

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, males and females followed the same growth trajectory. 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 trunk (Jirků, 2007). Estimation of SSD is thus not confounded by differences in body proportions between sexes (see, e.g., Kratochvíl et al., 2003 for 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) as well as 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 the 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 12L:12D light cycle. Each female was individually housed in a standardized plastic box with sand substrate, a shelter and 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 five weeks. Four times a week we checked for the presence of hatchlings, and their snout-to-vent length
(SVL) and body mass were measured immediately. Hatchlings were raised individually housed under the same conditions as their mothers. Crickets (*Gryllus assimilis*) of appropriate size dusted with vitamins (Roboran, Univit, Olomouc-Klášterň Hradisko, Czech Republic) were provided twice a week *ad libitum*. Water enriched by calcium (Vitacalcin, Zentiva, Prague, Czech Republic) was always provided but was replaced for one day in every two weeks by water supplemented with vitamins A, D₃ and E (Combinal A+D₃ 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, 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.

Surgery was conducted on sexually mature but not fully grown animals between the ages 12 to 16 weeks. Animals were anaesthetized by combining intramuscular injection of ketamine (Narkamon 5%, Spofa a.s., Prague, Czech Republic; 130 μg/g 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 T 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 INC, Somerville NJ, USA) and covered with Glubran ®2 surgical glue (GEM S.r.l., Viareggio, Italy). Tonic-release T implants were constructed as described in Cox et al. (2005) and Golinski et al. (2011).

Implants consisted of Silastic® tubing (Dow Corning, 0.058” i.d., 0.077” o.d.) with total length of 4 mm and lumen of approximately 1.5 mm. After recovery from the surgery (after ca. one month), each Mated female was allowed to mate with an adult male from our breeding colony. The males were present in female cages for one day per month. All remaining groups were kept in isolation until circulating T 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, at approximately 28 weeks after surgery, we collected blood plasma from each individual to determine levels of circulating T 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 sacrificed 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 T levels at the Institute of
Endocrinology (Prague, Czech Republic) from plasma samples using the method published by
Hampel (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 [125I]tyrosine
methyl ester derivative as a tracer. Intra-assay 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. (2005):

\[ y = D \exp \left( - \exp (\mu_{\text{max}} e^{\lambda t} / D (\lambda - t) + 1) \right) \] (1),

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

For comparison of body condition we used Fulton’s index \( \left( \frac{\text{body mass}}{\text{body length}^3} \right) \),
which was recently recommended by 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, \( \ln(\text{Final Body Mass}) = -11.9116 + 3.347 \ln(\text{final SVL}) \) among
all experimental geckos. The scaling coefficient \( 3.347 \pm 0.251 \) (SE) is not significantly
different from 3.

All data analyses were performed in Statistica 10.0 (StatSoft Inc. 2011).
Results

Validation of manipulations with hormone levels, reproductive and growth status

In the overwhelming majority of experimental animals, levels of plasma T measured 28 weeks after manipulation met our expectations and validated our experimental procedures. Intact males exhibited large variation in circulating levels of T, with values ranging from 3.30 to 144.22 ng per ml of blood plasma (n = 13, mean 39.63 ng/ml, Fig. 1). Implantation with exogenous T 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 T, 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 T 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 reproduction 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, F1, 73 = 1.922, p = 0.170; males: N = 37, mean ± S.E. 63.85 ± 0.45 mm; females: N = 38, 62.88 ± 0.50 mm), but sexes differed in body mass (ANOVA, F1, 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 eight weeks after the surgeries (Fig. 2). The asymptotic SVL computed from the Gompertz function and the final SVL are highly correlated (R² = 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 time of experiment termination. Furthermore, the final SVL of Intact males in the present study are 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 Fischer LSD test, p = 0.224) but reached a smaller final SVL than did Testosterone and Ovariectomized females, which formed a second homogenous group (Post-hoc Fischer 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 LSD: p < 0.025 in all comparisons). Intact and Testosterone females reached comparable intermediate final body mass (Post-hoc Fisher 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 LSD: p < 0.003 in all cases). The pattern did not change when female body mass with reproductive organs were included into this analysis. 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 LSD: p < 0.022 in all cases). Intact females did not differ from Testosterone females (Post-hoc Fisher LSD: p = 0.984). Ovariectomized females possessed the highest body condition (Post-hoc Fisher 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 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 largest follicles immediately preceding ovulation, while post-ovulatory females contained smaller follicles at varying stages of vitellogenesis.

Discussion

The predictions from neither the “gonadal androgen” hypothesis nor the “reproductive cost” hypothesis were supported by the experimental manipulations in *P. picta*. 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 T 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 et al. (2009, 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. They speculated that this bipotential effect of T on growth could involve direct effects on the endocrine growth axis. In the light of our results in *P. picta* and after critical re-evaluation of the studies mentioned in Cox et al. (2009) and other resources not included into their study (Civants, 2002; Duncan et al. 2011; L. Kubička, A. Golinski, H. John-Alder and L. Kratochvíl, subm. manuscript), 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 non-natural levels of T, or to indirect consequence of altered behaviour or social status in manipulated individuals (see e.g. also Cox and John-Alder, 2005; Cox et al., 2006).
In seven studies, the effect of T on growth and body size was tested by measuring differences between gonadally intact control males and those supplemented with exogenous T. Treatment with T reduced structural growth (SVL) or growth rate (mm/day) in most of these studies: in hatchlings of iguanians *Urosaurus ornatus* and *Sceloporus virgatus* (Hews et al., 1994; Abell, 1998); marginally insignificant (p = 0.06) effect in adults of iguanian *Sceloporus undulates* (Klukowski et al., 1998); and in juveniles of lacertid *Psammodromus algirus* (Civantos, 2002). Another study on *Psammodromus algirus* reported that “individuals treated with T 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*, T 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 T proved to be lethal in neonatal males of the same species (Lerner and Mason, 2001). Overall, it seems that T 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 negative effect on growth in manipulated individuals (see e.g. 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 T on growth here), but they may reflect just general negative effects of supra-physiological levels of T.

Other studies had tested effects of castration on male structural body size or growth and whether the effect is reversed by replacement of exogenous T in castrated males (reviewed in Table 1). The replacement of exogenous T 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 testes. In two of the reviewed studies (Cox et al. 2009; L. Kubička, A. Golinski, H. John-Alder and L. Kratochvíl, submitted manuscript), castration alone did not have any significant effect on male growth in comparison to control males, but administration of exogenous T 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 T in castrated males with caution and we keep effects of castration alone on male structural body size as a more reliable test of the control of growth by male gonads. We noticed 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 common environment with males having
elevated androgen levels). In three other studies where 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 lab 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, e.g., also discussion in 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 (see, e.g., Cox et al., 2006). Hormonal treatment can also influence other aspects of lizard behaviour or physiology yielding indirect effects on male lizard growth or body size. For example, Klukowski et al. (1998) documented changes in activity pattern among males of the iguanian lizards induced by increased T 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 not only maintained 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 male-larger body length 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 about 12 mW (Starostová et al., 2012), i.e., about 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 about 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 did the isolated Intact females, which had
deposited energy to fat reserves mainly in the liver, tail and abdominal fat bodies \((\text{personal observation})\). 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 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 did 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 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 that 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 it also has dramatic effects on female growth and other aspects of physiology. Interestingly, although castration and T replacement had no effect on final body size in males of \(P. picta\), T 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 T levels interfering with normal function of the ovaries. The ovaries in all Testosterone females were small and with no enlarged vitellogenic follicles. On the other hand, 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 allow 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, e.g., Lerner and Mason, 2001; see e.g. Malison et al. 1988 or Govoni et al., 2008 for evidence in other vertebrates), which precludes evaluating whether estrogens or progestins may affect female growth in the opposite direction in female- versus male-larger lizard species (see e.g. Cutler, 1997 for the support of 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 squamate species, that manipulated hormonal levels in females either by ovariectomy or by addition of exogenous T. 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. (1985) reported no significant effect of T treatment on growth in females or castrated males (see our note in Table 1), which might be caused by non-functional T implants (plasma T 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*; this study), T was only elevated in females of female-larger species. The results from our study suggest that both ovariectomy and elevation of T 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 T levels had no effect on final structural body size in males of this species (Fig. 3A), thus suggesting that the observed effect of elevated T 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 T 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 T levels cannot be ruled out until it is tested in
future studies by examining growth in ovariectomized females with and without T 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 at 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 T 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

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D$</td>
<td>limit of $y$</td>
</tr>
<tr>
<td>SSD</td>
<td>sexual size dimorphism</td>
</tr>
<tr>
<td>SVL</td>
<td>snout-to-vent length</td>
</tr>
<tr>
<td>T</td>
<td>testosterone</td>
</tr>
</tbody>
</table>
\[ y = \ln(\text{actual SVL/SVL at the time of hatching}) \]

\[ \lambda = \text{length of the lag phase of growth in days} \]

\[ \mu_{\text{max}} = \text{relative maximum growth rate (day}^{-1}) \]

**Acknowledgements**

We thank Henry John-Alder for continuous support, Robert Cox for inspiring discussions and two anonymous reviewers for comments. The experiment was conducted with the approval, and under the supervision, of the Ethical Committee of our institution, permit number 34711/2010-30.

**Funding**

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**References**


Figure legends:

Fig. 1 Concentration of plasma testosterone levels in experimental animals 28 weeks after surgery. Median and inner quartiles are shown, the abbreviation M stands for males, F for 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.

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.

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

<table>
<thead>
<tr>
<th>Species</th>
<th>SSD</th>
<th>Effect of C</th>
<th>Effect of C + T</th>
<th>Prevention of direct social contact</th>
<th>Field/Lab</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thamnophis sirtalis</em></td>
<td>F</td>
<td>+</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>no</td>
<td>lab</td>
<td>Crews et al., 1985</td>
</tr>
<tr>
<td><em>Anolis sagrei</em></td>
<td>M</td>
<td>0</td>
<td>+</td>
<td>no</td>
<td>lab</td>
<td>Cox et al., 2009</td>
</tr>
<tr>
<td><em>Urosaurus ornatus</em></td>
<td>0/M</td>
<td>-</td>
<td>not known</td>
<td>no</td>
<td>lab</td>
<td>Hews et al., 1994</td>
</tr>
<tr>
<td><em>Sceloporus virgatus</em></td>
<td>F</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>no</td>
<td>field</td>
<td>Cox and John-Alder, 2005</td>
</tr>
<tr>
<td><em>Sceloporus undulatus</em></td>
<td>F</td>
<td>0/+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>no</td>
<td>field</td>
<td>Cox et al., 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>not known&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-</td>
<td>yes</td>
<td>lab</td>
<td>Duncan, 2011</td>
</tr>
<tr>
<td><em>Sceloporus jarrovii</em></td>
<td>M</td>
<td>-</td>
<td>+</td>
<td>no</td>
<td>field</td>
<td>Cox and John-Alder, 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>yes</td>
<td>lab</td>
<td>Cox et al., 2006</td>
</tr>
<tr>
<td><em>Aeluroscalabotes felinus</em></td>
<td>F</td>
<td>0</td>
<td>-</td>
<td>yes</td>
<td>lab</td>
<td>L. Kubička, A. Golinski, H. John-Alder and L. Kratochvílí, subm. manuscript</td>
</tr>
<tr>
<td><em>Paroedura picta</em></td>
<td>M</td>
<td>0</td>
<td>0</td>
<td>yes</td>
<td>lab</td>
<td>this study</td>
</tr>
</tbody>
</table>

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.

<sup>a</sup>Castrated juvenile males treated with T grew to the same body size as did castrated males, but both groups attained much larger size than did sham-operated juvenile males.

<sup>b</sup>The interaction between growth rate and initial SVL was significant. Castration increased growth rate only in males with larger SVL at the beginning of the experiment.

<sup>c</sup>The positive effect of castration on growth was evident only in long-term observation.

<sup>d</sup>Only castrated males with and without T implant were compared, no control group was included into the experiment.
Table 2. Summary of effects of ovariectomy (OVX) or addition of exogenous testosterone (T) on female structural growth in squamate reptiles.

<table>
<thead>
<tr>
<th>Species</th>
<th>SSD</th>
<th>Manipulation</th>
<th>Effect of manipulation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Anolis sagrei</em></td>
<td>M</td>
<td>OVX</td>
<td>+</td>
<td>Cox and Calsbeek, 2009</td>
</tr>
<tr>
<td><em>Sceloporus jarrovii</em></td>
<td>M</td>
<td>OVX</td>
<td>+</td>
<td>Cox, 2006</td>
</tr>
<tr>
<td><em>Eublepharis macularius</em></td>
<td>M</td>
<td>OVX</td>
<td>+</td>
<td>Tousignant and Crews, 1995</td>
</tr>
<tr>
<td><em>Paroedura picta</em></td>
<td>M</td>
<td>OVX</td>
<td>+</td>
<td>this study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>+</td>
<td>this study</td>
</tr>
<tr>
<td><em>Aeluroscalabotes felinus</em></td>
<td>F</td>
<td>T</td>
<td>-</td>
<td>L. Kubička, A. Golinski, H. John-Alder and L. Kratochvíl, subm. manuscript</td>
</tr>
<tr>
<td><em>Sceloporus virgatus</em></td>
<td>F</td>
<td>T</td>
<td>-</td>
<td>Abell, 1998</td>
</tr>
<tr>
<td><em>Sceloporus undulatus</em></td>
<td>F</td>
<td>T</td>
<td>-</td>
<td>Duncan, 2011</td>
</tr>
<tr>
<td><em>Thamnophis sirtalis</em></td>
<td>F</td>
<td>T</td>
<td>-</td>
<td>Lerner and Mason, 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>-</td>
<td>Crews et al., 1985</td>
</tr>
<tr>
<td><em>Urosaurus ornatus</em></td>
<td>0/M</td>
<td>T</td>
<td>-</td>
<td>Hews and Moore, 1995</td>
</tr>
</tbody>
</table>

Note: Positive or negative effects of manipulation are given relative to controls.

*aThe 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).

*bThe authors pointed to potential pharmacological doses of T.
Intact Testosterone Castrated Male treatment groups

Snout-vent length (mm)
Intact Testosterone Castrated Male treatment groups

Fulton's index (g mm$^{-3}$)
Intact Mated Testosterone Ovariectomized Female treatment groups

Snout-vent length (mm)
The figure shows a box plot comparing Fulton's index (g mm$^{-3}$) across four female treatment groups: Intact, Mated, Testosterone, and Ovariectomized. The groups are represented as follows:

- **Intact** treatment group shows a box plot with a median close to 0.000030.
- **Mated** treatment group has a box plot with a median slightly higher than the Intact group.
- **Testosterone** treatment group displays a box plot with a median higher than both the Intact and Mated groups, and similar to the Ovariectomized group.
- **Ovariectomized** treatment group shows a box plot with a median significantly higher than the other groups.

The box plots are labeled with letters indicating significant differences: 'a', 'b', and 'c'.
Figure showing the mean ovary mass (g) for different female treatment groups: Intact, Mated, and Testosterone. The graph displays box plots for each group, with significant differences marked by letters a, b, and c.