RESEARCH ARTICLE

Fecundity Selection and the Evolution of Reproductive Output and Sex-Specific Body Size in the *Liolaemus* Lizard Adaptive Radiation

Daniel Pincheira-Donoso · Tom Tregenza

Abstract Fecundity is a primary component of fitness. Theory predicts that the evolution of fecundity through increased brood size results from fecundity selection favouring larger female size to accommodate more offspring and to store more energy. This is expected to generate asymmetric selection on body size between the sexes, ultimately driving evolution of female-biased sexual size dimorphism. Additionally, it has been predicted that the intensity of fecundity selection increases when the opportunities for reproduction are reduced by the limiting thermal effects of increasing latitude-elevation (i.e. decreasing environmental temperatures) on the length of the reproductive season. This later factor would be particularly strong among ectotherms, where reproduction is heavily temperature-dependent. However, this integrative perspective on reproductive evolution by fecundity selection has rarely been investigated. Here, we employ a comparative approach to investigate these predictions in *Liolaemus*, a prominent lizard radiation. As expected, *Liolaemus* reproductive output (i.e. offspring number per reproductive episode) increases predictably with increasing female size. However, contrary to predictions, we found that increased fecundity does not translate into female-biased SSD, and that combined latitude-elevation does not impose a detectable effect on fecundity. Finally, our allometric analyses reveal that SSD scales with body size, which supports the occurrence of Rensch's rule in these lizards. We discuss the evolutionary implications of our results, and the assumptions of the investigated hypotheses.

Introduction

The evolutionary dynamics of reproductive variation involve complex selection-mediated interactions among life history parameters that coevolve to increase reproductive success (Sinervo 2000; Roff 2002; Fairbairn 2006). Once the sexual stage of a reproductive episode has taken place, the action of traits functionally linked to the optimization of fecundity (i.e. offspring number per reproductive episode) is expected to impose a critical impact on fitness. Darwin (1874) suggested that selection for increasing female body size plays a central role in the evolution of fecundity, a scenario broadly known as fecundity selection or the fecundity advantage hypothesis (Shine 1988; Andersson 1994; Cox et al. 2003). The rationale behind this idea is that, first, larger female size results in more internal space to accommodate more developing embryos (Williams 1966), and second, it increases the capacity for storing energy to be invested in reproduction (Calder 1984). Therefore, the primary prediction of this hypothesis is that increased fecundity results from selection for larger female size, when variation in

D. Pincheira-Donoso (✉)
Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Streatham Campus, Exeter, Devon EX4 4PS, UK
e-mail: D.PincheiraDonoso@exeter.ac.uk

T. Tregenza
Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Cornwall Campus, Penryn, Cornwall TR10 9EZ, UK

offspring number depends on variation in female size. This prediction does not apply to species where genetic constraints preclude variation in offspring number per reproductive episode, such as in anoles or several geckonids (Fitch 1985; Vitt 1986; Shine 2005; Losos 2009). As expected, a positive relationship between female size and offspring number has consistently been observed in ectotherm lineages (Fitch 1970; Thornhill and Alcock 1983; Seigel and Ford 1987; Stearns 1992; Andersson 1994; Cox et al. 2003; Shine 2005; Cox et al. 2007; Stephens and Wiens 2009). These observations have given rise to the additional prediction that the effect of directional fecundity selection on female body size creates asymmetric selection between the sexes, resulting in the evolution of female-biased sexual size dimorphism (Darwin 1874; Cox et al. 2003; Stephens and Wiens 2009).

An additional dimension was later incorporated to the fecundity selection hypothesis by Tinkle et al. (1970) and Fitch (1978, 1981). These authors suggested that the intensity of fecundity selection on female body size would increase as a function of reduced opportunities for reproductive frequency caused by environmental constraints. Specifically, among terrestrial ectotherms (primarily squamate reptiles, i.e. lizards and snakes), reproductive frequency is seriously compromised by increasing latitude and elevation, which mirrors decreasing environmental temperatures and hence, shorter reproductive seasons (Fitch 1970; Cox et al. 2003; Shine 2005; Powell and Russell 2007; Pincheira-Donoso et al. 2008a). In high latitudes and elevations, reproductive frequency is also expected to be reduced by the evolution of viviparity, which usually occurs in these environments (Blackburn 2000; Schulte et al. 2000; Shine 2005), and which reduces the opportunities for multiple reproductive episodes per season by increasing the time invested in the retention of a single brood (Cox et al. 2003; Shine 2005). Therefore, according to this view, the strength of fecundity selection increases in species that reproduce infrequently to maximize the reproductive output in each reproductive event (Tinkle et al. 1970; Fitch 1981; Cox et al. 2003). From these ideas on fecundity evolution has been derived the subsequent macroecological prediction that the intensity of fecundity selection increases in species occupying increasingly higher latitudes and elevations (Tinkle et al. 1970; Fitch 1981; Cox et al. 2003).

Despite the significance of these hypotheses for our understanding of the evolution of fecundity and its implications for the evolution of female-biased sexual size dimorphism (SSD) among terrestrial vertebrates (where male-biased SSD predominates), and despite fecundity being a primary component of fitness (Losos 2009; Charlesworth and Charlesworth 2010), only a few studies, scattered across animal lineages, have explored these

predictions (Fairbairn and Shine 1993; Shine 1994; Head 1995; Cox et al. 2007; Stephens and Wiens 2009; Frýdlová and Frynta 2010). Indeed, although most research on these hypotheses comes from lizards, only six studies on these reptiles have appeared in three decades (Cox et al. 2007), two of which (Fitch 1978, 1981) were published when phylogenetic analyses were not incorporated into evolutionary research. Among the four known phylogenetic studies, only one (Cox et al. 2003) provides an integrative analysis on reproductive output, female-biased SSD and indicators of reproductive opportunity. Based on a large-scale approach involving 302 species from different lineages and areas of the world, this study showed a significant covariation between fecundity and female-biased SSD, but weak associations between fecundity and environmental constraints. The remaining three studies (Braña 1996, Zamudio 1998; Frýdlová and Frynta 2010) focus on the interaction between female-biased SSD and reproductive output, resulting in contradictory findings. However, it seems surprising that the use of prominent adaptive radiations as model systems to investigate these predictions has largely been neglected. Some major advantages offered by adaptive radiations are for example, that (1) exploitation of different environments is likely to reflect adaptive shifts resulting from recent spatial or temporal colonization of new selective conditions; (2) different expressions of homologous traits should reflect recent events of evolutionary divergence caused by the impact of selection on ancestral genetic architectures; and (3) all these changes occur under the same phylogenetic and historical context, and are therefore more directly comparable.

Here, we investigate the predictions that fecundity selection drives the evolution of larger female size to increase reproductive output through inferences from associations of offspring number per reproductive episode with female size and the patterns of SSD among species, and finally, whether the intensity of this force increases as reproductive opportunities are reduced by decreasing environmental temperatures. In addition, a quantitative analysis of the allometry of SSD as a function of body size was conducted to investigate the prediction that SSD increases with increasing body size in species where males are larger, while SSD decreases with increasing body size in species where females are larger, as established by Rensch's rule (Fairbairn 1997; Blanckenhorn et al. 2007a). We use the South American *Liolaemus* lizard radiation as a model system. Consisting of +210 species adapted to one of the widest environmental and ecological ranges recorded for a living lizard genus, this lineage represents one of the most extraordinary examples of adaptive radiations known among vertebrates (Pincheira-Donoso et al. 2008b, 2009). Ecologically, *Liolaemus* species range from the Atacama Desert (the driest place on earth) to austral rain forests and

Patagonia, and altitudinally from sea level to over 5,000 m (Cei 1993; Pincheira-Donoso et al. 2008b). As a result of this radiation, a considerable diversity of life history strategies have evolved within this genus (Schulte et al. 2000; Pincheira-Donoso et al. 2008b), providing a unique opportunity to investigate these predictions.

Materials and Methods

Data Collection and Variable Estimations

Our study comprises data collected for 73 *Liolaemus* species (Supplementary Table 1) belonging to all six main clades forming the genus and encompassing the entire geographic range occupied by these lizards (Schulte et al. 2000; Pincheira-Donoso et al. 2008b). Therefore, our dataset covers the entire diversity of selective conditions to which these lizards are currently exposed. To investigate the fecundity selection hypothesis in these reptiles, we collected data for body size, sexual size dimorphism (SSD), geographical distribution, reproductive mode, and fecundity for each species.

Firstly, SSD was obtained from measures of male and female snout-vent length (SVL). This variable is the standard proxy for body size in lizards (Meiri 2008), and has been shown to correlate with several life-history, ecological and morphological traits (Pough 1973; Peters 1983; Pough et al. 2004; Shine 2005). To obtain SVL for each sex we calculated means from the largest two-thirds of the entire sample per species comprising only adults (e.g. Losos et al. 2003; Pincheira-Donoso et al. 2008a), instead of the maximum recorded SVL. Although maximum SVL has been extensively used in lizard research, it has been shown that the use of occasional extreme values may result in body size overestimations (Brown et al. 1999). In contrast, the use of intermediate percentiles between the maximum record and the mean from the entire adult sample provides accurate estimates of asymptotic size, hence reducing size overestimations (Brown et al. 1999). We calculated a quantitative measure of SSD with the standard formula ln(male SVL/female SVL). This formula has been extensively used to obtain SSD across different taxa (Fairbairn 2007). After reviewing the statistical features of a number of formulae commonly used to calculate SSD, Smith (1999) concluded that the ln(M/F) formula offers quantitative attributes that make it superior to most alternative indices (e.g. this index is directional and symmetrical around zero; see Smith 1999; Fairbairn 2007, for details). After SSD values were calculated we arbitrarily changed the sign of the dimorphism from negative to positive when sexual dimorphism is female-biased, and vice versa (given that this SSD index is numerically

symmetric, it does not have any effect on the results of quantitative analyses, as it does not affect the quantitative magnitude of SSD).

Geographical distribution data were used as proxy for environmental thermal constraints on reproduction (Tinkle et al. 1970; Fitch 1981; Cox et al. 2003). Given that decreasing environmental temperature results from the combined effect of increasing latitude and elevation (Lutgens and Tarbuck 1998; Ashton 2002; Cruz et al. 2005; Pincheira-Donoso et al. 2008a), we combined both geographical parameters into a single variable, the adjusted latitudinal midpoint (ALM) calculated for each species, following Cruz et al. (2005). ALM is calculated on the assumption that temperatures in altitudinal transects decline 0.65°C for each 100 m of increased elevation (Lutgens and Tarbuck 1998; Cruz et al. 2005). Cruz et al. (2005) obtained a corrected latitudinal value for latitude and elevational thermal covariation with the formula $y = 0.009x - 6.2627$, where x represents the altitudinal midpoint for each species, and y the corrected temperature for latitude which is added to the latitudinal midpoint for each species. This returns the final ALM values for South American areas where *Liolaemus* lizards occur (Cruz et al. 2005; Pincheira-Donoso et al. 2007b, 2008a). In addition, previous studies have suggested that the opportunities for reproduction may also be compromised by the evolution of viviparity, as this derived reproductive mode requires longer retention of embryos in the females' body, and hence, it results in less frequent reproductive episodes and in higher overall costs associated with reproduction (Fitch 1978, 1981; Shine 2005; Cox et al. 2007). The fact that viviparity has consistently evolved among *Liolaemus* species occupying higher latitudes and elevations (Schulte et al. 2000; Pincheira-Donoso et al. 2008b) supports our primary assumption that species occurring in colder environments experience reduced opportunities for reproduction. Therefore, the use of latitude and elevation (through ALM) seems an appropriate variable to explore the problem of fecundity selection. Our lizard model system offers a unique opportunity to explore this prediction as the species range from extremely hot environments to extremely cold and elevated areas, encompassing an ideal range of variation (see above).

Finally, we obtained measures of fecundity from counts of offspring number per reproductive episode. Although brood mass also provides a primary indicator of energy allocation in reproduction (e.g. Roff 2002), this information is almost entirely unavailable for *Liolaemus* species. However, since brood mass and offspring number in lizards have been observed to be described by a negative genetic correlation (Sinervo 2000; Pianka and Vitt 2003; Shine 2005), where departures from this trade-off result in lower fitness mediated by stabilizing selection on the opposing

interaction of both parameters (Sinervo 2000), they both are appropriate alternative indexes of reproductive investment and success. Indeed, a given amount of energy allocated to reproduction can be divided either into a few large offspring or into several smaller ones (Shine 2005). Therefore, we recorded offspring number from both living and preserved specimens, and from the literature. Apart from published information, the sources of our data were direct observations of females ovipositing or giving birth, and of oviductal eggs and developing embryos counted from preserved specimens. Given that previous observations suggest that some *Liolaemus* species may have evolved communal nesting behaviours (Espinoza and Lobo 1996), we discarded data collected from field observations of nests to avoid risks of inflated brood size per female. For each species, we recorded the mean clutch/litter size and the range of intraspecific variation.

Statistical Analyses and Phylogenetic Control

Prior to statistical analyses, all variables were ln-transformed to reduce skew and homogenize variances (Zar 2009). We then investigated the effects of fecundity selection using regression analyses. It is broadly accepted that species in comparative analyses cannot be assumed to be independent data points, as their descent from common ancestors may result in phylogenetically related species expressing similar traits. This ultimately may increase the likelihood of observing inflated estimates towards the adaptive explanation, and hence, significant associations between predictor and response traits may be an artifact of phylogenetic dependence among species (Felsenstein 1985; Harvey and Pagel 1991). Therefore, we employed phylogenetic approaches to control for potential phylogenetic effects and infer correlated evolution between traits. However, it has also been suggested that reporting results from both conventional non-phylogenetic and phylogenetic analyses might provide a more powerful and inclusive approach (e.g. Harvey and Rambaut 2000; Schluter 2000). Hence, we have conducted all our regression analyses using both approaches.

While the test of the primary prediction of fecundity selection requires direct regressions of brood size on female SVL, tests of the additional hypotheses (brood size varies in response to SSD and ALM) require body size control on brood size, as variation in fecundity has consistently been shown to be influenced by body size (Tinkle et al. 1970; Fitch 1981; Peters 1983; Shine 1994; Cox et al. 2003; Shine 2005; also, this study). Residuals from ordinary least-square regressions (OLS) of body size (female SVL in this case) on the rest of variables are often calculated to obtain size-effect-free variables (Green 2001). However, it has been shown that a number of relevant

assumptions may not hold for residuals, and that the residual index is an ad-hoc sequential procedure with no demonstrated statistical justification (Garcia-Berthou 2001; Green 2001; Freckleton 2009). Therefore, we used an alternative approach where female SVL is added as a predictor in all regression analyses, which results in multiple regression models (with two predictors) where only one of them is the actual predictor of interest for these hypotheses (SSD and ALM). Hence, for both multiple regression analyses we employed a test for added predictors, to quantify whether the predictive contributions of SSD and ALM, respectively, on brood size are significant once female SVL is in the model (Tabachnick and Fidell 2007). This approach decomposes the regression model and evaluates explicitly whether the amount of variance explained (R_2^2 in Table 1) by SSD and ALM is significant (P_2 in Table 1) when body size is controlled for, and hence, it does not only takes into account the total variance and the overall significance of the full model with both predictors (Tabachnick and Fidell 2007).

To perform phylogenetic analyses, we used a *Liolaemus* phylogeny for 44 of the 73 species in our dataset, derived from two major phylogenetic hypotheses presented by Espinoza et al. (2004) and Abdala (2007) (Supplementary Fig. S1). Phylogenetic research on evolutionary relationships within *Liolaemus* has consistently shown the existence of a major monophyletic clade nested within the genus, characterized by the unique feature of a patch of enlarged scales on the posterior surface of the thigh (linked to a muscular hypertrophy), usually termed the *boulengeri* complex (e.g. Schulte et al. 2000; Espinoza et al. 2004; Abdala 2007; Pincheira-Donoso et al. 2007a). We used Espinoza et al.'s (2004) phylogeny as the basis for our *Liolaemus* phylogeny, but replaced the monophyletic *boulengeri* clade with Abdala's (2007) phylogeny for this complex, as it contains a larger number of species included in our dataset (e.g. Weiblen et al. 2000). Because the phylogeny is based on combined molecular and morphological data (Espinoza et al. 2004; Abdala 2007), branch lengths were set equal to 1.0, and a speciational Brownian motion model of evolutionary change was used (Martins and Garland 1991; Garland et al. 1993; Espinoza et al. 2004). Using this phylogeny, we employed Felsenstein's standardized phylogenetic independent contrasts (PIC) approach (Felsenstein 1985) as implemented in the software COMPARE version 4.6b (Martins 2004). We calculated standardized PIC for our variables to infer correlated evolution between traits, and between traits and environment. With PIC, the degree of covariation between variables reflects the extent to which these variables are functionally related during evolutionary change (e.g. evolutionary dependence between two traits is inferred if large changes in the contrasts of one variable are paralleled by

Table 1 Summary of non-phylogenetic and phylogenetic (phylogenetic independent contrasts, IC) regression analyses

Analysis	N	r	R^2	$F(df)$	P	Test for added X_i	
						R_2^2	P_2
Non-phylogenetic							
Brood size versus SVLF	73	0.34	0.12	9.23(1,71)	<0.01	–	–
Brood size versus SSD (+SVLF)	73	0.36	0.13	5.31(2,70)	<0.01	0.02	0.25
Brood size versus ALM (+SVLF)	73	0.34	0.12	4.55(2,70)	0.01	0.00	0.96
Phylogenetic IC							
Brood size versus SVLF	43	0.44	0.19	9.85(1,42)	<0.01	–	–
Brood size versus SSD (+SVLF)	43	0.48	0.23	6.13(2,41)	<0.01	0.04	0.15
Brood size versus ALM (+SVLF)	43	0.46	0.21	5.44(2,41)	<0.01	0.02	0.32

In the column containing the analyses performed, the first variable is always the predictor and the second is the response variable. Abbreviations are the same detailed in the texts. The test for added X quantifies the magnitude of the effect that adding a predictor (SSD in this case) to the multivariate model containing body size (SVLF in this case) only. R_2^2 refers to the incremental magnitude of variance explained when adding SSD, and the significance value P_2 whether the addition of SSD results in a significant increment of the predictive power on brood size (see Tabachnick and Fidell 2007). For both non-phylogenetic and phylogenetic analyses, P_2 shows that SSD does not predict variation in Brood size when size is controlled for. Abbreviations as specified in the text

large changes in the contrasts of the other). Regressions based on PIC were forced through the origin (Felsenstein 1985; Harvey and Pagel 1991; Garland et al. 1992). The same test for added predictors described for non-phylogenetic analyses was employed in these phylogenetic regressions.

Finally, to analyze the evolutionary trajectory of body size variation between the sexes, we investigated whether the magnitude of SSD increases as an allometric function of increasing SVL (e.g. Stuart-Fox 2009). This association, termed Rensch's rule, states that SSD increases with increasing body size in species where males are larger, while SSD decreases with increasing body size in species where females are the larger sex (Abouheif and Fairbairn 1997; Fairbairn 1997; Blanckenhorn et al. 2007a). Statistical analyses of Rensch's rule have been conducted using regressions of SSD indexes against mean body size of the species, or of the sexes (see Fairbairn 1997, for a review). However, this approach violates the fundamental assumption that variables y and x have to be mathematically independent, as size estimates are in both the response variable (SSD) and in the predictor (mean size); therefore, this is an inappropriate alternative (LaBarbera 1989; Fairbairn 1997). The interdependence of variables is circumvented by employing a log–log regression of male size against female size (Fairbairn 1997; Blanckenhorn et al. 2007a). In addition, because the y (male size) and x (female size) variables are both estimated with error, and are measured in a similar way and using similar scales, ordinary least-square regression (OLS, model I) is an inappropriate technique as it will tend to underestimate both the slope (β) and the confidence interval (CI) around β. Under these circumstances, major axis regressions (MA, model II) offer an accurate approach to test the null

hypothesis of isometry $\beta = 1.0$ (Sokal and Rohlf 1995; Fairbairn 1997; Blanckenhorn et al. 2007a). When male size is plotted on y and female size on x, an allometric pattern consistent with Rensch's rule occurs when variance in x is less than in y, and hence, when a hyperallometric or positive allometric relationship is observed (Fairbairn 1997; Blanckenhorn et al. 2007a). Standard errors (SE) and 95% confidence intervals calculated on error in both x and y for MA slopes, are obtained (Blanckenhorn et al. 2007b). Because phylogenetic relationships are also expected to bias the allometric relationship between male and female size, we employed phylogenetic analyses based on phylogenetic independent contrasts (see above), for which we performed MA regressions. To illustrate the fact that OLS regressions tend to underestimate β, we provide results from both OLS and MA regressions (Table 2). These analyses were conducted using an Excel spreadsheet prepared and maintained by W. U. Blanckenhorn.

Results

Our quantitative non-phylogenetic and phylogenetic analyses revealed similar results. As expected by the fecundity selection hypothesis, conventional non-phylogenetic analyses showed that offspring number per reproductive episode increases predictably as a function of increasing female size (Table 1; Fig. 1a). Phylogenetic analysis on PIC reveals the same relationship, and suggests that events of evolutionary change on female size are accompanied by evolutionary change in brood size (Table 1; Fig. 1b). However, contrary to expectations, the tendency for increasing clutch size among species does not covary predictably with a reversal in the form of sexual size

Table 2 Regression analyses of the allometry of sexual size dimorphism in *Liolaemus*, estimated from a log–log regression of ln(male SVL) on ln(female SVL)

	LS regressions				MA regressions		
	N	R^2	Intercept	β	N	Intercept	β
Non-phylogenetic	73	0.92	0.166	0.97 ± 0.034	73	-0.013	1.014 ± 0.024
Phylogenetic	44	0.9	0.001	1.11 ± 0.057	44	0.002	**1.178 ± 0.035***

Results from both conventional and phylogenetic least-square (LS) and major axis (MA) regressions are shown

* $P < 0.05$

dimorphism, and hence, higher fertility is not predicted by female-biased SSD (Table 1). Although the multiple regression model (with SSD and female SVL to control for size effects) is significant, a decomposition of this model with a test for added predictors (in this case, SSD to the model containing female SVL) reveals that the magnitude of explained variance only increases in 2% with SSD, a non-significant predictive contribution ($P_2 = 0.25$; Table 1). Hence, SSD does not predict variation in brood size when body size is controlled for. Consistent with this finding, our phylogenetic test on PIC shows the same result, where evolutionary change from male-biased SSD to female-biased SSD is not accompanied by evolutionary change in brood size when body size is controlled for ($P_2 = 0.15$; Table 1).

These findings are consistent with our analyses of Rensch's rule. Major axis regressions on phylogenetically controlled data reveal that variance in male size (*y*-axis) is larger than the corresponding variance in female size (*x*-axis), resulting in hyperallometric scaling between the sexes, consistent with Rensch's rule, as $\beta = 1$ does not overlap the 95% CI (Table 2; Fig. 2). Therefore, these results suggest that a large evolutionary change in male size is accompanied by a correlated smaller change in female size. Our MA regression on non-phylogenetic data also reveals hyperallometric scaling of ln(male) on ln(female) SVL, although it does not significantly differ from the isometric slope $\beta = 1$ (Table 2; Fig. 2).

Finally, contrary to predictions, our analyses on the impact of geographical gradients on reproduction reveal that increasing environmental constraints on the lengths of the reproductive seasons resulting from increasing latitude and elevation have no effect on the variation in offspring number among species distributed along a broad geographical and thermal gradient, a pattern that remains when employing non-phylogenetic and phylogenetic regressions. As shown in Table 1, the significance ($P < 0.05$) of the conventional and phylogenetic multiple regression models containing ALM are almost entirely explained by body size, as in both cases the addition of ALM fails to improve the fit of the model ($P_2 = 0.96$ and $P_2 = 0.32$, for conventional and phylogenetic analyses respectively).
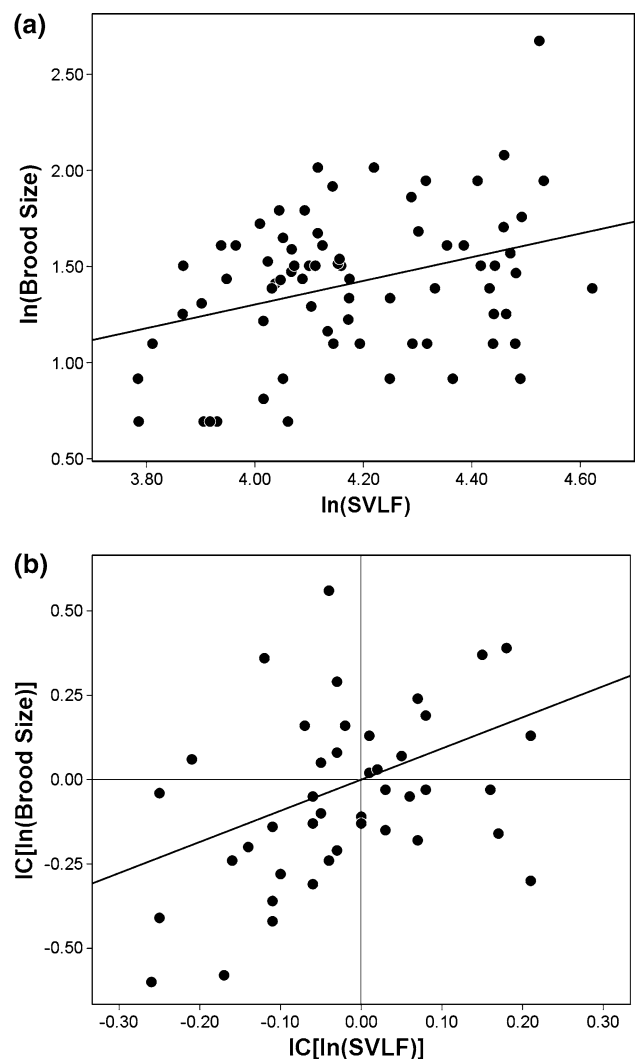

Fig. 1 Relationships between variation in brood size and female SVL among *Liolaemus* species. Increasing brood size is consistently predicted by increasing female ln(SVL) under conventional (**a**) and phylogenetic (independent contrasts, IC) analyses (**b**)

Discussion

Our findings in *Liolaemus* lizards are consistent with the primary prediction of the fecundity selection hypothesis, that increasing fecundity results from larger body size in
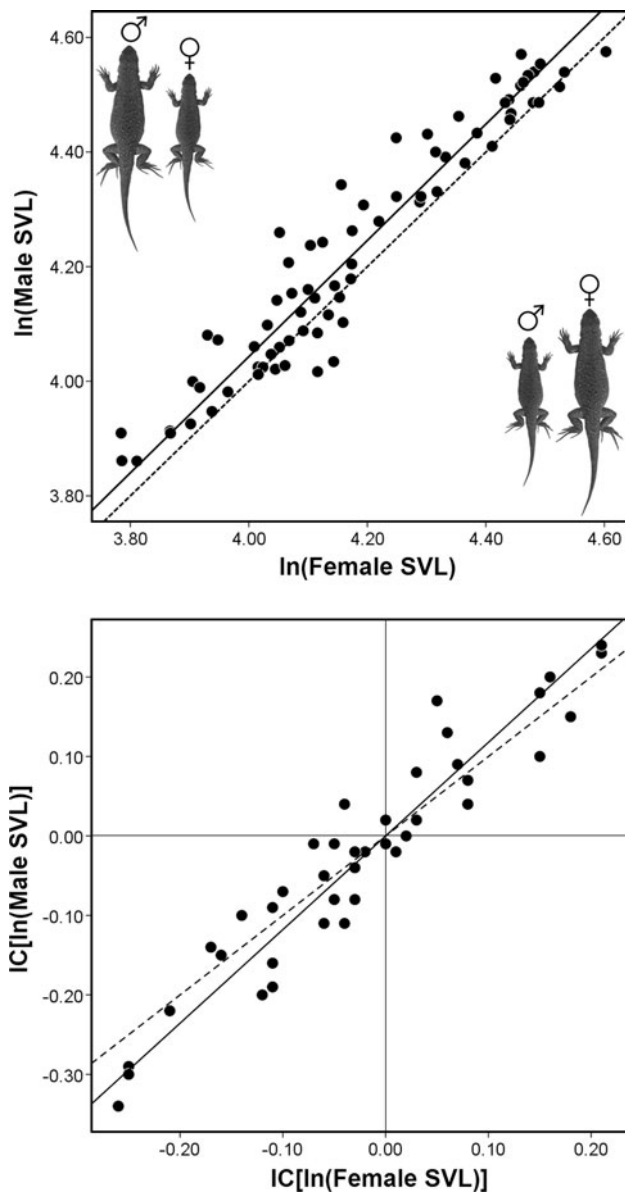
Fig. 2 Allometric scaling of sexual size dimorphism as a function of the sexes body size (SVL) based on major-axis regressions of raw data (*top*) and of phylogenetic independent contrasts (IC, *bottom*) of ln(male) on ln(female) SVL. The *dashed line* describes a reference isometric scaling between the sexes ($\beta = 1.0$), and the *solid line* describes the hyperallometry (existent but non-significant for raw data, and significant for phylogenetic data) observed for actual measures of body size in *Liolaemus* species ($\beta > 1.0$, see Table 2 for quantitative results), as predicted by Rensch's rule

females (Williams 1966; Fitch 1981; Seigel and Ford 1987; Cox et al. 2007). However, our data failed to support the derived prediction that fecundity selection is associated with female-biased SSD in this lineage, where male-biased SSD is the norm. These observations are consistent with previous studies involving lizard lineages from different areas of the world (Zamudio 1998; Frýdlová and Frynta 2010; see Shine 1994, for similar results in snakes), but

contrast with some other findings (Fitch 1978, 1981; Braña 1996; Cox et al. 2003; see Stephens and Wiens 2009, for similar results in turtles). Thus, our study contributes to a growing body of evidence suggesting that female-biased SSD cannot reliably be predicted by means of the effect of fecundity selection on female size inferred from the association between brood size and SSD. Indeed, even studies that statistically support this hypothesis of SSD have arrived at a similar conclusion. For example, despite Cox et al. (2003) observing that female-biased SSD covaries with female fecundity among lizards in general, they concluded that the low explanatory power of their analyses only provided weak evidence for the idea that fecundity selection is linked to the evolution of female-biased SSD, and therefore, that fecundity selection would not be a general explanation for patterns of SSD in these organisms. In addition, Fitch's (1978, 1981) results supporting this prediction rely on non-phylogenetic analyses, and hence, it is difficult to determine whether his findings were an artifact of the assumption of data independence implicit in his approach. On the other hand, our analyses also failed to support the established macroecological expectation that the strength of fecundity selection increases in colder climates, where the opportunities for reproduction are compromised by shorter reproductive seasons and lower environmental temperatures. Among *Liolaemus*, variation in brood size does not vary predictably with spatial distribution as predicted by this hypothesis. This finding is consistent with the only known phylogenetic study where this prediction has been tested (Cox et al. 2003).

Fecundity Selection and the Evolution of Sexual Size Dimorphism

The expression of female-biased SSD associated with higher female fecundity in lineages where male-biased SSD is the norm has often been interpreted as the evolutionary outcome of fecundity selection (see Cox et al. 2003; Fairbairn et al. 2007, for reviews). The rationale behind this view is that fecundity selection creates directional selection for larger female but not male body size. However, as shown in several studies, signals of fecundity selection (higher fecundity associated with larger female size) are not consistently associated with female-biased SSD. Indeed, our results in *Liolaemus* reveal that, despite higher fecundity being associated with larger female size, increases in fecundity are not associated with evolutionary change toward female-biased SSD (Table 1). This observation is compatible with our additional analyses on the magnitude of evolutionary change between the sexes (Fig. 2). We found in *Liolaemus* that variation in SSD is consistent with Rensch's rule, and hence, that larger female size (and hence higher fecundity) is associated with

reduced magnitude of female-biased SSD and increased magnitude of male-biased SSD (e.g. Fairbairn 1997; Blanckenhorn et al. 2007a). Therefore, both sources of evidence suggest that the evolution of female-biased SSD in *Liolaemus* lizards is not explained by the hypothesis that fecundity selection results in increased female size compared to male size, as also concluded by Cox et al. (2003) for lizards in general.

The relationship between fecundity selection and female-biased SSD can be viewed from two angles. First, where fecundity selection stands as an appropriate explanation for some cases of female-biased SSD, when significant relationships between brood size and female-biased SSD (mediated by a positive covariation between female SVL and brood size), are observed. Second, where female-biased SSD is interpreted as the result of strong fecundity selection on female size. In this later case, if a general prediction is that strong fecundity selection results in female-biased SSD, then this prediction implies that in species with male-biased SSD fecundity selection is weak. Therefore, an obvious question is whether sexually monomorphic or male-biased sexually dimorphic species should be interpreted as the result of weak or no fecundity selection. Intrinsic differences in reproductive (and often ecological) roles between the sexes make males and females different targets of different forms and mechanisms of selection in the same space and time, which often results in sex-specific regions of fitness maximization within the same species. Hence, observations of male-biased SSD or sexual size monomorphism do not necessarily reflect negligible fecundity selection. Indeed, even substantial fecundity selection might only sometimes translate into female-biased SSD. For example, the energetic and fitness costs associated with increasingly stronger directional fecundity (in females) and sexual (in males) selection are likely to make comparatively more advantageous for male sexually selected trait values, such as head size in male lizards (Husak et al. 2009), to continually increase. In contrast, female lifetime fitness in the context of fecundity selection depends on a balance between current and future investment in reproduction (Williams 1966; Reznick 1985), with disproportionate investment per reproductive episode incurring in potentially very high fitness costs. Hence, the response of each sex to these different mechanisms of directional selection might result in both sexes increasing the trait under selection, but more freely in males. Also, when the costs of expressing increased trait values are similar between both sexes (e.g. costs of reproduction are independent of fecundity in females), different mechanisms of selection (fecundity and sexual) described by the same fitness function (directional), may cause body size evolution in the same direction, potentially eclipsing the expression of SSD.

Alternatively, female-biased SSD can also evolve in response to negative directional sexual selection for smaller male size (the small-male advantage hypothesis; Zamudio 1998). According to this hypothesis, smaller male size can be favoured in species where low population densities result in females being spatially more dispersed, and hence, where male-male agonistic encounters are likely to be less common. Therefore, male fitness increases with smaller sizes that provide higher mobility, early maturation, and time and energy budgets that enhance mate searching (Trivers 1972; Ghiselin 1974; Cox et al. 2003). Although the lack of appropriate data precludes this hypothesis from being tested in *Liolaemus*, some qualitative observations suggest that it may not be the generality for this genus. Indeed, some *Liolaemus* species where females are larger can be quite abundant, although in other species, males do not show the more common signals of male-male sexual selection associated with positive directional selection, such as voluminous heads (e.g. see Vanhooydonck et al. 2010), which suggests that other forms of sexual selection (negative directional) may in fact operate on these males to create female-biased SSD.

Finally, when variation in SSD in a lineage is consistent with Rensch's rule, as observed in several organisms, including *Liolaemus* (Fairbairn 1997; Fairbairn et al. 2007; this study), the predicted relationship between increased female-biased SSD and higher fecundity becomes highly weak, as exemplified by *Liolaemus* lizards. In conclusion, we argue that the evolution of female-biased SSD can be an outcome of fecundity selection only under some circumstances, and that the degree of female-biased SSD does not necessarily reflect the strength of fecundity selection.

The Strength of Fecundity Selection

The hypothesis that the intensity of fecundity selection increases as a function of reduced opportunities for reproduction is appealing. As previously discussed (Tinkle et al. 1970; Fitch 1978, 1981; see also Cox et al. 2003), it is reasonable to expect that females of populations where the reproductive season is shorter will be subjected to stronger fecundity selection to optimize fecundity per reproductive episode. Therefore, at a first glance, our finding that shorter reproductive seasons do not result in increased fecundity in *Liolaemus* species (and in other studied lizards) may appear counterintuitive. However, these findings may actually have an explanation consistent with stronger fecundity selection.

This explanation might rely on the simplistic structure of the parameters involved in this macroecological prediction of fecundity selection. A fundamental requirement of the theory that fecundity selection is the driving mechanism behind a positive covariation between latitude-elevation

and brood size is that larger broods depend on larger female size (as often supported). Consequently, this theory assumes that female size increases with latitude and elevation, a pattern broadly known as Bergmann's rule. However, a number of studies reveal that this important assumption is consistently violated by empirical evidence in most lizards (Ashton and Feldman 2003; de Queiroz and Ashton 2004; Pincheira-Donoso 2010), including *Liolaemus* (Pincheira-Donoso et al. 2007b, 2008a), where Bergmann's rule is not supported. Hence, as latitude and elevation increase, the potential for increased brood size is limited by constraints of thermal selection on body size, making this prediction unlikely despite the intuitive appeal of the mechanistic idea behind it. Thus, indirectly, thermal selection on body size appears to prevent the evolution of larger broods in colder climates. Therefore, the evolution of variation in brood size in response to constraints on reproductive frequency might be context-dependent (i.e. relative to particular life history tradeoffs under particular selective environments). This implies that, although brood size does not increase in species experiencing stronger constraints on reproduction, in opposition to the expectations of the macroecological idea of fecundity selection, fecundity selection might in fact be stronger in colder climates. Indeed, in cold climate lizards (compared to warm climate species) the reproductive potential is thought to be reduced by at least three direct factors: (1) shorter reproductive seasons; (2) the evolution of viviparity, which has been shown to be the norm for cold climate *Liolaemus* species (Schulte et al. 2000), and which reduces the reproductive potential through prolonged brood retention (Shine 2005); and (3) more limited access to nutritive food to be translated into fitness through resource allocation to offspring. In support of this latter factor, it has been shown that the evolution of herbivory (i.e. low energy food) is also the norm for cold climate *Liolaemus* species, which might result from lower abundance of arthropods (relative to plant matter availability) in these areas (Espinoza et al. 2004). Moreover, in these species, the evolution of small size is thought to maximize plant matter digestion through maximization of thermoregulatory rates (Espinoza et al. 2004; Pincheira-Donoso et al. 2008a). Therefore, the observation that brood size does not differ between cold- and warm-climate species, despite the above limitations on reproduction experienced by the former, suggests that fecundity might actually be optimized in species from cold areas via stronger fecundity selection. If in these environments fecundity selection was weaker or negligible, brood size would otherwise be likely to experience a reduction as latitude and elevation increase (i.e. a four-offspring brood might be average in warm environments, but large in a cold area). Hence, it can be expected that variation in environmental temperatures along geographical gradients alone

would fail to capture the effects of fecundity selection on reproductive maximization.

References

Abdala, C. S. (2007). Phylogeny of the *boulengeri* group (Iguania: Liolaemidae, *Liolaemus*) based on morphological and molecular characters. *Zootaxa, 1538*, 1–84.

Abouheif, E., & Fairbairn, D. J. (1997). A comparative analysis of allometry for sexual size dimorphism: Assessing Rensch's rule. *American Naturalist, 149*, 540–562.

Andersson, M. (1994). *Sexual selection*. Princeton: Princeton University Press.

Ashton, K. G. (2002). Do amphibians follow Bergmann's rule? *Canadian Journal of Zoology, 80*, 708–716.

Ashton, K. G., & Feldman, C. R. (2003). Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. *Evolution, 57*, 1151–1163.

Blackburn, D. G. (2000). Reptilian viviparity: past research, future directions, and appropriate models. *Comparative Biochemistry and Physiology A, 127*, 391–409.

Blanckenhorn, W. U., Dixon, A. F. G., Fairbairn, D. J., Foellmer, M. W., Gibert, P., van der Linde, K., et al. (2007a). Proximate causes of Rensch's rule: does sexual size dimorphism in arthropods result from sex differences in development time? *American Naturalist, 169*, 245–257.

Blanckenhorn, W. U., Meier, R., & Teder, T. (2007b). Rensch's rule in insects: patterns among and within species. In D. J. Fairbairn, W. U. Blanckenhorn, & T. Szekely (Eds.), *Sex, size & gender roles. Evolutionary studies of sexual size dimorphism* (pp. 60–70). Oxford: Oxford University Press.

Braña, F. (1996). Sexual dimorphism in lacertid lizards: male head increase vs. female abdomen increase. *Oikos, 75*, 511–523.

Brown, R. P., Znari, M., El Mouden, E. L. H., & Harris, P. (1999). Estimating asymptotic body size and testing geographic variation in *Agama impalearis*. *Ecography, 22*, 277–283.

Calder, W. A. (1984). *Size, function and life history*. Massachusetts: Harvard University Press.

Cei, J. M. (1993). Reptiles del noroeste, nordeste y este de la Argentina. Herpetofauna de las selvas subtropicales, puna y pampas. Museo Regionale di Scienze Naturali di Torino, Torino.

Charlesworth, B., & Charlesworth, D. (2010). *Elements of evolutionary genetics*. Colorado: Roberts and Company.

Cox, R. M., Skelly, S. L., & John-Alder, H. B. (2003). A comparative test of adaptive hypotheses for sexual size dimorphism in lizards. *Evolution, 57*, 1653–1669.

Cox, R. M., Butler, M., & John-Alder, H. B. (2007). The evolution of sexual size dimorphism in reptiles. In D. J. Fairbairn, W. U. Blanckenhorn, & T. Szekely (Eds.), *Sex, size & gender roles. Evolutionary studies of sexual size dimorphism* (pp. 38–49). Oxford: Oxford University Press.

Cruz, F. B., Fitzgerald, L. A., Espinoza, R. E., & Schulte, J. A. (2005). The importance of phylogenetic scale in tests of

Bergmann's and Rapoport's rules: lessons from a clade of South American lizards. *Journal of Evolutionary Biology, 18,* 1559–1574.

Darwin, C. (1874). *The descent of man and selection in relation to sex* (2nd ed.). New York: Appleton.

de Queiroz, A., & Ashton, K. G. (2004). The phylogeny of a species-level tendency: species heritability and possible deep origins of Bergmann's rule in tetrapods. *Evolution, 58,* 1674–1684.

Espinoza, R. E., & Lobo, F. (1996). Possible communal nesting in two species of *Liolaemus* lizards (Iguania: Tropiduridae) from northern Argentina. *Herpetological Natural History, 4,* 65–68.

Espinoza, R. E., Wiens, J. J., & Tracy, C. R. (2004). Recurrent evolution of herbivory in small, cold-climate lizards: breaking the ecophysiological rules of reptilian herbivory. *Proceedings of the National Academy of Sciences, USA, 101,* 16819–16824.

Fairbairn, D. J. (1997). Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. *Annual Review of Ecology, Evolution, and Systematics, 28,* 659–687.

Fairbairn, D. J. (2006). Defining and measuring fitness. In C. W. Fox & J. B. Wolf (Eds.), *Evolutionary genetics. Concepts and case studies* (pp. 52–54). Oxford: Oxford University Press.

Fairbairn, D. J. (2007). The enigma of sexual size dimorphism. In D. J. Fairbairn, W. U. Blanckenhorn, & T. Szekely (Eds.), *Sex, size & gender roles. Evolutionary studies of sexual size dimorphism* (pp. 1–10). Oxford: Oxford University Press.

Fairbairn, D. J., & Shine, R. (1993). Patterns of sexual size dimorphism in seabirds of the southern hemisphere. *Oikos, 68,* 139–145.

Fairbairn, D. J., Blanckenhorn, W. U., & Szekely, T. (2007). *Sex, size & gender roles. Evolutionary studies of sexual size dimorphism.* Oxford: Oxford University Press.

Felsenstein, J. (1985). Phylogenies and the comparative method. *American Naturalist, 125,* 1–15.

Fitch, H. S. (1970). *Reproductive cycles in lizards and snakes* (Vol. 52, pp. 1–247). University of Kansas Museum of Natural History, Miscellaneous Publications.

Fitch, H. S. (1978). *Sexual size differences in the genus Sceloporus* (Vol. 51, pp. 441–461). University of Kansas Museum of Natural History, Miscellaneous Publications.

Fitch, H. S. (1981). *Sexual size differences in reptiles* (Vol. 70, pp. 1–72). University of Kansas Museum of Natural History, Miscellaneous Publications.

Fitch, H. S. 1985. *Variation in clutch and litter size in New World reptiles.* (Vol. 76, pp. 1–76). University of Kansas Museum of Natural History, Miscellaneous Publications.

Freckleton, R. P. (2009). The seven deadly sins of comparative analysis. *Journal of Evolutionary Biology, 22,* 1367–1375.

Frýdlová, P., & Frynta, D. (2010). A test of Rensch's rule in varanid lizards. *Biological Journal of the Linnean Society, 100,* 293–306.

Garcia-Berthou, E. (2001). On the misuse of residuals in ecology: testing regression residuals vs. the analysis of covariance. *Journal of Animal Ecology, 70,* 708–711.

Garland, T., Harvey, P. H., & Ives, A. R. (1992). Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Systematic Biology, 41,* 18–32.

Garland, T., Dickerman, A. W., Janis, C. M., & Jones, J. A. (1993). Phylogenetic analysis of covariance by computer simulation. *Systematic Biology, 42,* 265–292.

Ghiselin, M. T. (1974). *The economy of nature and the evolution of sex.* Berkeley: University of California Press.

Green, A. J. (2001). Mass/length residuals: measures of body condition or generators of spurious results? *Ecology, 82,* 1473–1483.

Harvey, P. H., & Pagel, M. D. (1991). *The comparative method in evolutionary biology.* Oxford: Oxford University Press.

Harvey, P. H., & Rambaut, A. (2000). Comparative analyses for adaptive radiations. *Philosophical Transactions of the Royal Society of London, B, 355,* 1599–1605.

Head, G. (1995). Selection on fecundity and variation in the degree of sexual size dimorphism among spider species (class Araneae). *Evolution, 49,* 776–781.

Husak, J. F., Lappin, A. K., & Van Den Bussche, R. A. (2009). The fitness advantage of a high-performance weapon. *Biological Journal of the Linnean Society, 96,* 840–845.

LaBarbera, M. (1989). Analyzing body size as a factor in ecology and evolution. *Annual Review of Ecology and Systematics, 20,* 97–117.

Losos, J. B. (2009). *Lizards in an evolutionary tree. Ecology and adaptive radiation of anoles.* California: University of California Press.

Losos, J. B., Butler, M., & Schoener, T. W. (2003). Sexual dimorphism in body size and shape in relation to habitat use among species of Caribbean *Anolis* lizards. In S. F. Fox, J. K. McCoy, & T. A. Baird (Eds.), *Lizard social behaviour* (pp. 356–380). Baltimore and London: John Hopkins University Press.

Lutgens, F. K., & Tarbuck, E. J. (1998). *The atmosphere. An introduction to meteorology.* New Jersey: Prentice Hall.

Martins, E. P. (2004). *COMPARE, version 4.6b. Computer programs for the statistical analysis of comparative data.* Distributed by the author at http://compare.bio.indiana.edu/. Department of Biology, Indiana University, Indiana.

Martins, E. P., & Garland, T. (1991). Phylogenetic analyses of the correlated evolution of continuous characters: a simulation study. *Evolution, 45,* 534–557.

Meiri, S. (2008). Evolution and ecology of lizard body sizes. *Global Ecology and Biogeography, 17,* 724–734.

Peters, R. H. (1983). *The ecological implications of body size.* Cambridge: Cambridge University Press.

Pianka, E. R., & Vitt, L. J. (2003). *Lizards. Windows to the evolution of diversity.* Berkeley, Los Angeles, London: University of California Press.

Pincheira-Donoso, D. (2010). The balance between predictions and evidence and the search for universal macroecological patterns: taking Bergmann's rule back to its endothermic origin. *Theory in Biosciences, 129,* 247–253.

Pincheira-Donoso, D., Scolaro, J. A., & Schulte, J. A. (2007a). The limits of polymorphism in *Liolaemus rothi:* molecular and phenotypic evidence for a new species of the *Liolaemus boulengeri* clade (Iguanidae, Liolaemini) from boreal Patagonia of Chile. *Zootaxa, 1452,* 25–42.

Pincheira-Donoso, D., Tregenza, T., & Hodgson, D. J. (2007b). Body size evolution in South American *Liolaemus* lizards of the *boulengeri* clade: a contrasting reassessment. *Journal of Evolutionary Biology, 20,* 2067–2071.

Pincheira-Donoso, D., Hodgson, D. J., & Tregenza, T. (2008a). The evolution of body size under environmental gradients in ectotherms: why should Bergmann's rule apply to lizards? *BMC Evolutionary Biology, 8,* 68.

Pincheira-Donoso, D., Scolaro, J. A., & Sura, P. (2008b). A monographic catalogue on the systematics and phylogeny of the South American iguanian lizard family Liolaemidae (Squamata, Iguania). *Zootaxa, 1800,* 1–85.

Pincheira-Donoso, D., Hodgson, D. J., Stipala, J., & Tregenza, T. (2009). A phylogenetic analysis of sex-specific evolution of ecological morphology in *Liolaemus* lizards. *Ecological Research, 24,* 1223–1231.

Pough, F. H. (1973). Lizard energetics and diet. *Ecology, 54,* 837–844.

Pough, F. H., Andrews, R. M., Cadle, J. E., Crump, M. L., Savitzky, A. H., & Wells, K. D. (2004). *Herpetology* (3rd ed.). New Jersey: Pearson, Prentice Hall.

Powell, G. L. & Russell, A. P. (2007). Life history implications for conservation and monitoring of lizards in Canada. In: C. N. L. Seburn & C. A. Bishop (eds.), *Ecology, conservation, and status of reptiles in Canada* (pp. 23–40). Herpetological Conservation 2.

Reznick, D. N. (1985). Costs of reproduction: an evaluation of the empirical evidence. *Oikos, 44*, 257–267.

Roff, D. A. (2002). *Life history evolution*. Sunderland: Sinauer Associates.

Schluter, D. (2000). *The ecology of adaptive radiation*. Oxford: Oxford University Press.

Schulte, J. A., Macey, J. R., Espinoza, R. E., & Larson, A. (2000). Phylogenetic relationships in the iguanid lizard genus *Liolaemus*: multiple origins of viviparous reproduction and evidence for recurring Andean vicariance and dispersal. *Biological Journal of the Linnean Society, 69*, 75–102.

Seigel, R. A., & Ford, N. B. (1987). Reproductive ecology. In R. A. Seigel, J. T. Collins, & S. S. Novak (Eds.), *Snakes. Ecology and evolutionary biology* (pp. 210–252). New York: Macmillan.

Shine, R. (1988). The evolution of large body size in females: a critique of Darwin's "fecundity advantage" model. *American Naturalist, 131*, 124–131.

Shine, R. (1994). Sexual size dimorphism in snakes revisited. *Copeia, 1994*, 326–346.

Shine, R. (2005). Life-history evolution in reptiles. *Annual Reviews of Ecology, Evolution and Systematics, 36*, 23–46.

Sinervo, B. (2000). Adaptation, natural selection, and optimal life-history allocation in the face of genetically based trade-offs. In T. A. Mousseau, B. Sinervo, & J. A. Endler (Eds.), *Adaptive genetic variation in the wild* (pp. 41–64). Oxford: Oxford University Press.

Smith, R. J. (1999). Statistics of sexual size dimorphism. *Journal of Human Evolution, 36*, 423–459.

Sokal, R. R., & Rohlf, F. J. (1995). *Biometry: the principles and practice of statistics in biological research*. California: W. H. Freeman.

Stearns, S. C. (1992). *The evolution of life histories*. Oxford: Oxford University Press.

Stephens, P. R., & Wiens, J. J. (2009). Evolution of sexual size dimorphisms in emydid turtles: ecological dimorphism, Rensch's rule, and sympatric divergence. *Evolution, 63*, 910–925.

Stuart-Fox, D. (2009). A test of Rensch's rule in dwarf chameleons (Bradypodion spp.), a group with female-biased sexual size dimorphism. *Evolutionary Ecology, 23*, 425–433.

Tabachnick, B. G., & Fidell, L. S. (2007). *Using multivariate statistics*. Massachusetts: Pearson International.

Thornhill, T., & Alcock, J. (1983). *The evolution of insect mating systems*. Massachusetts: Harvard University Press.

Tinkle, D. W., Wilbur, H. M., & Tilley, S. G. (1970). Evolutionary strategies in lizard reproduction. *Evolution, 24*, 55–74.

Trivers, R. (1972). Parental investment and sexual selection. In B. Campbell (Ed.), *Sexual selection and the descent of man, 1871–1971* (pp. 136–179). Chicago: Aldine.

Vanhooydonck, B., Cruz, F. B., Abdala, C. S., Moreno-Azocar, D. L., Bonino, M. F., & Herrel, A. (2010). Sex-specific evolution of bite performance in *Liolaemus* lizards (Iguania: Liolaemidae): the battle of the sexes. *Biological Journal of the Linnean Society, 101*, 461–475.

Vitt, L. J. (1986). Reproductive tactics of sympatric gekkonid lizards with a comment on the evolutionary and ecological consequences of invariant clutch size. *Copeia, 1986*, 773–786.

Weiblen, G. D., Oyama, R. K., & Donoghue, M. J. (2000). Phylogenetic analysis of dioecy in monocotyledons. *American Naturalist, 155*, 46–58.

Williams, G. C. (1966). *Adaptation and natural selection*. New Jersey: Princeton University Press.

Zamudio, K. R. (1998). The evolution of female-biased sexual size dimorphism: a population-level comparative study in horned lizards (*Phrynosoma*). *Evolution, 52*, 1821–1833.

Zar, J. H. (2009). *Biostatistical analysis*. New Jersey: Pearson International.