

Selection and constraints on offspring size-number trade-offs in sand lizards (*Lacerta agilis*)

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Abstract

The trade-off between offspring size and number is a central component of life-history theory, postulating that larger investment into offspring size inevitably decreases offspring number. This trade-off is generally discussed in terms of genetic, physiological or morphological constraints; however, as among-individual differences can mask individual trade-offs, the underlying mechanisms may be difficult to reveal. In this study, we use multivariate analyses to investigate whether there is a trade-off between offspring size and number in a population of sand lizards by separating among- and within-individual patterns using a 15-year data set collected in the wild. We also explore the ecological and evolutionary causes and consequences of this trade-off by investigating how a female's resource (condition)- vs. age-related size (snout-vent length) influences her investment into offspring size vs. number (OSN), whether these traits are heritable and under selection and whether the OSN trade-off has a genetic component. We found a negative correlation between offspring size and number within individual females and physical constraints (size of body cavity) appear to limit the number of eggs that a female can produce. This suggests that the OSN trade-off occurs due to resource constraints as a female continues to grow throughout life and, thus, produces larger clutches. In contrast to the assumptions of classic OSN theory, we did not detect selection on offspring size; however, there was directional selection for larger clutch sizes. The repeatabilities of both offspring size and number were low and we did not detect any additive genetic variance in either trait. This could be due to strong selection (past or current) on these life-history traits, or to insufficient statistical power to detect significant additive genetic effects. Overall, the findings of this study are an important illustration of how analyses of within-individual patterns can reveal trade-offs and their underlying causes, with potential evolutionary and ecological consequences that are otherwise hidden by among-individual variation.

Introduction

Life-history theory is characterized by trade-offs (Garland, 2014), such as energetic investment of limited resources into somatic maintenance vs. reproduction,

present vs. future reproduction and offspring size vs. number (OSN henceforth, Lessels, 1991; Stearns, 1992; Olsson & Shine, 1997). Specifically, OSN theory is based on the implicit assumption that offspring size is related to fitness, because larger offspring tend to have higher performance and reproductive output, and greater chances to survive (Ferguson & Fox, 1984; McGinley *et al.*, 1987; Sinervo, 1990; Einum & Fleming, 2000; Roff, 2002). Hence, if resources are unlimited, a female is expected to invest more resources per offspring to enhance her own reproductive success (Lack,

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1947; Smith & Fretwell, 1974; Brockelman, 1975; Grafen, 1988). However, under limited resources, OSN theory predicts that a mother cannot increase the size of individual offspring without a concomitant reduction in the number of offspring produced and, hence, a trade-off between these two traits will be inevitable (Smith & Fretwell, 1974; Stearns, 1992; Enum & Fleming, 2000). This reasoning is based on constraints at the physiological level; however, trade-offs can also occur at the genetic and morphological level, that is a trade-off may also be caused by a negative genetic correlation between two (or more) traits, or physical constraints due a female's body size or shape (Shine, 1992; Edward & Chapman, 2011; Ford & Seigel, 2015). As physiological and morphological trade-offs may have a genetic basis and different genotypes may differ in how they allocate resources, these explanations are not necessarily incompatible. However, only genetic trade-offs can translate into evolutionary trade-offs, and trade-offs at this level may have considerable effects on the rate and direction of evolution of the correlated traits (Stearns, 1992).

The trade-off between offspring size and number is one of the central concepts in life-history theory (Stearns, 1992). At the interspecific level, the theoretically expected negative correlation between these two traits has frequently been observed (Roff, 1992; Visman *et al.*, 1996; Christians, 2000; Walker *et al.*, 2008). However, within species, a wide range of phenotypic correlations between offspring size and number have been reported and the reason for this has been widely discussed (e.g. van Noordwijk & de Jong, 1986; Stearns, 1992; Moyes *et al.*, 2006, 2009). Variation in female 'quality' has long been recognized as a potential confounding factor, as an OSN trade-off could be masked by better 'quality' females laying both more and larger eggs, and *vice versa*. This type of quality effect is based on the assumption that differences in resource acquisition among individuals directly affect their fitness, and has often been corrected for by using maternal body size or condition as an indicator of individual quality (see Lim *et al.*, 2014; for a recent meta-analysis). Although frequently applied in studies investigating resource allocation trade-offs, this approach has received criticism (Moyes *et al.*, 2009; Wilson & Nussey, 2010; Cam *et al.*, 2013). Firstly, it is unlikely that a single trait captures the 'quality' of a female; it is more likely the result of a suite of fitness-related traits. Secondly, other factors than a female's resource-related size, such as her age-related size in species with indeterminate growth, could influence her reproductive strategy (Forslund & Pärt, 1995; Cam & Monnat, 2000; van de Pol & Verhulst, 2006; Ford & Seigel, 2015). One way to look for trade-offs, without having to consider potential confounding among-individual effects, is to analyse within-individual patterns of phenotypic covariance across measurements. This approach dates

back to Robinson's (1950) seminal paper on 'the ecological fallacy', that is drawing conclusions about a lower level (here trait associations within individuals; individual level) based on observations at a higher level (here trait associations among individuals; population level).

In this study, we use a 15-year data set on 353 female sand lizards (*Lacerta agilis*) and their 4626 offspring to investigate OSN trade-off patterns within individuals over multiple reproductive episodes in the wild. A negative relationship between clutch size and offspring size, and a positive relationship between clutch size and female size (mass), has previously been shown at the population level, using a smaller data set (5 years) on this population (Olsson & Shine, 1997). This led the authors to propose that larger females have more numerous but smaller offspring but, in contrast, they observed a positive relationship between maternal and offspring size. These conflicting findings could be due to confounded among- and within-individual effects or, as sand lizards continue to grow at a diminishing rate throughout life, entangled effects of a female's resource-related size and her age-related size. If energetic constraints were responsible for the trade-off observed in this population, increased resource availability should lead to more consistent offspring sizes. However, Olsson & Shine (1997) showed that the slope of the OSN trade-off was consistent among years of varying resource availability and when lizards were kept at *ad libitum* food availability in the laboratory, hence, suggesting a genetic basis for the relationship between these two traits.

The aim of this study was to further explore the OSN trade-off observed in this lizard population, as well as its ecological and evolutionary causes and consequences. We do this by analysing trait correlations within individual females and by addressing the following questions: (i) Is the OSN trade-off observed at the population level matched at the individual level, that is do individual females trade off offspring size and number? (ii) How does female size (resource vs. age related) influence investment into OSN? (iii) Which maternal investment trait, offspring size or number, is the primary target of selection? and (iv) Are these two maternal investment traits heritable, and does the OSN trade-off have a genetic component?

Materials and methods

The model system and study site – the Swedish sand lizard (*Lacerta agilis*)

The sand lizard (*L. agilis*) is a small ground dweller (max 20 g) with a distribution range that is approximately 8000 × 5000 km, one of the largest of any reptile (Bischoff, 1984). Our study population at

Asketunnan is situated approximately 50 km south of Gothenburg on the Swedish west coast (latitude 57°22', longitude 11°59'). Sand lizards grow at a diminishing rate through life; hence, body size and age are positively correlated (Olsson & Shine, 1996). Maturation is reached at an age of 2–3 years and, in Sweden, females lay a single annual clutch of 5–15 eggs. Each year (1987–1991 and 1998–2007), we followed the same field and laboratory protocols, which have previously been described in detail (e.g. Olsson, 1994; Olsson & Shine, 1997; Olsson *et al.*, 2000, 2011a,b; Ljungström *et al.*, 2015). In brief, lizards of known identity were monitored at our Asketunnan study site on every day that permitted lizard activity through April, May and early June. Asketunnan is a rocky peninsula approximately 500 × 400 m which during the study period contained a stable population of approximately 150–200 adult lizards which were easily monitored and tracked. In early June, females were brought to laboratory facilities at University of Gothenburg approximately 1 week before oviposition (which is obvious from egg contours visible on the sides of the body). Females were kept individually in cages (40 × 50 × 60 cm) with a spotlight at one end of the cage to allow thermoregulation and a flat rock with a wet patch of sand underneath to direct egg laying. Eggs were collected within hours of laying and incubated at 25 °C, the temperature with the highest hatching success and the least developmental asymmetries (Zakharov, 1989). After approximately 40 days, the eggs hatched at this temperature and the hatchlings were marked, measured and released at random sites at Asketunnan. Offspring survival was assessed annually as per our previous work (e.g. Olsson & Madsen, 2001; Ryberg *et al.*, 2004). We have shown before that by searching a 600-m corridor around Asketunnan (a peninsula), a distance five times the maximum recorded annual dispersal distance for an offspring in this population (Olsson *et al.*, 1996; Ryberg *et al.*, 2004), we remove the risk of having dispersal confound our estimates of mortality/survival.

Climatic data were purchased from the Swedish Bureau of Meteorology and Hydrology (SMHI) using data from the Varberg weather station (closest available to our field site, in the same coastal position, situated approximately 50 km south of Asketunnan). Varberg and Asketunnan are situated right on the coast, which is the main factor dictating cloud cover and rain fall, and thus basking opportunities for lizards in this area (Olsson & Shine, 1996; Olsson *et al.*, 2011a; Ljungström *et al.*, 2015). In order to estimate the environmental conditions the offspring experienced after release and prior to hibernation (the crucial period affecting survival), we calculated annual grand means of mean temperatures recorded per day for August–September to represent the activity period before hatchlings enter into hibernation.

Paternity analysis

For the years 1998–2007, paternity was confirmed using microsatellites. DNA was isolated from adult and offspring samples (blood and tissue) and was genotyped using 21 microsatellite loci (see detailed information on the genotyping methods in Olsson *et al.*, 2011b). Because of the low level of genetic variability in this population (Gullberg *et al.*, 1997), and the overlap of generations, it was necessary to use 17–21 microsatellite loci to assign paternity with high confidence. All of the adults and the offspring from years 2001 to 2007 were genotyped at 21 loci. The offspring from years 1998 to 2000 were genotyped with 17 loci except for offspring for which paternity between the two top candidate males could not be determined with 95% confidence; these offspring were genotyped at all 21 loci. Paternity analyses were conducted in Cervus 3.0 (Kalinowski *et al.*, 2007) for the 10 years of data 1998–2007.

A 4-year sliding window of adult genotypes was used to calculate the population allele frequencies for each year of offspring analysed. Genotyping error rate in the final data set is estimated to be < 1% based on mother–offspring comparisons, repeated genotyping of the same individuals collected in multiple years and from 30% of the data being independently scored by a second researcher. Paternity was assigned based on two simulation analyses, complete exclusion and 1% error rate. Candidate sires included all adult males sampled in previous years, the year the offspring were born and the subsequent year. Confidence levels of Delta (the difference between the LOD scores of the first and second most likely candidates) were set at 95% and 80%. The paternity assignment was classified using three ‘confidence codes’ based on the type of data that supported the assignment: (i) 95% confidence level of the mother–offspring–father Delta based on either the zero error rate or the 1% error rate simulation, (ii) eighty per cent confidence level of the mother–offspring–father Delta and additional evidence of partnership from field observations (witnessing copulations or mate guarding between the assigned sire and the mother) and (iii) eighty per cent confidence level of the mother–offspring–father Delta and evidence of the male contributing to the clutch due to siblings being independently assigned the same father with at least 80% confidence. If paternity could not be assigned based on these confidence codes, the individual was eliminated from subsequent analyses.

Because we have multigenerational data, many of the field-born adults that were sampled each year are likely to be offspring from adults sampled the same or previous years. Thus, parentage analyses were conducted on all field-born adults to identify potential mother–father pairs. Parentage analyses were conducted as based on a simulation of 1000 offspring, a zero error rate (complete exclusion) and assuming 85%

of candidate mothers and fathers sampled. All adults in the population were assumed to be potential parents. Parents identified with 95% confidence level of Delta were cross-checked with all available data on age, size and field location of the potential parents relative to the individual of interest to ensure the identified parental pairings were possible. We had high confidence in our ability to assign a parent if the parent had been sampled. Across years, the average probability of nonexclusion of a candidate parent if neither parent was known was 0.006, and the average probability of nonexclusion of a candidate parent if one parent was known was 5.87×10^{-05} . On average, 89% of the assignments had both a 95% confidence level of Delta in Cervus and additional evidence from either field observations or clutch information supporting the paternity assignment.

Statistical analyses

OSN patterns and female size effects among and within individuals

A female's mass is a composite of two components, her condition (usually expressed as a residual of body mass on some linear measure of body size) and her size due to continuous growth throughout life (snout-vent length), which may have separate effects on offspring size and number. Hence, to assess the relationship between offspring size and number among and within individuals, and to investigate the separate effects of the two components of a female's mass on these traits, we fitted a restricted maximum likelihood (REML) multivariate mixed model with clutch size, mean offspring mass (hereafter also referred to as offspring size), female condition (annual residuals of female mass after oviposition on snout-vent length) and snout-vent length (hereafter referred to as body size) as dependent variables. To model the among-individual effect and account for interannual variation, female ID (id) and year of breeding (year) were included as random effects. In summary, the following multivariate mixed model for the h th dependent variable of individual i in year j was applied:

$$Y_{hij} = u_h + \text{year}_{hj} + \text{id}_{hi} + e_{hi}, \quad (\text{Model 1})$$

where u_h is the population mean for each dependent variable, year_{hj} is the random effect of breeding year j for dependent variable h , id_{hi} is the random effect of female i for dependent variable h , and e_{hi} represents within-individual residuals. The random effect female ID id_{hi} and the within-individual residuals e_{hi} were modelled with unstructured covariance matrices to yield the among- and within-individual variances in all traits, as well as their among- and within-individual covariances. Year of breeding year_{hj} was modelled with a diagonal covariance matrix (level-specific variances and zero covariances) as the annual variances can be

assumed to be uncorrelated. To test whether total reproductive investment changed with age-related size, we also ran Model 1 with snout-vent length and total clutch mass as dependent variables. The significance of each covariance was tested by comparing the likelihood of the full model with the likelihood from a model in which the covariance was fixed at zero. The likelihoods were compared using an likelihood ratio test (LRT) with a single degree of freedom (Pinheiro & Bates, 2000).

The analyses described in this section were performed in ASReml 2.0 (VSN International; Gilmour *et al.*, 2006) and included 353 females with 561 clutches and 4626 individual offspring over 15 years (1987–1991 and 1998–2007). There were 133 females that bred in multiple years (85 bred twice, 30 bred in 3 years, 10 in 4 years, 7 in 5 years and 1 in 6 years) and 220 females bred once. Only females with at least two breeding attempts have within-individual residuals and hence contribute to estimation of trait covariation within individuals.

Determinants of offspring recruitment – selection on offspring size and number

To investigate which maternal investment trait, offspring size or number, is the primary target of selection, we ran a selection analysis by running a univariate mixed model using a female's seasonal number of recruits (REC) as response variable, and clutch size (CSIZE) and mean offspring mass (MOFFMASS) as predictors. Offspring recruitment may not only be determined by offspring size or number, but also by maternal body size and condition, timing of hatching and weather conditions late in the season (Adolph & Porter, 1993; Olsson & Shine, 1997). Hence, to control for potential effects of these variables on recruitment, female mass (FMASS) after oviposition (combined variable of body size and condition), oviposition date (LDAY; expressed in Julian days since 1 January) and annual grand means of mean temperatures recorded per day for August–September (STEMP; hereafter referred to as 'late summer temperature') were included as covariates in the model. Furthermore, to account for multiple records per year and per female, year of breeding (YEAR) was fitted as a covariate and female ID (id) as a random effect. To test for differences in the effects of mean offspring mass and clutch size on recruitment among years of breeding, we also fitted the interactions between these variables. Thus, we used the following mixed model for recruitment of individual i in year j :

$$\begin{aligned} \text{REC}_{ij} = & u + \text{CSIZE}_{ij} + \text{MOFFMASS}_{ij} + \text{YEAR}_j \\ & + \text{CSIZE}_{ij} * \text{YEAR}_j + \text{MOFFMASS}_{ij} * \text{YEAR}_j \\ & + \text{FMASS}_{ij} + \text{LDAY}_{ij} + \text{STEMP}_j + \text{id}_i + e_i, \end{aligned} \quad (\text{Model 2})$$

where u is the population mean and fixed and random effects are denoted in upper and lower case letters, respectively. Collinearity among the explanatory variables was investigated using variance inflation factors (VIFs). Following the approach by Zuur *et al.* (2010), we used a stringent VIF value of 3 as threshold for identifying collinearity issues. The VIFs for the continuous explanatory variables lay below this threshold. For the only categorical variable, year of breeding, all VIFs lay below 4, which is well below the value 10 generally used as a rule of thumb for identifying collinearity (see O'Brien, 2007; VIFs are displayed in Table S1). Based on these findings, collinearity among the explanatory variables is unlikely to affect the results of the selection analysis. Nonsignificant fixed effects were sequentially dropped from the model until remaining terms were significant ($\alpha = 0.05$) (but retained if their interaction was significant). All parameter estimates were solved for using REML algorithms implemented in SAS 9.3 (SAS Institute, Cary, NC, USA). Statistical significance of fixed effects was determined using Kenward-Roger F statistics and random effects were assessed LRTs, testing the difference in the $-2 \log$ likelihood between hierarchical models against a chi-square distribution with number degrees of freedom equal to the difference in number of estimated terms (Pinheiro & Bates, 2000).

The selection analysis was based on 3945 offspring from 465 clutches and 303 females over 12 years (1989–1991 and 1998–2006). Selection analysis using recruits as the female fitness measure has been criticized because it straddles two generations and, hence, the female's fitness is not independent of the genetic contribution from the male affecting offspring survival (e.g. Lande & Arnold, 1983; Arnold & Wade, 1984a,b; Wolf *et al.*, 2004). However, size-dependent offspring survival is at the very heart of OSN theory and is assumed to dictate selection on offspring size, with concomitant shifts in clutch size, which was our rationale for adopting this approach.

Heritability of offspring size, number and the basis of their trade-off

We used univariate animal models to investigate whether offspring size and number are heritable in this population. Animal models incorporate pedigree information into linear mixed models to estimate the additive genetic variance of the trait of interest in the population under study (Lynch & Walsh, 1998; Kruuk, 2004). The heritability analyses were based on 10 years (1998–2007) of data and the pedigree was formed by 3651 identities over three generations, incorporating 254 fathers and 271 mothers. Among the 449 clutches from 277 females included in the animal model for clutch size, there were 69 belonging to females with at least one known relative (i.e. with known mother) and 19 of these had more than one observation (11, 5, 2

and 1 female had 2, 3, 4 and 5 observations, respectively). In the analysis of mean offspring mass, 422 clutches from 264 females were included of which 65 belonged to females with at least one known relative and 17 of these had been observed more than once (10, 6 and 1 had 2, 3 and 5 observations, respectively). The rest of the clutches included in the analyses did not belong to females with known relatives, but they contribute to estimating the total phenotypic variance of clutch size vs. mean offspring mass more accurately.

Our univariate animal models with either mean offspring mass or clutch size as dependent variable had the following structure, representing the response of individual i in year j :

$$Y_{ij} = u + \text{FIXED}_{ij} + a_i + \text{pe}_i + \text{year}_j + e_i. \quad (\text{Model 3})$$

In this model, a_i is the additive genetic value of the individual and pe_i is its permanent environmental effect. This effect is estimated using repeated measures and includes sources of variation that lead to permanent differences among individuals, apart from those due to additive genetic effects (Kruuk, 2004). Random variation due to year of breeding year_j was also modelled, while controlling for the fixed effect FIXED_{ij} of maternal condition on mean offspring mass and snout-vent length on clutch size (as significant correlations between these traits were identified in multivariate analyses described above). LRTs were used to test significance of random factors, and the animal models were run in ASReml 2.0 (VSN International; Gilmour *et al.*, 2006).

The upper limit to heritability is generally considered to be set by the repeatability of a trait (Falconer & Mackay, 1996), although there are some situations in which this need not hold true (see Dohm, 2002). Hence, to assess the upper limit to heritability in offspring mass and clutch size, we also ran models substituting a_i and pe_i for the total individual phenotypic value $\text{ind}_i = a_i + \text{pe}_i$ and calculated repeatability as the ratio of among-individual phenotypic variance to the total variance [i.e. as $V_{\text{ind}}/(V_{\text{ind}} + V_{\text{year}} + V_e)$]. In addition, we tried to fit a bivariate animal model to investigate whether there is a genetic correlation, and hence trade-off, between these two traits. Unfortunately, this model failed to converge when attempting to fit the individual-specific permanent environment effect. Hence, as omitting this effect when repeated measures are present may upwardly bias the estimate of additive genetic variance (Kruuk & Hadfield, 2007), this model is not shown.

Results

OSN patterns and female size effects among and within individuals

Both among and within individuals, offspring size and number were negatively correlated (female ID, $\chi^2 = 4.9$,

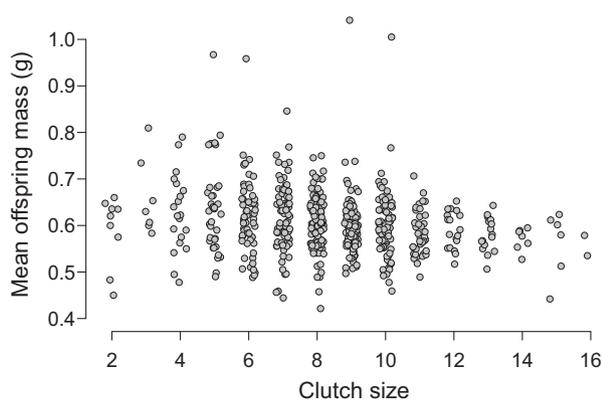


Fig. 1 Relationship between mean offspring mass and clutch size. Descriptive plot of mean offspring mass of individual sand lizard (*Lacerta agilis*) females vs. clutch size for the years 1987–1991 and 1998–2007 ($n_{\text{clutches}} = 561$, $n_{\text{females}} = 353$).

$P = 0.0272$, covariance \pm SE = -0.0175 ± 0.0082 , $r = -0.29$; residual, $\chi^2 = 11.2$, $P = 0.0008$, covariance \pm SE = -0.0209 ± 0.0067 , $r = -0.21$), meaning that females with a higher average clutch size have a lower average offspring size and *vice versa*, and that when a female has a larger clutch relative to her own mean she also has smaller offspring. The relationship between offspring size and number is visualized in Fig. 1, which shows a descriptive plot of mean offspring mass of individual females vs. their clutch size.

Investigating the separate effects of the two components of a female's mass, condition and snout-vent length, there was a positive correlation between condition and mean offspring mass within individuals ($\chi^2 = 10.46$, $P = 0.0012$, covariance \pm SE = 0.0121 ± 0.0042 , $r = 0.20$), but not among them ($\chi^2 = 0.05$, $P = 0.8162$). In contrast, condition had no effect on clutch size at either level (female ID, $\chi^2 = 0.04$, $P = 0.8414$; residual, $\chi^2 = 1.82$, $P = 0.1773$). Body size had no effect on mean offspring mass (female ID, $\chi^2 = 0.61$, $P = 0.4355$; residual, $\chi^2 = 2.26$, $P = 0.1327$), but was strongly positively correlated with clutch size among and within individuals (female ID, $\chi^2 = 55.5$, $P < 0.0001$, covariance \pm SE = 5.0720 ± 0.8115 ,

$r = 0.80$; residual, $\chi^2 = 58.06$, $P < 0.0001$, covariance \pm SE = 3.1469 ± 0.5245 , $r = 0.45$). Table 1 displays the within-individual covariance components for all the predictors of this analysis, and among-individual variances and covariances are shown in Table S2.

Overall, these results indicate that the positive effect of female mass on both offspring size and number, previously shown for this population (Olsson & Shine, 1997), is driven by a positive within-individual correlation between female condition and mean offspring mass and a positive correlation between female body size and clutch size at both levels. Furthermore, there was a positive relationship between body size and total clutch mass at both levels (female ID, $\chi^2 = 71.1$, $P < 0.0001$, covariance \pm SE = 4.2890 ± 0.5672 , $r = 0.92$; residual, $\chi^2 = 87.9$, $P < 0.0001$, covariance \pm SE = 1.7610 ± 0.2869 , $r = 0.50$, Table 1), indicating that total reproductive investment increases with age-related size.

Determinants of offspring recruitment – selection on offspring size and number

Running the full recruitment model with all predictors and covariates, the effect of late summer temperature could not be predicted, and the two interaction terms between offspring size and clutch size with year of breeding were not significant ($F_{11,427} = 0.68$, $P = 0.7591$ and $F_{11,438} = 0.97$, $P = 0.4736$, respectively); hence, these variables were sequentially dropped from the model. The results from the full recruitment model are displayed in Table S3. As the model no longer included any interactions with breeding year, and we were not interested in the effects of particular years, only to account for multiple records per year, year of breeding was fitted as a random factor in the proceeding analyses. This reduces the number of degrees of freedom used up at each level of the fixed effect and, hence, enhances statistical power. Running this reduced model, with year of breeding as a random factor, mean offspring mass and female mass were not significant predictors of offspring recruitment (mean offspring mass, $F_{1,459} = 1.10$, $P = 0.2952$; female mass, $F_{1,400} = 1.51$, $P = 0.2197$; see Table S4, for initial results from this model).

Table 1 Phenotypic matrix with among-individual variances along the diagonal, within-individual covariances in the lower triangle and their respective correlations in the upper triangle.

	M. offspring mass	Clutch size	Snout-vent length	Condition	T. clutch mass
M. offspring mass	–	–0.21	0.05	0.20	–
Clutch size	$-0.0209 \pm 0.0067^{**}$	–	0.45	0.01	–
Snout-vent length	0.0099 ± 0.0139	$3.1469 \pm 0.5245^{**}$	–	0.08	0.50
Condition	$0.0121 \pm 0.0042^{**}$	0.0254 ± 0.1409	0.3495 ± 0.2937	–	–
T. clutch mass	–	–	$1.7610 \pm 0.2869^{**}$	–	–

M. offspring mass = mean offspring mass, T. clutch mass = total clutch mass.

The covariance estimates are obtained from a multivariate mixed model (Model 1; $P < 0.05$; $**P < 0.01$).

When nonsignificant factors had been removed, the final model revealed that seasonal recruitment of offspring was positively affected by clutch size ($F_{1,445} = 5.56$, $P = 0.0179$, parameter estimate \pm SE = 0.0435 ± 0.0183) and early egg laying ($F_{1,208} = 16.71$, $P < 0.0001$, parameter estimate \pm SE = -0.0265 ± 0.0065). Furthermore, year of breeding explained a significant amount of the variation in recruited young left unexplained by these fixed effects ($\chi^2 = 22.3$, $P < 0.0001$, variance \pm SE = 0.0875 ± 0.0483). These results indicate that there is selection for clutch size and oviposition date in this population, but not for offspring mass. Visual inspection of our smoothing spline grid plot (Fig. 2) supports this result and shows that the trajectory for offspring recruitment on clutch size (XY, Fig. 2) was steeper compared to the mean offspring mass trajectory (ZY, Fig. 2). Normality of the predictors was assessed from graphics plots produced with Proc Univariate in SAS 9.3. All predictors showed mid-peak unimodal distribution with roughly equal tails and hence fulfilled the assumption of normality.

Heritability of offspring size, number and the basis of their trade-off

Running the animal model, there were significant phenotypic differences among females in both offspring size and number; however, no additive genetic basis for this variation was found (Table 2). Permanent environment effects explained a significant amount of the among-individual variation in mean offspring mass, after controlling for condition, but had no effect on clutch size (mean offspring mass, $\chi^2 = 4.64$, $P = 0.0312$; clutch size, $\chi^2 = 0.18$, $P = 0.6713$; Table 2). Furthermore, year of breeding contributed to the unexplained variation in both traits (clutch size, $\chi^2 = 8.84$, $P = 0.0029$; mean off-

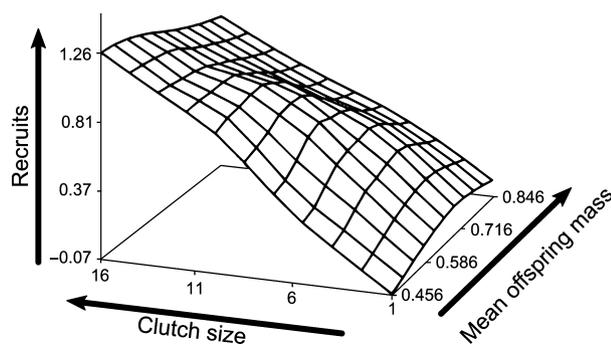


Fig. 2 Selection surface for mean offspring mass and clutch size. g3 grid plot with a smoothing spline curve fit over recruitment success (y-axis), clutch size (x-axis) and mean offspring mass (z-axis). The g3 grid was created with a joining function in SAS 9.3 with a smoothing parameter of 2.0.

spring mass, $\chi^2 = 17.37$, $P < 0.0001$; Table 2). Investigating the results from these analyses, presented in Table 2, permanent differences among individuals explained the majority of the phenotypic variation in offspring size, whereas additive genetic effects appear to explain most of the among-individual variation in clutch size. However, as only individuals with repeat measures that have at least one link in the pedigree can be used to separate additive and permanent environment effects, it is unclear whether there was sufficient power to separate these effects. The repeatabilities of mean offspring mass and clutch size were 0.34 ± 0.06 and 0.21 ± 0.06 , respectively.

Discussion

A trade-off between the two maternal investment traits, offspring size and number, has been commonly identified at the species level. However, at the intraspecific level, a whole range of phenotypic correlations between these two traits have been observed in terms of sign and magnitude. In this study, we attempted to unravel if, how and why offspring size and number are traded off in a population of sand lizards by separating among- and within-individual patterns using a 15-year data set collected in the wild.

OSN patterns and female size effects among and within individuals

In agreement with the findings of a previous study on this population, using a smaller 5-year data set to investigate population-level effects (Olsson & Shine, 1997), we found a negative correlation between offspring size and number both among and within individual sand lizard females. The negative within-individual correlation between these two traits is indicative of a trade-off and implies that females in this population do not produce a consistent clutch size or offspring size across reproductive events. An interesting question is therefore what causes a change in the relationship between these two traits? Olsson & Shine (1997) predicted the trade-off to be due to heavier females laying more but smaller eggs. In contrast, they found a positive population-level relationship between female mass and both offspring size and number. Here, we explore these results further by analysing how the components that make up a female's mass, her condition (resource-related size) and size due to continuous growth throughout life (age-related size; snout-vent length) separately affect the sizes of offspring and clutches.

Breaking down female mass into its two components, we found that female body size was only related to clutch size, whereas female condition was only related to offspring mass, such that when females were larger or in better condition relative to their own mean, they

Table 2 Quantitative genetics of mean offspring mass and clutch size in female sand lizards from univariate animal models (mean offspring mass, $n_{\text{clutches}} = 422$, $n_{\text{females}} = 264$; clutch size, $n_{\text{clutches}} = 449$, $n_{\text{females}} = 277$). The results are obtained from Model 3.

Variable	Repeatability	Estimate \pm SE				P
Mean offspring mass	0.3414 \pm 0.0640					
Fixed effects			d.f. (nom)	d.f. (den)	Wald F	
Condition		0.0091 \pm 0.0020	1	408.8	21.61	< 0.0001
Random effects			χ^2	d.f.		
V_{ind}		0.1301 \times e⁻² \pm 0.2835 \times e⁻³	33.68	1		< 0.0001
V _A		0.3080 \times e ⁻⁴ \pm 0.6160 \times e ⁻³	0.00	1		n.e.
V_{PE}		0.1271 \times e⁻² \pm 0.6261 \times e⁻³	4.64	1		0.0312
V_Y		0.2653 \times e⁻³ \pm 0.1608 \times e⁻³	17.37	1		< 0.0001
V _R		0.0022				
Clutch size	0.2166 \pm 0.0625					
Fixed effects			d.f. (nom)	d.f. (den)	Wald F	
SVL		0.2780 \pm 0.0136	1	408.8	21.61	< 0.0001
Random effects			χ^2	d.f.		
V_{ind}		0.6407 \pm 0.1977	15.66	1		< 0.0001
V _A		0.4663 \pm 0.4317	1.3	1		0.2542
V _{PE}		0.1793 \pm 0.4374	0.18	1		0.6713
V_Y		0.1397 \pm 0.0963	8.84	1		0.0029
V _R		2.1698				

n.e., nonestimable.

Significant terms are highlighted in bold.

produced more or larger offspring, respectively. These findings suggest that physical constraints (size of body cavity) limit the number of eggs that a female can produce and that the OSN trade-off appears due to resource constraints as a female continues to grow throughout life and, hence, produces more eggs. Our finding that being in better condition allows a female to have heavier offspring supports this reasoning, but this effect is apparently not strong enough to override the OSN trade-off. Hence, there is a possibility that clutch size and offspring mass are negatively genetically correlated, which in turn may have considerable effects on the rate and direction of evolution of these two traits.

To obtain an index for female body condition, we separated effects of female structural body size from the size associated with energy reserves using the residuals from a linear regression of body mass on snout-vent length (Jakob *et al.*, 1996; Hayes & Shonkwiler, 2001). Although this is one of the most common methods of measuring body condition in vertebrate taxa, it has been the subject of controversy (e.g. Green, 2001; Hayes & Shonkwiler, 2006; Peig & Green, 2009; but see Ardia, 2005; and Schulte-Hostedde *et al.*, 2005). We chose this approach as our data set lacked information on actual energy reserves/body composition of individual females, and hence our index of body condition reflects not only fat reserves, but also protein and water stores. As protein can be catabolized to meet energy demands during periods of low food resources, this index is, however, likely to reflect interindividual differences in condition. In agreement with our findings, an increase in clutch size as a simple consequence of a female's physical limitation has previously been

demonstrated in both lizards and snakes (Shine, 1992; in den Bosch & Bout, 1998; Kratochvil & Frynta, 2006; Pizzatto *et al.*, 2007; Ford & Seigel, 2015) and so has positive effects of maternal body condition on offspring size (e.g. Madsen & Shine, 1996; Brown & Shine, 2007). Classic OSN theory assumes that a female can increase her relative fitness by investing more resources into her eggs when it is theoretically possible. This leads us to question why larger/older females in this population invest in more eggs when this appears to lead to smaller offspring, that is why is clutch size not constrained to the advantage of larger offspring?

Determinants of offspring recruitment – selection on offspring size and number

In our long-term data set, the relative importance of clutch size for maternal recruitment was still significant when phenology (i.e. oviposition date) was taken into account. However, in contrast to classic OSN assumptions (Lack, 1947; Smith & Fretwell, 1974; Brockelman, 1975; Grafen, 1988), offspring size did not predict recruitment rate. Several environmental factors have been shown to induce selection on offspring size, including intra- and interspecific competition, predation, food availability and abiotic stress (Marshall & Keogh, 2008). In this study population, intra- and interspecific competition is weak, food resources are commonly abundant, and predation is most likely size invariant [any small or large lizard hatchling (range approximately 0.7–1.2 g) is an easy target for predators]. Furthermore, the study site is exposed to high interannual variability in local weather conditions,

which presumably leads to variable selection pressures on body size among years, and therefore no single offspring size is likely to be optimal across time. This argument is supported by previous findings for this population, showing variation in size-dependent offspring survival among years of varying potential for resource acquisition (Olsson & Madsen, 2001). Hence, offspring size is likely to have little contemporary effect on offspring fitness in this population, which has also been reported for other reptile populations (Sinervo *et al.*, 1992; Congdon *et al.*, 1999; Husak, 2006; Warner & Shine, 2007; Uller *et al.*, 2011).

Unexpectedly, these findings suggest that females are not disadvantaged by having smaller offspring. So, why do smaller/younger individuals have relatively larger eggs and why do females invest relatively more resources into their offspring when they are in better condition when this does not benefit their fitness? Selection for larger offspring early in life could answer the first question; however, as annual recruitment was used as proxy for fitness, our observations of no selection on offspring size and directional selection on clutch size are invariant of a female's age. Hence, the most parsimonious answer to these questions is that the number of eggs produced is constrained by body size and investment into offspring size is just a consequence of resource deposition into a set number of eggs. Theoretically, this suggests that smaller/younger females could attain higher fitness by trading in one large for two smaller eggs. However, as there is likely to be a set minimal offspring size below which survival is not possible, these females may not have a large enough body cavity to produce another viable egg. This is supported by our finding that although there is a trade-off between mean offspring mass and clutch size, total clutch mass is positively correlated with snout-vent length, hence, indicating that a female's body size sets a limit to her total reproductive investment. These findings correspond well with earlier arguments by Bernardo (1996) that question the focus on offspring fitness in evolutionary studies of offspring size, and highlight the importance of a mother's ecological context in shaping the evolution of offspring size phenotypes.

Historical evolutionary inertia, when a species has spent most of its evolutionary history outside of its current distribution range, may also provide some answers. In this case, the Swedish sand lizard dispersed from continental Europe to Scandinavia via land bridges submerged around 9000 BP (Gullberg *et al.*, 1998), and previous work by us on other aspects of evolutionary divergence between Swedish and continental populations shows outbreeding effects at laboratory crossings *in lieu* of population divergence and local adaptation arguments (Olsson *et al.*, 2004, 2005). Hence, in their previous environment, offspring size may have had an effect on recruitment and, hence, smaller females may have been able to compensate for a smaller clutch size

by producing larger more viable young. As the lizards in this population have no parental care and are subject to high predation pressure, we have focused our study on only fecundity selection. However, as offspring size and number could potentially affect the survival of a mother, this may not tell the full story.

In addition to clutch size, we found interannual variability in weather conditions during the breeding season to be a strong determinant of offspring recruitment. Spring temperature has previously been shown to affect the timing of oviposition (Olsson *et al.*, 2011a,b; Ljungström *et al.*, 2015) and here we found earlier clutches to have the highest recruitment rate, as in a number of other lizard taxa (e.g. Warner & Shine, 2007; Wapstra *et al.*, 2010; Le Henanff *et al.*, 2013). One plausible explanation is that early-born offspring have longer time to accumulate reserves before autumn and therefore survive winter better. This idea runs counter to findings by Adolph & Porter (1993), showing that the strongest negative effect on offspring survival is activity and, as a corollary, exposure to predators. However, in this study we found no effect of late season temperature, and hence activity, on offspring recruitment. Thus, early clutches appear to be favoured by a greater opportunity for resource accumulation.

Heritability of offspring size, number and their trade-off

To summarize the findings from the previous sections, female lizards in this population displayed a trade-off between offspring size and number and there was directional selection on clutch size but not on offspring size. Evolution towards larger clutches is determined by two things: the amount of additive genetic variation in clutch size and, if the trade-off is genetically determined, the amount of additive genetic variation in mean offspring mass. With a genetic basis for the trade-off and sufficient additive genetic variation in both traits, we would expect clutch size to increase over evolutionary time, with a concomitant decrease in offspring size limited by the minimal size for offspring survival.

The results from our heritability analyses showed significant phenotypic variation among females in both investment traits; however, no additive genetic component of this variation could be detected. However, effects that lead to permanent differences among individuals explained the majority of the variation in mean offspring mass, which suggests that offspring size is not only determined by a female's clutch size and condition in a particular year, but that other effects, such as a female's own birth year, mother or clutch size, also impose fixed differences among individuals. Although we did not detect a significant additive genetic or permanent environment component of variance in clutch size, our results indicate that genetic effects are respon-

sible for most of the among-individual variation observed in this trait. These findings support our previous discussion, that is that clutch size is a less flexible trait set by the space available in a female's body cavity due to age-related growth and that offspring size is more variable and is influenced by a female's resources, as set by current or past conditions. These observations also correspond well with theoretical predictions of optimal offspring size and number in relation to female size (Hendry *et al.*, 2001).

A lack of additive genetic variation in offspring size and number and low repeatabilities (and hence low upper limits to heritability) could have two possible explanations: (i) strong selection on these life-history traits has led to low levels of additive genetic variation and (ii) our analyses do not have sufficient statistical power to detect significant additive genetic effects. Furthermore, a lack of statistical power (due to a low number of individuals with repeat measures that have at least one link in the pedigree) may also have affected our ability to separate additive genetic and permanent environment effects. Although we did not detect selection on offspring size in this study, the first point could be the result of selection on this trait in the evolutionary past, as discussed in the section above. Unfortunately, we were unable to estimate genetic and permanent environment components of the observed phenotypic OSN trade-off. However, our finding that the trade-off remains even when a female is in good condition and invests more resources into offspring size may indicate that this trade-off has a genetic component. Only two other studies have examined the genetic basis for an observed OSN trade-off in reptiles [lizards (Sinervo & Doughty, 1996) and snakes (Brown & Shine, 2007)], both of which indicate the existence of a negative genetic correlation between these two traits. As a genetic basis for the trade-off observed in this population could not be established, our current findings suggest that an evolutionary response to selection on clutch size may be constrained by low additive genetic variation in this trait.

Conclusions

Using a long-term data set on sand lizards collected in the wild, we found that females in this population do not produce a consistent clutch size or offspring size across reproductive events, but these traits experience a classic OSN trade-off. Furthermore, our findings suggest that the number of eggs produced is limited by a female's age-related size and, hence, that the OSN trade-off appears due to resource constraints as a female continues to grow throughout life and, hence, has larger clutches. In contrast to classic OSN predictions, directional selection on offspring size was not detected. Yet, smaller/younger females produced relatively larger offspring and higher resource availability

also lead to larger young. This suggests that investment into offspring size is just a consequence of resource deposition into a set number of eggs, or possibly a result of historical evolutionary inertia. We were unable to determine whether the observed OSN trade-off had a genetic basis; thus, our current findings suggest that an evolutionary response to selection on clutch size may be constrained by low additive genetic variation in this trait. The findings of this study are an important illustration of how analyses of within-individual patterns can reveal trade-offs and their underlying causes, with potential evolutionary and ecological consequences that are otherwise hidden by among-individual variation.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Variance inflation factors (VIF) for the predictors in the recruitment model (Model 2).

Table S2 Phenotypic matrix with among-individual variances and covariances (Model 1).

Table S3 Results from the full recruitment model (Model 2).

Table S4 Initial results from the reduced recruitment model (Model 2).

Data deposited at Dryad: doi: 10.5061/dryad.700jq

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