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.

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

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15 Size-number trade-off in sand lizards

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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 dataset 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) *versus* age-related size (snout-vent length) influences her investment into offspring size *versus* 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,

45   with potential evolutionary and ecological consequences that are otherwise hidden by among-

46   individual variation.

47

Introduction

Life history theory is characterized by trade-offs (Garland, 2014), such as energetic investment of limited resources into somatic maintenance *versus* reproduction, present- *versus* future reproduction and offspring-size *versus* 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, 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; Einum & Fleming, 2000). This reasoning is based on constraints at the physiological level, however, trade-offs can also occur at the genetic and morphological level, i.e., 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).

72 The trade-off between offspring size and number is one of the central concepts in life history

73 theory (Stearns, 1992). At the interspecific level the theoretically expected negative correlation

74 between these two traits has frequently been observed (Roff, 1992; Visman *et al*., 1996;

75 Christians, 2000; Walker *et al*., 2008). However, within species, a wide range of phenotypic

76 correlations between offspring size and number have been reported and the reason for this has

77 been widely discussed (e.g., van Noordwijk & de Jong, 1986; Stearns, 1992; Moyes *et al*., 2006,

78 2009). Variation in female "quality" has long been recognized as a potential confounding factor,

79 as an OSN trade-off could be masked by better "quality" females laying both more and larger

80 eggs, and *vice versa*. This type of quality effect is based on the assumption that differences in

81 resource acquisition among individuals directly affect their fitness, and has often been corrected

82 for by using maternal body size or condition as an indicator of individual quality (see Lim *et al*.,

83 2014 for a recent meta-analysis). Although frequently applied in studies investigating resource

84 allocation trade-offs, this approach has received criticism (Moyes *et al*., 2009; Wilson & Nussey,

85 2010; Cam *et al*., 2013). Firstly, it is unlikely that a single trait captures the "quality" of a female;

86 it is more likely the result of a suite of fitness-related traits. Secondly, other factors than a

87 female's resource-related size, such as her age-related size in species with indeterminate growth,

88 could influence her reproductive strategy (Forslund & Pärt, 1995; Cam & Monnat, 2000; van de

89 Pol & Verhulst, 2006; Ford & Siegel, 2015). One way to look for trade-offs, without having to

90 consider potential confounding among-individual effects, is to analyse within-individual patterns

91 of phenotypic covariance across measurements. This approach dates back to Robinson's (1950)

92 seminal paper on "the ecological fallacy", i.e. drawing conclusions about a lower level (here trait

93 associations within individuals; individual-level) based on observations at a higher level (here

94 trait associations among individuals; population-level).

95

96   In this study we use a 15-year dataset on 353 female sand lizards (*Lacerta agilis*) and their 4626

97   offspring to investigate OSN trade-off patterns within individuals over multiple reproductive

98   episodes in the wild. A negative relationship between clutch size and offspring size, and a

99   positive relationship between clutch size and female size (mass), has previously been shown at

100   the population level, using a smaller data set (5 years) on this population (Olsson & Shine, 1997).

101   This led the authors to propose that larger females have more numerous but smaller offspring but,

102   in contrast, they observed a positive relationship between maternal and offspring size. These

103   conflicting findings could be due to confounded among- and within-individual effects or, as sand

104   lizards continue to grow at a diminishing rate throughout life, entangled effects of a female's

105   resource-related size and her age-related size. If energetic constraints are responsible for the

106   trade-off observed in this population, increased resource availability should lead to more

107   consistent offspring sizes. However, Olsson and Shine (1997) showed that the slope of the OSN

108   trade-off was consistent among years of varying resource availability, and when lizards were kept

109   at *ad libitum* food availability in the laboratory, hence, suggesting a genetic basis for the

110   relationship between these two traits.

111

112   The aim of this study is to further explore the OSN trade-off observed in this lizard population

113   and its ecological and evolutionary causes and consequences. We do this by analysing trait

114   correlations within individual females and by addressing the following questions: (i) Is the OSN

115   trade-off observed at the population level matched at the individual level, i.e., do individuals

116   trade off offspring size and number? (ii) How does female size (resource- *versus* age-related)

117   influence investment into offspring size *versus* number? (iii) Which investment trait, offspring

118   size or number, is the primary target of selection? (iv) Are these two maternal investment traits

119   heritable, and does the OSN trade-off have a genetic component?

120

Methods

THE MODEL SYSTEM AND STUDY SITE – THE SWEDISH SAND LIZARD (*LACERTA AGILIS*)

The sand lizard (*Lacerta agilis*) is a small ground-dweller (max 20 g) with a distribution range that is ca 8000 x 5000 km, one of the largest of any reptile (Bischoff, 1984). Our study population at Asketunnan is situated ca 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, Olsson *et al.*, 2011b,c; 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 ca 500 x 400 meters which during the study period contained a stable population of ca 150-200 adult lizards which were easily monitored and tracked. In early June, females were brought to laboratory facilities at University of Gothenburg approximately one week before oviposition (which is obvious from egg contours visible on the sides of the body). Females were kept individually in cages (40 x 50 x 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

144 (Zakharov, 1989). After approximately 40 days the eggs hatched at this temperature and the

145 hatchlings were marked, measured and released at random sites at Asketunnan. For the years

146 1998-2007, maternity and paternity was confirmed using 21 microsatellites (Olsson *et al*.,

147 2011a). Offspring survival was assessed annually as per our previous work (e.g., Olsson &

148 Madsen 2001; Ryberg *et al*., 2004). We have shown before that by searching a 600 m corridor

149 around Asketunnan (a peninsula), a distance five times the maximum recorded annual dispersal

150 distance for an offspring in this population (Olsson *et al*., 1996; Ryberg *et al*., 2004), we remove

151 the risk of having dispersal confound our estimates of mortality/survival.

152

153 Climatic data was purchased from the Swedish Bureau of Meteorology and Hydrology (SMHI)

154 using data from the Varberg weather station (closest available to our field site, in the same coastal

155 position, situated ca 50 km south of Asketunnan). Varberg and Asketunnan are situated right on

156 the coast, which is the main factor dictating cloud cover and rain fall, and thus basking

157 opportunities for lizards in this area (Olsson & Shine, 1996; Olsson *et al.*, 2010; Ljungström *et*

158 *al.*, 2015). In order to estimate the environmental conditions the offspring experienced after

159 release and prior to hibernation (the crucial period affecting survival), we calculated annual grand

160 means of mean temperatures recorded per day for August-September to represent the activity

161 period before hatchlings enter into hibernation.

162

163 STATISTICAL ANALYSES

164

165 OSN PATTERNS AND FEMALE SIZE EFFECTS AMONG AND WITHIN INDIVIDUALS

166

167 To assess the relationship between offspring size and number among and within individuals, we

168 fitted a restricted maximum likelihood (REML) bivariate mixed model with clutch size and mean

169 offspring mass (hereafter also referred to as offspring size) as dependent variables and female ID

170 (*id*) as a random effect. Year of breeding (*YEAR*) was included as a fixed factor to control for the

171 effect of inter-annual variation on both variables. In summary, the following bivariate mixed

172 model for the *h*th dependent variable of individual *i* in year *j* was applied:

173

174 $Y_{hij} = u + YEAR_{hj} + id_{hi} + e_{hi}$ (Model 1)

175

176 The random effect female ID id_{hi} and the within-individual residuals e_{hi} were modelled with

177 unstructured covariance matrices to yield the among- and within-individual variances in both

178 traits, as well as their among- and within-individual covariances. Prior to running the bivariate

179 model, we studied statistical significance of fixed and random factors with univariate models for

180 mean offspring mass and clutch size, respectively. Significance of the fixed factor ($\alpha = 0.05$) was

181 assessed with conditional Wald F statistics and that of random factors with likelihood ratio tests

182 (LRT), testing the difference in the -2 log likelihood between hierarchal models against a chi-

183 square distribution with number degrees of freedom equal to the difference in number of

184 estimated terms (Pinheiro & Bates, 2000).

185

186 To investigate the effect of female mass on clutch size and mean offspring mass among and

187 within individuals, we ran Model 1 with female mass after oviposition and either of these two

188 variables as responses. Female mass is a combined component of two variables, the condition of

189 a female and her size due to continuous growth throughout life (snout-vent length), which may

190   have separate effects on offspring size and number. Hence, to further explore the relationship

191   between female mass and the OSN trade-off, Model 1 was also used to test the correlation

192   between clutch size and mean offspring mass and either female condition (annual residuals of

193   female mass after oviposition on snout-vent length) or snout-vent length (hereafter referred to as

194   body size). We also ran the model with snout-vent length and total clutch mass as dependent

195   variables, to test whether total reproductive investment changed with age-related size. For all

196   bivariate models, the significance of each covariance was tested by comparing the likelihood of

197   the full model with the likelihood from a model in which the covariance was fixed at zero. The

198   likelihoods were compared using an LRT with a single degree of freedom (Pinheiro & Bates,

199   2000). The univariate and bivariate analyses described in this section included 353 females with

200   561 clutches and 4626 individual offspring over 15 years (1987-1991 and 1998-2007). There

201   were 133 females that bred in multiple years (85 bred twice, 30 bred in 3 years, 10 in 4 years, 7 in

202   5 years and 1 in 6 years) and 220 females bred once. Only females with at least two breeding

203   attempts have within-individual residuals and hence contribute to estimation of trait-covariation

204   within individuals in the bivariate models. All analyses in this section were performed in ASReml

205   2.0 (VSN International; Gilmour *et al.*, 2006).

206

207   DETERMINANTS OF OFFSPRING RECRUITMENT – SELECTION ON OFFSPRING SIZE

208   AND NUMBER

209

210   The selection analysis was performed by running a univariate mixed model using a female's

211   seasonal number of recruits (*REC*) as response variable, clutch size (*CSIZE*) and mean offspring

212   mass (*MOFFMASS*) as predictors, and female mass (*FMASS*) after oviposition as covariate.

213   Offspring recruitment may not only be determined by offspring size or number, but also by

214   timing of hatching and weather conditions late in the season (Adolph & Porter, 1993; Olsson &

215   Shine, 1997). Hence, oviposition date (*LDAY*; expressed in Julian days since 1 January), annual

216   grand means of mean temperatures recorded per day for August-September (*STEMP*; hereafter

217   referred to as "late summer temperature"), and year of breeding (*YEAR*) were included as

218   covariates, and female ID (*id*) was fitted as a random effect to account for multiple measures per

219   female. To test for differences in the effects of mean offspring mass and clutch size on

220   recruitment among years of breeding, we also fitted the interactions between these variables.

221   Thus, we used the following mixed model for recruitment of individual *i* in year *j*:

222

223     $REC_{ij} = u + CSIZE_{ij} + MOFFMASS_{ij} + YEAR_j + CSIZE_{ij}*YEAR_j + MOFFMASS_{ij}*YEAR_j +$

224             $FMASS_{ij} + LDAY_{ij} + STEMP_j$

225             $+ id_i + e_i$ (Model 2)

226

227   where fixed effects are denoted in upper case, and random effects in lower case letters,

228   respectively. Non-significant fixed effects were sequentially dropped from the model until

229   remaining terms were significant ($\alpha = 0.05$) (but retained if their interaction was significant). All

230   parameter estimates were solved for using REML algorithms implemented in SAS 9.3. Statistical

231   significance of fixed effects was determined using Kenward-Roger F statistics and random

232   effects were assessed with LRTs as outlined for the univariate analyses in the section above. The

233   selection analysis was based on 3945 offspring from 465 clutches and 303 females over 12 years

234   (1989-1991 and 1998-2006). Selection analysis using recruits as the female fitness measure has

235   been criticized because it straddles two generations and, hence, the female's fitness is not

236   independent of the genetic contribution from the male affecting offspring survival (e.g., Lande &

237   Arnold, 1983; Arnold & Wade, 1984a,b; Wolf *et al*., 2004). However, size-dependent offspring

238   survival is at the very heart of OSN theory and is assumed to dictate selection on offspring size,

239   with concomitant shifts in clutch size, which was our rationale for adopting this approach.

240

241   HERTABILITY OF OFFSPRING SIZE, NUMBER AND THE BASIS OF THEIR TRADE-OFF

242

243   We used univariate animal models to investigate whether offspring size and number are heritable

244   in this population. Animal models incorporate pedigree information into linear mixed models to

245   estimate the additive genetic variance of the trait of interest in the population under study (Lynch

246   & Walsh, 1998; Kruuk, 2004). The pedigree was formed by 3651 identities over 3 generations,

247   incorporating 254 fathers and 271 mothers. Among the 449 clutches included in the animal model

248   for clutch size, there were 97 belonging to females with at least one known relative (i.e., with

249   known mother). In the analysis of mean offspring mass, 422 clutches were included out of which

250   88 belonged to females with at least one known relative. The rest of the clutches included in the

251   analyses did not belong to females with known relatives, but they contribute to estimating the

252   total phenotypic variance of clutch size *versus* mean offspring mass more accurately.

253   Our univariate animal models with either mean offspring mass or clutch size as dependent

254   variable had the following structure, representing the response of individual *i* in year *j*:

255

256    $Y_{ij} = u + FIXED_{ij} + a_i + pe_i + year_j + e_i$ (Model 3)

257

258   In this model a_i is the additive genetic value of the individual and pe_i is its permanent

259   environmental effect. This effect is estimated using repeated measures and includes sources of

260   variation that lead to permanent differences among individuals, apart from those due to additive

261   genetic effects (Kruuk, 2004). In the offspring size analysis 164, 63, 24, 6, 6 and 1 females were

262   observed 1, 2, 3, 4, 5 and 6 times respectively (in different years), and for clutch size these

263   numbers were 171, 64, 26, 9, 6 and 1. Random variation due to year of breeding $year_j$ was also

264   modelled, while controlling for the fixed effect $FIXED_{ij}$ of maternal condition on mean offspring

265   mass and snout-vent length on clutch size (as significant correlations between these traits were

266   identified in bivariate analyses described above). LRTs were used to test significance of random

267   factors, and the animal models were run in ASReml 2.0 (VSN International; Gilmour *et al.*,

268   2006).

269

270   The upper limit to heritability is set by the repeatability of a trait. Hence, to assess the upper limit

271   to heritability in offspring mass and clutch size, we also ran models substituting a_i and pe_i for the

272   total individual phenotypic value $ind_i = a_i + pe_i$, and calculated repeatability as the ratio of

273   among-individual phenotypic variance to the total variance (i.e., as $V_{ind} / (V_{ind} + V_{year} + V_e)$). In

274   addition, we tried to fit a bivariate animal model to investigate whether there is a genetic

275   correlation, and hence trade-off, between these two traits. Unfortunately, this model failed to

276   converge when attempting to fit the individual-specific permanent environment effect. Hence, as

277   omitting this effect when repeated measures are present may upwardly bias the estimate of

278   additive genetic variance (Kruuk & Hadfield, 2007), this model is not shown.

279

280   **Results**

281

282   OSN PATTERNS AND FEMALE SIZE EFFECTS AMONG AND WITHIN INDIVIDUALS

283

284   Running the univariate models with either clutch size or mean offspring mass as dependent

285   variables year of breeding had a significant effect on both variables (clutch size, *F $_{14,485.7}$ = 3.38*,

286   *P < 0.0001*; mean offspring mass, $F_{14,481.9} = 8.79$, *P < 0.0001*), and random variation among

287   females explained a significant amount of the residual variation in clutch size ($X^2 = 45.3$, *P <*

288   *0.0001*, variance $\pm$ S.E. $= 2.1196 \pm 0.3954$), and in mean offspring mass ($X^2 = 33.5$, *P < 0.0001*,

289   variance $\pm$ S.E. $= 0.0016 \pm 0.0003$). The bivariate model for the relationship between offspring

290   size and number revealed a negative among-individual correlation ($X^2 = 3.9$, *P = 0.0489*,

291   covariance $\pm$ S.E. $= -0.0155 \pm 0.0081$, r $= -0.27$), meaning that females with a higher average

292   clutch size have a lower average offspring size and *vice versa*. Within individuals, clutch size was

293   negatively correlated with mean offspring mass, hence, when females have larger clutches

294   relative to their own mean they also have smaller offspring ($X^2 = 11.0$, *P < 0.0001*, covariance $\pm$

295   S.E. $= -0.0220 \pm 0.0069$, r $= -0.22$; Table 1). These relationships are visualized in Figure 1, which

296   shows a descriptive plot of mean offspring mass of individual females *versus* their clutch size.

297

298   Testing for the effect of female mass among and within individuals, female mass had a positive

299   effect on clutch size at both levels (female ID (among-individuals), $X^2 = 28.6$, *P < 0.0001*,

300   covariance $\pm$ S.E. $= 1.4502 \pm 0.3099$, r $= 0.70$; residual (within-individuals), $X^2 = 27.3$, *P <*

301   *0.0001*, covariance $\pm$ S.E. $= 1.1008 \pm 0.2403$, r $= 0.33$) and on mean offspring mass at the

302   individual level (female ID, $X^2 = 0.44$, *P = 0.5071*; residual, $X^2 = 5.9$, *P = 0.0151*, covariance $\pm$

303   S.E. $= 0.0159 \pm 0.0066$, r $= 0.17$). Investigating the separate effects of the two components of

304   female mass, condition and snout-vent length, there was a positive correlation between condition

305   and mean offspring mass within individuals ($X^2 = 8.42$, *P = 0.0037*, covariance $\pm$ S.E. $= 0.0122 \pm$

306   0.0043, r $= 0.20$), but not among them ($X^2 = 0.6$, *P = 0.4385*). However, condition had no effect

307   on clutch size at either level (female ID, $X^2 = 0.02$, *P = 0.8875*; residual, $X^2 = 0.04$, *P = 0.8414*).

308   Body size had no effect on mean offspring mass (female ID, $X^2 = 0.03$, *P = 0.8624*; residual, X^2

309   *= 0.29*, *P = 0.5902*), but was strongly positively correlated with clutch size among and within

310   individuals (female ID, $X^2 = 55.8, P < 0.0001$, covariance $\pm$ S.E. = 5.5446 $\pm$ 0.8759, r = 0.81;

311   residual, $X^2 = 48.5, P < 0.0001$, covariance $\pm$ S.E. = 2.9174 $\pm$ 0.5136, r = 0.44).

312

313   As this study focuses on within-individual effects, the within-individual covariance components

314   from all bivariate analyses are displayed in Table 1. Overall, these results indicate that the

315   positive effect of female mass on mean offspring mass within individuals is driven by differences

316   in condition, and that the positive effect of female mass on clutch size at both levels is

317   determined by differences in body size. Furthermore, there was a positive correlation between

318   body size and total clutch mass at both levels (female ID, $X^2 = 71.1, P < 0.0001$, covariance $\pm$

319   S.E. = 4.2890 $\pm$ 0.5672, r = 0.92; residual, $X^2 = 87.9, P < 0.0001$, covariance $\pm$ S.E. = 1.7610 $\pm$

320   0.2869, r = 0.50, Table 1), suggesting that total reproductive investment increases with age-

321   related size.

322

323   DETERMINANTS OF OFFSPRING RECRUITMENT – SELECTION ON OFFSPRING SIZE

324   AND NUMBER

325

326   Running the full recruitment-model with all predictors and covariates, the two interaction terms

327   between offspring size and clutch size with year of breeding were not significant ($F_{11,426} = 0.68$,

328     $P = 0.7591$ and $F_{11,437} = 0.97$, $P = 0.4736$, respectively) and, hence, were sequentially dropped

329   from the model. As the model no longer included any interactions with breeding year, and we

330   were not interested in the effects of particular years, only to account for multiple records per year,

331   year of breeding was fitted as a random effect in the proceeding analyses. This reduces the

332   number of degrees of freedom used up at each level of the fixed effect and, hence, enhanced

333   statistical power. Mean offspring mass and female mass were not significant predictors of

334   offspring recruitment (mean offspring mass, $F_{1,457} = 1.15$, $P = 0.2835$; female mass, $F_{1,399} =$

335   1.05, $P = 0.3072$), nor was the effect of late summer temperature ($F_{1,8.68} = 2.35$, $P = 0.1610$).

336

337   The final model revealed that seasonal recruitment of offspring was positively affected by clutch

338   size ($F_{1,445} = 5.56$, $P = 0.0179$, parameter estimate $\pm$ S.E. $= 0.0435 \pm 0.0183$) and early egg-

339   laying ($F_{1,208} = 16.71$, $P < 0.0001$, parameter estimate $\pm$ S.E. $= -0.0265 \pm 0.0065$). Furthermore,

340   year of breeding explained a significant amount of the variation in recruited young left

341   unexplained by these fixed effects ($X^2 = 22.3$, $P < 0.0001$, variance $\pm$ S.E. $= 0.0875 \pm 0.0483$).

342   These results indicate that there is selection for clutch size and oviposition date in this population,

343   but not for offspring mass. Visual inspection of our grid plot (Fig. 2) supports this result and

344   shows that the trajectory for offspring recruitment on clutch size (XY, Fig. 2) was steeper

345   compared to the mean offspring mass trajectory (ZY, Fig. 2).

346

347   HERTABILITY OF OFFSPRING SIZE, NUMBER AND THE BASIS OF THEIR TRADE-OFF

348

349   The heritability analyses were based on 10 years (1998-2007) of data, with 422 clutches from 264

350   females for the analysis of offspring size, and 449 clutches from 277 females for that of clutch

351   size. In accordance with the results from the non-genetic univariate models presented above,

352   there were significant phenotypic differences among females in both traits, however, this

353   variation did not have an additive genetic basis (Table 2). Permanent environment effects

354   explained a significant amount of the among-individual variation in mean offspring mass, after

355   controlling for condition, but had no effect on clutch size (mean offspring mass, $X^2 = 4.64$, $P =$

356   0.0312; clutch size, $X^2 = 0.18$, $P = 0.6713$; Table 2). Furthermore, year of breeding contributed to

357   the random unexplained variation in both traits (clutch size, $X^2 = 8.84$, $P = 0.0029$; mean

358   offspring mass, $X^2 = 17.37, P < 0.0001$; Table 2). Investigating the results from these analyses,

359   presented in Table 2, the majority of the phenotypic variation observed among individuals in

360   offspring size is explained by permanent differences among individuals, but additive genetic

361   effects appear to explain most of the variation among individuals in clutch size. The

362   repeatabilities of mean offspring mass and clutch size were 0.34 ± 0.06 and 0.21 ± 0.06

363   respectively.

364

365   **Discussion**

366

367   A trade-off between the two maternal investment traits; offspring size and number, has been

368   commonly identified at the species level. However, at the intraspecific level, a whole range of

369   phenotypic correlations between these two traits have been observed in terms of sign and

370   magnitude. In this study we attempted to unravel if, how and why offspring size and number are

371   traded off in a population of sand lizards by separating among- and within-individual patterns

372   using a 15-year dataset collected in the wild.

373

374   OSN PATTERNS AND FEMALE SIZE EFFECTS AMONG AND WITHIN INDIVIDUALS

375

376   In agreement with the findings of a previous study on this population, using a smaller 5-year data

377   set to investigate population-level effects (Olsson & Shine, 1997), we found a negative

378   correlation between offspring size and number both among and within individuals. The negative

379   within-individual correlation indicates that there is indeed a trade-off between these two traits in

380   sand lizard females. This finding also implies that females in this population do not produce a

381   consistent clutch size or offspring size across reproductive events, which lead us to question what

382   causes a change in the relationship between these traits? In contrast to their predictions, Olsson

383   and Shine (1997) found a positive relationship between female and offspring mass in these

384   lizards. Here we explore these results further by analysing how the components that make up a

385   female's mass, her condition (resource-related size) and size due to continuous growth

386   throughout life (age-related size; snout-vent length), separately affect the sizes of offspring and

387   clutches.

388

389   Running our analyses we found a positive correlation between female mass and both the size and

390   number of offspring, however, this relationship only occurred within individual females.

391   Breaking down female mass into its two component variables, female body size had a positive

392   effect on clutch size at both levels, but not on offspring size, and female condition only had an

393   effect on offspring size (positive) within individuals. These results indicate that the positive effect

394   of female mass on mean offspring mass detected within individuals is driven by differences in

395   condition, and that the positive effect of female mass on clutch size at both levels is determined

396   by differences in body size. These findings suggest that physical constraints (size of body cavity)

397   limit the number of eggs that a female can produce and that the OSN trade-off appears due to

398   resource constraints as a female continues to grow throughout life and, hence, produces more

399   eggs. Our finding that being in better condition allows a female to have heavier offspring

400   supports this reasoning, but this effect is apparently not strong enough to override the OSN trade-

401   off. Hence, there is a possibility that clutch size and offspring mass are negatively genetically

402   correlated, which in turn may have considerable effects on the rate and direction of evolution of

403   these two traits.

404

405 An increase in clutch size as a simple consequence of a female's physical limitation has

406 previously been demonstrated in both lizards and snakes (Shine, 1992; in den Bosch & Bout,

407 1998; Kratochvil & Frynta, 2006; Pizzatto *et al.*, 2007; Ford & Seigel, 2015), and so has positive

408 effects of maternal body condition on offspring size (eg., Madsen & Shine, 1996; Brown &

409 Shine, 2006). Classic OSN theory assumes that a female can increase her relative fitness by

410 investing more resources into her eggs when it is theoretically possible. This leads us to question

411 why larger/older females in this population invest in more eggs when this appears to lead to

412 smaller offspring, i.e., why is clutch size not constrained to the advantage of larger offspring?

413

414 DETERMINANTS OF OFFSPRING RECRUITMENT – SELECTION ON OFFSPRING SIZE

415 AND NUMBER

416

417 In our long term dataset, the relative importance of clutch size for maternal recruitment was still

418 significant when phenology (i.e., oviposition date) was taken into account. However, in contrast

419 to classic OSN assumptions (Lack, 1947; Smith & Fretwell, 1974; Brockelman, 1975; Grafen,

420 1988), offspring size did not predict recruitment rate. Several environmental factors have been

421 shown to induce selection on offspring size, including intra- and interspecific competition,

422 predation, food availability, and abiotic stress (Marshall & Keough, 2008). In this study

423 population, intra- and interspecific competition is weak, food resources are commonly abundant,

424 and predation most likely size invariant (any small or large lizard hatchling (range ca. 0.7-1.2 g)

425 is an easy target for predators). Furthermore, the study site is exposed to high inter-annual

426 variability in local weather conditions, which presumably leads to variable selection pressures on

427 body size among years, and therefore no single offspring size is likely to be optimal across time.

428   This argument is supported by previous findings for this population, showing variation in size-

429   dependent offspring survival among years of varying potential for resource acquisition (Olsson &

430   Madsen, 2001). Hence, offspring size is likely to have little contemporary effect on offspring

431   fitness in this population, which has also been reported for other reptile populations (Sinervo *et*

432   *al.*, 1992; Congdon *et al.*, 1999; Husak, 2006; Warner & Shine, 2007; Uller *et al.*, 2011).

433

434   Unexpectedly, these findings suggest that females are not disadvantaged by having smaller

435   offspring. So, why do smaller/younger individuals have relatively larger eggs and why do

436   females invest relatively more resources into their offspring when they are in better condition

437   when this does not benefit their fitness? Selection for larger offspring early in life could answer

438   the first question, however, as annual recruitment was used as proxy for fitness, our observations

439   of no selection on offspring size and directional selection on clutch size are invariant of a

440   female's age. Hence, the most parsimonious answer to these questions is that the number of eggs

441   produced is constrained by body size and investment into offspring size is just a consequence of

442   resource deposition into a set number of eggs. Theoretically, this suggests that smaller/younger

443   females could attain higher fitness by trading in one large for two smaller eggs. However, as

444   there is likely to be a set minimal offspring size below which survival is not possible, these

445   females may not have a large enough body cavity to produce another viable egg. This is

446   supported by our finding that although there is a trade-off between mean offspring mass and

447   clutch size, total clutch mass is positively correlated with snout-vent length, hence, indicating that

448   a female's body size sets a limit to her total reproductive investment. Historical evolutionary

449   inertia, when a species has spent most of its evolutionary history outside of its current distribution

450   range, may also provide some answers. In this case, the Swedish sand lizard dispersed from

451   continental Europe to Scandinavia via land bridges submerged around 9000 BP (Gullberg, Olsson

452   & Tegelström, 1998), and previous work by us on other aspects of evolutionary divergence

453   between Swedish and continental populations show outbreeding effects at laboratory crossings *in*

454   *lieu* of population divergence and local adaptation arguments (Olsson *et al*., 2004; Olsson *et al*.,

455   2005). Hence, in their previous environment, offspring size may have had an effect on

456   recruitment and, hence, smaller females may have been able to compensate for a smaller clutch

457   size by producing larger more viable young.

458

459   In addition to clutch size, we found inter-annual variability in weather conditions during the

460   breeding season to be a strong determinant of offspring recruitment. Spring temperature has

461   previously been shown to affect the timing of oviposition (Olsson *et al*., 2011b,c; Ljungström *et*

462   *al*., 2015) and here we found earlier clutches to have the highest recruitment rate, as in a number

463   of other lizard taxa (e.g., Warner & Shine, 2007; Wapstra *et al*., 2010; Le Henanff *et al*., 2013).

464   One plausible explanation is that early-born offspring have longer time to accumulate reserves

465   before autumn and therefore survive winter better. This idea runs counter to findings by Adolph

466   and Porter (1993), showing that the strongest negative effect on offspring survival is activity and,

467   as a corollary, exposure to predators. However, in this study we found no effect of late season

468   temperature, and hence activity, on offspring recruitment. Thus, early clutches appear to be

469   favored by a greater opportunity for resource accumulation.

470

471   HERITABILITY OF OFFSPRING SIZE, NUMBER AND THEIR TRADE-OFF

472

473   To summarize the findings from the previous sections, female lizards in this population displayed

474   a trade-off between offspring size and number and there was directional selection on clutch size

475   but not on offspring size. Evolution towards larger clutches is determined by two things; the

476   amount of additive genetic variation in clutch size and, if the trade-off is genetically determined,

477   the amount of additive genetic variation in mean offspring mass. With a genetic basis for the

478   trade-off and sufficient additive genetic variation in both traits, we would expect clutch size to

479   increase over evolutionary time, with a concomitant decrease in offspring size limited by the

480   minimal size for offspring survival.

481

482   The results from our heritability analyses showed significant phenotypic variation among females

483   in both investment traits, however, this variation did not have an additive genetic component.

484   However, effects that lead to permanent differences among females explained the majority of the

485   variation among individuals in mean offspring mass. This suggests that offspring size is not only

486   determined by a female's clutch size and condition in a particular year, but that other effects,

487   such as birth year, mother or clutch size, also impose fixed differences among individuals.

488   Although we did not detect a significant additive genetic or permanent environment component

489   of variance in clutch size, our results still indicate that genetic effects are responsible for most of

490   the among-individual variance observed in this trait. These results support our previous

491   discussion, i.e., that clutch size is a less flexible trait set by the space available in a female's body

492   cavity due to age-related growth, and that offspring size is more variable and is influenced by a

493   female's resources, as set by current or past conditions. These observations correspond well with

494   theoretical predictions of optimal offspring size and number in relation to female size (Hendry *et*

495   *al.*, 2001).

496

497   A lack of additive genetic variance in offspring size and number and low repeatabilities (and

498   hence low upper limits to heritability) could have two possible explanations; 1) strong selection

499   on these life history traits has led to low levels of additive genetic variation, 2) our analyses do

500   not have sufficient statistical power to detect significant additive genetic effects. Although we did

501   not detect selection on offspring size in this study, the first point could be the result of selection

502   on this trait in the evolutionary past, as discussed in the section above. Unfortunately, we were

503   unable to estimate genetic and permanent environment components of the observed phenotypic

504   OSN trade-off. However, our finding that the trade-off remains even when a female is in good

505   condition and invests more resources into offspring size may indicate that this trade-off has a

506   genetic component. Only two other studies have examined the genetic basis for an observed OSN

507   trade-off in reptiles (lizards (Sinervo & Doughty, 1996) and snakes (Brown & Shine, 2007)), both

508   of which indicate the existence of a negative genetic correlation between these two traits. As a

509   genetic basis for the trade-off observed in this population could not be established, our current

510   findings suggest that an evolutionary response to selection on clutch size may be constrained by

511   low additive genetic variance in this trait.

512

513   **Conclusions**

514

515   Using a long term dataset on sand lizards collected in the wild, we found that females in this

516   population do not produce a consistent clutch size or offspring size across reproductive events,

517   but that these traits experience a classic OSN trade-off. Furthermore, our findings suggest that the

518   number of eggs produced is limited by a female's age-related size and, hence, that the OSN trade-

519   off appears due to resource constraints as a female continues to grow throughout life and, hence,

520   has larger clutches. In contrast to classic OSN predictions, directional selection on offspring size

521   was not detected. Yet, smaller/younger females produced relatively larger offspring and higher

522   resource availability also lead to larger young. This suggests that investment into offspring size is

523   just a consequence of resource deposition into a set number of eggs, or possibly a result of

524   historical evolutionary inertia. We were unable to determine whether the observed OSN trade-off

525   had a genetic basis, thus, our current findings suggest that an evolutionary response to selection

526   on clutch size may be constrained by low additive genetic variation in this trait. The findings of

527   this study are an important illustration of how analyses of within-individual patterns can reveal

528   trade-offs and their underlying causes, with potential evolutionary and ecological consequences

529   that are otherwise hidden by among-individual variation.

530

531   **Acknowledgements**

532

537

538   **References**

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697

698 **Legends to figures**

699 Fig. 1. Relationship between mean offspring mass and clutch size. Descriptive plot of mean

700 offspring mass of individual sand lizard (*Lacerta agilis*) females versus clutch size for

701 1987–1991 and 1998–2007 ($n_{clutches}$ = 561, $n_{females}$ = 353).

702

703 Fig. 2. Selection surface for mean offspring mass and clutch size. g3 grid plot over recruitment

704 success (Y-axis), clutch size (X-axis), and mean offspring mass (Z-axis). The g3 grid was

705 created with a joining function in SAS 9.3 with a smoothing parameter of 2.0.

706