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# Geographic variation of life-history traits in the sand lizard, *Lacerta agilis*: testing Darwin's fecundity-advantage hypothesis

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## Abstract

The fecundity-advantage hypothesis (FAH) explains larger female size relative to male size as a correlated response to fecundity selection. We explored FAH by investigating geographic variation in female reproductive output and its relation to sexual size dimorphism (SSD) in *Lacerta agilis*, an oviparous lizard occupying a major part of temperate Eurasia. We analysed how sex-specific body size and SSD are associated with two putative indicators of fecundity selection intensity (clutch size and the slope of the clutch size–female size relationship) and with two climatic variables throughout the species range and across two widespread evolutionary lineages. Variation *within* the lineages provides no support for FAH. In contrast, the divergence *between* the lineages is in line with FAH: the lineage with consistently female-biased SSD (*L. a. agilis*) exhibits higher clutch size and steeper fecundity slope than the lineage with an inconsistent and variable SSD (*L. a. exigua*). *L. a. agilis* shows lower offspring size (egg mass, hatchling mass) and higher clutch mass relative to female mass than *L. a. exigua*, that is both possible ways to enhance offspring number are exerted. As the SSD difference is due to *male* size (smaller males in *L. a. agilis*), fecundity selection favouring larger females, together with viability selection for smaller size in both sexes, would explain the female-biased SSD and reproductive characteristics of *L. a. agilis*. The pattern of intraspecific life-history divergence in *L. agilis* is strikingly similar to that between oviparous and viviparous populations of a related species *Zootoca vivipara*. Evolutionary implications of this parallelism are discussed.

## Introduction

In many species, males and females differ in their characteristic body size. This sexual size dimorphism (SSD) exhibits pronounced variation at all taxonomic levels, and this variation is a prominent issue in evolutionary studies (reviewed by Fairbairn *et al.*, 2007). It is now widely agreed that fecundity selection in females and sexual selection in males are the major evolutionary forces selecting for larger body size in most organisms (e.g. Andersson, 1994; Blanckenhorn, 2000; Fairbairn *et al.*, 2007). Recent studies furthermore stressed a substantial role of proximate factors such as differential

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growth constraints and phenotypic plasticity of the sexes (Fairbairn, 2005; Cox & John-Alder, 2007; Cox & Calsbeek, 2010).

The fecundity-advantage hypothesis (FAH) explains larger female size relative to male size as a correlated response to selection for female fecundity (Darwin, 1874; Shine, 1988; Reeve & Fairbairn, 1999). Its basic assumption, a strong correlation of female size with fecundity within populations, is fulfilled in most ectotherms. In experimental populations of fruit flies, *Drosophila melanogaster*, selection for fecundity lead to an increase in female size and SSD. Moreover, this correlated response of SSD to selection for female fecundity was stronger than the direct response to selection for large female size (Reeve & Fairbairn, 1996, 1999).

In comparative studies, the rate of fecundity selection is roughly estimated using clutch or litter size (i.e. fecundity in a single reproductive episode), or the slope of the regression of clutch size on female size (fecundity slope). FAH predicts a more female-biased SSD in species/populations with higher clutch size and/or steeper fecundity slope (Shine, 1994; Braña, 1996; Cox *et al.*, 2003). Yet FAH has been tested via comparative studies by far less frequently than sexual selection, especially for vertebrate taxa. Specifically for reptiles, although they are a model group for studying the evolution of SSD (e.g. Blanckenhorn, 2000; Cox *et al.*, 2007) and reproductive strategies (Vitt & Pianka, 1994; Shine, 2005), the data are scarce and discrepant. Braña (1996) found a strong positive correlation between female-biased SSD and fecundity slope among eight species of lacertid lizards. For lizards in general, female size-corrected clutch size and fecundity slope explained a rather small portion of the total variation in SSD across species (Cox *et al.*, 2003). The results of a comparable study in snakes are also equivocal (Shine, 1994). Among closely related iguanid species, Zamudio (1998) and Pincheira-Donoso & Tregenza (2011) found no correlation between fecundity and SSD. For animals in general, studies explicitly addressing FAH are also not numerous and render mixed results (Myers, 1978; Wiklund & Karlsson, 1988; Head, 1995; Lislevand *et al.*, 2009; Herczeg *et al.*, 2010; Puniamoorthy *et al.*, 2012; Winkler *et al.*, 2012). Furthermore, the vast majority of the comparative studies listed above dealt with the variation among species, whereas only few (Herczeg *et al.*, 2010; Winkler *et al.*, 2012; Puniamoorthy *et al.*, 2012; see also Zamudio, 1998) investigated intraspecific variation.

Intraspecific variation is important because it links macroevolutionary patterns to microevolutionary processes that lead to the phenotypic diversity we wish to understand. Wide-ranging species present promising models for simultaneously evaluating the role of various factors shaping phenotypic diversity, because the variation of target traits can be documented for numerous geographically distinct populations exhibiting diverse combinations of putative predictors. However, comprehensive rangewide studies of geographic variation in widespread species are rare, even for fundamentally important traits such as adult body size, offspring size and fecundity (Roitberg, 2007; Roitberg *et al.*, 2013; references therein).

Here, we test FAH by investigating geographic variation in female reproductive output and its relation to SSD, in the sand lizard *Lacerta agilis* L. This species occupies much of the temperate Palaearctic (Fig. 1) and possesses the second largest range among terrestrial reptiles. Several morphologically and geographically distinct subspecies are recognized (Bischoff, 1984; Blanke, 2010; Fig. 1), five of them being supported as monophyletic units (henceforth intraspecific lineages, clades) by extensive molecular genetic studies (Kalyabina-Hauf & Ananjeva, 2004; Joger *et al.*, 2007; Andres *et al.*, 2014).

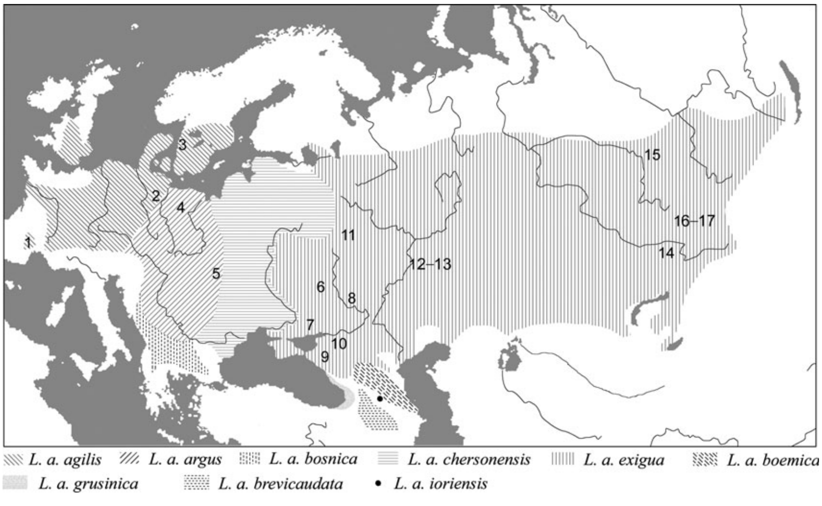

**Fig. 1** Geographic distribution of different subspecies of *Lacerta agilis* (after Roitberg, 2007) and our study samples. We considered *L. a. garzoni* (the westernmost isolate of the species range) and *L. a. argus* as parts of *L. a. agilis* (the *agilis/argus* clade of Andres *et al.*, 2014) in all analyses.

*L. agilis* exhibits pronounced geographic variation in SSD: in Western Europe (clade *L. a. agilis*), females are on average 10% larger than males, whereas in the North Caucasian form *L. a. boemica*, males are clearly larger than females (Roitberg, 2007). The other clades show intermediate patterns. In the eastern clade (*L. a. exigua*), which has the widest range (Fig. 1), SSD is quite variable, but there is no overall trend towards larger females (Roitberg, 2007). Thus, *L. a. agilis* and *L. a. exigua*, the two most widespread clades, do differ in their characteristic SSD. Therefore, it is interesting to compare their fecundity patterns. According to FAH, *L. a. agilis* is expected to have larger clutches (Prediction 1) and a steeper increase of clutch size with female body length (Prediction 2) than *L. a. exigua*. Alternatively, if the SSD differences between the two lineages are largely due to sexual selection or other, potentially stochastic processes, no considerable reproductive divergence is expected. Although the original version of FAH considers only offspring *number*, it is reasonable to extend it to another related aspect of female reproductive output, namely total clutch mass (Shine, 1988). Accordingly, we further expect the lineage with more female-biased SSD (*L. a. agilis*) to have higher clutch mass relative to female body mass (Prediction 3).

Within clades, a previous study (Roitberg, 2007) revealed a clinal increase of female size relative to male size with latitude (*L. a. exigua*) and altitude (*L. a. boemica*). In view of FAH, such clines, that is more female-biased SSD in colder climates, could arise as an adaptive compensation of a decrease in reproduction frequency (Tinkle *et al.*, 1970; Fitch, 1981; Cox *et al.*, 2003). As adult body size is a phenotypically plastic trait in ectotherms (e.g. Jetz *et al.*, 2009), and body growth can be differently constrained in males and females (Cox & John-Alder, 2007; Bonnet *et al.*, 2011), climate can also directly affect SSD. Based on Adolph & Porter's (1996) model and growth data for low- and high-elevation populations of *L. a. boemica* (Roitberg & Smirina, 2006a), the considered SSD clines can be parsimoniously explained in terms of differential trade-offs between growth and reproduction (e.g. Cox & John-Alder, 2007) and viewed as a direct phenotypic response to environmental constraints (Roitberg & Smirina, 2006a; Roitberg, 2007; see Discussion). Therefore, for climate-driven SSD clines, we have to distinguish between an adaptive (FAH) and a plastic response to environmental differences. Although a comparative study generally cannot differentiate between genetic adaptation and phenotypic plasticity, some patterns of among-trait and trait-climate relationships may be more indicative of one of the two scenarios. For example, if geographic SSD variation is related to a climatic gradient, but not to variation in female reproductive output, FAH is an unlikely explanation. If fecundity selection is a major determinant of the geographic SSD variation within clades, then clutch size (Prediction 1b) and fecundity slope (Prediction 2b) should be associated with more female-biased SSD.

## Materials and methods

### Samples and characters

We analysed variation in body size and female reproductive output using the following traits: snout–vent length (SVL) of adult males, adult females and currently reproducing females; clutch size (number of eggs per clutch); mean egg mass per clutch; mean hatchling mass per clutch; relative clutch mass, RCM (RCM = CM/PM where CM is clutch mass taken as total mass of freshly laid eggs, and PM is post-oviposition female mass).

SVL is the primary measure of overall body size in lizards and snakes (Roitberg *et al.*, 2011 and references therein). Body mass is generally less suitable for comparative studies as it typically varies with reproductive status, fat storage, digestive state and state of the tail. However, post-oviposition female mass (except when females miss a large part of the tail) and especially hatchling mass are free from these faults. Moreover, hatchling mass is clearly a more suitable estimator of offspring size than SVL, because inaccuracy of body length measurement in hatchlings is too large relative to true natural variability (Massot *et al.*, 1992; Roitberg *et al.*, 2013). This problem is particularly relevant in our study because the data come from different researchers and, hence, may additionally include inter-observer bias (Roitberg *et al.*, 2011).

Clutch size, the number of offspring in a single reproductive bout, is the most frequently studied reproductive parameter. In many lizards, including *L. agilis*, clutch size is strongly correlated with maternal body size (e.g. Olsson, 1993; this study), thus fulfilling the principal assumption of FAH.

The RCM metric is widely used as measure of reproductive investment in reptiles (e.g. Shine, 1992) because most species, including *L. agilis*, display no parental care after laying eggs. RCM also estimates the physical burden the female carries out (Qualls & Shine, 1995).

Egg mass and hatchling mass present two different estimates of offspring size. Offspring size is important because clutch size is a function of clutch mass and mean offspring mass, and because offspring size is often strongly fitness related.

All data on mass and a larger part of the clutch size data were obtained via monitoring of gravid females that were caught from the wild and held in captivity for a few days or weeks under standard conditions until oviposition (Rykena, 1988a; with minor modifications in other studies, e.g. Olsson & Madsen, 2001). The post-oviposition female mass, clutch mass and egg mass were measured within 24 h after oviposition, and hatchling mass was recorded within 24 h after hatching.

A part of the clutch size data was obtained by counting enlarged vitellogenic follicles, oviductal eggs or (a minor fraction of data) *corpora lutea*. These counts were recorded on autopsied females, along with measuring maternal SVL. These data come from museum samples or from earlier studies (e.g. Amat *et al.*, 2000; Saveliev *et al.*, 2006). No animals were killed for the present study. Additional individual-based data on clutch size and maternal SVL were extracted from published scatterplots (Olsson, 1993) or tables (e.g. Juszczyk, 1974). Furthermore, we included in our analyses published mean values and other summary statistics for the relevant characters.

In total, we summarized adult SVL data from 1410 males and 1701 females, maternal SVL/clutch size data from 637 reproducing females/clutches, offspring size data from over 1000 eggs and ca. 1000 hatchlings and clutch mass/post-oviposition mass data from 66 females. The data on sex-specific adult body size, maternal size and fecundity (main data set) come from 17 geographically distinct samples across the species range (Fig. 1; Table 1). The data on mean offspring size and clutch mass are limited to 5–8 (≥ 2 for each lineage) geographically distinct samples.

Climatic data (monthly mean minimum and maximum temperatures, and monthly mean precipitation) for the 76 study localities were obtained through the WorldClim database version 1.4, which is based on weather conditions recorded from 1950 to 2000. The spatial resolution is approximately 900 m × 900 m for Central Europe and somewhat lower for the other Eurasian regions (Hijmans *et al.*, 2005).

## Data analysis

Within localities, samples from different years were pooled to increase sample sizes and to apply a standard approach across all data. Whenever reasonable sample sizes were available, we used strictly local samples, both for original and published data. When local sample sizes were too small, however, we pooled them into compound samples for larger geographic areas and used in our analyses weighted means for the study traits and unweighted means for climatic variables.

Mean SVL of adult individuals of each sex in a study sample was used as the main estimator of characteristic sex-specific body size. Yet in species with continuing post-maturational growth, the size distribution of adults in a particular sample can be affected by a variety of proximate factors that may interfere with geographic variation (for instance, temporal variation in the proportion of newly matured animals). To partly overcome this problem, most analyses were performed for mean values and also for the 80th percentiles of the size distributions. Higher percentiles have a lower statistical power than the means, but they are less biased by the factors mentioned before (see Roitberg, 2007 for more details and references). Like in previous studies (Roitberg & Smirina, 2006b; Roitberg, 2007), the figures of sex-specific SVL and SSD Index based on the two statistics showed rather concordant geographic variation (Spearman rank correlation coefficient [$r_s$]: male SVL, $r_s = 0.89$; female SVL, $r_s = 0.91$; SSD Index, $r_s = 0.94$; $N = 17$, $P < 0.001$ for all cases), arguing for plausibility of using means in this study.

SSD was quantified with the index: *SDI* = (*size of larger sex / size of smaller sex*) – *1*, conventionally expressed as positive if females are larger and negative if males are larger (Lovich & Gibbons, 1992). We chose this index because it generates values that are intuitive, directional, properly scaled and symmetrical around zero (Lovich & Gibbons, 1992); SDI also has good statistical properties (Smith, 1999).

To avoid the problem of statistical nonindependence of data collected from siblings, the mean value for each clutch was used to analyse variation in egg mass and hatchling mass in this and most other studies (e.g. Olsson & Shine, 1997a; Olsson & Madsen, 2001; Li *et al.*, 2013). However, considering each hatchling as an independent observation did not significantly bias the results (Sorci & Clobert, 1999; Roitberg *et al.*, 2013; this study). Hence, we included population means derived with this approach (Rykena, 1988b) in our geographic variation analyses.

For egg mass, hatchling mass and RCM, we included in our analyses data from laboratory-raised animals (Rykena, 1988b). The following points were argued for this inclusion: (i) within a comprehensive study of a model *L. agilis* population (Olsson & Shine, 1997a), mean hatchling mass did not differ significantly in wild vs. laboratory-raised females; (ii) patterns of among-clade differences in the above traits found for laboratory-raised animals (Rykena, 1988b) were similar to those for pregnant females from the wild (see Results).

Considering the critique of using ratios (e.g. Packard & Boardman, 1988) and regression residuals (Garcia-Berthou, 2001), we controlled for confounding effects of correlated traits using ANCOVA whenever possible. Yet in a few situations, we used ratios or residuals in the absence of alternatives. First, we used the ratio RCM as a single dependent variable, because for many study samples, the numerator (clutch mass) or the denominator (post-oviposition female mass) values were not available. See Roitberg *et al.* (2013) for further justifications of using RCM. Second, we used residuals of an ordinary least squares regression of mean clutch size on mean female SVL to measure relative (size-specific) fecundity. Adjusted means from ANCOVA on individual-based data would hardly be a better alternative here because heterogeneity of variances persisted even in log-transformed data.

**Table 1** Body length and reproductive characteristics for 17 geographically distinct populations of the lizard *Lacerta agilis*.

| Study sample (see Fig. 1 for locations) | | | Adult male SVL (mm) | | | | | | Adult female SVL (mm) | | | | | | SSD | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ID | Sample details | Clade | *N* | Min | Max | Mean | SD | P80 | *N* | Min | Max | Mean | SD | P80 | SSD_x | SSD_p80 |
| 1 | Spain, Eastern Pyrenees | ag | 36 | 64 | 90 | 76.02 | 6.16 | 81.0 | 83 | 70 | 99 | 84.11 | 6.21 | 89.5 | 0.106 | 0.088 |
| 2 | Germany | ag | 158 | 55 | 85 | 68.80 | 6.17 | 75.0 | 160 | 59 | 96 | 75.60 | 7.60 | 82.0 | 0.097 | 0.093 |
| 3 | South Sweden | ag | 44 | | | 70.00 | 5.97 | 77.5 | 45 | | | 78.20 | 4.70 | 85.0 | 0.117 | 0.097 |
| 4 | Poland | ag | 32 | 57 | 84 | 69.28 | 7.86 | 78.0 | 64 | 59 | 89 | 73.31 | 7.52 | 80.0 | 0.058 | 0.026 |
| 5 | Ukraine, Transcarpathians | ag | 26 | 61 | 90 | 75.99 | 7.15 | 81.3 | 25 | 60 | 91 | 78.94 | 8.97 | 87.4 | 0.039 | 0.075 |
| 6 | Ukraine, Kharkiv Region | ex | 21 | 64 | 87 | 79.10 | 6.43 | 84.6 | 65 | 60 | 98 | 76.94 | 7.54 | 82.0 | −0.028 | −0.032 |
| 7 | Ukraine, Donetsk Region | ex | 40 | 62 | 104 | 86.86 | 8.68 | 91.2 | 50 | 69 | 105 | 86.47 | 9.53 | 96.4 | −0.005 | 0.034 |
| 8 | Ukraine, Luhansk Region | ex | 43 | 60 | 93 | 82.37 | 7.21 | 88.9 | 45 | 64 | 104 | 80.81 | 9.24 | 88.6 | −0.019 | −0.004 |
| 9 | Russia, Ciscaucasia 1 | ex | 40 | 60 | 99 | 79.60 | 6.96 | | 40 | 63 | 95 | 77.10 | 6.96 | | −0.032 | |
| 10 | Russia, Ciscaucasia 2 | ex | 302 | 63 | 111 | 79.73 | 10.56 | 90.0 | 296 | 63 | 108 | 78.37 | 10.12 | 87.0 | −0.017 | −0.034 |
| 11 | Russia, Ryazan Region | ex | 333 | 60 | 112 | 78.64 | 12.69 | 88.4 | 380 | 60 | 112 | 80.43 | 13.96 | 94.0 | 0.023 | 0.063 |
| 12 | Russia, Middle Volga 1 | ex | 92 | 62 | 105 | 88.88 | 11.16 | 98.0 | 96 | 64 | 100 | 83.56 | 9.32 | 91.0 | −0.070 | −0.077 |
| 13 | Russia, Middle Volga 2 | ex | 62 | 61 | 89 | 75.60 | 7.70 | 83.0 | 106 | 60 | 87 | 77.04 | 6.42 | 83.0 | 0.018 | 0.005 |
| 14 | North-eastern Kazakhstan | ex | 13 | 67 | 89 | 81.23 | 6.94 | 88.2 | 34 | 65 | 86 | 77.74 | 6.10 | 83.0 | −0.045 | −0.063 |
| 15 | Russia, Tomsk Region | ex | 127 | 61 | 94 | 79.69 | 6.34 | 84.4 | 121 | 62 | 105 | 83.44 | 9.09 | 91.4 | 0.047 | 0.083 |
| 16 | Russia, North Altai | ex | 5 | 68 | 87 | 79.98 | 7.96 | 87.0 | 37 | 66 | 95 | 79.30 | 7.55 | 87.2 | −0.009 | 0.003 |
| 17 | Russia, Altai Reserve | ex | 36 | 61 | 95 | 80.44 | 8.17 | 88.6 | 54 | 66 | 104 | 79.57 | 8.72 | 87.0 | −0.011 | −0.018 |

| Sample ID | Clutch size (CS) | | | | | Maternal SVL (SVL, mm) | | | | CS-SVL correlation | | Fecundity slope | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *N* | Min | Max | Mean | SD | Min | Max | Mean | SD | Estimate | SE | Estimate | SE |
| 1 | 37 | 4 | 15 | 9.16 | 2.81 | 71 | 99 | 85.04 | 6.70 | 0.779 | 0.106 | 0.420 | 0.045 |
| 2 | 52 | 5 | 19 | 9.31 | 2.97 | 65 | 93 | 76.90 | 5.69 | 0.657 | 0.107 | 0.523 | 0.056 |
| 3 | 83 | 4 | 14 | 8.22 | 2.15 | 61 | 94 | 80.88 | 6.12 | 0.587 | 0.090 | 0.352 | 0.032 |
| 4 | 14 | 6 | 14 | 9.64 | 2.37 | 63 | 87 | 77.00 | 6.06 | 0.850 | 0.152 | 0.391 | 0.060 |
| 5 | 16 | 4 | 14 | 9.44 | 2.68 | 60 | 91 | 79.22 | 8.07 | 0.836 | 0.147 | 0.332 | 0.049 |
| 6 | 45 | 4 | 14 | 7.40 | 2.18 | 61 | 98 | 77.86 | 7.95 | 0.821 | 0.087 | 0.274 | 0.024 |
| 7 | 14 | 3 | 10 | 6.71 | 2.27 | 69 | 102 | 83.91 | 9.73 | 0.806 | 0.171 | 0.241 | 0.043 |
| 8 | 34 | 4 | 12 | 7.62 | 2.20 | 70 | 98 | 82.38 | 7.93 | 0.892 | 0.080 | 0.278 | 0.022 |
| 9 | 22 | 4 | 11 | 7.64 | 2.17 | 68 | 100 | 83.66 | 9.61 | 0.751 | 0.148 | 0.226 | 0.033 |
| 10 | 46 | 2 | 12 | 8.52 | 2.46 | 60 | 105 | 83.86 | 10.48 | 0.551 | 0.126 | 0.234 | 0.030 |
| 11 | 45 | 4 | 12 | 7.47 | 1.65 | 63 | 102 | 80.42 | 10.11 | 0.606 | 0.121 | 0.163 | 0.020 |
| 12 | 53 | 5 | 12 | 7.92 | 1.63 | 74 | 100 | 87.79 | 6.06 | 0.685 | 0.102 | 0.269 | 0.027 |
| 13 | 64 | 3 | 8 | 5.30 | 1.32 | 65 | 87 | 78.69 | 5.12 | 0.616 | 0.100 | 0.257 | 0.026 |
| 14 | 31 | 3 | 11 | 5.97 | 1.99 | 65 | 86 | 77.65 | 6.20 | 0.720 | 0.129 | 0.321 | 0.041 |
| 15 | 41 | 2 | 13 | 7.37 | 2.85 | 62 | 105 | 83.39 | 10.35 | 0.750 | 0.106 | 0.276 | 0.029 |
| 16 | 21 | 4 | 9 | 6.05 | 1.56 | 69 | 90 | 78.89 | 6.98 | 0.701 | 0.164 | 0.224 | 0.037 |
| 17 | 19 | 3 | 13 | 6.53 | 2.99 | 68 | 97 | 81.68 | 9.86 | 0.803 | 0.144 | 0.303 | 0.044 |

Clades (subspecies): AG, *L. a. agilis*; EX, *L. a. exigua*.

Data sources, ID: 1 – Amat & Carretero, orig. data; 2 – Roitberg, orig. data; 3 – Olsson (1993) (clutch size – maternal SVL), Olsson (1988) (sex-specific SVL); 4 – Juszczyk (1974) (clutch size – maternal SVL), Borczyk & Paśko (2011) (sex-specific SVL); 5 – Roitberg, orig. data; 6 – Zinenko, orig. data; 7–8 – Kotenko, orig. data; 9 – Ushkalova (1976); 10 – Lukina (1966); 11 – Zharkova (1973); 12–13 – Eplanova, orig. data; 14 – Berdibayeva (1989, 1990); 15–16 – Kuranova & Bulakhova, orig. data; 17 – Yakovlev, orig. data.

Fecundity slope was calculated as the slope of the linear regression of clutch size on female SVL because the use of log-transformed data (King, 2000) generally did not increase the determination coefficient ($r^2$) of the model within our study samples. Following Shine (1994), Braña (1996) and Cox *et al.* (2003), we used the reduced major axis regression because the ordinary least square regression underestimates the slope when the independent variable has a measurement error or natural variation (e.g. Harvey & Pagel, 1991).

To simultaneously analyse categorical (lineage) and continuous (phenotypic variables and two climatic vectors, see below) effects on the variation among population means of a target trait, we used general linear models and selected the best model using Akaike's information criterion for finite samples (AICc). We started with a full model, and then we consecutively simplified it by removing the least significant predictor. We also checked whether our final model was affected by the order in which we removed the nonsignificant predictors.

The above analyses of fecundity-related traits treated sample means as data points. This approach would not be suitable for egg mass, hatchling mass and particularly RCM, because the mass traits were available for much fewer study samples than the fecundity data. At the same time, offspring size and RCM did not show substantial heterogeneity of sample variances. Therefore, we employed ANOVA designs for individual-based data with a reproductive trait as the dependent variable, and clade and sample (nested within clade) as the categorical effects. For a few samples that were presented with summary statistics only, we generated surrogate individual-based data, following the suggestion of Larson (1992).

A principal components analysis (PCA) was used to summarize the geographic variation of climatic parameters: the 36 intercorrelated temperature and precipitation variables were reduced to a smaller set of orthogonal vectors, which include a major portion of the total variation. These principal components were then used as predictor variables in the GLM procedures.

To investigate the possibility that an effect of climate on a study trait inferred from the above models is an artefact of isolation by distance, we tested for a relationship between among-sample distances for a study trait, climatic vectors and geographic distances. The statistical correlation between matrices of phenetic, climatic and geographic distances was evaluated using simple and partial Mantel tests. The software used was ZT (Bonnet & Van de Peer, 2002), and the number of permutations was 10000.

When necessary, variables were log-transformed to meet the requirements of parametric tests.

### Considering the effects of evolutionary lineage

Phylogeographic studies (Kalyabina-Hauf & Ananjeva, 2004; Joger *et al.*, 2007; Andres *et al.*, 2014) provided reasonably dense covering of border areas between the major clades, thereby all our study samples could be readily assigned to particular lineages based on their geographic locations. The above studies confirmed *L. a. agilis* and *L. a. exigua* to be distinct lineages. Applicability of established comparative phylogenetic procedures for the intraspecific variation, which may well include reticulated evolution, is still debated (e.g. Stone *et al.*, 2011; see also Díaz *et al.*, 2012). Considering this lack of consensus and the fact that the number of lineages in our study is two, we included clade identity as a predictor in our analyses to partial out the evolutionary pathway effects, as did Díaz *et al.* (2012).

### Methodological caveats

Numerous factors unrelated to geographic variation, such as local and temporal fluctuations in the abiotic (e.g. temperature and humidity) and/or biotic (e.g. food resources) environment, can affect statistical characteristics of body size and reproductive traits in a particular study sample (Fitch, 1985; Shine, 2005). Further biases can come from pooling data of several independent researchers. They may differ in measuring routine, type of material (living vs. preserved specimens), and in collecting and monitoring of gravid females. The biases from the first two factors are expected to be within 2 mm or so (Vervust *et al.*, 2009; Roitberg *et al.*, 2011), and this is much lower than the observed variation within and among our study samples. Means of clutch size computed for different oocyte stages (enlarged follicles vs. oviductal eggs vs. laid eggs) usually do not differ significantly in oviparous lizards (for *L. agilis*: Amat *et al.*, 2000; this study). Finally, and most importantly, the confounding factors considered here are unlikely to create a regular pattern shaped by a large number of independently collected data units. Only such robust patterns are considered in our discussion.

A more general problem is how mean clutch size and fecundity slope relate to the fecundity selection we wish to estimate. Fecundity slope is a measure of selective advantage of larger body size (Braña, 1996); it estimates ongoing (present) selection. Mean clutch size estimates the response to such fecundity selection in the past. Yet this 'past' may be quite recent if we compare conspecific populations. Moreover, the above difference is of minor importance in comparative studies such as ours, as especially for shaping SSD divergence *between* clades, 'recent' selection seems to be as important as ongoing selection. In presenting our evidence of these two types, we are assuming that (i) fecundity is heritable and (ii) clutch size appropriately estimates the life-time fecundity.

Shine (1994) discussed several conditions under which estimates of the fecundity selection intensity from fecundity slope, and estimates of the life-time fecundity from clutch size, are likely to be biased. A 'negative' bias, that is underestimated life-time fecundity of larger relative to smaller females, occurs if not only clutch size but also reproductive frequency (number of clutches per year) increases with female body size. A 'positive' bias occurs if larger females are bigger because they mature at later ages, thus missing earlier reproductive opportunities. However, in *L. agilis*, due to environmental constraints of seasonal climate, the predominant number of clutches per year is one and the modal age at first reproduction is about 2 years in a wide range of environments (Baranov *et al.*, 1976; Blanke, 2010). Therefore, in the study populations, clutch size is likely to be the by far most variable component, and thus a reasonable estimator, of the life-time fecundity.

## Results

### Climatic variation across the study sites

The first axis of the principal components analysis on the climatic variables (PC1-clim) explained 51.1% of the total variance among localities (Fig. S1). PC1-clim is strongly and positively loaded with all monthly temperature and precipitation parameters outside the warmest quarter (Fig. S1); PC1-clim is highly correlated with mean annual temperature ($r_s = 0.89$, $n = 17$, $P < 0.001$) and mean temperature of the coldest ($r_s = 0.99$, $P < 0.001$) but not the warmest ($r_s = 0.04$, $P = 0.87$) quarter.

In contrast, the second principal component (PC2-clim, 35.0% of the total variance, Fig. S1) is heavily loaded with the monthly values of the warmest season (April–September), with consistently positive loadings of temperatures and consistently negative loadings of precipitation (Fig. S1). PC2-clim is tightly related to the mean temperature of the warmest quarter ($r_s = 0.96$, $n = 17$, $P < 0.001$) but not the coldest quarter ($r_s = 0.10$, $n = 17$, $P = 0.70$).

### Between-clade differences for single reproductive characteristics

Consistent with Prediction 2, the fecundity slopes of all five West European samples (*L. a. agilis*) were steeper than those of the 12 eastern clade samples (*L. a. exigua*) (Fig. 2), and the difference between the two groups is significant (Mann–Whitney *U*-test, $Z = -3.16$, $P = 0.002$).

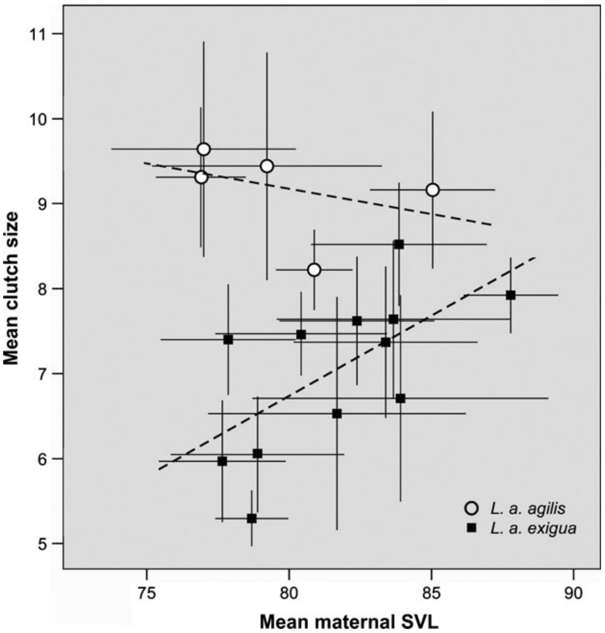

**Fig. 3** Clutch size and maternal SVL (means ± 2 SE) for 17 study samples of *Lacerta agilis*. Slopes of the regression differ at $P = 0.06$ between the subspecies (see text for details).

Mean clutch size was clearly higher in *L. a. agilis* than in *L. a. exigua* (Mann–Whitney *U*-test, $Z = -3.06$, $P = 0.002$; Fig. 3). Mean clutch size relative to mean maternal SVL differed in the same direction (Fig. 3), the differences being significant both in a separate slope model (clade, $F_{1,13} = 5.24$, $P = 0.039$; clade × maternal SVL, $F_{1,13} = 4.26$, $P = 0.060$; maternal SVL, $F_{1,13} = 1.08$, $P = 0.318$) and in ANCOVA (clade, $F_{1,14} = 28.25$, $P < 0.001$; maternal SVL, $F_{1,14} = 3.28$, $P = 0.092$). These results confirm Prediction 1. Note that whereas in the eastern clade the relationship of clutch size and maternal SVL among geographically distinct samples was highly concordant ($r_s = 0.66$, $N = 12$, $P = 0.014$), the western form did not show such a relationship at all (Fig. 3). The between-clade difference in the geographic relationship of the two traits is marginally significant

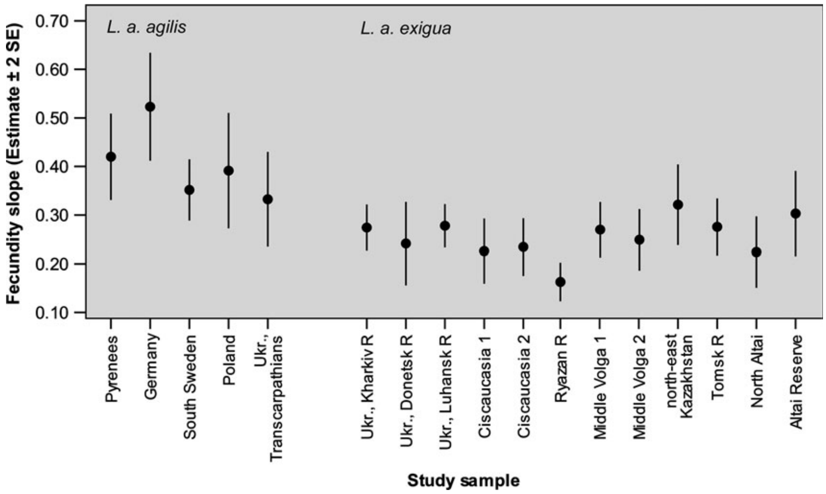

**Fig. 2** Slopes (±2 standard errors) of reduced major axis regression of clutch size on female SVL in 17 regional samples of *Lacerta agilis*.

($P = 0.06$ in the above separate slope model). Considering this pattern, we defined mean relative fecundity as the residuals of the regression of mean clutch size on mean maternal SVL calculated using the *L. a. exigua* samples only (Fig. 3). This derived character was used in our subsequent analyses as a predictor of SSD.

*L. a. agilis* also exhibited clearly lower egg and hatchling sizes, but higher relative clutch mass than *L. a. exigua* (Fig. 4; the latter pattern confirms Prediction 3). The differences are highly significant in all cases (Table 2), the effect of clade being always much stronger than that of sample (Table 2).

### Simultaneously estimating the effects of different predictors on SSD and sex-specific body size

For SSD, the initial set of predictors included clade, residual clutch size, fecundity slope and two climatic vectors (PC1-clim and PC2-clim) (Table 3). Contrary to Predictions 1b and 2b, neither mean size-specific clutch

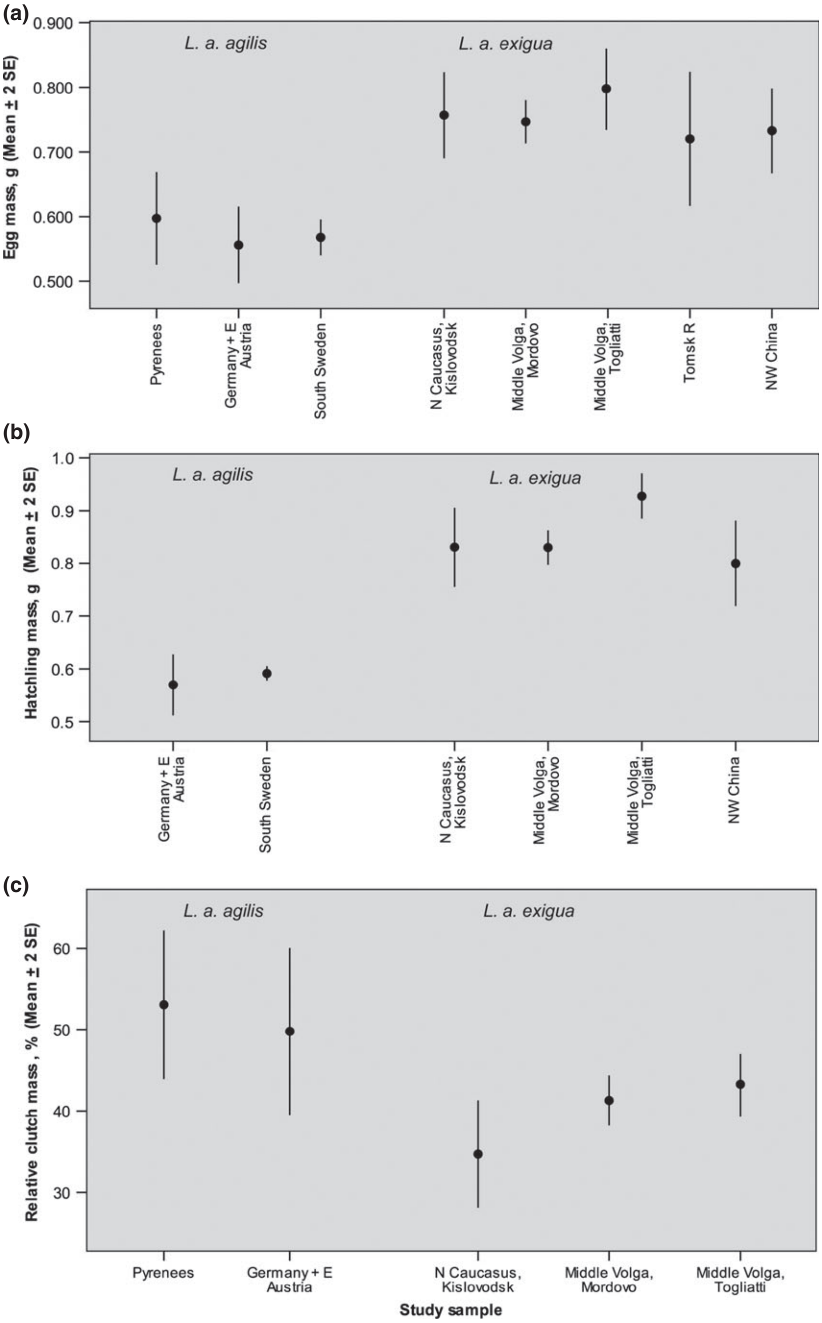

**Fig. 4** Variation in investment per offspring and total reproductive investment in local and regional samples of *Lacerta agilis*. Sources: Pyrenees, F. Amat & M.A. Carretero, orig. data; Germany + E Austria, Rykena (1988b); South Sweden, Olsson & Shine (1997a), Olsson & Madsen (2001); Ciscaucasia, Kislovodsk, Rykena (1988b); Middle Volga, Mordovo, G. V. Eplanova, orig. data; Middle Volga, Togliatti, G. V. Eplanova, orig. data; Tomsk Region, V.N. Kuranova & N.A. Bulakhova, orig. data; NW China, Li *et al.* (2013).

**Table 2** Nested ANOVAS for the total reproductive investment and the investment per offspring. See text for details.

| | *d.f.1* | *d.f.2* | *MS* | *F* | *P* | % variance (partial eta × 100) |
|---|---|---|---|---|---|---|
| Mean egg mass | | | | | | |
| Corrected model | 7 | 140 | 0.192 | 16.14 | 0.000 | 44.7 |
| Clade | 1 | 140 | 0.621 | 52.11 | 0.000 | 27.1 |
| Sample (Clade) | 6 | 140 | 0.011 | 0.90 | 0.496 | 3.7 |
| Mean hatchling mass | | | | | | |
| Corrected model | 5 | 210 | 0.707 | 100.92 | 0.000 | 70.6 |
| Clade | 1 | 210 | 1.252 | 178.71 | 0.000 | 46.0 |
| Sample (Clade) | 4 | 210 | 0.062 | 8.92 | 0.000 | 14.5 |
| Relative clutch mass | | | | | | |
| Corrected model | 4 | 61 | 0.199 | 3.56 | 0.011 | 18.9 |
| Clade | 1 | 61 | 0.633 | 11.29 | 0.001 | 15.6 |
| Sample (Clade) | 3 | 61 | 0.079 | 1.41 | 0.250 | 6.5 |

size nor fecundity slope remained in the best models; these results hold true also when clutch size was not corrected for maternal SVL and/or when reduced major axis estimators of fecundity slope were replaced with those from ordinary least square regressions. The two best models with nearly equally low AICc scores included the predictors clade and PC2-clim (74.6% of the total geographic SSD variance), or clade only (68.6%). In the former model, PC2-clim explained much less variance than clade (Table 3), but the model including only PC2-clim still explained a quite substantial portion of the total geographic variance (52.7%; see also Fig. 5d).

For sex-specific body size (mean SVL of adults), the initial set of predictors included those for SSD and additionally the mean SVL of the other sex (Table 3). The best model for male size included the predictors clade and female size. The best model for female size included male size and PC2-clim, whereas the effect of clade was not significant (Table 3); that is, despite the geographic body size variation being strongly correlated between the sexes, the SSD differences between *L. a. agilis* and *L. a. exigua* are primarily due to a shift in male size (Fig. 6).

## Investigating the effects of spatial autocorrelations

Mantel tests revealed a significant correlation between the matrix of among-sample distances for PC2-clim and the matrix of geographic distances (Geo) for the whole data set and for the subset of *L. a. exigua* samples (Appendix A). SSD was significantly associated with PC2-clim and with Geo (both with and without control for the opposite variable) across clades but not within

**Table 3** Best models for geographic variation of SSD and sex-specific body length in *Lacerta agilis*. See text for details.

| | *d.f.1* | *d.f.2* | *MS* | *F* | *P* | % variance (partial eta × 100) | AICc |
|---|---|---|---|---|---|---|---|
| Initial model: SSD BY Clade WITH Residual clutch size, Fecundity slope, PC1-clim, PC2-clim | | | | | | | |
| Corrected model | 2 | 14 | 0.018 | 20.59 | 0.000 | 74.6 | −63.87 |
| Clade | 1 | 14 | 0.010 | 12.08 | 0.004 | 46.3 | |
| PC2-clim | 1 | 14 | 0.003 | 3.33 | 0.089 | 19.2 | |
| Corrected model | 1 | 15 | 0.032 | 32.75 | 0.000 | 68.6 | −63.73 |
| Clade | 1 | 15 | 0.032 | 32.75 | 0.001 | 68.6 | |
| Corrected model | 1 | 15 | 0.025 | 16.73 | 0.001 | 52.7 | −56.78 |
| PC2-clim | 1 | 15 | 0.025 | 16.73 | 0.001 | 52.7 | |
| Initial model: ln(male SVL) BY Clade WITH ln(female SVL), Residual clutch size, Fecundity slope, PC1-clim, PC2-clim | | | | | | | |
| Corrected model | 2 | 14 | 0.034 | 36.25 | 0.000 | 83.8 | −63.87 |
| Clade | 1 | 14 | 0.030 | 32.11 | 0.000 | 69.6 | |
| ln(female SVL) | 1 | 14 | 0.018 | 19.67 | 0.001 | 58.4 | |
| Initial model: ln(female SVL) BY Clade WITH ln(male SVL), Residual clutch size, Fecundity slope, PC1-clim, PC2-clim | | | | | | | |
| Corrected model | 2 | 14 | 0.010 | 16.61 | 0.000 | 70.4 | −69.69 |
| ln(male SVL) | 1 | 14 | 0.020 | 32.65 | 0.000 | 70.0 | |
| PC2-clim | 1 | 14 | 0.007 | 11.18 | 0.005 | 44.4 | |

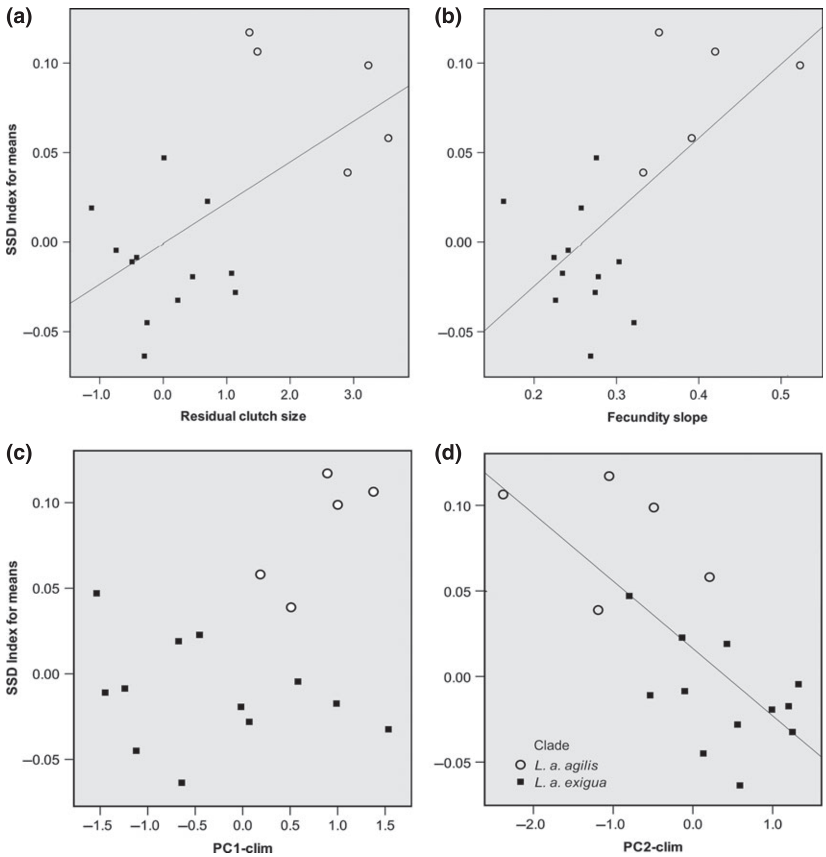

**Fig. 5** Sexual size dimorphism in *Lacerta agilis* plotted against the two main predictors of the fecundity-advantage hypothesis. (a, b), and against the two climatic vectors (c, d) explaining ca. 86% of the geographic variation for 36 climate parameters (see Fig. S1 for component structure). Regression lines are shown whenever the slopes are significantly different from zero; no significant relationships were found within clades.

clades (Appendix A). Taking into account that the geographic ranges of *L. a. agilis* and *L. a. exigua* show a distinct longitudinal separation (Fig. 1), the effects of climate, and particularly geographic proximity, remained confounded with that of lineage in our across-clades analyses. Therefore, the present results do not seem to provide strong evidence for the true effects of geographic separation on geographic SSD variation.

## Discussion

### Variation in sexual size dimorphism within clades

Geographic SSD variation *within* the studied lineages provides no support for the fecundity-advantage hypothesis: neither mean clutch size nor fecundity slope explains a substantial fraction of this variation, once the effect of clade is considered (Table 3). Thus, Predictions 1b and 2b are not confirmed. A marginally significant effect of PC2-clim, that is more female-biased SSD in areas with warmer and drier summers (Fig. 5d), found in this study, accords well with latitudinal/altitudinal clines previously reported for this species (Roitberg, 2007). This pattern can be most parsimoniously explained using the Adolph & Porter (1996) model. Their model predicts a nonlinear relationship between the length of the activity season and the age at first reproduction, which consequently affects the minimum and average body size of adults. In the year of life in which the warm climate individuals enter reproduction, individuals from colder environments cannot attain an appropriate body size within the available season so as to reproduce properly. Therefore, they invest available energy into further growth and start reproduction in the following year but at a larger size. As reproduction is expected to more strongly inhibit body growth in females than in males, earlier maturation might be responsible for smaller female relative to male size in warmer regions (see Roitberg & Smirina, 2006a for indirect evidence in *L. a. boemica*). In this model, the emerging SSD cline is a direct response to environmental constraint rather than

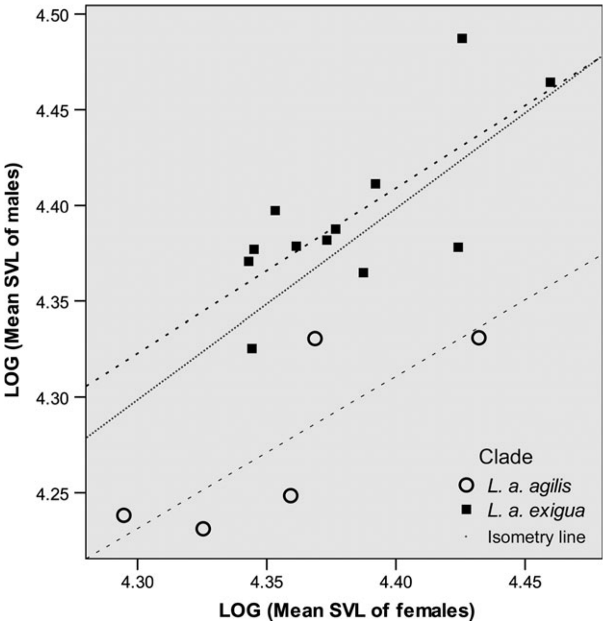

**Fig. 6** Plot of log-transformed male size on log-transformed female size for 17 study samples of *Lacerta agilis*. Isometry line (male size = female size) is also indicated (small hatched). Hatched lines are fit for the two clades (*L. a. agilis*, $r^2 = 0.661$, $n = 5$, $P = 0.094$; *L. a. exigua*, $r^2 = 0.548$, $n = 12$, $P = 0.006$).

genetic divergence. Specifically for *L. a. exigua*, the lack of considerable genetic differentiation over the range (Kalyabina-Hauf & Ananjeva, 2004; Andres *et al.*, 2014) and the lack of significant correlation between the SSD and geographic distances (Appendix A; geographic distances corrected for climatic distances are considered as proxies for genetic isolation – Jetz *et al.*, 2009) are consistent with this hypothesis. Pronounced SSD differences between conspecific populations due to plasticity were revealed in several reptile species (e.g. Madsen & Shine, 1993). Note that for the variation due to plasticity, FAH predictions are unlikely to hold *a priori* (because this model is explicitly selectionistic), and this may explain the discrepancy between the within-clade and between-clade patterns.

## Reproductive divergence between *L. a. agilis* and *L. a. exigua*

In contrast to the within-clade variation, the pattern of reproductive divergence *between* the studied clades is straight in line with the FAH predictions: the clade with consistently female-biased SSD (*L. a. agilis*) exhibits higher mean clutch size (both raw values and when corrected for female size) and higher fecundity slope than the clade with an inconsistent and variable SSD (*L. a. exigua*) (Predictions 1 and 2; Fig. 2; Fig. 3). *L. a. agilis* also shows clearly lower egg and hatchling sizes, but higher relative clutch mass than *L. a. exigua* (Prediction 3; Fig. 4). These patterns, as well as the SSD differences between the study clades, are likely due to genetic divergence, as they persist over a range of environments (Fig. 5c, 5d), and factor clade consistently explained much more of the total geographic variation than factor sample (Tables 2 and 3). This hypothesis is in line with considerable differentiation between *L. a. agilis* and *L. a. exigua* revealed by molecular genetic studies (see Introduction). Assuming that divergence between two clades reflects a single evolutionary event, the revealed association of female-biased SSD with higher mean relative fecundity and steeper fecundity slope could arise via stochastic processes (like founder effects, etc) rather than being an adaptive response to fecundity selection. Another possibility is that the reproductive pattern found in *L. a. agilis* is an ancestral state that persists largely due to phylogenetic conservatism. Below we present arguments that the FAH-related scenario is clearly more plausible than those based on these two alternative hypotheses.

A higher clutch size of *L. a. agilis* results not merely from a shift within an offspring size-number trade-off, as in most other reports on geographic life-history variations in lizards (e.g. Sinervo, 1990; Olsson & Shine, 1997a; Díaz *et al.*, 2012), but additionally from an increase in total reproductive investment (Fig. 4). Thus, the two possible ways to enhance the number of offspring per reproductive bout are exerted, and this argues for stronger fecundity selection in *L. a. agilis* than in *L. a. exigua*. Note that offspring mass does not tend to be negatively correlated to relative clutch mass among individual females within populations (Table S1; see also Marco & Pérez-Mellado, 1998), so that the revealed pattern of reproductive divergence is unlikely due to genetic or developmental constraints. Therefore, when considering the origin via stochastic processes, we have to assume two independent traits have evolved in the FAH-predicted directions. Such a scenario seems quite unlikely for traits so tightly related to fitness. The next paragraph presents evidence that phylogenetic conservatism is also an unlikely explanation for the life-history profile of *L. a. agilis*.

## Trends of life-history evolution within *Lacerta agilis*

Scarce data for the North Caucasian clade *L. a. boemica*, which occupies a basal position within the species phylogeny (Kalyabina-Hauf & Ananjeva, 2004; Joger *et al.*, 2007; Andres *et al.*, 2014; see also Roitberg, 1987 for morphological evidence), argue that its reproductive pattern might be even more distinct from that of *L. a. agilis* than the pattern of *L. a. exigua*. Egg and hatchling sizes of *L. a. boemica* (Warnecke, 2000; Kidov *et al.*, 2014) approach or exceed the upper values of *L. a. exigua*, whereas clutch size (Khonyakina, 1970; Kidov *et al.*, 2014) is close to the lowest values of population means in the eastern clade. Thus, out-group comparisons argue that small offspring size and higher fecundity of *L. a. agilis* are derived states, that is they are likely a result of recent evolution rather than common ancestry. This is also true for the consistently female-biased SSD of *L. a. agilis* because the SSD of *L. a. boemica* (as well as lacertid lizards in general – Cox *et al.*, 2007, fig. 4.1) is predominantly male biased (Roitberg, 2007).

The sequence *L. a. boemica* – *L. a. exigua* – *L. a. agilis* also exhibits another important trend: a decrease in the duration of egg incubation at a given temperature (Rykena, 1988a; Zakharov, 1989). A shorter incubation time in *L. a. agilis* is viewed as crucially important in wet and cool summer climates of Western Europe (Rykena, 1987; Olsson *et al.*, 1996; Olsson & Shine, 1997b). Indeed, in several other lizards, hatching date was found to be even more strongly fitness related than hatchling body size (Qualls & Shine, 2000; Warner & Shine, 2007). Taking into account that among individuals incubation time is often negatively correlated with hatchling size (e.g. Sinervo, 1990; Olsson *et al.*, 1996; Díaz *et al.*, 2012), smaller hatchling size in *L. a. agilis* could be a correlated response to selection for faster embryo development. Even in this case, we have to suggest stronger fecundity selection in the western clade to explain a higher relative clutch mass. Moreover, small offspring size – regardless through which evolutionary mechanism it has evolved – should enhance the potential for FAH because it naturally increases the fecundity slope if traded-off against offspring number.

### Putative determinants of sex-specific body size and SSD differences between *L. a. agilis* and *L. a. exigua*

Even though fecundity selection in *L. a. agilis* is likely stronger than in *L. a. exigua*, FAH cannot *solely* explain the more female-biased SSD of *L. a. agilis* because the SSD difference is primarily due to a shift in male rather than female size. Such a pattern, which corresponds to Rensch's rule (Fairbairn, 1997), is usually explained via geographic differences in sexual selection (Pearson *et al.*, 2002; Tamate & Maekawa, 2006; Puniamoorthy *et al.*, 2012). Yet the currently available evidence for sexual selection for larger male size being weaker in *L. a. agilis* than in *L. a. exigua* is poor. Roitberg (2007) suggested that in Western Europe, population density tends to be lower than in more eastern parts of the species range. At the same time, even in the female-larger *L. a. agilis*, in which weaker selection for larger male size is expected, larger males are more successful in agonistic interactions (Olsson, 1992) and have higher reproductive success (Gullberg *et al.*, 1997). Unfortunately, no comparable data exist for *L. a. exigua*. The prevalence of bite scars, which is often used as a proxy of the intensity of male–male agonistic encounters in snakes and lizards (e.g. Shine & Fitzgerald, 1995; Hasegawa, 2003), does not differ significantly between the study clades (*L. a. agilis*, 0.0%, $N = 62$; *L. a. exigua*, 4.2%, $N = 47$; E. S. Roitberg, unpublished data).

Instead of or in addition to the sexual selection hypothesis, the pattern of sex-specific body size differences between *L. a. agilis* and *L. a. exigua* could be explained by geographic differences in viability selection. This major component of lifetime selection is known to often counterbalance sexual selection in males and fecundity selection in females, which both usually favour larger size (Wikelski & Trillmich, 1997; Blanckenhorn, 2000; Preziosi & Fairbairn, 2000; Cox & Calsbeek, 2010; Puniamoorthy *et al.*, 2012). A comprehensive review by Siepielski *et al.* (2011) showed that at the temporal scale, viability selection varies in direction more frequently than sexual and fecundity selection. It seems therefore reasonable to assume that viability selection also varies geographically. We can thus hypothesize that viability selection favouring smaller adult body size, in correspondence with environmental growth constraints, is stronger in Western Europe than in more eastern parts of the species range. These forces are expected to similarly act on both sexes, but in females they are counterbalanced by positive fecundity selection (cf. Kratochvíl & Frynta, 2002; Roitberg, 2007; Stuart-Fox, 2009). Consistent with this hypothesis, male *L. a. agilis* are much smaller than male *L. a. exigua*, whereas female *L. a. agilis* are similar-sized but clearly more fecund than their eastern counterparts. The latter life-history profile in *L. a. agilis*, combined with a higher total reproductive investment (RCM), can well be viewed as a response to fecundity selection under conditions where increasing maternal body size is suppressed by viability selection and/or growth constraints. This hypothesis seems to be more consistent with our results than the hypothesis involving sexual selection (the latter cannot explain the pattern of reproductive divergence).

Yet as with sexual selection, evidence for macrogeographic differences in viability selection is poor. One can suggest that cool and humid summer climates in most of the species' range in Western Europe, thought to be suboptimal for egg incubation (Rykena, 1987; Olsson *et al.*, 1996; Olsson & Shine, 1997b), might also reduce energy acquisition opportunities for adult sand lizards (relative to those in more continental Eurasia). We stress that the three putative determinants of the smaller male size of *L. a. agilis* may well act in concert: an environmental factor like reduced energy acquisition opportunities can (i) directly constrain growth, (ii) decrease survival of large adults and (iii) reduce energetically costly social behaviour and thus mating advantages of large males. Regardless of which of the outlined mechanisms are or were involved, fecundity selection has likely contributed to the SSD differences between the study lineages.

### Other evolutionary implications of reproductive divergence within *Lacerta agilis*

The pattern of reproductive divergence within *L. agilis* is interesting in a more general context than FAH. A negative association of high total reproductive investment with low investment per individual offspring is not predicted by the classical life-history theory (Smith & Fretwell, 1974; for further references, see Caley *et al.*, 2001; Czesak & Fox, 2003), which modelled these two traits as independent. However, this association is predicted by a more recent model (Winkler & Wallin, 1987), and an evolutionary link of this kind has been revealed in a few empirical studies (Caley *et al.*, 2001 and references therein; Czesak & Fox, 2003). As the study systems whose reproductive divergence follow the Winkler & Wallin model should exhibit a pronounced differentiation in fecundity (and apparently, in fecundity selection), they are particularly promising for testing FAH. Moreover, we predict the existence of some link between the two models: regardless whether small offspring size has evolved due to fecundity selection or for other reasons, it does enhance the potential for FAH because it naturally increases the fecundity slope.

Below we argue that *Lacerta agilis* with its allies, that is species of the tribe Lacertini (Arnold *et al.*, 2007), can be a study system of this kind. In this group, a strong FAH-predicted association between the SSD and reproductive patterns was found (eight sympatric species from NW Spain were studied – Braña, 1996). Further, the reproductive divergence among these species is likely in line with the Winkler & Wallin model: both relative clutch mass (positively) and mean egg mass relative to female mass (negatively) are correlated with the extent of retention of eggs in maternal oviducts (Braña *et al.*, 1991). The latter trait is an important variable of reptilian life history that reflects the position of the individual female, population or species along the *oviparity–viviparity continuum* (Shine, 1983; Braña *et al.*, 1991). Remarkably, within one of these species (*Zootoca vivipara*), which exhibits reproductive bimodality, the pattern of reproductive divergence between viviparous (smaller offspring mass and higher relative clutch mass) vs. oviparous (larger offspring mass and lower RCM) populations (Roitberg *et al.*, 2013) is similar to that between *L. a. agilis* and *L. a. exigua* (this study). This parallelism lends additional support for the Winkler & Wallin model.

In the above context, it is noteworthy that according to Dobrowolska (1985), freshly laid eggs of *L. a. agilis* have more developed embryos than those of *L. a. exigua*, the difference constituting 10–15 days. Rollinat (1905, cited by Angel, 1946) found advanced embryos of 10 mm length in freshly laid eggs of *L. a. agilis* and spoke of a tendency to ovoviviparity. These reports are in line with two other studies that revealed a short incubation time in the latter form (Rykena, 1988a; see also Sobocinski & Maślak, 1996). It is, therefore, likely that the extent of egg retention in *L. a. agilis* is consistently higher than in *L. a. exigua*, and the life-history profile of *L. a. agilis* revealed in the present study (smaller offspring size, higher RCM and female-biased SSD) might be an evolutionary correlate of a shift along the *oviparity–viviparity continuum*. Note that a more female-biased SSD in viviparous vs. related oviparous taxa is the most consistent pattern found in all studies of broad-scale SSD variation in squamate reptiles (Fitch, 1981; Shine, 1994; Cox *et al.*, 2003).

## Conclusion

As predicted by the fecundity-advantage hypothesis, the lineage with consistently female-biased SSD (*L. a. agilis*) exhibited higher clutch size and steeper fecundity slope than the lineage with an inconsistent and variable SSD (*L. a. exigua*). Although weak if taken alone, this evidence increases in importance if one considers the following points: (i) the higher clutch size of *L. a. agilis* is unlikely due to genetic correlations or stochastic processes because it is achieved via *two independent* ways, namely by decreasing mean offspring size and at the same time increasing the total investment. (ii) The higher clutch size and female-biased SSD of *L. a. agilis* is unlikely due to phylogenetic conservatism because the most basal clade (*L. a. boemica*) apparently has even larger offspring, even smaller size-specific clutch size and even smaller female relative to male size than *L. a. exigua*. (iii) A similar pattern of life-history divergence was found between oviparous and viviparous populations of another species of Lacertinae, as well as among species of this subfamily. Lack of association between SSD and clutch size within the studied lineages may reflect a greater role of purely phenotypic component at this level of variation. Further investigations, particularly long-term common garden experiments (Ferguson & Talent, 1993; Madsen & Shine, 1993) involving populations which belong to different clades or experience contrasting climatic environments would provide a more rigorous test of the scenario suggested by our comparative study.

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## Appendix A

Summary of Mantel tests applied to the geographic SSD variation in *Lacerta agilis* within and between the study clades. Simple Mantel tests were used to examine the correlation between phenotypic distance (SSD) with geographic (Geo) or climatic (PC2-clim) distance, and the partial Mantel test was used to examine the correlation between differences in SSD and climatic distance controlled for geographic distance (PC2-clim | Geo Distance) and vice versa (Geo | PC2-clim Distance).

| | All samples, *n* = 17 | | *L. a. agilis*, *n* = 5 | | *L. a. exigua*, *n* = 12 | |
|---|---|---|---|---|---|---|
| Mantel test | *r* | *P* | *r* | *P* | *r* | *P* |
| PC2-clim with Geo | **0.251** | 0.028 | 0.391 | 0.258 | **0.605** | 0.002 |
| SSD with PC2-clim | **0.410** | 0.002 | –0.337 | 0.142 | 0.094 | 0.234 |
| SSD with Geo | **0.324** | 0.009 | 0.171 | 0.275 | –0.056 | 0.388 |
| SSD with PC2-clim \| Geo | **0.359** | 0.005 | –0.445 | 0.067 | 0.161 | 0.154 |
| SSD with Geo \| PC2-clim | **0.250** | 0.026 | 0.349 | 0.142 | –0.143 | 0.140 |

Bold values represent significant correlations ($P < 0.05$).

## Supporting information

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

**Figure S1** Factor loadings and percents of trace associated with the first two principal components of among-sites variation in climatic parameters.

**Table S1** Pearson correlation coefficients between the total reproductive investment and the investment per offspring among individual females within populations.

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