

Female common lizards (*Lacerta vivipara*) do not adjust their sex-biased investment in relation to the adult sex ratio

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Abstract

Sex allocation theory predicts that facultative maternal investment in the rare sex should be favoured by natural selection when breeders experience predictable variation in adult sex ratios (ASRs). We found significant spatial and predictable interannual changes in local ASRs within a natural population of the common lizard where the mean ASR is female-biased, thus validating the key assumptions of adaptive sex ratio models. We tested for facultative maternal investment in the rare sex during and after an experimental perturbation of the ASR by creating populations with female-biased or male-biased ASR. Mothers did not adjust their clutch sex ratio during or after the ASR perturbation, but produced sons with a higher body condition in male-biased populations. However, this differential sex allocation did not result in growth or survival differences in offspring. Our results thus contradict the predictions of adaptive models and challenge the idea that facultative investment in the rare sex might be a mechanism regulating the population sex ratio.

Introduction

Fisher argued that the evolution of parental investment into sons and daughters should be influenced by fitness returns from differential investment into the sexes, such that higher fitness returns in one sex selects for stronger parental investment in that sex (Fisher, 1930; Charnov, 1982; Frank, 1990). Higher fitness returns in one sex can be the consequence of demographic disequilibria if the rare sex benefits from a lower intrasexual competition (Fisher, 1930). Werren & Charnov (1978) showed that a facultative investment in the rare sex could also be selected for in species being subjected to variations in adult population sex ratios (ASRs). In their article, Werren & Charnov (1978) modelled species whose generations are overlapping and in which females adjust their sex-biased investment in relation to the local ASR.

In such species, conditions favouring a facultative investment in the rare sex are (i) variable ASRs experienced by the parents and (ii) predictability in ASRs experienced by offspring later in their life (Werren & Charnov, 1978; Bensch *et al.*, 1999).

Fisher's theory was originally phrased as a differential investment in males and females (Fisher, 1930; Frank, 1990), thus including both sex ratio (the proportion of one sex) and sex allocation (the proportion of resources invested in one sex). It is therefore indispensable to assess sex ratio and sex allocation tactics as well as the sex-specific offspring fitness when testing the predictions of Werren and Charnov's model. The ability of species with genotypic sex determination (GSD) to adjust their sex ratio has been questioned due to constraints imposed by the Mendelian segregation of sex chromosomes (Frank, 1990; Oddie, 1998). Case studies in birds and mammals provided strong support for adaptive adjustment of the sex ratio at birth (reviewed by Cockburn *et al.*, 2002), but evidence is lacking in many species (Krackow, 1995; Ewen *et al.*, 2004). Studies in lizards (Reptilia, Squamata) suggest several potential mechanisms by which females might adjust their sex-biased investment. In GSD species,

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mothers could control sex ratio via gamete selection and differential abortion of embryos (Blackburn, 1998; Calsbeek & Sinervo, 2004). These mechanisms might be under environmental control through the effects of gonadotropin and steroid hormones (Krackow, 1995; Sinervo, personal communication), and could therefore be influenced by variation in ASR. Furthermore, studies have demonstrated mechanisms of temperature-dependent sex determination in lizards (Robert & Thompson, 2001; Shine, 2002; Wapstra *et al.*, 2004), suggesting that mothers can control the sex ratio by selecting the temperature at which their offspring develop. Finally, mother lizards can allocate more resources to one sex by biasing the amount of yolk or steroid hormones deposited in the egg (e.g. Olsson & Shine, 2001; Painter *et al.*, 2002). This differential allocation might influence the number and/or the quality of the recruiting offspring, and thus the fitness returns of the sex-specific parental investment.

Here, we used the common lizard (*Lacerta vivipara* Jacquin, 1787) as a model system to experimentally test Werren and Charnov's predictions. Like the species envisioned by Werren & Charnov (1978), the common lizard is characterized by overlapping generations that share small and nonexclusive home ranges (Massot *et al.*, 1992). In natural populations variation in local ASRs can result from sexual differences in survival and movement (Massot *et al.*, 1992). Furthermore, female common lizards can perceive ASR variation of their environment by optical or chemical cues (Bauwens *et al.*, 1987). In this viviparous species, the sex at birth is determined (at least partly) genetically by a modified ZZ/ZW chromosomal system (Chevalier *et al.*, 1979). Transfer of maternal metabolites and steroids during egg formation (synchronized with mating) and during embryonic development (through a primitive chorioallantoic placenta) allow for maternal effects (Panigel, 1956; Gavaud, 1986). Females are the heterogametic sex (ZW) and have thus the potential to control sex-biased investment through pre-ovulation control of chromosome segregation, selective sex-specific abortion of the ovulated follicles, and sex-biased investment into the eggs. Maternal control of offspring life history traits has been demonstrated in this species (e.g. Meylan & Clobert, 2004) and sex-specific prenatal effects on offspring morphology are known (Uller *et al.*, 2004). In the common lizard, the clutch sex ratio at birth varies with environmental conditions (pp. 69–83 in Leturque, 2002). Higher habitat humidity in the study of Lorenzon *et al.* (2001) was associated with more male-biased sex ratios at birth and experimentally increased maternal corticosterone levels led to more female-biased clutches in the experiment by Meylan & Clobert (2004). These results indicate that sex ratios can be adjusted in response to external cues. Because experimental treatments were applied during the last month of gestation in both studies, sex-specific mortality of embryos might have been the adjustment mechanism (Leturque, 2002).

We first studied spatial and interannual variations in local ASR in a natural population of the common lizard monitored from 1989 to 2001 (Clobert *et al.*, 1994). In this population, the mean ASR is female-biased (Massot *et al.*, 1992). Using information on natural ASR variation, we then experimentally tested for facultative maternal investment in the rare sex. We simulated a temporary deviation of the ASR from its natural (female-biased) situation. This approach corresponds to the 'dynamic tests' of Fisherian sex ratio adjustment theory (Bull & Charnov, 1988) and matches the population dynamics envisioned in 'perturbation models' (Werren & Charnov, 1978; West & Godfray, 1997). We created populations with a female-biased ASR (control) and populations with a male-biased ASR (treatment) during a first breeding episode. We then translocated all individuals into populations with female-biased ASR (control) for a second breeding episode. According to the model developed by Werren & Charnov (1978), we predict that mothers should bias their sex ratio and/or sex allocation towards the rare sex.

Materials and methods

Study species

The common lizard *L. vivipara* is a small Lacertidae [adult snout-vent length (SVL): 50–70 mm]. In early spring, males emerge on average 1 month earlier than females from hibernation and mating occurs in April. Females can start reproducing at the age of 1 year and almost all females reproduce at the age of 2 years (Boudjemadi *et al.*, 1999). Parturitions last from the beginning of June until the end of July. Females lay on average five transparent, soft-shelled eggs (range: 1–12). The offspring hatch within the same day and thereafter are autonomous. Individuals were marked with a unique code using toe clipping.

Adult sex ratio variations in a natural population

We define the ASR as the proportion of males among adult individuals. As part of a long-term population monitoring, a site located at the Mont Lozère (1420 m above sea level, 44°30'N, 3°45'E) has been studied from 1989 to 2001 (Clobert *et al.*, 1994). The study population was monitored each year during late spring and during late summer, and the code, position and sex of the reproducing (aged more than 1-year old) individuals were identified. The study site was divided in neighbourhoods of 20 by 20 m to monitor interannual and spatial variations in the local ASRs. The neighbourhood's area corresponds to the maximum home range area of female lizards breeding in this population and is a pertinent choice to study social environments in this species (Clobert *et al.*, 1994). The repeated monitoring was used to calculate the year-specific capture probabilities sepa-

rately for males and females in our study site. Sex-specific capture probabilities were estimated with Cormack–Jolly–Seber models [$\Phi(t)$, $P(t)$] fitted on the mark-recapture data including sex as a factor, using a likelihood-based approach (Lebreton *et al.*, 1992), and assuming similar capture probabilities among neighbourhoods. We checked with GOF tests that there was no significant heterogeneity in capture probabilities using the Test 2 + Test 3 of the Cormack–Jolly–Seber model in RELEASE (Lebreton *et al.*, 1992) (males: $\chi^2_{13} = 5.16$, $P = \text{n.s.}$; females: $\chi^2_{33} = 30.43$, $P = \text{n.s.}$). Subsequently we estimated the number of males and females present in each neighbourhood in late spring by dividing the number of adult males or females seen in each neighbourhood by our estimates of sex-specific capture probability. The estimated number of females per neighbourhood varied from 0 to 34 (mean = 6.35 ± 0.41 SE, $n = 197$). Because we were interested in ASR variations experienced by breeding females, neighbourhoods where no adult female were seen were excluded from the analyses.

Experimental procedures

The experimental study was carried out in semi-natural populations at the Ecological Research Station of Foljuif (60 m above sea level, 48°17'N, 2°41'E) using lizards from natural populations of the Mont Lozère area. Experimental populations were housed in 12 outdoor enclosures (10 × 10 m). The enclosure size corresponds to the female's core home range size. Enclosures were located in a natural meadow and were surrounded by plastic walls to prevent lizards from escaping [see Boudjemadi *et al.* (1999) for more details]. Lizards were able to disperse by using a 20 m long dispersal corridor ending in a pitfall trap. Dispersing lizards were collected daily and introduced into a new population of the same ASR treatment. Less than 10% of the individuals dispersed during the study and dispersal probability were not affected by treatments, except in adult females during the first summer (Le Galliard & Fitze, unpublished results). The female dispersal behaviour did not affect their reproductive characteristics (differences between resident and dispersing females in fecundity: $P = 0.18$, clutch sex ratio: $P = 0.52$, offspring morphology: all $P > 0.67$), and resident and dispersing females were therefore analysed together.

In June–July 2002, six randomly chosen enclosures were populated with an ASR biased towards females. This treatment corresponds to the average ASR observed in natural populations and thus serves as a control. In another six enclosures, we initiated populations with a male-biased ASR (treatment, see Table 1). Yearling and juvenile sex ratios were held constant in all populations (1 : 1) as well as the proportion of yearling, juvenile and adult lizards. The initiated population structure corresponds to the equilibrium population structure observed

Table 1 Mean age and sex structure per treatment in six treatment (male-biased) and six control (female-biased) populations at the start of the study. Data are mean \pm SD.

Age class	Treatment populations		Control populations	
	Males	Females	Males	Females
Juveniles	21.3 \pm 0.5	21.8 \pm 0.7	21 \pm 0.6	22.3 \pm 1.1
Yearlings	6 \pm 0	6 \pm 0	6 \pm 0	6 \pm 0
Adults	14 \pm 0	4 \pm 0	4 \pm 0	14 \pm 0

in the wild (Massot *et al.*, 1992). The ASR of the control populations (ASR = 0.22) corresponds to the average ASR of the natural population studied, while the ASR of the treatment populations (ASR = 0.78) corresponds to the extreme ASRs observed in the same natural population. Before release, SVL was measured to the nearest mm and body mass was measured to the nearest mg.

In early June 2003, all surviving lizards were captured. The ASR was still different between treatment and control populations (treatment: 0.80 ± 0.03 SE; control: 0.37 ± 0.04 SE; $\chi^2_1 = 53.50$, $P < 0.001$). Females were housed in individual terraria (25 × 15 × 15 cm) under standardized conditions (Le Galliard *et al.*, 2003b). Terraria were checked daily for freshly laid clutches at 9:00 and 14:00 o'clock. Approximately 1 h after clutch detection, viable offspring ($n = 549$), dead offspring ($n = 62$) and late aborted embryos ($n = 50$) were measured for SVL, tail length and body mass, and they were sexed by counting the number of ventral scales (Lecomte *et al.*, 1992). This method correctly determines the offspring sex in 96% of the cases, as evidenced using three cohorts of juveniles that were again sexed at the age of 1 year, where sex-attribution is unambiguous ($n = 525$ recaptures; Le Galliard, unpublished data). Aborted embryos were distinguished from dead offspring (offspring that did not hatch) by the developmental stage: dead offspring were melanin-pigmented and fully developed with a similar body shape as viable offspring (Dufaure & Hubert, 1961). The same person took all measurements.

Animals were then released into new, unknown enclosures containing a female-biased ASR and thus corresponding to the control situation simulated during the first year of this experiment. This 'common garden' experiment allowed for the measurement of the benefits of sex-biased offspring investment, independent of any direct effects of the ASR (e.g. survival or growth effects) 1 year after the ASR perturbation. Two days after hatching, both the living offspring (532 of 549) and their mothers from the first year of the experiment were released into eight new enclosures. Offspring and mothers were released with a homogeneous sample of adult males and yearlings. In each enclosure, 18 adult females, 10 adult males and 12 yearlings (sex ratio 1 : 1) were introduced to create a female-biased ASR (36% of adult males). Within each enclosure, the offspring sex ratio was held constant (sex ratio: 0.48 ± 0.038 SE) and the

proportions of offspring originating from control and treatment populations were similar (proportion of offspring originating from control populations: 0.08 ± 0.02 SE; $\chi^2_7 = 10.92$, $P = 0.14$). At the end of May 2004, all surviving lizards were recaptured and females were housed in individual terraria. Clutch sex ratio and offspring morphology were measured at birth using the same procedures as described above.

Statistical analysis

We analysed the results of the experiment using mixed-effects models in SAS (Littell *et al.*, 1996). Sex ratio (proportion of males in the clutch) and sex-specific survival probability of the offspring were modelled with the GLIMMIX procedure using a logit link and a binomial error term. The goodness of fit of the models was checked with a Pearson chi-square test (McCullagh & Nelder, 1989). Offspring morphology (SVL, tail length and body condition) and sex-specific body growth rates of offspring were analysed with general linear mixed models using the MIXED procedure. The latter models' assumptions (normality and homogeneous variance of residuals) were fulfilled in all cases. All models contained at least the following factors: ASR treatment, mother age class (juvenile, yearling or adult), SVL of the mother and their interactions as fixed effects, as well as the random effect of the enclosure nested within treatment and the random effect of the family nested within enclosure and treatment. For survival and growth rates of the first generation of offspring, we included potential differences between release enclosures by adding release enclosure as a random factor. The final models were selected after backward elimination of nonsignificant terms.

All offspring sexed at birth were included in the analysis of the sex ratio at birth ($n = 651$ observations from 117 families). We tested for potential differences among the offspring's developmental classes (late aborted embryos, dead offspring and viable offspring) by including a categorical variable with three levels into the model. The clutch sex ratio 1 year after birth was analysed as the proportion of surviving male offspring per number of surviving offspring ($n = 66$ families). For the analyses of offspring SVL, tail length and body condition (body mass adjusted for body length by including SVL as a covariate in the analysis), late aborted embryos were excluded and a categorical factor (dead or viable) was included into the model to distinguish between the two developmental classes.

Results

Adult sex ratio variations

The local ASRs varied from 0 to 0.77 in the natural population (average ASR = 18 ± 0.18 SD, 22 neighbourhoods surveyed during 12 years). In a total of 16

neighbourhoods, sufficient data was available for the analysis of local ASR variations over five subsequent years. ASRs varied significantly among years and neighbourhoods (logistic regression, neighbourhood: $\chi^2_{15} = 41.24$, $P < 0.001$; year: $\chi^2_4 = 68.97$, $P < 0.0001$). In 21 instances, ASRs could be measured during two consecutive years within the same neighbourhood. Local ASRs during two consecutive years were positively correlated [ANOVA, see Lessels & Boag (1987), $F_{20,21} = 3.16$, $P < 0.01$, intra-class correlation coefficient: $r = 0.52$]. Similar interannual variation was detected at larger spatial scales, but spatial variation in ASR was only significant at scales below 25 m. When analysing fluctuations in the ASR at the population level during 12 years, ASR in year t was positively correlated with ASR in year $t + 1$ ($F_{10,11} = 3.35$, $P < 0.05$, $r = 0.54$).

Sex ratio adjustment during the manipulation

We modelled sex ratio at birth and sex ratio 1 year after birth with mixed effects logistic regressions. The goodness of fit test of these models showed no evidence of extra-binomial variation (sex ratio at birth: $\chi^2_{639} = 622.4$, $P = \text{n.s.}$; sex ratio 1 year after birth: $\chi^2_{149} = 151.0$, $P = \text{n.s.}$). The sex ratio at birth in our semi-natural populations did not differ from the Mendelian expectation for a species with genotypic sex determination [average clutch sex ratio = 0.48 (0.43, 0.53) (95% CI) males per clutch]. The sex ratio at birth was 0.53 (0.38, 0.68) in male-biased populations and 0.48 (0.41, 0.55) in control populations. The differences between ASR treatments were not significant ($F_{1,10} = 0.92$, $P = \text{n.s.}$). Mother SVL, mother age and the interactions between these factors and the ASR treatment did not affect the sex ratio at birth (all $P > 0.30$). The sex ratio at birth was however significantly different among developmental classes ($F_{2,532} = 4.29$, $P < 0.05$). The sex ratio was 0.71 (0.55, 0.83) in aborted embryos, 0.51 (0.38, 0.64) in dead offspring, and 0.46 (0.41, 0.52) in viable offspring. These differences in sex ratio among developmental stages were not affected by the ASR treatment (interaction ASR treatment \times developmental class: $F_{2,530} = 0.04$, $P = \text{n.s.}$). Furthermore, the clutch sex ratio 1 year after birth was not significantly affected by the ASR treatment ($F_{1,8} = 0.42$, $P = \text{n.s.}$). The clutch sex ratio 1 year after birth was 0.50 (0.21, 0.79) in treatment populations and 0.40 (0.31, 0.50) in control populations.

The absence of a clutch sex ratio adjustment in response to the ASR manipulation may be the result of a lack of power due to the relatively low number of clutches in male-biased populations ($n = 20$). Therefore, the minimum detectable effect size (defined according to Quinn & Keough (2002) as the sex ratio contrast between ASR treatments associated with a power of 0.80) was calculated using Monte Carlo simulations (1000 data sets with a clutch size distribution within each population similar to our sample). The minimum

detectable effect size at birth was a clutch sex ratio contrast of approximately 0.17 between ASR treatments, suggesting that our statistical tests had reasonable power.

Sex allocation adjustment during the manipulation

The ASR treatment had no effect on the sexual dimorphism in SVL (effect of sex \times ASR treatment: $F_{1,497} = 1.38$, $P = \text{n.s.}$) and in tail length at birth ($F_{1,496} = 0.001$, $P = \text{n.s.}$, Fig. 1), but influenced the sexual dimorphism for body condition at birth ($F_{1,495} = 9.24$, $P < 0.01$). Male offspring had higher body condition in male-biased populations than in control populations (contrast = 18.3 mg adjusted mass \pm 6.84 SE, $P < 0.01$), while female offspring showed no difference between ASR treatments (contrast = 5.39 mg adjusted mass \pm 6.48 SE, $P = \text{n.s.}$, Fig. 1). This difference was not due to intersexual differences in body mass between the ASR treatments (effect of sex \times ASR treatment on body mass: $F_{1,498} = 0.12$, $P = \text{n.s.}$).

Despite the differences in body condition at birth between male-biased and control populations, the ASR treatments had no long-lasting effects on the performances of the offspring as measured by their sex-specific annual survival probability (effect of sex \times ASR treatment: $F_{1,506} = 0.13$, $P = \text{n.s.}$, $n = 532$) and growth rates (effect of sex \times ASR treatment: $F_{1,120} = 12.47$, $P = 0.12$; size at birth: $F_{1,120} = 13.78$, $P < 0.001$; date of birth: $F_{1,120} = 7.72$, $P < 0.01$, $n = 141$; see Table 2).

Sex-biased maternal investment after the manipulation

Of the 890 juvenile, yearling and adult females that were introduced in our experimental populations in 2002, 71 survived until the second reproductive episode in 2004 (1 year after the ASR manipulation), and 38 gave birth to offspring that could be sexed at birth ($n = 162$ offspring). The clutch sex ratio at birth of the surviving females was on average 0.48 (0.40, 0.56) (95% CI), and did not differ between ASR treatments ($F_{1,10} = 0.41$, $P = 0.53$). The clutch sex ratio was 0.50 (0.40, 0.60) for females originating from control populations and 0.46 (0.32, 0.59) for females originating from male-biased populations. The mother's SVL, age and the interactions between these factors and the ASR treatments did not affect the sex ratio of the clutch (all $P > 0.08$).

Sex allocation was studied by analysing the SVL, tail length and body condition of offspring born 1 year after the ASR perturbation (Table 3). After backward elimination of the nonsignificant terms, the ASR treatment had no effect on offspring SVL (effects of ASR treatment: $F_{1,10} = 0.52$, $P = \text{n.s.}$; sex: $F_{1,121} = 17.60$, $P < 0.0001$; sex \times ASR treatment: $F_{1,121} = 0.41$, $P = \text{n.s.}$; mother age: $F_{2,121} = 4.74$, $P < 0.01$, $n = 161$), tail length (effects of ASR treatment: $F_{1,10} = 0.63$, $P = \text{n.s.}$; sex: $F_{1,121} = 2.92$, $P = 0.09$; sex \times ASR treatment: $F_{1,121} = 0.01$, $P =$

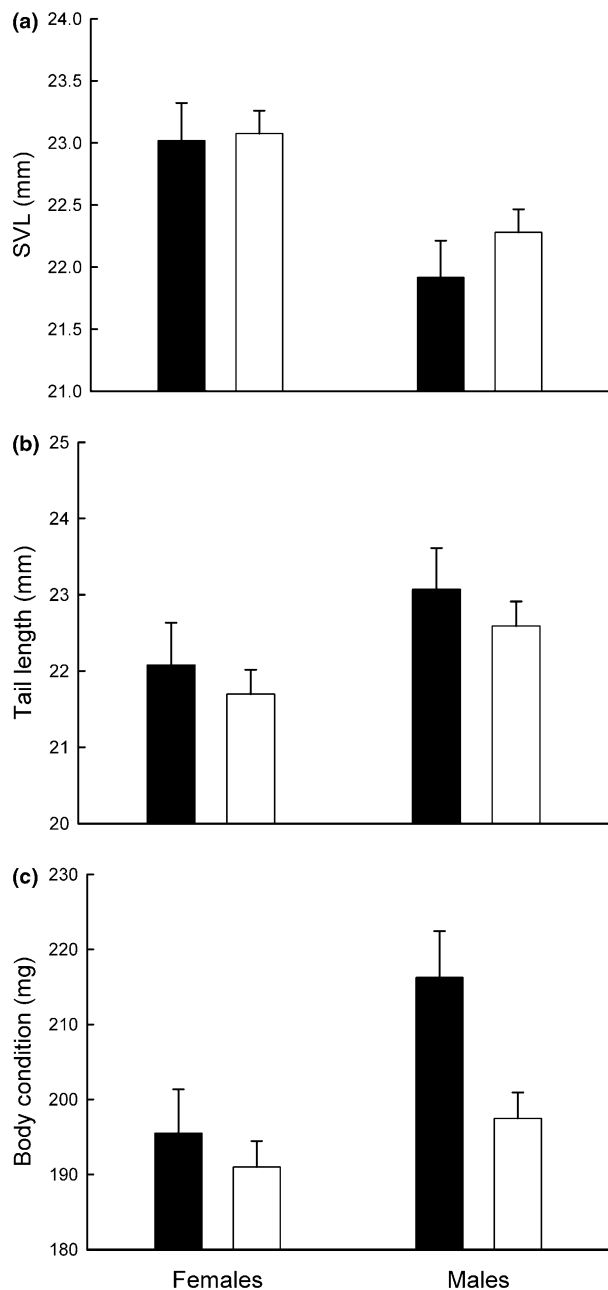


Fig. 1 Direct effects of the ASR treatment on SVL (a), tail length (b) and body condition (c). Least-squares mean (\pm SE) of male and female offspring in treatment (filled bars, male-biased) and control (empty bars, female-biased) populations are presented (models described in the text). Males were smaller at birth ($F_{1,498} = 224.70$, $P < 0.0001$), had a longer tail length ($F_{1,497} = 64.48$, $P < 0.0001$), and a higher body condition ($F_{1,495} = 44.35$, $P < 0.0001$) than females, but body mass did not differ between the sexes ($F_{1,498} = 0.12$, $P = 0.73$).

n.s.; mother age: $F_{2,121} = 5.31$, $P < 0.01$) and body condition (effects of ASR treatment: $F_{1,10} = 0.51$, $P = \text{n.s.}$; sex: $F_{1,119} = 8.14$, $P < 0.01$; sex \times ASR treatment:

Table 2 Mean annual survival probability and growth rate of offspring per ASR treatment and sex. Data are the least-squares mean (\pm SE) of the models discussed in the text.

	Treatment populations		Control populations	
	Males	Females	Males	Females
Annual survival	0.27 \pm 0.09	0.27 \pm 0.10	0.25 \pm 0.03	0.31 \pm 0.03
Annual growth rate (mm)	29.3 \pm 1.64	27.2 \pm 1.76	27.2 \pm 0.62	28.8 \pm 0.56

Table 3 Mean SVL, tail length and body condition of offspring born during the second reproductive episode per ASR treatment (population of the mother during the first reproductive episode) and sex. Mothers were all translocated in control populations during 1 year after the end of the sex ratio manipulation. Data are least-squares mean (\pm SE) of the models discussed in the text.

	Treatment populations		Control populations	
	Males	Females	Males	Females
Offspring SVL (mm)	20.85 \pm 0.52	21.5 \pm 0.52	21.18 \pm 0.45	22.06 \pm 0.45
Offspring tail length (mm)	20.05 \pm 0.96	19.3 \pm 0.96	20.44 \pm 0.86	19.73 \pm 0.86
Offspring body condition (mg)	156.6 \pm 11.16	152.8 \pm 11.08	168.7 \pm 10.19	155.9 \pm 10.2

$F_{1,119} = 2.60$, $P = 0.11$; mother age: $F_{2,119} = 6.60$, $P < 0.01$; offspring development class: $F_{1,119} = 7.86$, $P < 0.01$; SVL: $F_{1,119} = 116.7$, $P < 0.0001$).

Discussion

We found significant variation in the ASR both at the scale of a mother's home range and between years in the studied natural population of the common lizard. Furthermore, the local ASR of year t predicted the ASR of year $t + 1$ within the same population. Thus, the two conditions for the evolution of facultative investment in the rare sex were met: (i) variable local ASRs experienced by mothers and (ii) predictability of the local variation in the ASR. Nevertheless, we experimentally demonstrated that mothers did not adjust their clutch sex ratio during and after the ASR manipulation, although the individuals used in our experiment stemmed from the same geographic area as the natural population does. Contrary to the predictions made by sex allocation theory, females produced sons with a higher body condition in male-biased compared to female-biased populations. However, this sex-biased maternal allocation had no detectable long-lasting effects on the sex-specific growth and survival of the offspring.

The significance of the obtained results could be weakened if the social organization would have been disrupted due to the semi-natural conditions during our experiment. However, our experimental setting is not

likely to have confounded this study, because the population density and the ASRs were within the range of their natural variation (Massot *et al.*, 1992; Clobert *et al.*, 1994). Further, dispersal rates, the timing of the dispersal, the mating system, density dependence, as well as selection on life history traits were found to be unaltered in the enclosures compared to the natural situation (Le Galliard *et al.*, 2003a; Laloi *et al.*, 2004; Lecomte *et al.*, 2004; Le Galliard *et al.*, 2004). Finally, the mean sex ratio at birth observed in this study is similar to the one observed in natural populations and is not changed by transplantation of the lizards (Clobert *et al.*, 1994; Boudjemadi *et al.*, 1999).

Patterns of sex ratio

Evidence for an adjustment of the sex ratio at birth in favour of the rare sex is scarce and ambiguous in vertebrates (Trivers, 1985; Cockburn *et al.*, 2002). Luummaa *et al.* (1998) found facultative sex ratio allocation into the rare sex in rural populations of humans in Finland (see also Ranta *et al.*, 2000), Olsson & Shine (2001) observed a similar trend in viviparous skinks from Tasmania, and Byholm *et al.* (2002) described a mechanism balancing local offspring sex ratio towards the rarer sex in goshawks. However, these empirical studies did not test the assumptions of the sex ratio models and could also be plagued by several confounding factors correlated with local sex ratios in the wild. Another investigation of facultative sex ratio adjustment in a natural reed warbler population strongly supported the assumptions of the sex ratio models, but did not detect a sex ratio adjustment in relation to the ASR (Bensch *et al.*, 1999). Further, two experimental studies in vertebrates failed to detect adjustments of the sex ratio at birth in response to a perturbation of the population sex ratio (Brown, 1982; Bond *et al.*, 2003). Our results are in line with the latter studies, suggesting that a sex ratio adjustment in relation to the population sex ratio might be rare in vertebrates.

Given the fact that the basic assumptions of Werren and Charnov's model (1978) are fulfilled and given the ability of the common lizard to control the clutch sex ratio (Leturque, 2002), our results indicate that the selective benefits of a facultative adjustment into the rare sex might be too weak. We suggest that benefits of facultative investment into the rare sex might be diminished by frequency dependence, intersexual interactions, and the costs of sex ratio adjustment. First, the advantages of a facultative investment in the rare sex are frequency-dependent and restricted in space (Frank, 1990). Benefits might therefore be lost if most females are employing this strategy (Bensch *et al.*, 1999) and if offspring settle outside their natal home range (Ranta *et al.*, 2000). Simulations have shown that sex allocation can be locally maladaptive when mothers lack information about the environment experienced by their

offspring (Ranta *et al.*, 2000). However, our population monitoring indicates that a significant proportion of the juveniles are recaptured within the scale (25×25 m) at which spatial variations in the ASR occur and can be experienced by their mothers (proportion of recruits aged 1 year = 65.9%, $n = 1040$; 2 years = 68.2%, $n = 918$). Therefore, our results are unlikely to be explained by the fact that mothers and offspring frequently experience different ASRs and thus by the spatial structure of the population.

Furthermore, Fisherian models assume that intrasexual competition is the critical determinant of the lifetime reproductive success of each sex (Frank, 1990). It is therefore beneficial to produce the rare sex that enjoys a lower intrasexual competition. This assumption neglects the role of intersexual interactions. We found that sexual aggression by males induced lower survival, lower fecundity and delayed parturitions in female common lizards inhabiting male-biased populations (Le Galliard & Fitze, unpublished results). Thus, daughters produced in areas where sex ratios have been distorted towards males suffer reduced reproductive success, which selects against a facultative investment towards daughters in areas where the ASR is male-biased.

Finally, there might be constraints associated with a flexible adjustment of the sex ratio (Krackow, 1995). The Mendelian inheritance of the sex chromosomes might be too rigid and biasing the sex ratio at birth might be costly (Oddie, 1998). For example, sex ratio skews can have negative effects on the rare sex within the clutch. In the viviparous common lizard, offspring are influenced by hormonal interactions with their siblings *in utero*, and it has recently been found that a prenatal exposure to steroids produced by the common sex within the clutch reduces the fitness of the rare sex (Uller *et al.*, 2004). Such negative effects of skewed sex ratios at birth generate selection for sex ratio homeostasis (Uller, 2003).

Patterns of sex allocation

Sex allocation theory also predicts that mothers should invest more resources in the rare sex (Olsson & Shine, 2001). Contrasting to these expectations, mothers produced more corpulent sons in male-biased than in female-biased populations, which might be explained by several mutually exclusive hypotheses. First, there might exist a direct correlation of maternal condition with sex-biased investment, such that higher maternal condition is associated with higher investment in sons (Trivers & Willard, 1973). This hypothesis is however not supported since we found no difference in maternal condition between treatments (post-parturition body condition, $F_{1,10} = 0.03$, $P = \text{n.s.}$), no differential survival of the females according to treatment and body condition at the start of our manipulation (effect of treatment \times body condition on annual survival probability in juvenile females: $F_{1,168} = 0.39$, $P = \text{n.s.}$; in yearling

and adult females: $F_{1,164} = 0.07$, $P = \text{n.s.}$), and no relationship between clutch sex ratio at birth and maternal condition ($F_{1,104} = 1.09$, $P = \text{n.s.}$).

Alternatively, treatment effects could have been mediated by the quality of the females' mating partners (Sheldon, 2000) since competition among males for access to females should be stronger and the scope for female choice should be bigger in male-biased than in female-biased populations (Kvarnemo & Ahnesjö, 1996). As a result, females may have copulated with more attractive partners of higher quality in male-biased populations. To investigate this question, we used paternity data obtained during our study (Fitze *et al.*, unpublished results). Females of the two ASR treatments did not mate with males of significantly different SVL ($F_{1,10} = 0.79$, $P = \text{n.s.}$), tail length ($F_{1,10} = 0.49$, $P = \text{n.s.}$) or body condition ($F_{1,10} < 0.01$, $P = \text{n.s.}$). However, females tended to mate with older males in male-biased populations ($F_{1,10} = 4.54$, $P = 0.06$), suggesting that male offspring condition might have changed due to sex-biased maternal investment depending on the mate's age [see Sheldon (2000) for a review].

A last explanation may be direct fitness advantages gained when producing more corpulent sons in male-biased populations. However, sex-specific differences in offspring condition between treatments had no detectable consequences on the performances of the offspring later in their life. Hence, it is difficult to conclude what the advantages of differential maternal investment are. To clarify this point, additional experiments (e.g. reciprocal transplants of offspring from male-biased and female-biased populations) should investigate in more details the fitness consequences of differential maternal investment.

Conclusions

We experimentally showed that mothers neither adjusted their clutch sex ratio nor the sex-specific offspring survival towards the rare sex, but found that mothers produced sons with a higher body condition in male-biased populations. We suggest that benefits of facultative investment in the rare sex might be diminished by frequency-dependent selection, intersexual interactions, and the costs of sex ratio adjustment. For example, as male frequency increases, intrasexual competition between females decreases and females suffer from stronger intersexual competition, which may select against the production of daughters. Although such modifications of the social system could generate selective pressures on sex-biased maternal investment, current models fail to take them into account (Frank, 1990). Our results thus urge for the development of more detailed models to better predict facultative maternal investment in the rare sex.

Our results are in line with a recent review that suggests that facultative primary sex ratio variation is not a consistent biological phenomenon (Ewen *et al.*, 2004).

This implies that facultative maternal investment in the rare sex may not be a widespread strategy used to compensate perturbations of the population sex ratio. Instead, short-term changes in sex-specific migration and mortality rates, or frequency dependent selection on heritable variation in sex-biased investment, may be the principal mechanisms maintaining the usually stable population sex ratios.

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References

- Bauwens, D., Nuijten, K., van Wezel, H. & Verheyen, R.F. 1987. Sex recognition by males of the lizard *Lacerta vivipara*: an introductory study. *Amph. Rept.* **8**: 49–57.
- Bensch, S., Westerdahl, H., Hansson, B. & Hasselquist, D. 1999. Do females adjust the sex of their offspring in relation to the breeding sex ratio? *J. Evol. Biol.* **12**: 1104–1109.
- Blackburn, D.G. 1998. Resorption of oviductal eggs and embryos in squamate reptiles. *Herp. J.* **8**: 65–71.
- Bond, M.L., Wolff, J.O. & Krackow, S. 2003. Recruitment sex ratios in gray-tailed voles (*Microtus canicaudus*) in response to density, sex ratio, and season. *Can. J. Zool.* **81**: 1306–1311.
- Boudjemadi, K., Lecomte, J. & Clobert, J. 1999. Influence of connectivity on demography and dispersal in two contrasting habitats: an experimental approach. *J. Anim. Ecol.* **68**: 1207–1224.
- Brown, L.P. 1982. Can guppies adjust the sex ratio? *Am. Nat.* **120**: 694–698.
- Bull, J.J. & Charnov, E.L. 1988. How fundamental are Fisherian sex ratios? *Oxf. Surv. Evol. Biol.* **5**: 98–135.
- Byholm, P., Ranta, E., Kaitala, V., Lindén, H., Saurola, P. & Wikman, M. 2002. Resource availability and goshawk offspring sex ratio variation: a large-scale ecological phenomenon. *J. Anim. Ecol.* **71**: 994–1001.
- Calsbeek, R. & Sinervo, B. 2004. Within-clutch variation in offspring sex determined by differences in sire body size: cryptic mate choice in the wild. *J. Evol. Biol.* **17**: 464–470.
- Charnov, E.L. 1982. *The Theory of Sex Allocation*. Princeton University Press, Princeton.
- Chevalier, M., Dufaure, J.P. & Lecher, P. 1979. Cytogenetical study of several species of *Lacerta*, with particular reference to sex chromosome. *Genetica* **50**: 11–18.
- Clobert, J., Massot, M., Lecomte, J., Sorci, G., de Fraipont, M. & Barbault, R. 1994. Determinants of dispersal behavior: the common lizard as a case study. In: *Lizard Ecology. Historical and Experimental Perspectives* (L. J. Vitt & E. R. Pianka, eds), pp. 183–206. Princeton University Press, Princeton.
- Cockburn, A., Legge, S. & Double, M.C. 2002. Sex ratios in birds and mammals: can the hypotheses be disentangled? In: *Sex Ratios. Concepts and Research Methods* (I. C. Hardy, ed.), pp. 266–286. Cambridge University Press, Cambridge.
- Dufaure, J.P. & Hubert, J. 1961. Table de développement du lézard vivipare: *Lacerta (Monotoca) vivipara* Jacquin. *Archives d'Anatomie Microscopique Morphologique et Expérimentale* **50**: 309–328.
- Ewen, J.G., Cassey, P. & Møller, A.P. 2004. Facultative primary sex ratio variation: a lack of evidence in birds? *Proc. R. Soc. Lond. B* **271**: 1277–1282.
- Fisher, R.A. 1930. *The Genetical Theory of Natural Selection*. Oxford University Press, Oxford.
- Frank, S.A. 1990. Sex-allocation theory for birds and mammals. *Ann. Rev. Ecol. Syst.* **21**: 13–55.
- Gavaud, J. 1986. Vitellogenesis in the lizard *Lacerta vivipara*. II Vitellogenin synthesis during the reproductive cycle and its control by ovarian steroids. *Gen. Compar. Endocrin.* **63**: 11–23.
- Krackow, S. 1995. Potential mechanisms for sex ratio adjustment in mammals and birds. *Biol. Rev.* **70**: 225–241.
- Kvarnemo, C. & Ahnesjö, I. 1996. The dynamics of operational sex ratios and competition for mates. *Trends Ecol. Evol.* **11**: 404–408.
- Laloi, D., Richard, M., Lecomte, J., Massot, M. & Clobert, J. 2004. Multiple paternity in clutches of common lizard *Lacerta vivipara*: data from microsatellite markers. *Mol. Ecol.* **13**: 719–723.
- Le Galliard, J.-F., Ferrière, R. & Clobert, J. 2003a. Mother-offspring interactions affect natal dispersal in a lizard. *Proc. R. Soc. Lond. B* **270**: 1163–1169.
- Le Galliard, J.-F., Le Bris, M. & Clobert, J. 2003b. Timing of locomotor impairment and shift in thermal preferences during gravidity in a viviparous lizard. *Funct. Ecol.* **17**: 877–885.
- Le Galliard, J.-F., Clobert, J. & Ferrière, R. 2004. Physical performance and darwinian fitness in lizards. *Nature* **432**: 502–505.
- Lebreton, J.D., Burnham, K.P., Clobert, J. & Anderson, D.R. 1992. Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. *Ecol. Monog.* **62**: 67–118.
- Lecomte, J., Clobert, J. & Massot, M. 1992. Sex identification in juveniles of *Lacerta vivipara*. *Amph. Rept.* **13**: 21–25.
- Lecomte, J., Boudjemadi, K., Sarrazin, F., Cally, K. & Clobert, J. 2004. Connectivity and homogenisation of population sizes: an experimental approach in the common lizard. *J. Anim. Ecol.* **73**: 179–189.
- Lessels, C.M. & Boag, P.T. 1987. Unrepeatable repeatabilities: a common mistake. *The Auk* **104**: 116–121.
- Leturque, H. 2002. *Evolution of Sex Ratio and Dispersal in Structured Populations*. PhD thesis, University of Montpellier II, Montpellier.
- Littell, R.C., Milliken, G.A., Stroup, W.W. & Wolfinger, R.D. 1996. *SAS Systems for Mixed Models*. SAS Institute, Cary, North Carolina.
- Lorenzon, P., Clobert, J. & Massot, M. 2001. The contribution of phenotypic plasticity to adaptation in *Lacerta vivipara*. *Evolution* **55**: 392–404.
- Luummaa, V., Merilä, J. & Kause, A. 1998. Adaptive sex ratio variation in pre-industrial human (*Homo sapiens*) populations? *Proc. R. Soc. Lond. B* **265**: 563–568.

- Massot, M., Clobert, J., Pilorge, T., Lecomte, J. & Barbault, R. 1992. Density dependence in the common lizard: demographic consequences of a density manipulation. *Ecology* **73**: 1742–1756.
- McCullagh, P. & Nelder, J.A. 1989. *Generalized linear models*, 2nd edn. Chapman & Hall, London.
- Meylan, S. & Clobert, J. 2004. Maternal effects on offspring locomotion: influence of density and corticosterone elevation in the lizard *Lacerta vivipara*. *Physiol. Biochem. Zool.* **77**: 450–458.
- Oddie, K. 1998. Sex discrimination before birth. *Trends Ecol. Evol.* **13**: 130–131.
- Olsson, M. & Shine, R. 2001. Facultative sex allocation in snow skink lizards (*Niveoscincus microlepidotus*). *J. Evol. Biol.* **14**: 120–128.
- Painter, D., Jennings, D.H. & Moore, M.C. 2002. Placental buffering of maternal steroid hormone effects on fetal and yolk hormone levels: a comparative study of a viviparous lizard, *Sceloporus jarrovi*, and an oviparous lizard, *Sceloporus graciosus*. *Gen. Compar. Endocrin.* **127**: 105–116.
- Panigel, M. 1956. Contribution à l'étude de l'ovoviviparité chez les reptiles: gestation et parturition chez le lézard vivipare *Zooteca vivipara*. *Ann. Sci. Nat. Zool.* **18**: 569–668.
- Quinn, G.P. & Keough, M.J. 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.
- Ranta, E., Lummaa, V., Kaitala, V. & Merilä, J. 2000. Spatial dynamics of adaptive sex ratios. *Ecol. Lett.* **3**: 30–34.
- Robert, K.A. & Thompson, M.B. 2001. Viviparous lizards selects sex of embryos. *Nature* **412**: 698–699.
- Sheldon, B.C. 2000. Differential allocation: tests, mechanisms and implications. *Trends Ecol. Evol.* **15**: 397–401.
- Shine, R. 2002. Co-occurrence of multiple, supposedly incompatible modes of sex determination in a lizard population. *Ecol. Lett.* **5**: 486–489.
- Trivers, R. 1985. *Social Evolution*. Benjamin/Cummings, Menlo Park.
- Trivers, R.L. & Willard, D.E. 1973. Natural selection of parental ability to vary the sex ratio of offspring. *Science* **177**: 90–92.
- Uller, T. 2003. Viviparity as a constraint on sex-ratio allocation. *Evolution* **57**: 927–931.
- Uller, T., Massot, M., Richard, M., Lecomte, J. & Clobert, J. 2004. Long-lasting fitness consequences of prenatal sex ratio in a viviparous lizard. *Evolution* **58**: 2511–2516.
- Wapstra, E., Olsson, M., Shine, R., Edwards, A., Swain, R. & Joss, J.M.P. 2004. Maternal basking behaviour determines offspring sex in a viviparous lizard. *Biol. Lett.* **271**: S230–S232.
- Werren, J.H. & Charnov, E.L. 1978. Facultative sex ratios and population dynamics. *Nature* **272**: 349–350.
- West, S.A. & Godfray, H.C.J. 1997. Sex ratio strategies after perturbation of the stable age distribution. *J. Theor. Biol.* **186**: 213–221.

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