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Sex-specific fitness returns are too weak to select for non-random patterns of sex allocation in a viviparous snake

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Abstract When environmental conditions exert sex-specific selection on offspring, mothers should benefit from biasing their sex allocation towards the sex with the highest fitness in a given environment. Yet, studies show mixed support for such adaptive strategies in vertebrates, which may be due to mechanistic constraints and/or weak selection on facultative sex allocation. In an attempt to disentangle these alternatives, we quantified sex-specific fitness returns and sex allocation (sex ratio and sex-specific mass at birth) according to maternal factors (body size, age, birth date, and litter size), habitat, and year in a viviparous snake with genotypic sex determination. We used data on 106 litters from 19 years of field survey in two nearby habitats occupied by the meadow viper *Vipera ursinii ursinii* in south-eastern France. Maternal reproductive investment and habitat quality had no differential effects on the growth and survival of sons and daughters. Sex ratio at birth was balanced despite a slight female-biased mortality before birth. No sexual mass dimorphism between offspring was evident. Sex allocation was almost random apart for a trend towards more male-biased litters as females grew older, which could be explained by an inbreeding avoidance strategy. Thus, a weak selection for facultative sex allocation seems sufficient to explain the almost equal sex allocation in the meadow viper.

Keywords Selection · Sex allocation · Sexual size dimorphism · Sex ratio · Snakes

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Introduction

In vertebrates, differential allocation of resources to sons versus daughters (i.e. sex allocation) is a source of variation in lifetime reproductive success between parents (Charnov 1982). Sex ratio theory predicts that an optimal population sex allocation is maintained by frequency-dependent selection around a level determined by the costs of producing each sex and the costs of controlling sex allocation (Fisher 1930; Pen and Weissing 2002). However, when variable environmental conditions have differential effects on the fitness of sons and daughters, mothers should benefit from biasing their sex allocation towards the sex with the highest fitness in each environment (e.g. Pen and Weissing 2002; Trivers and Willard 1973). Empirical studies with birds and mammals have provided support for such adaptive facultative sex allocation (reviewed by Cockburn et al. 2002). Yet, it is now increasingly apparent that patterns of facultative sex allocation are not necessarily adaptive in vertebrates (e.g. see Brommer et al. 2003; Cockburn et al. 2002; Ewen et al. 2004). On one hand, the adaptation of sex allocation strategies is limited by

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mechanistic constraints (e.g. Krackow 1995; Pen and Weissing 2002; Uller 2003). On the other hand, selection on facultative sex allocation may be too weak in species with weak sexual dimorphism, structured life cycles, and complex social systems (Cockburn et al. 2002; Pen and Weissing 2002).

Squamate reptiles (lizards and snakes) are good model systems with which to investigate these alternatives because they have simple patterns of parental investment into the offspring, they have less complex social systems than birds and mammals, they show contrasted sex determination mechanisms and life cycles, and the fitness consequences of sex allocation can be assessed relatively easily in the field (reviewed in Wapstra and Warner 2010; Wapstra et al. 2007). In squamates with sex chromosomes (many lizards and all snakes), mechanisms of sex ratio control include gamete selection and differential abortion of embryos (e.g. Blackburn 1998), but also mixed genetic and temperature-dependent sex determination (e.g. Shine et al. 2002; Wapstra and Warner 2010). Changes in the amount of yolk or steroid hormones deposited into the eggs during vitellogenesis and maternal hormonal transfers to embryos during gestation allow females to control their investment in offspring (reviewed in Sinervo 1999). However, despite the opportunity that squamates offer and the apparent simplicity of their social systems, their sex allocation strategies show contrasting patterns across studies like in other vertebrates (Wapstra and Warner 2010;

Wapstra et al. 2007). A good example of contradictory findings comes from conflicting reports of facultative sex allocation towards the rarer sex in lizards, sometimes even within the same species (Allsop et al. 2006; Le Galliard et al. 2005c; Olsson and Shine 2001; Robert et al. 2003; Warner and Shine 2007). Unfortunately, we know at present too little about the fitness costs and benefits of facultative sex allocation to ascribe these differences to a lack of selection or to the presence of mechanistic constraints (Uller and Olsson 2006).

Among squamates investigated so far, snakes are characterized by genotypic sex determination, weak sexual size dimorphism at birth, a typically female-biased sexual size dimorphism at adulthood, and slower demographic tactics than in lizards (Shine 2003; Wapstra et al. 2007). Relevant factors for differential sex allocation in snakes include maternal length and condition (Blouin-Demers and Weatherhead 2007; Dunlap and Lang 1990; Lemen and Voris 1981; Weatherhead et al. 1998), maternal age (Madsen and Shine 1992), and thermal conditions during gestation (Burger and Zappalorti 1988; Dunlap and Lang 1990). Some patterns of facultative sex ratio adjustment reported so far with snakes may be adaptive (Table 1 for an overview). For example, the larger sex at birth could benefit more from an increase in maternal investment and be overproduced by larger mothers or mothers in better condition (Trivers and Willard 1973; Weatherhead et al. 1998), which seems to be the case in some species

Table 1 Overview of previous studies of facultative sex allocation (sex ratio, % of males) in snakes (Squamata, Serpentes)

Study species	Reproductive mode	Adult SSD	Factors of variation	Main results
Elaphe obsoleta[a]	Oviparity	Male biased	Laying date	Sex ratio increases slightly
Nerodia sipedon[b]	Oviparity	Female biased	Incubation temperature	Sex ratio decreases significantly
Nerodia sipedon[c]	Oviparity	Female biased	Laying date	Sex ratio unchanged
			Maternal size	Sex ratio decreases significantly
Notechis scutatus[d]	Oviparity	Not reported	Maternal size	Sex ratio unchanged
Pituophis melanoleucus[e]	Oviparity	No dimorphism	Incubation temperature	Sex ratio increases significantly
Enhydrina schistose[f]	Viviparity	Female biased	Maternal size	Sex ratio decreases significantly
Thamnophis sirtalis[b]	Viviparity	Female biased	Maternal size	Sex ratio increases significantly
Vipera berus[g]	Viviparity	Female biased	Maternal size/condition	Sex ratio unchanged
			Litter size	Sex ratio unchanged
			Litter number (age)	Sex ratio decreases significantly

SSD Sexual size dimorphism

[a] Blouin-Demers and Weatherhead (2007)

[b] Dunlap and Lang (1990)

[c] Weatherhead et al. (1998)

[d] After data in Table 1 of Shine and Bull (1977)

[e] Burger and Zappalorti (1988) [note that Burger and Zappalorti (1988) used thermal conditions outside the natural range experienced by the species and that this study might not be taken as evidence of sex ratio manipulation (G. Blouin-Demers, personal communication)]

[f] Lemen and Voris (1981)

[g] Madsen and Shine (1992)

(Table 1). Similarly, the larger sex at adulthood may benefit more from being born earlier in the season because of potential advantages in body size later in life. Thus, this sex should be overproduced in earlier clutches and in warmer environments (Uller and Olsson 2006; Weatherhead et al. 1998). For example, Blouin-Demers and Weatherhead (2007) have reported weak seasonal changes in sex ratios at birth in snakes and this scenario could also explain thermal effects on sex allocation because offspring typically hatch earlier in warmer environments (Table 1). Finally, local competition among males may be important in snakes with a male-male mate competition breeding system and a male natal philopatry (Madsen and Shine 1992). Unfortunately, fitness returns of sex allocation strategies have never been tested in snakes to our knowledge, and few of the previous studies have investigated sex-specific investment, though it can be easily assessed by measuring offspring mass at birth (Madsen and Shine 1992; Wapstra et al. 2007).

Here, we studied sex allocation strategies and sex-specific fitness returns in the meadow viper (*Vipera ursinii ursinii*, Bonaparte 1835) using long-term mark-recapture data collected in a natural population in south-eastern France since 1979. The meadow viper is a late maturing snake (age at maturation = 3–6 years) with a weak sexual size dimorphism at birth, a weak sexual size dimorphism at adulthood, similar dispersal distances between the sexes, no apparent sexual differences in juvenile and sub-adult survival, and a balanced sex ratio at birth (Baron 1997; Baron et al. 1996, 2010). Sex may be determined genetically since heteromorphic sex chromosomes have been reported (Saint Girons 1977). Previous studies did not investigate facultative sex allocation (Baron et al. 1996, 2010), but were used to formulate two major predictions. Firstly, our study site consists of two separated habitats that do differ in terms of female body size and size-dependent reproductive performances, but not annual survival of males and females (Baron 1997; Baron et al. 1996). This suggests that body size may be more sensitive to habitat quality in females than males, and that females should be overproduced in the better habitat. To confirm this, we tested whether sex-specific growth trajectories and sex allocation differ between the two habitats. Secondly, juvenile performances vary between birth cohorts in the meadow viper due to the combined effects of prenatal and postnatal factors (Baron et al. 2010). There is significant annual variation in growth early in life mediated by body condition at birth. Also, there is strong annual variation in juvenile survival, positive survival selection on offspring mass at birth, and negative survival selection on offspring condition at birth. Maternal effects on offspring fitness traits are indirect and mediated by offspring traits at birth, and therefore maternal reproductive investment (Baron et al. 2010). Thus, if maternal reproductive investment in the offspring had differential effects on the performance of juvenile males and females, we would expect facultative sex allocation in response to annual conditions and maternal condition. To confirm the assumption and prediction of this scenario, we tested for sex-specific fitness effects of maternal reproductive investment and facultative sex allocation according to maternal factors (body size, age, birth date, and litter size) and annual conditions. Our results show that selection for facultative sex allocation was weak and that sex allocation was almost random.

Materials and methods

Study site and data collection

The study area is located on Mont-Ventoux, south-eastern France (ca. 1,430 m a.s.l., 44°18′N, 5°26′E) and consists of open meadows with juniper trees and rock outcrops. It was divided into two contiguous study sites of similar size (ca. 2–3 ha): a north-facing and largely mesophilic hillside (habitat A) and a south-facing and more xerophilic hillside (habitat B). The two habitats are separated by a 10-m-wide road, which strongly limits dispersal movements, and differ consistently in insolation, humidity and food availability (J.-P. Baron, personal observation). Females from habitat A grow larger, breed more frequently, and produce larger clutches due to their larger body size (Baron et al. 1996, unpublished data). From 1979 onwards, meadow vipers of all age and sex classes were sought, captured, and individually marked upon first capture by scale clipping during twice-yearly capture campaigns (see Baron et al. 1996 for a detailed description of field methods). Each capture session lasted 10–14 days and was carried out during the mating season before ovulation (May) and during late gestation before parturition (late August or early September depending on climatic conditions).

Collection of sex allocation data was done in 1983–1988 and 1994–2009 by capturing gravid female during late gestation and transporting them to a laboratory located near the field site for the measurement of their snout-vent length (SVL). Females were then maintained in individual cages (350 × 180 × 210 mm) provided with a shelter, free access to water and a heat source enabling optimal thermoregulation. Parturition was checked for daily and occurred on average 13.2 days (±8.9 SD) after the capture date. After hatching, most healthy offspring (n = 360 out of 380) and some stillborn (n = 22 out of 32) were sexed unambiguously according to the number of their sub-caudal scales (Baron 1997). Healthy offspring were measured for their body mass at birth and released in the field with their mother at the last capture location of the mother. From 1994 onwards, healthy offspring were marked and

subsequently recaptured and measured for SVL during standard recapture sessions, thus providing data on their growth and survival.

We knew the exact age of 13 female snakes because they were born in the laboratory. In addition, we could reliably estimate the age of nine other females that were observed as sub-adults (i.e. within 2 years following their birth). Such individuals were assigned to a particular age class using a discriminant function (k-nearest neighbour method; Venables and Ripley 2002) that was calibrated with a data set of animals born in the laboratory and included only four age groups (k = 4 corresponding to age classes 0, 1, 2, and ≥3 years). Snakes encountered for the first time at an age older than 2 years could not be reliably assigned to an age class because size and mass distributions overlap substantially over age classes of older individuals.

Analysis of sex-specific fitness effects

We first analysed sex-specific growth data from all meadow vipers to test for sexual and habitat differences in demographic performances that are determined by body size at the adult stage (Baron et al. 1996). We calculated SVL in a large sample of known-age individuals (n = 561) and used a non-linear von Bertalanffy growth model. Growth duration was defined as the number of active days since birth date and was calculated, as described by Blouin-Demers et al. (2002), as the number of days elapsed between birth date (or mean birth date of the birth year when birth date was unknown) and focal capture date minus the number of days spent in hibernation and emergence, taken to occur from 1 October to 30 April in our study population. The von Bertalanffy model has three parameters (SVL at birth, exponential growth rate and asymptotic SVL) and was fitted with the non-linear mixed effects model procedure assuming random individual variation in asymptotic SVL (Pinheiro and Bates 2000). Fixed effects of habitat, sex and their interaction for exponential growth rate and asymptotic SVL were investigated.

We also studied sex-specific growth and survival for juvenile and sub-adult meadow vipers based on a data set of offspring marked at birth described in Baron et al. (2010). Previously, we investigated offspring growth early in life (from birth to the age of sexual maturation) based on a linear mixed-effect (LME) model and found positive effects of offspring condition at birth. Also, we used capture-mark-recapture models to model annual survival and uncovered no sexual dimorphism in juvenile and sub-adult survival but significant effects of offspring mass at birth and offspring condition at birth (Baron et al. 2010). Here, we used these two statistical models to test for sex-specific fitness effects and ask two main questions: does the positive effect of body condition at birth on offspring growth early in life differ between the sexes, and do the effects of body mass and body condition at birth on juvenile survival differ between the sexes? Details on the statistical procedures, sample size and hypotheses tests are provided elsewhere (Baron et al. 2010).

Analysis of sex allocation

We analysed sex ratio data separately for stillborn and healthy offspring. The goodness-of-fit of a binomial distribution was tested with Pearson's χ^2 statistics, the deviation from a balanced sex ratio was quantified with a binomial test, and confidence intervals were obtained with a profile likelihood method (Wilson and Hardy 2002). We also tested for consistency of sex ratio bias among females using data from mothers scored several times during their life. We used a maximum likelihood (ML) method with Laplace approximation to fit a generalized mixed effects model (GLMM) including a binomial error terms, a logit link function and a random female identity effect (glmmML procedure, see Broström 2003). Since this analysis showed very weak variation among females (see "Results"), we next analysed sex ratios in all litters with generalized linear models (GLM) for binomial data (glm procedure). We used the GLM to test the effects of several prenatal factors (see last paragraph) on sex ratio at birth.

We also studied maternal energetic investment in sons and daughters by analysing the body mass at birth of healthy offspring. In meadow vipers, body mass at birth provides a relevant measure of maternal investment since the energetic costs of reproduction scales linearly with total litter mass (J.-P. Baron et al., unpublished data) and body mass at birth is positively correlated with juvenile survival (Baron et al. 2010). We used a LME model with the lme procedure and analysed body mass at birth with sex as a factor following Pinheiro and Bates (2000). Potential prenatal factors (see next paragraph) and their interaction with sex were included as fixed effects. A random effect of birth year and family identity nested with birth year was included to account for variation between years and litters. Assumptions of normality and homogeneity of variances were checked and fulfilled. In this model, a biased sex investment is indicated by a significant sex effect and a facultative sex investment is indicated by a significant interaction between sex and another factor.

Prenatal factors included in the analyses of sex ratio and sex-specific investment were additive effects of habitat (categorical factor) and of maternal SVL, total litter size or total clutch size, and birth date (continuous factors). Influential terms were selected by a backward elimination of non-significant factors. We addressed separately the effect of mother's age (continuous factor) on sex allocation in a subset of known-age females (n = 22 litters). Finally,

we analysed temporal variation in sex allocation using 13 study years where more than four litters were available. Fixed factors were tested with likelihood ratio tests (LRT) in glm and with conditional F-tests based on the usual (restricted ML) conditional estimate of variance in lme using the standard type I error critical level ($\alpha = 0.05$). All analyses were implemented in R 2.10.0 software (http://cran.r-project.org).

Results

Sex-specific fitness effects

Growth trajectories of meadow vipers differed significantly between habitats (growth rate, $F_{1,311} = 21.75$, $P < 0.0001$; asymptotic body size, $F_{1,311} = 23.81$, $P < 0.001$) but not between the sexes for exponential growth rates (sex, $F_{1,309} = 1.11$, $P = 0.29$; sex by habitat interaction, $F_{1,308} = 1.91$, $P = 0.06$) and for asymptotic body size (sex, $F_{1,310} = 3.23$, $P = 0.07$; sex by habitat interaction, $F_{1,307} = 0.003$, $P = 0.95$). Meadow vipers grew faster and reached a larger asymptotic body size in habitat A than in habitat B (contrast in natural logarithm of exponential growth rate $= -0.19 \pm 0.04$ SE; contrast in asymptotic body size $= +36.5$ mm ± 7.47 SE; Fig. 1). The marginal sex by habitat interaction for growth rates and the marginal sex effect for asymptotic body size were suggestive of a slightly stronger habitat effect on growth rates in males and a slightly larger asymptotic body size in females, respectively. However, these trends were not significant in an analysis of growth trajectories restricted to offspring marked at birth (sex by habitat interaction, $F_{1,115} = 0.08$, $P = 0.78$; sex, $F_{1,115} = 0.05$, $P = 0.16$).

In addition, our analysis of sex-specific fitness effects for body growth and survival of offspring revealed no major sexual differences. Firstly, the positive effect of body condition at birth on body growth early in life did not differ between the sexes (sex × body condition, $F_{1,68} = 0.05$, $P = 0.83$; $n = 38$ individuals and $n = 109$ observations). Secondly, we found no evidence for sex-specific effects of body mass at birth ($\chi_1^2 = 1.54$, $P = 0.21$, $n = 147$ offspring from seven birth cohorts) and of body condition at birth ($\chi_1^2 = 0.95$, $P = 0.33$) on juvenile survival [these LRT were obtained from the best mark-recapture model 29 of Table 2 in Baron et al. (2010) after including a sex effect].
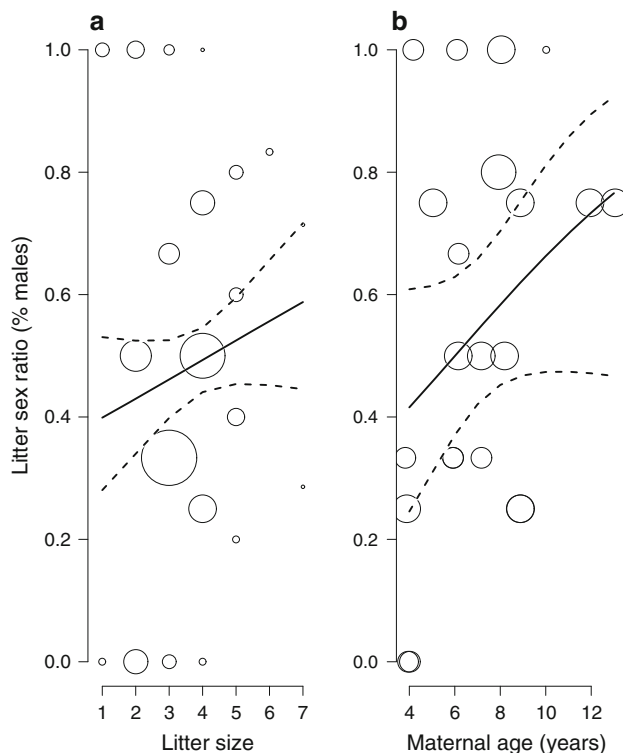
Sex ratios

In stillborn offspring sexed at hatching, we counted 16 females and six males but could not sex ten stillborn offspring. This sex ratio distribution was marginally female
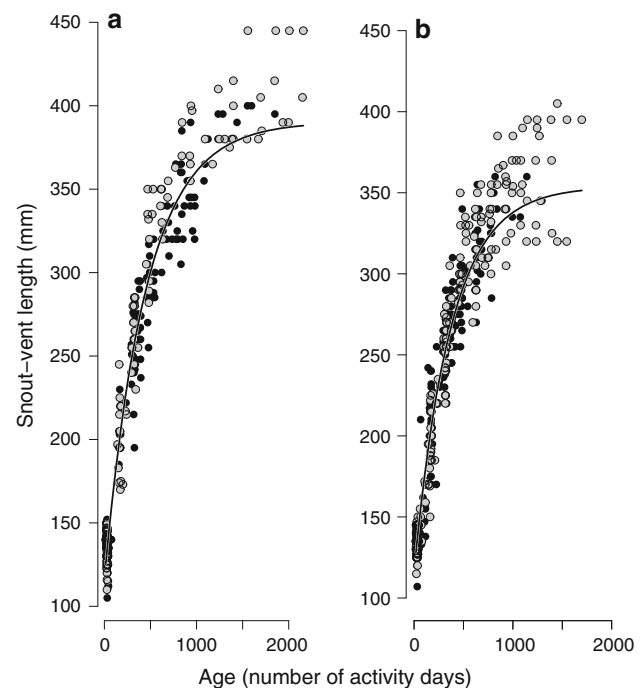
Fig. 1a, b Effects of sex and habitat on body growth of meadow viper *Vipera ursinii ursinii*. Body growth does not differ between males (*black circles*) and females (*grey circles*, all $P > 0.06$) in habitat A (**a**) and in habitat B (**b**). However, snakes grow faster and larger in habitat A ($P < 0.0001$). Data are snout-vent length versus number of days of activity since birth (i.e. excluding hibernation and emergence periods, there are about 150 activity days per year). *Regression lines* correspond to the mean von Bertalanffy growth curve in each habitat

biased (two-sided binomial test, $P = 0.05$), but would not have been biased if sex ratios had been perfectly balanced in unsexed stillborns ($P = 0.11$). Subsequent analyses of sex ratio variation were conducted with healthy offspring only ($n = 360$ offspring, $n = 106$ litters, 19 years). The sex ratio distribution was well fitted by a binomial distribution (Pearson's χ^2 statistic, $\chi_{105}^2 = 105.71$, $P = 0.46$) and sex ratios did not differ from equality (two-sided binomial test, $P = 0.71$). Female meadow vipers produce an average of 3.39 healthy offspring (± 1.27 SD) out of which 1.66 (± 1.19 SD) were males [sex ratio $= 0.49$ (0.44, 0.54) 95% CL]. We analysed consistency in sex ratio bias among females with repeated observations of litter sex ratio with a GLMM (20 females, 52 litters), which indicated weak inter-individual variation (bootstrap test of the null hypothesis of no inter-individual variance: $P = 0.37$). Furthermore, sex ratios did not change with birth date ($\chi_1^2 = 0.13$, $P = 0.71$), habitat ($\chi_1^2 = 0.27$, $P = 0.60$), maternal SVL ($\chi_1^2 = 0.26$, $P = 0.60$), litter size ($\chi_1^2 = 2.22$, $P = 0.14$; Fig. 2a) or number of stillborn offspring ($\chi_1^2 = 2.14$, $P = 0.15$). Temporal variation in sex ratios was also small and non-significant ($\chi_{12}^2 = 8.76$, $P = 0.72$). However, we found a nearly significant effect

Fig. 2 Effects of litter size (number of healthy offspring; **a**) and mother's age (**b**) on sex ratio at birth (proportion of males) in litters of the meadow viper. Sex ratio at birth does not change with litter size ($n = 106$ litters, $P = 0.14$) but tends to increase with mother's age ($P = 0.07$). Note that the *size* of each *point* is proportional to the number of litters (**a;** range 1–13) or litter size (**b;** range 1–5). Symbols are staggered in **b** for purposes of presentation. *Unbroken curves* correspond to the prediction from a logistic regression and *dashed curves* correspond to point-wise confidence bounds

of mother's age on sex ratio at birth ($\chi^2_1 = 3.22$, $P = 0.07$). The proportion of males in the litter tended to increase with maternal age (logit slope $= 0.169 \pm 0.098$ SE; Fig. 2b).
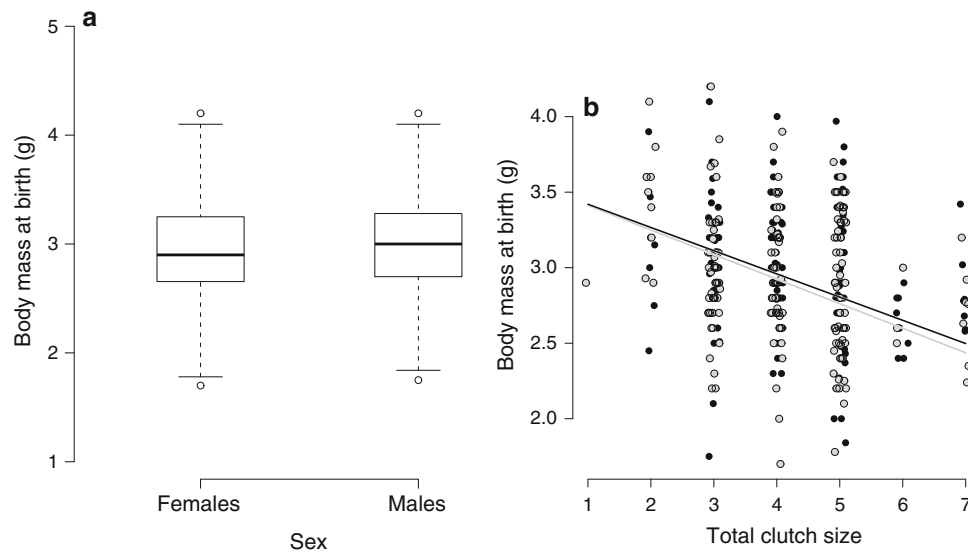
Sex-specific investment

Sons and daughters did not differ in mean body mass at birth indicating that maternal investment was well balanced (mass of sons, 2.99 g $\pm$ 0.43 SD, $n = 169$; mass of daughters, 2.94 g $\pm$ 0.44 SD, $n = 179$; Fig. 3a, Table 2 for statistical results). In addition, there was no differential investment in sons and daughters according to habitat, maternal SVL, clutch size, or birth date (Table 2 for interactions with sex), though we found significant main effects of maternal size (positive effect) and clutch size (negative effect) on mean offspring mass and almost significant effects of birth date (Table 2, Fig. 3b for main effects). The age of the mother did not influence offspring mass differently between sons and daughters either (interaction term sex × maternal age, $F_{1,47} = 0.05$, $P = 0.82$). Finally, despite strong inter-annual variation in mean

offspring mass (year, $F_{12,77} = 2.54$, $P = 0.007$), sexual differences remained similar between years (interaction term sex × year, $F_{12,202} = 0.78$, $P = 0.67$).

Discussion

In the meadow viper, sex ratios did not deviate from a binomial distribution and the mean sex ratio was not different from equality. This result was expected because females are heterogametic and the sex at conception is determined by the random segregation of sex chromosomes (Saint Girons 1977). More importantly, our results indicate almost equal sex allocation since prenatal factors had no significant effects on sex ratio and sex-specific body mass at birth, apart from the effect of maternal age on sex ratio. Sex ratio biases were inconsistent among females. Furthermore, maternal size did not influence sex allocation like in the closely related adder but contrary to three other published studies on snakes (Table 1). Finally, sex allocation did not change seasonally, between years, or between habitats. Since vipers give birth earlier in the season during warmer summers (J.-P. Baron and J.-F. Le Galliard, unpublished data), these data suggest weak climatic effects on sex allocation. Seasonal changes in sex allocation were also non-significant in other snakes, but effects of thermal conditions during embryogenesis were detected in two other oviparous snake species (Table 1). These thermal effects on sex ratio were mediated by differential mortality during incubation (Burger and Zappalorti 1988; Dunlap and Lang 1990). We report weak evidence of female-biased pre-natal mortality here. This pattern may result from sex-specific sensitivity to thermal stress during gestation (e.g. Lourdais et al. 2004) and may be a predicted consequence of female heterogamety; specifically, Z-linked deleterious alleles are expressed more in female than male snakes (Olsson et al. 2005). A reverse pattern caused by Y-linked alleles has been seen in primates and other male-heterogametic species (Trivers 1972). Yet, this result is surprising because male-biased pre-natal mortality is seen in other snakes (Burger and Zappalorti 1988; Dunlap and Lang 1990; Weatherhead et al. 1998). Irrespective of the explanation for this sex-biased mortality, however, the weak and non-significant seasonal and annual variation in sex allocation found here could be due to the fact that meadow vipers have a stricter control of thermal conditions during embryonic development than oviparous snake species and can buffer some of the consequences of natural variation in thermal conditions.

Some constraints may act against the evolution of facultative sex allocation strategies in the meadow viper. Four relevant limits to facultative sex allocation are: a random genetic sex determination (Krackow 1995); the negative

Fig. 3 Sex-specific investment into offspring (**a**) and the trade-off between total clutch size (number of eggs) and offspring mass in sons and daughters (**b**). Body mass at birth did not differ between sexes ($P = 0.23$) and offspring mass at birth decreased similarly with total clutch size in sons (*black circles* and *black line*) and daughters (*grey circles* and *grey line*). *Box plots* represent the median, the quartiles, the whiskers at 1.5 times the interquartile range and the outliers of body mass at birth ($n = 345$). The *regression lines* in **b** were obtained from the model from Table 2 where sex and the interaction between sex and clutch size were included

Table 2 Variation in sex-specific investment in litters of the meadow vipers estimated by the body mass of offspring at birth (345 offspring, 105 litters, 19 years)

Factor			
Fixed effects[a]	Estimate ± SE	$F_{[ndf,ddf]}$	P value
Intercept (g)	2.232 ± 0.419	$28.35_{[1,240]}$	<0.0001
Habitat (habitat B)	-0.098 ± 0.079	$1.54_{[1,82]}$	0.22
Maternal SVL (mm)	0.004 ± 0.001	$19.02_{[1,83]}$	<0.0001
Total clutch size (egg number)	-0.157 ± 0.030	$25.78_{[1,83]}$	<0.0001
Birth date (days since 1 August)	-0.009 ± 0.004	$3.85_{[1,83]}$	0.053
Sex (males vs. females)	0.036 ± 0.030	$1.44_{[1,239]}$	0.23
Sex × Habitat	0.052 ± 0.070	$0.55_{[1,236]}$	0.45
Sex × Maternal SVL	-0.001 ± 0.001	$2.59_{[1,238]}$	0.11
Sex × Total litter size	0.041 ± 0.032	$1.68_{[1,237]}$	0.20
Sex × Birth date	0.0015 ± 0.003	$0.20_{[1,235]}$	0.65
Random effects	Estimate ± 95% CL	LRT	P value
Year	$\sigma = 0.18\ [0.10, 0.30]$	12.75	0.0004
Family within year	$\sigma = 0.27\ [0.22, 0.33]$	138.30	<0.0001
Residual	$\sigma = 0.25\ [0.22, 0.27]$		

None of the pre-natal factors influenced sex-specific body mass (see interaction terms between sex and covariates)

SVL snout-vent length, *CL* confidence limit, *LRT* likelihood ratio test

[a] Estimates and test statistics for fixed effects were obtained from a linear mixed-effect model that included random variation between years and between litters within a year

effects of sex ratio biases for the fitness of juveniles, for example due to hormonal interactions between male and female embryos (Brana 2008; Uller 2003); the costs of sex ratio control (Pen and Weissing 2002); and a life history characterized by small litters of large offspring and substantial costs of reproduction (Baron et al. 1996; Pen and Weissing 2002). The occurrence of sex ratio biases and facultative sex allocation strategies in other snakes suggest

that genetic sex determination may not be a major constraint in this group (Table 1). Similarly, we are not aware of negative effects of sex ratio biases for the fitness of juveniles in snakes (Weatherhead et al. 2006). Constraints due to the costs of sex ratio control and the life cycle of the species are more plausible. One potential mechanism by which mothers can adjust their sex ratio is differential embryonic mortality during development, which entails a significant relative reduction of the litter size. Furthermore, abortions may be associated with substantial losses of energy if females lack the ability to resorb eggs and embryos (Blackburn 1998; Bonnet et al. 2008). One way to bypass these constraints could be for mothers to adjust the sex-specific reproductive investment rather than the sex ratio, which may require less costly mechanisms (Cockburn et al. 2002; Krackow 1995). For example, studies of mammals have shown that females can adjust sex-specific investment when rearing their young (Cameron 2004; Koskela et al. 2009; Robert et al. 2010). Female snakes do not exhibit parental care but could adjust energetic investment in eggs and embryos before birth since maternal investment has strong fitness effects on offspring (e.g. Baron et al. 2010). Here, the mass investment into the offspring was influenced by classic prenatal factors such as maternal length (strong positive effect), litter size (strong negative effect), and birth date (small negative effect). Thus, if sons benefited differentially from a change in reproductive investment than daughters, sex allocation theory would predict the evolution of facultative investment towards that sex according to maternal length, litter size and/or birth date (Pen and Weissing 2002).

However, our data on sex-specific fitness effects indicate no evidence of selection for differential maternal investment into the offspring and therefore suggest that a balanced sex allocation may be optimal in this species (Charnov 1982; Fisher 1930; Pen and Weissing 2002). We previously found no direct effects of maternal length, litter size or birth date on offspring fitness traits, and significant life history differences between habitats in adults (Baron et al. 1996, 2010). Maternal effects on offspring fitness traits were all indirect and mediated by offspring body mass at birth, which had a positive effect on juvenile survival, and by offspring body condition at birth, which had a positive effect on juvenile growth but a negative effect on juvenile survival (Baron et al. 2010). Here, we show that none of the fitness effects of offspring traits at birth were sex specific and that no sex-specific differences in growth trajectories existed between habitats contrary to previous suggestions based on a smaller sample size (Baron et al. 1996). These results clearly indicate that they are more demographic differences between years, habitats, or matrilines than between the sexes in meadow vipers. In general, sexual differences in demographic traits and sensitivity to environmental conditions are mirrored by

sexual differences in body mass, morphology, dispersal behaviour, and reproductive behaviour (reviewed in Le Galliard et al. 2005a). Since sexual differences in morphology are small in meadow vipers, finding no fitness benefit of facultative sex allocation is not surprising. This weak selection for facultative sex allocation may be sufficient to explain the evolution of a balanced and almost equal sex allocation in the meadow viper (Cockburn et al. 2002; Pen and Weissing 2002). Snakes with stronger sexual size dimorphism and non-random patterns of sex allocation should be studied in the future to look for the fitness consequences of their facultative sex allocation.

The tendency for older females to produce more sons is a surprising result of our study. This maternal age effect explained only 10% of the deviance in sex ratio at birth and should be confirmed when additional data on known-age females are obtained. Still, this trend requires some explanation because maternal age is rarely accounted for in other sex allocation studies. If this correlation with age was due to differential selection of "high quality" females, we would also expect that better quality females produce more sons and that sex ratio biases are consistent among females, which was not the case here. A correlation between maternal age and sex ratio at birth is also expected if reproductive performance changes with age and reproductive investment has differential effects on the fitness of sons and daughters (Hewison et al. 2002; Trivers and Willard's hypothesis, Trivers and Willard 1973), or if one sex is more costly to produce and costs of reproduction change with maternal age (terminal investment hypothesis; Cockburn et al. 2002; Cooch et al. 1997). However, no sexual size dimorphism at birth was found suggesting that males are not more costly to produce than females, and no sex-specific fitness effects of maternal reproductive investment were detected (see above). Also, the capacity of a female to invest in reproduction is indicated by her body size and condition before reproduction (Baron et al. 1996). Yet, age effects on sex ratio at birth were not caused by body size (GLM, size effect, $\chi_1^2 = 0.15$, $P = 0.70$; age effect, $\chi_1^2 = 6.18$, $P = 0.01$) and sex ratio at birth was not influenced by maternal body condition before reproduction (GLM, early spring body condition effect, $\chi_1^2 = 0.20$, $P = 0.66$, $n = 38$). Local mate competition would predict female-biased sex ratios in older mothers because daughters primarily compete with their mother for resources while sons compete for mates (e.g. Madsen and Shine 1992; Table 1), which runs counter to the sex allocation strategy of meadow vipers. A possible explanation is that these age effects are due to inbreeding avoidance where younger mothers under-produce males to avoid the risks of future inbred mating with their sons and between their siblings. Recapture data suggest a strong natal philopatry in both sexes and the risks of inbreeding should be significant

in this population in the absence of mate choice and multiple mating strategies (J.-P. Baron, personal observation).

In small populations, randomness in the sex ratio at birth and non-randomness due to facultative sex allocation can generate fluctuations in the adult sex ratio and influence the extinction risk (Ferrer et al. 2009; Le Galliard et al. 2005b). Understanding sex allocation strategies of meadow vipers is important because this species is critically endangered, and our study population is small and threatened by human activities. This analysis suggests that most demographic effects of sex allocation will be driven by random variation in sex ratio at birth around equality, which should enhance extinction risks if the mating system is not too polygynous (reviewed in Legendre 2004). The trend towards male-biased litters in older females may further increase extinction risks if population declines are associated with an increase in the mean age of females or if age structure fluctuates (see Ferrer et al. 2009 for a case study). Future studies should try to incorporate sex allocation strategies into stochastic demographic models of the meadow viper.

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