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Female field voles with high testosterone and glucose levels produce male-biased litters

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The proximate physiological mechanisms producing the parental ability to vary offspring sex ratio in many vertebrates remain elusive. Recently, high concentrations of maternal testosterone and glucose and low concentrations of maternal corticosterone have been suggested to explain male bias in offspring sex ratio. We examined how these factors affect secondary offspring sex ratio in nondomesticated field voles, *Microtus agrestis*, while controlling for maternal age, testosterone level of the male and body condition of both the female and the male. We found that females with high preconception serum testosterone and glucose levels produced a male-biased litter, whereas there was no association between maternal corticosterone level and litter sex ratio. Older females produced a bias towards sons, but neither their body condition nor paternal testosterone level correlated with litter sex ratio. Finally, females mated with a high body-condition male tended to deliver a male-biased litter. Our results suggest that several physiological traits of the mother may simultaneously be related to offspring sex ratio in mammals.

Keywords: age; body condition; corticosterone; field vole; life history; litter size; mammal; *Microtus agrestis*; sex allocation; sex-biased mortality; stress

Parents are predicted to adjust their offspring sex ratio according to the fitness payoffs of each sex (Trivers & Willard 1973). Facultative sex ratio adjustment in relation to parental characteristics and ecological conditions has been suggested for many birds (e.g. Ellegren et al. 1996; Komdeur et al. 1997; Nager et al. 1999; Badyaev et al. 2002; Griffin et al. 2005) and mammals (Sheldon & West 2004; Cameron 2004; Rosenfeld & Roberts 2004; Schino 2004; Gomendio et al. 2006). Despite this we still face problems identifying the proximate physiological mechanisms responsible for vertebrate sex ratio determination (Krackow 1995).

There has been increasing interest recently in hormones as proximate mechanisms of offspring sex ratio variation. Grant (1998) and James (1996, 1997, 2004, 2006a) have argued that maternal testosterone level during fertilization may influence offspring sex such that females with high level of testosterones produce more sons. Recent studies have found evidence for such a link in birds (Veiga et al. 2004; Pike & Petrie 2005a; Rutkowska & Cichoń 2006). In mammals, evidence for male-biased litters among females with high testosterone levels is much weaker (James 1996, 2004, 2006a; Manning et al. 2002). Only one study has previously directly addressed this question. Grant & Irwin (2005) found that in bovines high follicular testosterone concentration was associated with the fertilized ovum being a male.

Maternal stress hormones also have been suggested to skew offspring sex ratio in vertebrates (Lane & Hyde 1973; Pratt & Lisk 1989). Direct evidence for this hypothesis is scarce, but in birds high levels of maternal stress hormones, such as corticosterone and its precursor progesterone, are found to result in female-biased offspring sex ratios (Correa et al. 2005; Love et al. 2005; Pike & Petrie 2005b, 2006; Bonier et al., in press). In mammals, only one study has previously examined whether the

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prebreeding concentration of stress hormones affects offspring sex ratio. Geiringer (1961) found that in albino rats stressed mothers produced female-biased litters. It is currently unclear how stress hormones may bias offspring sex ratio. Induced abortion of generally more sensitive males is one potential explanation (see references in Love et al. 2005).

In addition to these hormonal mechanisms, a high level of maternal circulating glucose has been suggested as a physiological mechanism explaining male-biased offspring sex ratios (Cameron 2004); in mice a bias towards males resulted from an elevated maternal glucose level (Machado et al. 2001). This hypothesis invokes female-biased early mortality being responsible for male-biased secondary offspring sex ratio in females with high glucose levels. This is based on findings showing that the development and growth of female conceptus is retarded in the presence of an excess of glucose (Gutiérrez-Adán et al. 2001; Larson et al. 2001; Jimenéz et al. 2003a; Pérez-Crespo et al. 2005). Indirect evidence for this hypothesis comes from studies showing that female mice and opossums fed with a high-fat diet, leading potentially to increased glucose levels (Folmer et al. 2003), produced male-biased litters (Austad & Sunquist 1986; Rosenfeld et al. 2003).

James (1996, 2004, 2006a) also has suggested that in mammals male-biased offspring sex ratio may be related to high paternal testosterone level. This hypothesis has not yet been tested. Such an effect could be found if male testosterone level correlates with sperm sex ratio because males are the heterogametic sex. However, there is currently no evidence for this (Tiido et al. 2005). On the other hand, females may produce male-biased litters when mated with a high-quality male (Ellegren et al. 1996; Pike & Petrie 2005b), of which high testosterone level can be a signal (Mills et al. 2007).

We examined whether maternal levels of circulating testosterone, corticosterone and glucose and paternal levels of testosterone were associated with secondary litter sex ratio in a small mammal, the field vole, *Microtus agrestis*. We simultaneously tested the predictions that (1) parents with high levels of testosterone around fertilization produce male-biased litters, (2) females with high stress levels, measured as corticosterone concentration, produce female-biased litters, and (3) females with high baseline levels of glucose produce male-biased litters. To evaluate whether such effects, particularly the effects of glucose and corticosterone, might have operated via differential mortality of male and female embryos rather than primary sex ratio determination (i.e. during fertilization), we also examined how the above-mentioned factors correlated with litter size.

METHODS

Study Species, Study Design and General Methods

We used first-generation laboratory-born offspring of field voles that were trapped from the wild in southwestern Finland during spring 2005. Before pairing, voles were housed in same-sex pairs in standard plastic mouse cages ($48 \times 37.5 \times 21$ cm; Tecniplast, Varese, Italy), maintained on a 16:8 h light:dark cycle at approximately 20°C and fed with ad libitum Rat/Mouse Breeding Diet pellets (Altromin GmbH, Lage, Germany), oats and potatoes. Dry peat and hay were used as bedding.

The study was conducted during autumn 2005 in three successive trials, including 20, 16 and 6 pairings (42 females and 24 males). Females were paired with a male randomly but with no formation of sibling pairs. In females, pairing was preceded by the collection of, in the following order: faecal and vaginal samples for measuring corticosterone and the phase of the oestrus cycle, respectively, and blood samples for measuring testosterone and glucose (see below). The rationale for measuring corticosterone from faecal samples instead of from blood was to avoid the short-term confounding (e.g. due to handling) of baseline corticosterone level and to maintain a proper haematogical status of females (Harper & Austad 2000). Immediately before vaginal and blood samples, we also measured the body mass of females using electronic scales and the head width using a calliper. After sampling, which was always conducted between 0800 and 1000 hours, the females were housed individually in separate cages. On the next day, males had their blood sampled to measure testosterone, were weighed and had their head width measured before being paired with the females. All measurements and blood sampling were made by O. Huitu, with all measures being taken to minimize discomfort to the animals. The voles were paired for 14 days to ensure copulation/fertilization before the males were removed. The gravidity status of females was monitored visually daily thereafter.

To obtain a more reliable estimate of female baseline blood glucose, we placed females into hay-only cages without food before sampling. Because fasting time differed between the females (mean $\pm$ SD $= 157 \pm 28$ min), their blood glucose levels were corrected in the analyses for the time spent fasting by linear regression ($\beta \pm$ SE $= -0.049 \pm 0.023$, $t_{28} = -2.09$, $P = 0.046$; no evidence for nonlinearity was found). To obtain faecal samples for corticosterone analyses, these cages were lined on the bottom with tissue to collect faecal pellets and absorb urine. Approximately 20 faecal pellets were collected into a microtube and immersed in 1 ml of 95% ethanol. Samples were immediately stored at −20°C until further analyses.

After this, blood samples were collected from the submandibular area of voles using 5-mm Goldenrod Animal Lancets (Medipoint Inc., Mineola, NY, U.S.A.). Approximately 150–200 µl of blood was drawn into heparinized 75-µl capillary tubes (Hirschmann Laborgeräte, Eberstadt, Germany). As a single sample, such an amount of blood can be safely drawn from animals with body masses comparable to those of the field voles studied (Wolfensohn & Lloyd 1994; Table 1). One drop of blood per female was used for the determination of blood glucose level with a digital instrument (Glucocard II, Arkray Inc., Shiga, Japan). Capillary tubes were centrifuged for 5 min at 10 000 rpm (Mikro 12–24; Hettich, Tuttlingen, Germany), after which plasma was separated and stored in microtubes at −20°C until hormonal analyses. Because the levels of circulating testosterone and corticosterone may

Table 1. Summary statistics of the raw data on the selected explanatory variables studied

	Females		Males	
	Mean±SD	Min–max	Mean±SD	Min–max
Litter size	4.2±1.8	1–8	—	—
Age (days)	133.4±19.8	90–167	—	—
Body mass (g)	38.2±6.8	26.8–57.2	46.0±8.0	30.9–60.4
Testosterone (pg/ml)	88.3±44.9	2.5–228	2152±1888	239–6600
Corticosterone (ng/mg)	0.7±1.2	0.1–6.4	—	—
Glucose (mmol/L)	7.2±3.6	3.1–18.3	—	—

potentially vary according to the reproductive state of females (Millspaugh & Washburn 2004; Cavigelli et al. 2005), we recorded the oestrus cycle phases of females with vaginal cytology by the inspection of the shape of vaginal epithelium cells after sampling for blood (LeFevre & McClintock 1988).

We also recorded female age at the beginning of pairing because previous studies have found an association between maternal age and offspring sex ratio, although the results have been contradictory because advanced maternal age has been related to both female- and male-biased offspring sex ratio (Schlager & Roderick 1968; Thomas et al. 1989; Côté & Festa-Bianchet 2001; Rosenfeld et al. 2003; Isaac et al. 2005). Because female quality is also predicted to affect offspring sex ratio (Trivers & Willard 1973), perhaps independent of her hormonal status (James 2006a), we recorded the body condition of females. Paternal body condition was also recorded because females may adjust their offspring sex ratio according to male quality (e.g. Ellegren et al. 1996). As a proxy of individual body condition, we used standardized residuals from linear ordinary least squares regression of body mass on body size, measured as head width (Schulte-Hostedde et al. 2005).

Pups born ($N = 129$) from 28 litters were sexed visually by means of the anogenital distance under a microscope. This procedure usually took less than 1 min per litter. During the sexing procedure, pups were kept warm and showed no signs of distress. Litters were monitored daily thereafter to record possible pup mortality. The sex of all pups that died prior to weaning age ($N = 18$, 14% of all pups born, including a litter of eight pups that was found dead immediately after the birth) was verified by necropsy. Apart from weekly measurements of body mass, no other procedures were applied to the pups during the study. The secondary offspring sex ratio (mean ± SD proportion of males (males/litter size)) was 0.40 ± 0.29 and significantly female biased compared to the expected random binomial distribution with a mean of 0.5 (exact binomial test: $z = -2.65$, $P = 0.013$). This result corresponds well to our previous data on offspring sex ratio in field voles (Hansson 1987).

Three of the females who reproduced had reproduced once before and may thus confound our analyses. However, we found no difference between primiparous and multiparous females in litter size (regression with negative binomial errors: $\chi^2_1 = 0.76$, $P = 0.38$) and sex ratio (logistic regression: $\chi^2_1 = 1.87$, $P = 0.17$) or maternal age (general linear model (GLM): $\chi^2_1 = 0.64$, $P = 0.43$), testosterone levels (GLM: $\chi^2_1 = 0.95$, $P = 0.33$), corticosterone levels (GLM: $\chi^2_1 = 0.53$, $P = 0.47$) or glucose levels (GLM: $\chi^2_1 = 2.15$, $P = 0.14$). Thus, multiparous females were included in the analyses to obtain maximal sample size.

Means ± SD of the selected variables studied are given in Table 1. Correlations between individual body condition and hormonal measures suggest that in both sexes body condition was uncorrelated with hormonal measures (Table 2). The absence of strong multicollinearity (see

Table 2. Correlation matrix between predictor variables in females (F) and males (M)

	Condition (F)	Glucose (F)	Testosterone (F)	Corticosterone (F)	Testosterone (M)
Glucose (F)	0.041 *0.84*				
Testosterone (F)	−0.138 *0.48*	0.047 *0.81*			
Corticosterone (F)	−0.116 *0.56*	−0.124 *0.53*	0.204 *0.30*		
Testosterone (M)	−0.083 *0.68*	−0.234 *0.23*	−0.285 *0.14*	−0.297 *0.13*	
Condition (M)	0.033 *0.87*	−0.240 *0.22*	0.057 *0.77*	0.262 *0.18*	0.096 *0.63*
Age (F)	−0.346 *0.07*	0.395 *0.038*	−0.062 *0.76*	0.057 *0.77*	−0.386 *0.043*

Coefficients are Pearson's correlation coefficients, except in the cases of corticosterone where Spearman's correlation coefficients were used due to nonnormal distribution of the data. Values given in italics represent *P* values for the corresponding correlation coefficients.

below) helps us to contrast the effects of parental condition with the effects of hormonal measures per se on offspring sex ratio. The study was approved by the Lab-Animal Care & Use Committee of the University of Turku (license no. 1534/05).

Serum Testosterone Assay

Plasma testosterone was measured by a direct radioimmunoassay (DiaSorin, Stillwater, Minnesota, U.S.A.) using antibody-coated tubes and iodine-labelled T tracer according to the manufacturer's instructions. The analytical sensitivity of the assay was 20 pg/ml at the 95% confidence interval. This was calculated as the apparent concentration of analyte, which was distinguishable from the zero calibrator, i.e. two standards deviations below zero. The intraassay variations were less than 8.1% and the interassay variations below 7.6%.

Faecal Corticosterone Assay

Faecal samples were collected into calibrated tubes for the measurement of wet mass. After adding 1 ml of 95% ethanol, the samples were frozen in −20°C until suspended with an RW 16 homogenizer (IKA®; Werke GmbH & Co. KG, Staufen, Germany). Ethanol was evaporated before lyophilization (Freeze dryer Beta 2–16; Christ, U.S.A.) and the dry mass of the sample was recorded. Pulverized samples were then suspended in 1 ml of 100% ethanol with ultrasonication (MSE ultrasonic disintegrator). Corticosterone was extracted with boiling at 75°C for 20 min. After centrifugation at 12 000*g* for 5 min, the supernatants were collected and the faecal material was resuspended in ethanol. The extraction phase was repeated and the supernatants of each sample were pooled. Ethanol was evaporated and the extract was reconstituted with ultrasonication (MSE ultrasonic disintegrator) in 200 µl of phosphate-buffered saline (pH 7.4) containing 1% bovine serum albumin (Sigma Aldrich, Steinheim, Germany). After reconstitution, the samples were centrifuged at 12 000*g* for 5 min and the supernatants collected and stored in −20°C until analysis. Faecal corticosterone content was analysed using a commercial double antibody ^{125}I radioimmunoassay kit (MP BioMedicals, Orangeburg, NY, U.S.A.) according to the procedure described by the manufacturer. The lower limit of assay sensitivity was 7.7 ng/ml when defined as two SD above zero. The intraassay coefficient of variation (CV) was below 10.2% and the interassay CV below 7.2%. The results are expressed as the concentration of corticosterone (ng) per dry mass of faeces (mg) analysed per individual.

Statistical Analyses

Litter sex ratio in relation to parental testosterone and maternal glucose and corticosterone concentrations was examined using a logistic regression where the number of male pups was the response with the litter size as a denominator, fitted with binomial errors and logit link function (Allison 1999). The full model controlled for the body condition of both parents, maternal age at the beginning of pairing and litter size (Williams 1979; Huck et al. 1990; Clark et al. 1991). Trial number was included as a random factor to control for possible intertrial variation due to unknown reasons, and hence Satterthwaite's formula was used to estimate the denominator degrees of freedom of fixed effects (Littell et al. 1996). No interactions were investigated because of, due to our small sample size ($N = 28$), the risk of overfitting the models (Peduzzi et al. 1996). Model reduction by backward elimination of nonsignificant fixed effects using F tests was applied to obtain a minimal final model. In logistic regression analysis with small sample size, the conventional elimination criteria using a P value of 0.05 may lead to biases in model selection and thus parameter estimation (Steyerberg et al. 2000). We thus used a P value of 0.15 for an elimination criterion (see also Hosmer & Lemenshow 1989).

To explore the possibility that parental hormonal measures and maternal glucose concentration might have affected litter sex ratio through sex-selective foetal loss, we examined whether these parental traits were related to litter size. Because litter size is a count variable, we applied a general linear mixed model as described above, fitted with negative binomial errors and logit link function, to explicitly estimate a dispersion parameter (Allison 1999; Littell et al. 1996). Because the variance estimate of trial number was negative (i.e. effectively zero), it was excluded from the model. Backward model selection was done with likelihood-ratio tests that compare the change in deviance between two nested models, distributed as χ^2 statistics with a number of degrees of freedom equal to the difference in the number of parameters in the two models.

Because each male mated with up to three females in successive pairings, we examined whether male identity, included as a random factor, was related to litter sex ratio and size. Likewise, because the voles used were from the same litters, we also included female and male family identities (male identity above was nested within the male family identity) as random factors in our model. However, because both of these random effects were nonsignificant ($P > 0.14$) in both models using one-sided t tests, they were omitted from the global models to obtain parsimony and to avoid overfitting of the models.

Prior to these analyses, the potential dependence of female testosterone and corticosterone concentrations on oestrus cycle phase (Millspaugh & Washburn 2004; Cavigelli et al. 2005) was examined by general linear models, where oestrus cycle was treated as a four-level fixed effect (prooestrus, oestrus, oestrus–metoestrus and metoestrus). Female testosterone level appeared to be associated with oestrus cycle phase ($F_{1,24} = 2.77$, $P = 0.064$), whereas corticosterone level was unrelated to oestrus cycle ($F_{1,24} = 0.48$, $P = 0.70$). Moreover, female glucose level was not related to oestrus cycle ($F_{1,24} = 0.83$, $P = 0.49$). Hence, variation due to oestrus cycle was removed from the maternal testosterone level and the residual values of this variable were used in the following analyses.

Multicollinearity among explanatory variables was assessed with variance inflation factors and tolerance values. The largest variance inflation factor was 1.72 and the

lowest tolerance value 0.58, suggesting no bias in the standard errors of regression coefficients. All analyses were conducted with SAS statistical software version 9.1 (SAS Institute Inc., Cary, NC, U.S.A.).

RESULTS

Litter Sex Ratio

Females with high serum testosterone and glucose levels delivered male-biased litters (Table 3; Fig. 1), whereas maternal corticosterone level was not associated with litter sex ratio (Table 3). The effects of maternal testosterone and glucose levels correspond to 2 and 19% increases in the proportion of males in a litter per increase of 1 pg/ml and 1 mmol/L of testosterone and glucose, respectively. Older females and those who delivered large litters also produced male-biased litters, as an increase of 1 day in female age and one extra pup born resulted in 5 and 57% increases in the proportion of males in a litter, respectively (Table 3). Moreover, litter sex ratio was not associated with female body condition (Table 3). Male testosterone level was not related to litter sex ratio, but females mated with a male of high body condition tended to deliver male-biased litters (Table 3).

Litter Size

Females with high glucose levels produced smaller litters (Table 4, Fig. 2). The magnitude of this effect corresponds to a 6% reduction of litter size per increase of 1 mmol/L of maternal glucose level. The parental testosterone level and body condition and the maternal corticosterone level and age were unrelated to litter size (Table 4).

DISCUSSION

Our results show that the proportion of male pups in a litter was positively associated with levels of maternal circulating testosterone and glucose just prior to breeding, old females delivered proportionally more male pups than did young females, and female corticosterone level and body condition were unrelated to litter sex ratio. Furthermore, paternal testosterone level was not associated with litter sex ratio, but females who mated with males of high body condition tended to produce male-biased litters. Of the parental variables studied only maternal glucose level was associated with litter size, as females with high levels of glucose produced smaller litters.

Effects of Parental Variables on Litter Sex Ratio

The result that females with high serum testosterone levels produced male-biased litters is in line with previous studies in birds (Veiga et al. 2004; Pike & Petrie 2005a; Rutkowska & Cichoń 2006). In mammals, evidence for an association between female testosterone level prior to conception and male-biased offspring sex ratio is primarily indirect (James 1996, 2004, 2006a; Manning et al. 2002). Only Grant & Irwin (2005) directly investigated such a link. They found that in domestic heifers high follicular testosterone levels correlated positively with the odds of the fertilized ovum being a male. Our study is thus the first to show an association between high maternal circulating testosterone level and male-biased litter sex ratio in a nondomestic mammal. The exact mechanisms responsible for this association, however, remain unknown. Maternal testosterone level potentially influences differently the fertilization success of X- and Y-bearing spermatozoa by favouring the mobility and/or survival of Y-bearing spermatozoa. Although mammalian oocytes are generally believed to be neutral towards X- and Y-bearing sperm (Zuccotti et al. 2005), elevated testosterone concentration in ovarian follicle fluid also may affect differently the receptivity of the ovum to X- and Y-bearing spermatozoa (Grant & Irwin 2005).

This study is also the first to simultaneously contrast the effects of maternal and paternal testosterone concentrations on litter sex ratio under a study design where the females were paired randomly with different males in similar environmental conditions, thus excluding assortative mating and environmental correlations as confounding factors. In contrast to James' (1996, 2004, 2006a) prediction, paternal testosterone level was not related to litter sex ratio. Therefore, maternal testosterone level appears to play a more important role in litter sex ratio variation than paternal testosterone level; alternatively, female field voles do not bias their litter sex ratio according to male testosterone level. This result is expected

Table 3. Effects of parental traits on secondary litter sex ratio

Explanatory variable	$df_{num,den}$	F	P	Odds ratio (95% CI)
Female glucose	**1,22**	**4.00**	**0.058**	**1.19 (0.99–1.43)**
Female testosterone	**1,22**	**4.70**	**0.041**	**1.02 (1.00–1.03)**
Female age	**1,22**	**8.96**	**0.007**	**1.05 (1.02–1.09)**
Litter size	**1,22**	**3.60**	**0.071**	**1.57 (0.91–2.68)**
Male condition	**1,22**	**2.99**	**0.098**	**1.36 (0.97–1.91)**
Female condition	1,21	0.32	0.58	1.17 (0.66–2.10)
Female corticosterone	1,20	0.54	0.47	1.18 (0.74–1.90)
Male testosterone	1,19	0.04	0.84	1.00 (1.00–1.00)

Variables given in bold face are included in the final minimal model. Odds ratio estimates, which measure effect size, are given with 95% confidence intervals (CI).

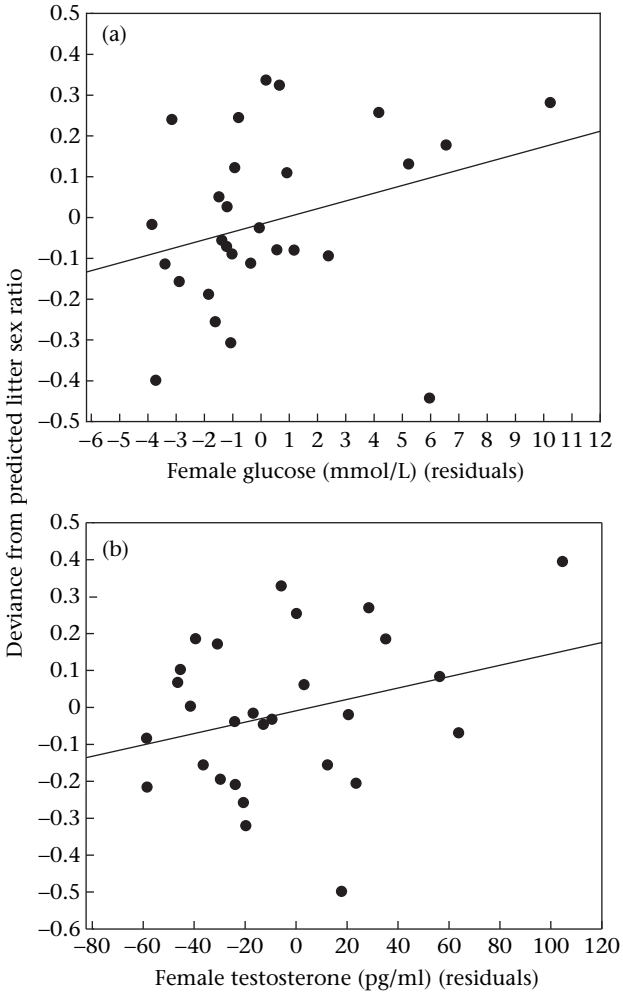

Figure 1. Effects of female (a) serum glucose and (b) testosterone levels on litter sex ratio. Points represent the deviation of observed litter sex ratio from the predicted litter sex ratio obtained from the model excluding the independent variable in question.

because paternal testosterone level is unlikely (except through sex-biased sperm) to affect directly the fertilization success of Y- and X-bearing spermatozoa. Even though sperm sex ratio varies according to male testosterone level, females still might have better control over the sex ratio of sperm fertilizing the ova as described above.

We also found evidence for a high level of female serum glucose being associated with a male-biased litter, supporting the previous findings from in vitro and in vivo investigations of developing embryos (Gutiérrez-Adán et al. 2001; Larson et al. 2001; Jimenéz et al. 2003a; Pérez-Crespo et al. 2005). Inbred laboratory mice also exhibited a male-biased offspring sex ratio as a result of an elevated maternal glucose level (Machado et al. 2001). Our results thus provide the first demonstration that high female glucose level is associated with offspring sex ratio bias towards males also in a nondomestic mammal.

In contrast to previous studies (Geiringer 1961; Love et al. 2005; Pike & Petrie 2005a, 2006; Bonier et al., in press), we found no evidence for the proposed association between a high female corticosterone level and a female-biased litter sex ratio. One potential explanation for this might be that maternal stress did not exceed some threshold level required to induce stress-related responses on litter sex ratio, and, hence, manipulative experiments may be required to establish such an association.

Potential shortcomings of the hormonal variables measured here deserve mentioning. Some loss of resolution may be introduced by our inability to take measurements precisely at the time of conception or during very early development of offspring due to difficulties of repeated sampling and accurately determining the time of conception. However, 86% of the females gave birth within 3 days of the expected mean duration of pregnancy, suggesting that in most cases fertilization occurred shortly after the hormonal measures were taken. In addition, in field voles we do not currently know how well the maternal serum levels of testosterone and glucose or faecal corticosterone level match with the corresponding levels in reproductive organs or in the follicular or amniotic fluids specifically (see Grant 2007). In mammals, serum glucose and testosterone levels should be constant through the circulation system, but whether these concentrations reflect those, for example, in follicular fluid remain unexplored.

In agreement with some previous studies, we found that older females delivered male-biased litters. In sexually dimorphic species where males are heavier, including field voles (Table 1), such an association may indicate an increased reproductive effort due to declining residual reproductive value with advancing maternal age (Charlesworth & Leon 1976). Alternatively, instead of investing more in reproduction in terms of the production of proportionally more sons, older females may be better mothers due to their prior experience (Cameron et al. 2000). This explanation seems unlikely for our data because most of the females used here were primiparous. We also found that

Table 4. Effects of parental variables on litter size

Explanatory variable	β (95% CI)	*df*	χ^2	*P*
Female glucose	−0.059 (−0.108 to −0.010)	**1**	**6.17**	**0.013**
Female condition	0.100 (−0.031–0.231)	1	2.17	0.14
Male condition	0.081 (−0.064–0.226)	1	1.18	0.28
Female testosterone	−0.001 (−0.004–0.003)	1	0.12	0.73
Female age	−0.001 (−0.010–0.008)	1	0.06	0.81
Male testosterone	−0.000 (−0.0001–0.0001)	1	0.00	0.95
Female corticosterone	0.000 (−0.133–0.133)	1	0.00	1.00

Variables given in bold face are included in the final minimal model. Regression estimates are given with 95% confidence intervals (CI).

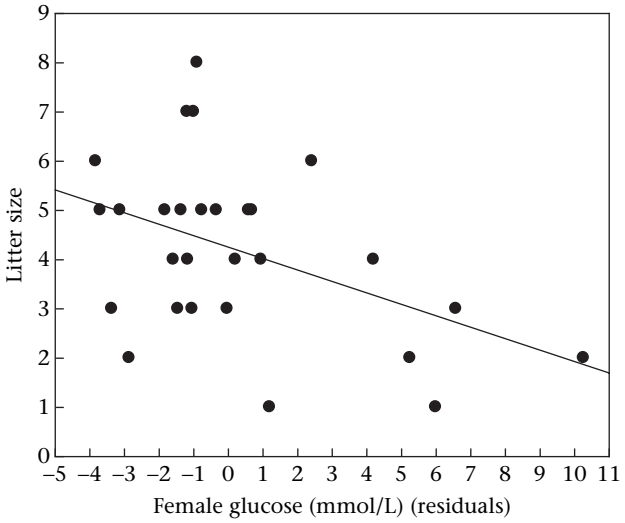

Figure 2. Association between female serum glucose level and litter size.

large litter size was related to a bias towards males, which is in contrast to some previous work (Huck et al. 1990; Clark et al. 1991). It is difficult to assess the reasons for these different outcomes because currently the mechanisms (e.g. female-biased embryonic mortality in large litters) or ecological background for why litter sex ratio should vary with litter size are unclear (Sheldon & West 2004).

Maternal body condition and quality might affect offspring sex ratio (Trivers & Willard 1973; Sheldon & West 2004; Cameron 2004), but we did not find such a relationship in field voles (see also Koskela et al. 2004). Our results could thus indicate that in field voles maternal quality is not adequately described by body condition only. Because small rodents commonly exhibit infanticidal behaviour towards newborn pups (Wolff 1985), maternal quality might be best measured by her capacity to exhibit maternal aggression against intruders. This type of behaviour is strongly influenced by hormonal levels, in particular testosterone and progesterone (Lonstein & Gammie 2002). Furthermore, Clarke & Faulkes (1997) and Beehner et al. (2005) have shown that in mammals top-ranked females have elevated testosterone levels, suggesting also that in female mammals testosterone level may be linked to dominance rank and female quality. This idea is in line with studies in ungulates where offspring sex ratios have been found to correlate more strongly with the behavioural measures of female dominance than with the morphological or physiological measures of female condition (Sheldon & West 2004). However, in primates such associations are not found (Brown & Silk 2002; Silk et al. 2005). In addition, high testosterone level might be detrimental to female body condition (Pike & Petrie 2005a), thereby potentially confounding the prediction that high-quality females should produce more sons (James 2006a). In our study female body condition was unrelated to testosterone level, suggesting that maternal testosterone level affected litter sex ratio independent of body condition. Another explanation for the absence of the effect of maternal body condition on litter sex ratio may be that, by subsisting on a high-quality laboratory diet, all females were in sufficiently good physiological condition for sex ratios to not be affected.

Finally, we found a tendency of females paired with a male of high body condition to deliver a male-biased litter. Theory suggests that females mated with a high-quality male should produce proportionally more sons if male quality is heritable and affects more the fitness of sons than that of daughters (Trivers & Willard 1973). While we do not currently know the heritability of male body condition in field voles, our result is compatible with the idea of offspring sex ratio bias according to male quality. We also do not know whether body condition has sex-dependent fitness benefits in field voles in the wild, but at least in this study female condition was unrelated to one estimate of fitness, litter size. Nevertheless, good body condition may be advantageous in the wild, particularly to males, because it may increase their survival and success in male–male competition for mates.

Effects of Parental Variables on Litter Size

Of those parental variables measured, only maternal serum glucose level was associated with litter size, as females with high levels of glucose produced smaller litters. In field voles, pregnancy and preimplantation loss occur (Brambell & Hall 1939). Furthermore, Machado et al. (2001) showed that in mice an elevated maternal glucose level resulted in increased rates of resorptions. Hence, our findings that high maternal glucose levels predicted both male-biased litter sex ratio (while controlling for litter size) and small litter size are compatible with the idea that high maternal glucose levels may have induced female-biased embryonic mortality in field voles, leading to a higher proportion of sons born. Instead, an absence of such an association between maternal testosterone and litter size suggests that, as predicted, testosterone may be involved in primary sex ratio determination in field voles.

CONCLUSION

Our results support the hypotheses that high maternal testosterone and glucose levels act as proximate mechanisms biasing secondary offspring sex ratio towards males in field voles. Although male testosterone level did not affect litter sex ratio, females paired with a male of high body condition tended to deliver male-biased litters. Our results thus suggest that several different maternal and to a lesser extent paternal traits may affect offspring sex ratio in mammals. The factors studied here independently correlated with litter sex ratio in the predicted directions, but this does not exclude the possibility of contrasting effects or even important interactions among the traits investigated. Furthermore, the discussion on whether pre- or postconceptional mechanisms mainly are responsible for secondary offspring sex ratio variation in mammals remains lively (Boklage 2005; James 2006b). As we found only maternal glucose level to be related to litter size, potentially indicating a sex-biased early mortality, our

findings suggest that preconceptional mechanisms might be more important in influencing secondary sex ratio variation in field voles. This does not, however, exclude the possibility that both of these mechanisms play roles in secondary sex ratio in mammals (Jimenéz et al. 2003b).

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