Behav Ecol Sociobiol (2010) 64:371–379
DOI 10.1007/s00265-009-0853-z

ORIGINAL PAPER

Secondary sex ratios do not support maternal manipulation: extensive data from laboratory colonies of spiny mice (Muridae: *Acomys*)

Marcela Nováková · Barbora Vašáková · Hana Kutalová · Katarina Galeštoková · Klára Průšová · Petr Šmilauer · Radim Šumbera · Daniel Frynta

Received: 24 February 2009 / Revised: 23 May 2009 / Accepted: 2 September 2009 / Published online: 6 October 2009

Abstract Spiny mice of the genus *Acomys* (Muridae) represent a very suitable mammalian model for studying factors influencing the secondary sex ratio (SSR). The maternal effort in these rodents is extremely biased in favour of the prenatal period and, therefore, maternal manipulation of the SSR is potentially more advantageous. We studied the SSR in four populations/species of spiny mice kept in family groups consisting of two closely related females, one non-relative male and their descendants. The groups were established from founding animals aged about 3 months (maturing age) and were allowed to breed freely for several months. Each litter was sexed after birth, and relevant data were thoroughly recorded. Altogether, data were collected on 1684 litters: 189 of *Acomys sp.* from Iran, 203 of *A. cilicicus*, 875 of *A. cahirinus*, and 417 of *A. dimidiatus*. We recorded the sex of 4048 newborns of which 1995 were males and 2053 were females. The overall sex ratio was close to 1:1 (49.2%). Generalized linear mixed models and/or generalized linear models were constructed to evaluate the effect of four life history and eight social variables on the sex ratio. No consistent effects of these variables on the sex ratio were found and, interestingly, none of the variables associated with maternal life history had any effect on the sex ratio. Three factors associated with group composition (i.e. the number of immature males, the number of immature females and the number of breeding females) did have significant effects on the sex ratio, but these effects were not consistent across the studied species. In conclusion, our evaluation of this large dataset revealed that the sex ratio in spiny mice is surprisingly stable.

Keywords Parental effort · Rodents · Sex allocation · Sex ratio · Social behaviour

Introduction

The secondary sex ratios (SSRs) of newborn mammals have attracted enormous research effort since Darwin (1871). There are several theoretical backgrounds for maternal manipulation that would result in a biased sex ratio of the progeny. These include the Fisherian theory, which assumes the allocation of equal investment to male and female progeny (Fisher 1930), the model of local resource competition, which expects SSRs to be biased in favour of the dispersing sex (Clark 1978) and the maternal condition hypothesis, which predicts a higher proportion of males under favourable conditions and, conversely, a higher proportion of females under unfavourable conditions (Trivers and Willard 1973; Carranza 2002; Cameron and

Communicated by G.Wilkinson

M. Nováková · H. Kutalová · K. Galeštoková · K. Průšová · D. Frynta (✉)
Department of Zoology, Faculty of Science, Charles University, Viničná 7,
128 44 Prague 2, Czech Republic
e-mail: frynta@centrum.cz

M. Nováková
e-mail: marc.novakova@centrum.cz

B. Vašáková · R. Šumbera
Department of Zoology, Faculty of Science,
University of South Bohemia,
Branišovská 31,
370 05 České Budějovice, Czech Republic

P. Šmilauer
Department of Ecosystem Biology, Faculty of Science,
University of South Bohemia,
Branišovská 31,
370 05 České Budějovice, Czech Republic

Linklater 2002; for a review, see Cameron 2004). Current theoretical studies combine the above models as well as other selective forces into complex mathematical models for predicting the sex ratios (e.g. Wade et al. 2003; Wild and West 2007).

Empirical results support the view that SSR is usually close to equality; nevertheless, significant deviations from the 1:1 ratio have been repeatedly reported (Austad and Sunquist 1986; Gosling 1986; Labov et al. 1986; Huck et al. 1990; Perret and Colas 1997; Creel et al. 1998; Côté and Festa-Bianchet 2001; Cameron 2004; Sheldon and West 2004; Kaňková et al. 2006). These deviations have been attributed either to the above-mentioned ultimate causes or to proximate mechanisms that are by definition not mutually exclusive. Of the proximate mechanisms, the most promising are (1) the level of circulating steroid hormones (James 1996, 1998, 2004, 2006; Grant 2007), (2) developmental asynchrony of sexes in blastocyst growth (Krackow 1995, 1997; Krackow and Burgoyne 1998; Krackow et al. 2003) and (3) circulating glucose level (Gutiérrez-Adán et al. 2001; Cameron 2004; Kimura et al. 2005; Cameron et al. 2008; Helle et al. 2008).

In recent years, the evidence for the adaptive maternal SSR adjustment model has been questioned both on empirical (see below) and theoretical grounds (Krackow 2002). Festa-Bianchet (1996) accentuated the high frequency of contradictory results and also the selective publication success of papers presenting positive results. In this respect, he shared the scepticism of an earlier paper of Clutton-Brock and Iason (1986). A similar conclusion is also supported by a thorough review by Cockburn et al. (2002). Interestingly, an empirical study analysing extensive datasets from wild savannah baboons (*Papio cynocephalus*) has clearly demonstrated that significant results are a product of stochastic biases that arise in small samples (Silk et al. 2005). The only effects on mammalian SSR clearly supported by recent reviews are those of maternal nutritional status around the time of conception (Cameron 2004; Rosenfeld and Roberts 2004; Sheldon and West 2004).

Among mammals, rodents are a suitable model for studying sex ratios and influencing factors, since they may be easily kept under laboratory conditions, thereby providing an opportunity to gather sufficient sample sizes required for relevant statistical analysis (for review, see Sikes 2007). Classical laboratory rodents, such as mice (*Mus musculus*; Krackow and Hoeck 1989; Krackow and Gruber 1990; Kaňková et al. 2007), rats (*Rattus norvegicus*; Bird and Contreras 1986), golden hamsters (*Mesocricetus auratus*; Labov et al. 1986, Huck et al. 1990) and guinea pigs (*Cavia porcellus*; Peaker and Taylor 1996) are traditional models for empirical studies. Nevertheless, SSRs have been studied in many other species as well, such as coypus (*Myocastor coypus*; Gosling 1986), Mongolian gerbils (*Meriones unguiculatus*; Clark et al. 1991), wood rats (*Neotoma floridana*; McClure 1981), wood mice (*Apodemus sylvaticus*; Frynta and Žižková 1994) and voles (*Microtus agrestis*, *M. oeconomus*; Hansson 1987; Ims 1994). Unfortunately, most of the published studies rely solely on correlations between sex ratio and parameters of maternal life history. Experimental studies have mostly focussed on the manipulation of food intake and food quality (e.g. Huck et al. 1986; Wright et al. 1988; Koskela et al. 2004; Rosenfeld et al. 2003; Cameron et al. 2008; Fountain et al. 2008). The effects of true social factors, such as dominance (golden hamsters: Pratt and Lisk 1989), population density (voles: *Microtus townsendii*, Lambin 1994; *M. oeconomus*, Aars et al. 1995), group size (house mice: Wright et al. 1988) and/or group composition (marmots (*Marmota flaviventris*): Armitage 1987, Mongolian gerbils: Scheibler et al. 2005), on sex ratios have been addressed less frequently (but see Cameron 2004 for review in other mammals). Although social factors have complex consequences that are usually difficult to interpret on a proximate level, they cannot be ignored. The considerable variation in densities and social circumstances that affects breeding females of most rodent species even under natural conditions has to be taken into account.

In the study reported here, we focussed on the sex ratio in spiny mice of the genus *Acomys* and its relationships to social and life history variables. Spiny mice are social animals, and under laboratory conditions they should be kept in families consisting of an adult male, multiple females and their descendants (Young 1976), which mimics their wild social system. Their societies are not anonymous, and individual recognition has been demonstrated unequivocally (Porter et al. 1986). In groups consisting of related individuals, communal care for the young comprising allosuckling is frequent; however, mothers are able to recognize their own offspring (Porter et al. 1980). In addition to the ease of keeping and breeding spiny mice under laboratory conditions, their newborns, unlike those of many other rodents, can be easily and reliably sexed according to external characters. Moreover, spiny mice are likely to be predisposed to maternal manipulation of the sex ratio for the following three reasons.

(1) In contrast to many other muroid rodents, after an extended gestation period (36–42 days, which is nearly twice as long as that for laboratory mice), spiny mice produce only small litters (most frequently consisting of two or three newborns, range1–7; Frynta et al. unpublished results) consisting of relatively large and well-developed newborns (Brunjes 1990). Maternal effort is therefore high in the prenatal period compared with that in many other muroid rodents. Thus, we consider that maternal manipulation of the sex ratio

prior to parturition is more advantageous than sex-selective parental infanticide of newborns.

(2) The large body size of newborns relative to maternal body size (the mean litter weight at birth represents 20–25% of maternal weight; Dieterlen 1961) facilitates the potential effect of litter size on offspring quality. Resources available per individual offspring are considerably reduced even by the increment of a single newborn. Small litters thus may be predisposed for the production of the sex exhibiting a more closer relationship between consumed maternal resources and fitness prospects (Trivers and Willard 1973). Nevertheless, the assumptions of the Trivers–Willard hypothesis (Hewison and Gaillard 1999; Carranza 2002; Cameron and Linklater 2002; Blanchard et al. 2005) predicting an association between parameters related to maternal condition and allocation of maternal investment to the sexes have not been empirically tested in spiny mice yet. Two of these three principal assumptions (mothers in better condition can produce weanlings in better condition; adult males benefit more from a good condition than adult females) are most probably satisfied in spiny mice due to high maternal investment and strong male-male competition, while the validity of the third one (correlation between weanling and adult condition) is likely, but remains unproved. Spiny mice live in environments with high spatiotemporal variation in resource availability (semideserts, seasonal savannas and woodlands). Under natural conditions, the reproductive value of the offspring produced in good and bad conditions may differ considerably, and thus alternative reproductive strategies that switch according to actual ecological and nutritional conditions may evolve.

(3) Spiny mice are social rodents in which social circumstances may also contribute to fitness. The reproductive fate of rodent female may be determined by the presence or absence of maternal kin in the neighbourhood (e.g. Lambin and Yoccoz 1998). Both sexes, but especially males, may be limited by the presence of older cohort of the same sex. Our data from laboratory colonies suggest that adolescent males are driven away by the territorial male while females are tolerated. Local resource competition between the mother and female offspring can, therefore, be a reasonable expectation. The number of females within a family may indicate the expected cost of bearing additional daughters. Consequently, we hypothesized positive association between the number of females in the family group and the sex ratio. Conversely, an excess of juvenile and/or adolescent males in the environment may reduce the reproductive prospect of additional male offspring. Thus, a negative association between the number of immature males and sex ratio should be expected.

Spiny mice are, therefore, a suitable model for critically testing the predictions of the sex ratio theory. We have analysed the data on SSR collected in captive colonies of spiny mice belonging to four closely related species. The aim of our study was to assess (1) deviations from the one to one and/or Fisherian ratios, (2) effects of life history variables and (3) social composition of the breeding groups on SSR.

Material and methods

Spiny mice of the genus *Acomys* are small rock-dwelling rodents inhabiting Africa and Middle East. Despite their earlier systematic placement, spiny mice are more related to gerbils than to true murids belonging to the subfamily Murinae, which are represented by rats and house mice (Steppan et al. 2004).

Our laboratory colonies of spiny mice were of the following origin: *Acomys cahirinus*, Abu Simbel archaeological site, southern Egypt (22º 22′ N, 31º 38′ E); *A. cilicicus*, east of Silifke, southern Turkey (36º 26′ N, 34º 06′ E); *Acomys. sp*., Zagros, southwestern Iran (28º 56′ N, 52º 32′ E); *A. dimidiatus*, laboratory strain, Prague zoo, probably from Israel or Sinai. Phylogenetic analysis of mitochondrial control region sequences in these colonies revealed that all of the studied populations/species belong to the *cahirinus/dimidiatus* group. The former two populations/species belong to the clade of *A. cahirinus* sensu lato inhabiting North Africa and the eastern Mediterranean region (Crete, Cyprus, Kilikian coast in Anatolia), while the latter two belong to the *A. dimidiatus* sensu lato clade ranging from Sinai, throughout the Arabian Peninsula and along the coast of the Gulf of Oman from Iran to Pakistan. The specific/subspecific status of the Iranian population, referred to here as *Acomys sp.*, needs further clarification (Frynta and Průšová unpublished results).

The animals were kept in terrariums (60×50×40 cm or 70×60×40 cm) or in rodent standard plastic cages (VELAZ T4 , 55×32×18 cm) under standard laboratory conditions. Wood shavings were used as bedding material, a clay flowerpot with a lateral opening served as a shelter and tree branches for climbing and gnawing were provided as environmental enrichment. Food (standard diet for rats and mice ST1; VELAZ, Czech Republic, supplemented with a mixture of grains, dry bread, apples and herb leaves) and water were available ad libitum.

The spiny mice were kept in family groups consisting of two closely related females (full sisters or uterine sisters), one non-relative male and their descendants. The groups were established from founding animals about 3 months of age (maturing age) and then allowed to breed freely for several months. Manipulation of group structure only occurred in the case of male-to-male aggression between the male founder and his mature male offspring (if so, young males were removed). The experimental groups were regularly checked (either daily or every other day), and each litter was sexed immediately after its detection. Compared to many other rodents, sexing in the spiny mice does not rely solely on ambiguous anogenital distances, as female nipples are clearly visible, even in newborns. Thus, the sexing error approaches zero in these animals. The putative mother was identified, and other circumstances (see below) were thoroughly recorded.

The recorded data included the sex ratio itself (expressed as the proportion of males in the litter), maternal life history variables, such as (1) parity, (2) age in days, (3) postpartum estrus (the litter was considered as conceived postpartum when delivered before day 41 after the previous one) and (4) litter size, and social variables, such as (5) time from the founding of the group (in months), (6) litter order (from the group perspective), (7) number of adult males actually present in the group (aged ≥90 days), (8) number of adult females (see 7), (9) number of breeding females (i.e. those that already gave birth) in the group—coded as the presence of either one or more than one breeding female in the group and further referred to as breeding females, (10) maternal status (first breeding founder, second breeding founder, their daughters), (11) number of immature males and (12) number of immature females (aged ≤90 days for both sexes). It should be noted that maternal body weight was assessed but not included because it was missing in an additional 342 cases. Nevertheless, the statistical models referred below were not substantially affected by the inclusion of maternal body weight, and this factor remained non-significant.

Statistical analysis

We estimated generalized linear mixed models (GLMM) and/or generalized linear models (GLM) in which litter sex ratio was treated as a dependent variable with a binomial distribution and the logit link function was adopted. As the models require complete sets of explanatory variables, all litters with at least one missing value were excluded (n=212). Therefore, the numbers of litters and newborns used for the computation of the overall sex ratios exceed those included in GLMM analyses.

We first computed GLMMs in which maternal identity was included as a random factor to avoid pseudoreplications (Krackow and Tkadlec 2001). The significance of this random effect was tested using the log-likelihood ratio test based on χ^2 distribution, and the effect was found to be non-significant. This allowed us to further use the simpler GLMs instead of GLMMs.

As no effect of any factor associated with maternal identity (i.e. maternal parity, age, postpartum estrus and status) was found to be significant in either the complete GLMM analysis or in any separate GLMM analysis of data concerning individual species, we removed these variables from subsequent GLMs. We then incorporated all remaining explanatory variables into main effects GLMs computed separately for each species and pooled the data. Finally, we computed GLM allowing interactions between species and explanatory variables. The above full models were then reduced to variables with $P<0.1$ (see Results).

The size of the effects is presented either graphically and/or as the percentage point difference in sex ratio from the nominal value of 50% due to a unit change of the predictor (CPU). The calculations were performed using the R statistical package ® development core team 2005). Traditional chi-square tests were also conducted to test deviations of the observed sex ratios from the expected equality. Although this approach is theoretically less appropriate than the above-mentioned one and may inflate the significance, it is more intuitive and allows for the inclusion of all records.

Results

We recorded the sex of 4048 newborns of which 1995 were males and 2053 were females. Thus, the overall sex ratio was very close to 1:1 (49.2%, χ^2=0.831, P=0.36). Among the studied species, only *Acomys sp.* from Iran exhibited a significant deviation from the balanced sex ratio (42.5% males; Table 1).

Generalized linear mixed models The initial full GLMM evaluating the effect of all examined factors—i.e. four maternal life history (maternal parity, age, postpartum estrus and litter size) and eight social variables (time from the founding of the group, litter order, number of adult males, number of adult females, number of breeding females, maternal status, number of immature males, number of immature females)—revealed significant effects of species (*A. cilicicus* CPU=9.02, *A. cahirinus* CPU=7.89, *A. dimidiatus* CPU=9.55) and litter size (CPU=-1.89) on the sex ratio. Table 2 presents details on the statistics. The effect of the number of immature males approached the chosen α level of 0.05 (CPU=0.75; $F_{(1,963)}$=3.21, P=0.0734).

Table 1 Secondary sex ratio in study population categorized according to species

Species	Number of litters	Males	Females	Total number of newborns	Sex ratio (%)
A. cahirinus	875	988	979	1967	50.2
A. cilicicus	203	241	241	482	50.0
A. dimidiatus	417	612	625	1237	49.5
Acomys sp.	189	154	208	362	42.5*
Total	1684	1995	2053	4048	49.2

*$P<0.05$

Table 2 Results of analyses using the GLMM and GLM models for testing the secondary sex ratio in four species/populations of the genus *Acomys*

Model	CPU	LCI	UCI	*F*	*df*	Residual *df*	*P*
GLMM							
Species				3.18	3	487	0.0238
A. cilicicus	9.02	1.41	16.22				
A. cahirinus	7.89	1.63	13.92				
A. dimidiatus	9.55	2.95	15.81				
Litter size	−1.89	−3.53	−0.24	5.19	1	963	0.0229
Main effect GLM							
Immature males	0.81	0.13	1.49	6.49	1	1679	0.0109
Litter size	-1.61	−3.15	−0.06	4.15	1	1675	0.0415
GLM with interactions							
Immature females × species				9.34	4	1646	<0.0001
Immature females × *A. cilicicus*	-4.64	−6.67	−2.60				
Immature females × *A. cahirinus*	0.95	−0.16	2.05				
Immature females × *A. dimidiatus*	-2.02	−3.63	−0.41				
Immature females × *Acomys sp.*	3.95	0.84	7.03				
Immature males × species				6.73	3	1654	0.0002
Immature males × *A. cilicicus*	8.88	4.43	13.20				
Immature males × *A. cahirinus*	1.08	−2.75	4.90				
Immature males × *A. dimidiatus*	2.22	−1.86	6.26				
Breeding females × species				2.96	4	1650	0.0184
Breeding females × *A. cilicicus*	1.08	−11.09	13.13				
Breeding females × *A. cahirinus*	-9.73	−16.40	−2.69				
Breeding females × *A. dimidiatus*	11.22	3.05	18.80				
Breeding females × *Acomys sp.*	-6.73	−18.83	6.23				
Immature males	-1.19	−4.85	2.49	6.55	1	1661	0.0105
Separate GLMs							
A. cilicicus							
Immature males	7.46	5.07	9.82	20.57	1	201	<0.0001
Immature females	-4.93	−6.90	−2.94	26.62	1	200	<0.0001
A. cahirinus							
Breeding females	−10.21	-16.73	−3.32	5.81	1	867	0.0159
A. dimidiatus							
Immature females	−1.89	-3.39	−0.38	6.05	1	414	0.0139
Breeding females	12.19	4.31	19.47	5.92	1	415	0.0150
Acomys sp.							
Immature females	2.70	0.06	5.33	4.04	1	187	0.0444

Only significant effects for reduced models are shown

CPU, percentage point difference in sex ratio from the nominal value of 50%, due to a unit change of the predictor; LCI, lower bound of 95% confidence interval (CI); UCI, upper bound of 95% CI; GLMM, generalized linear mixed models; GLM, generalized linear models

Main-effects GLM We then excluded the factors associated with maternal identity and performed the GLM analysis. The effect of the number of immature males reached significance ($F_{(1,1661)}$=6.55, P=0.0105), while the significance of effects of the species ($F_{(3,1658)}$=2.56, P=0.0530) and litter size ($F_{(1,1655)}$=3.23, P=0.0722) decreased. When the model was further reduced to include only these three factors, the analysis revealed significant effects of the number of immature males (CPU=0.81) and litter size (CPU=-1.61), but not of the species.

Generalized linear model allowing interactions with species When the interactions between the factors included in the original GLM and the species identity were allowed, three of the factors appeared to be significant. The final model (including effects with $P<0.1$; see Table 2) revealed significant effects of the number of immature females × species interaction (Fig. 1), the number of immature males × species interaction (Fig. 2), the number of immature males (CPU= −1.19) and the number of breeding females × species interaction. The effects of the species ($F_{(3,1658)}$=2.56, P= 0.0530) and litter size ($F_{(1,1657)}$=3.27, P=0.0707) dropped below the significance level.

Separate GLMs for each species We also performed separate analyses for each species. Only the results of the final reduced models that revealed the following significant factors are provided: in *A. cahirinus* the number of breeding females (CPU=−10.21); in *A. cilicicus*, the number of immature males (CPU=7.46) and the number of immature females (CPU=-4−93); in *A. dimidiatus*, the number of breeding females (CPU=12.19) and the number of immature females (CPU= −1.89); in *Acomys sp.*, the number of immature females (CPU=2.70). For detailed statistics see Table 2.

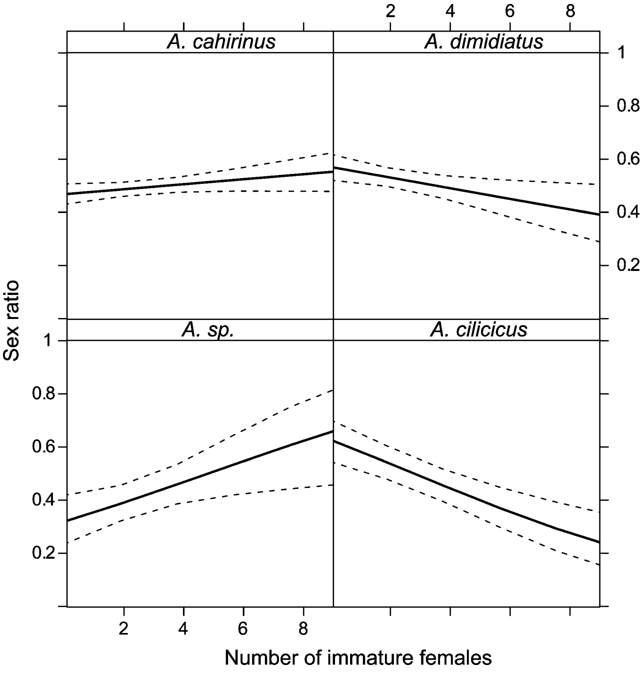

Fig. 1 The predictions of the generalized linear model (GLM) for the number of immature females and species interaction

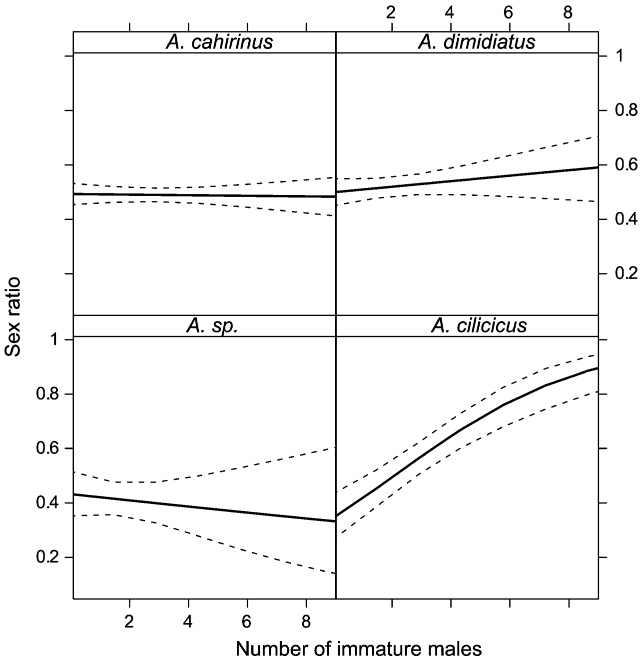

Fig. 2 The predictions of the GLM for the number of immature males and species interaction

In *A. cahirinus*, the species represented by the largest dataset, we also calculated partial GLMs, including those for only litters consisting of two and three newborns (i.e. most common litter sizes), respectively. This analysis was performed to avoid the possible interaction of the effects of differential cost by sex and by litter size. No significant effect was revealed by these partial analyses.

Discussion

The SSRs found in our dataset were very close to parity in three of the four populations/species studied. Interestingly, the only population exhibiting a slightly female-biased sex ratio was represented by the smallest sample size. Overall, these results were unsurprising, as balanced ratios at birth in other mammals have been frequently reported (Clutton-Brock and Iason 1986). Nevertheless, this phenomenon cannot be viewed as an unavoidable consequence of chromosomal sex determination. Although mammalian sex ratios are primarily determined by the sperm carrying the sex chromosomes and, consequently, are not biased to any large extent at the time of conception, episodes of sex-specific selective mortality of zygotes and embryos could lead to considerable deviations from parity, especially during early ontogeny (Kirby et al. 1967; Milki et al. 2003). These processes provide a good chance for maternal manipulation of the SSR (compare Grant and Irvin 2009). Therefore, the evolutionary maintenance of unbiased sex ratios requires the presence of a specific stabilizing mechanism, as assumed, for example, by Fisher (1930) who expected natural selection to favour an equal allocation of investment in male and female progeny. In any case, the demonstration of unbiased sex ratios does not mean a falsification of the hypothesis of maternal sex ratio adjustment (Wild and West 2007).

Despite our extensive datasets, we detected only a few significant and consistent effects on sex ratios in spiny mice. Surprisingly, the final models included only three variables, all of which were associated with the composition of the group: the number of breeding females, the number of immature males and the number of immature females. Thus, if any factor plays a role in sex ratio adjustment in these species, it has probably something to do with the actual social environment. This hypothesis corresponds quite well with the recent finding that individual families of spiny mice differ in levels of faecal cortisol metabolites (Nováková et al. 2008). Conversely, a significant interaction with species is revealed in all of the three variables included in our final models. The same factors (e.g. number of immature females; Fig. 1) showed even opposite effects in separate analyses of particular species/populations of spiny mice. In other words, the detected effects are not consistent across the studied species. Although information on the ecology of spiny mice under natural conditions is scarce, these species have fairly comparable requirements, and we can provide no straightforward explanation for the observed inconsistency. Therefore, these results need to be interpreted with caution.

None of the remaining nine explanatory variables appeared to be significant. Our results provide some support for the assumption that the effects of most factors of mammalian SSRs tend to be only small and biologically unimportant, while significant effects are mostly associated with small sample sizes and publication bias (e.g. Festa-Bianchet 1996; Cockburn et al. 2002; Silk et al. 2005, but see Cameron 2004).

Factors such as glucose level in the circulating blood around the time of conception (Cameron et al. 2008) and its correlates (such as fat content in diet; Rosenfeld et al. 2003; Rosenfeld and Roberts 2004) are currently considered to be promising proximate mechanisms of maternal SR manipulation. Not one of our variables provides a direct measure of maternal condition or metabolic status. It can be reasonably expected that some of the examined factors, such as postpartum conception, age and, possibly, social status, are related to maternal condition; however, we have no data on maternal glucose or fat levels, which are difficult to collect in large samples. Thus, covert effects of these variables cannot be excluded.

As there are both good theoretical reasons for maternal manipulation of the offspring sex ratios and data from reliable studies demonstrating the influence of various factors on sex ratios (see Introduction), we avoid drawing over-generalized conclusions from our particular study and instead focus on the peculiarities of the biology of the studied species.

True laboratory animals were selected in order to maximize the reproductive efforts for many generations. However, spiny mice are originally savanna and/or desert dwellers living in unpredictable or seasonal environments. They are therefore likely to be able to regulate their reproduction in response to actual resource availability (e.g. rainfall: Sicard and Fuminier 1996) and the corresponding prospect of the reproductive event. We may only speculate that since spiny mice as wild and more K-selected species (remember their large-sized precocial newborns) strictly avoid breeding whenever they perceive the environmental or social conditions as not fully favourable, there may be reduced variance in body condition and, consequently, no reason for maternal manipulation and/or any other maternal effect on the sex ratio of the progeny.

In conclusion, we found no consistent effects of the studied factors on the sex ratio in spiny mice. Although our correlation approach to the sex ratios has many inherent limitations, it still represents the only easy approach to obtain sufficient datasets from non-domestic mammals. Our results are not interpretable in terms of the most popular sex ratio theories (e.g. the Trivers–Willard hypothesis and/or local resource competition hypothesis). We found fairly balanced SSRs, and suspect that only some factors associated with group composition affect this trait in spiny mice.

Acknowledgements We thank J. Sádlová, L. Schwarzová, P. Kunzová, J. Flegr, M. Kaftan and J. Borek for their help in collecting ancestral stocks of spiny mice. We are grateful to Marco Festa-Bianchet and two anonymous reviewers for their helpful comments on earlier versions of the manuscript.

Ethical note Any harm to experimental animals was avoided. The experiments were performed in accordance with Czech law implementing all corresponding EU regulations and were approved by the Institutional Animal Care and Use Committee.

Financial declaration The project was supported by the Grant Agency of the Czech Academy of Sciences (project no. IAA6111410), Ministry of Education of the Czech Republic (MSMT 6007665801) and Grant Agency of the University of South Bohemia (project no. GAJU 39/2007). The personal costs of M.N. were covered from grant no. 206/05/H012 provided by The Grant Agency of the Czech Republic.

References

Aars J, Andressen HP, Ims RA (1995) Root voles: litter sex ratio variation in fragmented habitat. J Anim Ecol 64:459–472

Armitage KB (1987) Do female yellow-bellied marmots adjust the sex ratios of their offspring? Am Nat 129:501–519

Austad SN, Sunquist ME (1986) Sex-ratio manipulation in the common opossum. Nature 324:58–60

Bird E, Contreras RJ (1986) Maternal dietary sodium chloride levels affect the sex ratio in rat litters. Physiol Behav 36:307–310

Blanchard P, Festa-Bianchet M, Gaillard JM, Jorgenson JT (2005) Maternal condition and offspring sex ratio in polygynous ungulates: a case study of bighorn sheep. Behav Ecol 16:274–279

Brunjes PC (1990) The precocial mouse, *Acomys cahirinus*. Psychobiology 18:339–350

Cameron EZ (2004) Facultative adjustment of mammalian sex ratios in support of the Trivers-Willard hypothesis: evidence for a mechanism. Proc R Soc Lond B Biol Sci 271:1723–1728

Cameron EZ, Linklater WL (2002) Sex bias in studies of sex bias: the value of daughters to mothers in poor condition. Anim Behav 63: F5–F8

Cameron EZ, Lemons P, Bateman PW, Bennett NC (2008) Experimental alteration of litter sex ratios in a mammal. Proc Roy Soc B 275:323–327

Carranza J (2002) What did Trivers and Willard really predict? Anim Behav 63:F1–F3

Clark AB (1978) Sex ratio and local resource competition in a Prosimian primate. Science 201:163–165

Clark MM, Galef BG, Saal FSV (1991) Nonrandom sex composition of gerbil, mouse, and hamster litters before and after birth. Dev Psychobiol 24:81–90

Clutton-Brock TH, Iason GR (1986) Sex ratio variation in mammals. Q Rev Biol 61:339–374

Cockburn A, Legge S, Double MC (2002) Sex ratios in birds and mammals: can the hypotheses be disentangled? In: Hardy ICW (ed) Sex ratios: concepts and research methods. Cambridge, Cambridge University Press, pp 266–286

Côté SD, Festa-Bianchet M (2001) Offspring sex ratio in relation to maternal age and social rank in mountain goats (*Oreamnos americanus*). Behav Ecol Sociobiol 49:260–265

Creel S, Creel NM, Monfort SL (1998) Birth order, estrogens and sex-ratio adaption in African wild dogs (*Lycaon pictus*). Anim Reprod Sci 53:315–320

Darwin C (1871) The descent of man, and selection in relation to sex. John Murray, London

Dieterlen F (1961) Beitrage zur Biologie der Stachelmaus. *Acomys cahirinus dimidiatus* Cretzschmer. Z Säugetierkunde 26:1–13

Festa-Bianchet SA (1996) Offspring sex ratio studie of mammals: does publication depend upon the duality of the research or the direction of the results? Ecoscience 3:42–44

Fisher RA (1930) The genetical theory of natural selection. Clarendon, Oxford

Fountain ED, Mao J, Whyte JJ, Mueller KE, Ellersieck MR, Will MJ, Roberts RM, MacDonald R, Rosenfeld CS (2008) Effects of diets enriched in omega-3 and omega-6 polyunsaturated fatty acids on offspring sex-ratio and maternal behavior in mice. Biol Reprod 78:211–217

Frynta D, Žižková M (1994) Sex ratio in *Apodemus sylvaticus* (Rodentia: Muridae): a comparison of field and laboratory data. Acta Soc Zool Boh 57:183–192

Gosling LM (1986) Selective abortion of entire litters in the coypu: adaptive control of offspring production in relation to quality and sex. Am Nat 127:772–795

Grant VJ (2007) Could maternal testosterone levels govern mammalian sex ratio deviations? J Theor Biol 246:708–719

Grant VJ, Irvin RJ (2009) A simple model for adaptive variation in the sex ratios of mammalian offspring. J Theor Biol 258:38–42

Gutiérrez-Adan A, Granados J, Pintado B, De La Fuente J (2001) Influence of glucose on the sex ratio of bovine IVM/IVF embryos cultured in vitro. Reprod Fertil Dev 13:361–365

Hansson L (1987) Vole sex ratios: the importance of mating systems and maternal condition. Oikos 49:161–164

Helle S, Laaksonen T, Adamsson A, Paranko J, Huitu O (2008) Female field voles with high testosterone and glucose levels produce male-biased litters. Anim Behav 75:1031–1039

Hewison AJM, Gaillard JM (1999) Successful sons or advantaged daughters? The Trivers-Willard model and sex-biased maternal investment in ungulates. Trends Ecol Evol 14:229–234

Huck UW, Labov JB, Lisk RD (1986) Food restricting young hamsters (*Mesocricetus auratus*) affects sex-ratio and growth of subsequent offspring. Biol Reprod 35:592–598

Huck UW, Seger J, Lisk RD (1990) Litter sex ratios in the golden hamsters vary with time of mating and litter size and are not binomially distributed. Behav Ecol Sociobiol 26:99–109

Ims RA (1994) Litter sex ratio variation in laboratory of two geographically distinct strains of the root vole *Microtus oeconomus*. Ecography 17:141–146

James WH (1996) Evidence that mammalian sex ratios at birth are partially controlled by parental hormone levels at the time of conception. J Theor Biol 180:271–286

James WH (1998) Hypotheses on mammalian sex ratio variation at birth. J Theor Biol 192:113–116

James WH (2004) Hypotheses on how selection for some traits in rodents led to correlated responses in offspring sex ratios. J Theor Biol 228:1–6

James WH (2006) Possible constraints on adaptive variation in sex ratio at birth in humans and other primates. J Theor Biol 238:383–394

Kaňková Š, Šulc J, Nouzová K, Fajfrlík K, Frynta D, Flegr J (2006) Women infected with parasite *Toxoplasma* have more sons. Naturwissenschaften 94:122–127

Kaňková Š, Kodym P, Frynta D, Vavřinová R, Kuběna A, Flegr J (2007) Influence of latent toxoplasmosis on secondary sex index in mice. Parasitology 134:1709–1717

Kimura K, Spate LD, Green MP, Roberts RM (2005) Effects of D-glucose concentration, D-fructose, and inhibitors of enzymes of the pentose phosphate pathway on the development and sex ratio of bovine blastocysts. Mol Reprod Dev 72:207–207

Kirby DRS, McWhirter KG, Teitelbaum MS, Darlington CD (1967) A possible immunological influence on sex ratio. Lancet 1:139–140

Koskela E, Huitu O, Koivula M, Korpimaki E, Mappes T (2004) Sex-biased maternal investment in voles: importance of environmental conditions. Proc R Soc Lond B Biol Sci 271:1385–1391

Krackow S (1995) The developmental asynchrony hypothesis for sex ratio manipulation. J Theor Biol 176:273–280

Krackow S (1997) Further evaluation of the developmental asynchrony hypothesis of the sex ratio variation. Appl Anim Behav Sci 51:243–250

Krackow S (2002) Why parental sex ratio manipulation is rare in higher vertebrates (invited article). Ethology 108:1041–1056

Krackow S, Burgoyne PS (1998) Timing of mating, developmental asynchrony and the sex ratio in mice. Physiol Behav 63:81–84

Krackow S, Gruber F (1990) Sex ratio and litter size in relation to parity and mode of conception in three inbred strains of mice. Lab Anim 24:345–352

Krackow S, Hoeck HN (1989) Sex ratio manipulation, maternal investment and behaviour during concurrent pregnancy and lactation in house mice. Anim Behav 37:177–186

Krackow S, Tkadlec E (2001) Analysis of brood sex ratios: implications of offspring clustering. Behav Ecol Sociobiol 50:293–301

Krackow S, Schmidt TA, Elepfandt A (2003) Sexual growth dimorphism affects birth sex ratio in house mice. Proc R Soc Lond B Biol Sci 270:943–947

Labov JB, Huck UW, Vaswani P, Lisk RD (1986) Sex ratio manipulation and decreased growth of male offspring of undernourished golden hamsters (*Mesocricetus auratus*). Behav Ecol Sociobiol 18:241–249

Lambin X (1994) Sex ratio variation in relation to female philopatry in Townsend's voles. J Anim Ecol 63:945–953

Lambin X, Yoccoz NG (1998) The impact of population kin-structure on nestling survival in Townsend's voles, *Microtus townsendii*. J Anim Ecol 67:1–16

McClure PA (1981) Sex-biased litter reduction in food-restricted wood rats (*Neotoma floridana*). Science 211:1058–1060

Milki AA, Jun SH, Hinckley MD, Westphal LW, Giudice LC, Behr B (2003) Comparison of the sex ratio with blastocyst transfer and cleavage stage transfer. J Assist Reprod Genet 20:323–326

Nováková M, Palme R, Kutalová H, Janský L, Frynta D (2008) The effects of sex, age and commensal way of life on levels of fecal glucocorticoid metabolites in spiny mice (*Acomys cahirinus*). Physiol Behav 95:187–193

Peaker M, Taylor E (1996) Sex ratio and litter size in the guinea-pig. J Reprod Fertil 108:63–67

Perret M, Colas S (1997) Manipulation of sex ratio at birth and maternal investment in female mouse lemurs (*Microcebus murinus*), Primates. Appl Anim Behav Sci 51:275–283

Porter RH, Cavallaro SA, Moore JD (1980) Developmental parameters of mother-offspring interactions in *Acomys cahirinus*. Z Tierpsychol 53:153–170

Porter RH, Matochik JA, Makin JW (1986) Discrimination between full-sibling spiny mice. (*Acomys cahirinus*) by olfactory signatures. Anim Behav 34:1182–1188

Pratt NC, Lisk RD (1989) Effects of social stress during early pregnancy on litter size and sex ratio in the golden hamster (*Mesocricetus auratus*). J Reprod Fertil 87:763–769

R Development Core Team (2005) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Available at: www.R-project.org

Rosenfeld CS, Roberts RM (2004) Maternal diet and other factors affecting offspring sex ratio: a review. Biol Reprod 71:1063–1070

Rosenfeld CS, Grimm K, Livingston K, Brokman A, Lamberson WE, Roberts RM (2003) Striking variation in the sex ratio of pups born to mice according to whether maternal diet is high in fat or carbohydrate. Proc Natl Acad Sci USA 100:4628–4632

Scheibler E, Weinandy R, Gattermann R (2005) Social factors affecting litters in families of Mongolian gerbils, *Meriones unguiculatus*. Folia Zool 54:61–68

Sheldon BC, West SA (2004) Maternal Dominance, maternal condition, and offspring sex ratio in ungulate mammals. Am Nat 163:40–54

Sicard B, Fuminier F (1996) Environmental cues and seasonal breeding patterns in Sahelian rodents. Mammalia 60:667–675

Sikes RS (2007) Facultative sex ratio adjustment. In: Sherman PW, Wolff JO (eds) Rodent societies: an ecological & evolutionary perspective. The University Chicago Press, Chicago, pp 129–138

Silk JB, Willoughby, Brown (2005) Maternal rank and local resource competition do not predict birth sex ratios in wild baboons. Proc R Soc Lond B Biol Sci 272:859–864

Steppan SJ, Adkins RM, Anderson J (2004) Phylogeny and divergence-date estimates of rapid radiations in muroid rodents based on multiple nuclear genes. Syst Biol 53:533–553

Trivers RL, Willard DE (1973) Natural selection of parental ability to vary the sex ratio of offspring. Science 179:90–92

Wade MJ, Shuster SM, Demuth JP (2003) Sexual selection favors female-biased sex ratios: the balance between the opposing forces of sex-ratio selection and sexual selection. Am Nat 162:403–414

Wild G, West SA (2007) A sex allocation theory for vertebrates: combining local resource competition and condition-dependent allocation. Am Nat 170:E112–E128

Wright SL, Crawford CB, Anderson JL (1988) Allocation of reproductive effort in *Mus domesticus*: responses of offspring sex ratio and quality to social density and food availability. Behav Ecol Sociobiol 23:357–365

Young DAB (1976) Breeding and fertility of the Egyptian spiny mouse, *Acomys cahirinus*: effect of different environments. Lab Anim 10:15–24