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ORIGINAL PAPER

Varying levels of female promiscuity in four *Apodemus* mice species

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Abstract Sexual selection in most vertebrates is based on the evolution of fitness optimization strategies such as multiple-male mating (MMM). Several ecological correlates of MMM have been identified in bird and fish populations; however, only few studies have documented the effects of environmental change on promiscuity in mammals. In this study, the 127 pregnant females from four central European and ecologically diverse species of field mice (genus *Apodemus*) were studied to assess the role of ecological factors that may have shaped the evolution of particular mating systems. MMM was found in all analyzed species: in *Apodemus uralensis* and *Apodemus flavicollis*, up to two males could be identified as the fathers of a particular litter, while three males sired 9.1% of analyzed litters of *Apodemus sylvaticus* and 20.6% of *Apodemus agrarius*. Furthermore, there were obvious differences between species in relative testes size and the proportion of multiple sired litters during those seasons when the opportunity for multiple mating was high. The species with the smallest testes and the least promiscuous was *A. uralensis* (only 43.5% of multiple sired litters), while the species with the biggest testes and the most promiscuous was *A. agrarius* (69.2%). MMM was significantly associated with higher litter size in *A. flavicollis*, and the probability of MMM strongly increased with season in *A. agrarius* and with abundance in *A. uralensis*. These results indicate that ecological factors are associated with MMM rates in *Apodemus* field mice and more research is needed to fully understand the evolution of mating strategies at different levels of biological resolution.

Keywords Mating systems · Multiple paternity · Wood mice · Testis size · *Apodemus*

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Introduction

Since males invest less energy in reproduction than females in most vertebrate species, sexual selection results in the evolution of optimization strategies such as multiple-male mating (MMM) and female promiscuity, as many recent studies of genetic parentage analysis have confirmed. MMM is a relatively common reproductive strategy in fish, amphibians, and birds (e.g., Avise et al. 2002; Griffith et al. 2002; Westneat and Stewart 2003; Vieites et al. 2004; Liebgold et al. 2006) and has also been documented in various mammalian species (Clapham and Palsboll 1997; Stockley 2003; Wolff and Macdonald 2004). In fact, there is very little evidence that any female mammal would always benefit from unlimited and exclusive partnership with one male. However, the evolutionary origin and maintenance of promiscuous mating systems in animals is a source of ongoing debate (Arnquist and Kirkpatrick 2005; Albrecht et al. 2006). According to the prevailing view, both sexes benefit from pairing with more than one mate, with benefits for females being either direct (e.g., assurance of fertilization) or indirect (e.g., increased genetic quality of offspring; see Zeh and Zeh 1997; Griffith et al. 2002; Wolff and Macdonald 2004).

Several morphological traits are good correlates of a promiscuous mating system (defined by its genetic, rather than a social outcome) in vertebrates, namely relative testis size (Parker et al. 1997; Pitcher et al. 2005; Ramm et al. 2005; but see Munshi-South 2007), sperm morphology (Anderson et al. 2005; Immler et al. 2007; Kleven et al. 2008), and the size of seminal vesicles and anterior prostate (Ramm et al. 2005). Although the rates of female promiscuity and subsequent multiple paternity typically carry a strong phylogenetic signal, variation in the rates of promiscuity even among phylogenetically related species have been noted (e.g., Westneat and Stewart 2003). Comparative interspecific studies, particularly in birds, have identified several ecological factors associated with high proportions of litters with multiple paternity, including high population abundance, highly synchronized reproductive period, migratory behavior, and short life span (reviewed in Bennett and Owens 2002; Griffith et al. 2002). Studies of factors promoting MMM in other vertebrate taxa are less comprehensive (but see Wolff and Macdonald 2004).

The rates of MMM often vary among different species as well as between populations within one species and even among individuals within a population as a consequence of diverse social conditions. There is strong evidence for substantial variation in levels of multiple paternity in bird (Westneat and Stewart 2003) and fish populations (e.g., Reichard et al. 2008 and references therein). However, only a few studies have documented the effects of a changing environment on promiscuity in mammals (Say et al. 1999; Dean et al. 2006); for example, in a period of low population abundance, the rate of female promiscuity may be lower, either because males are unavailable or because accessible males are able to monopolize several females with synchronized estrus (Dean et al. 2006). On the other hand, high population abundance may enable dominant males to exert their control more effectively in species with rigid social hierarchies by preventing copulations from subordinates (Bronson 1979); therefore, multiple paternity is expected to be lower for this scenario as well.

Mice of the genus *Apodemus* have became popular models for various ecological and ethological studies as they are easy to handle, and there is extensive knowledge on species-specific interactions, particularly concerning aggression (Frynta et al. 1995; Čiháková and Frynta 1996; Suchomelová and Frynta 2000; Simeonovska-Nikolova 2006; Simeonovska-Nikolova 2007; Zgrabszynska and Pilacinska 2002) and grooming behavior in social contexts (Polechová and Stopka 2002; Stopka and Graciasová 2001). For each species, there are also species-specific sex ratios (Pelikán 1970), spatial organization, home range distribution (Zejda and Pelikán 1969; Wolton and Flowerdew 1985; Mazurkiewicz and Rajska-Jurgiel 1998; Vukicevic-Radic et al. 2006), and ectoparasite load (Morand et al. 2004; Stanko et al. 2007) in natural populations. All these parameters are known to be related to the frequency of social contact with different individuals of each sex and, thus, to social organization and mating systems. Nevertheless, there is only scarce direct evidence for mating system variation among and within *Apodemus* species. Multiple paternities were found in natural populations of *Apodemus sylvaticus* (Baker et al. 1999; Booth et al. 2007), *Apodemus agrarius* (Baker et al. 1999), *Apodemus flavicollis* (Gryczyńska-Siemiątkowska et al. 2008), and *Apodemus uralensis* (= *Apodemus microps*; Bryja and Stopka 2005). However, with the exception of the Bryja and Stopka's (2005) study, the sample sizes analyzed were very low (maximum 13 litters) and, thus far, no study has attempted to identify environmental or population factors associated with particular MMM levels in *Apodemus* species.

This study aims at identifying species specificity and within-species variation in the rate of MMM in four species of *Apodemus* to assess the role of ecological factors that might have shaped the evolution of species-specific mating systems. Since relative testis size is a good correlate of the strength of intermale (sperm) competition, this measure is considered useful for the prediction of mating system (Kenagy and Trombulak 1986; Heske and Ostfeld 1990; Breed and Taylor 2000). However, because there is only limited data on testes size variation in *Apodemus* species (Huminski 1969; Breed and Taylor 2000), we only used this measure for confirming the level of multimale mating. In addition, because social conditions can significantly affect mating system, we tested for the effects of season and abundance on the mean observed number of fathers per litter. Furthermore, we specifically tested the predictions that (1) multiple paternity is a result of forced copulations (i.e., it should be more frequent in smaller females that are assumed to have lower ability to defend males' attacks) and (2) that fertilization by several males leads to increased litter sizes.

Materials and methods

Sampling of pregnant females

One hundred twenty-seven females of four *Apodemus* species were sampled between April and September from

2003 to 2006 in southeastern Czech Republic (Southern Moravia) and southeastern Slovakia. Rodents were captured using line transects of 50 live or snap traps lied. Trapping sites were distributed among four main habitat types (poplar windbreaks, lowland forests and shrubs, cultivated or abandoned fields, and river valleys) that were comparable in both countries. Field mice of the genus *Apodemus* were the most abundant rodents in most of these habitats. Animals captured in live traps were killed by cervical dislocation. During dissection, the uterus from pregnant females was removed and individual embryos were extracted and stored in 96% ethanol. All *A. sylvaticus* (N=22 pregnant females) were trapped in the Czech Republic, while all *A. agrarius* females (N=34) were collected in Slovakia. Females of *A. flavicollis* were captured in lowland forests of the Czech Republic (N=3) as well as in forests and shrubs of Slovakia (N=22). Most *A. uralensis* (N=37) were caught in the agricultural landscape of the Czech Republic sites, while a few (N=9) was collected in similar habitats in Slovakia.

Relative size of testes

Extensive morphological measurements of various rodent species have been obtained by some of us from ecological studies of small mammal communities in the last 10 years in the southeastern Czech Republic (e.g., Bryja and Řehák 1998; Bryja et al. 2002; Heroldová et al. 2005; Heroldová et al. 2008) and Slovakia (e.g., Stanko et al. 2005; Krasnov et al. 2006), including left testes length (measured from fresh testes during dissection) in males of four *Apodemus* species. More than 80% of these males came from the same populations where the pregnant females were sampled for this study. The vast majority of testes were measured by J.B. and M.S. and no significant differences in the values obtained by two authors were observed. Only the males in full reproductive condition were measured; that is, individuals captured during the breeding season with a significantly enlarged visible epididymis and seminal vesicles. The length of left testis was recorded in millimeters (with the precision to 0.1 mm) and the mass of both testes was calculated using the equation $Y=0.001\times X^{2.58}$ (Ribble and Millar 1992) where Y is the mass of both testes and X is the left testis length. Relative testes sizes were calculated according to the rodent regression equation of Kenagy and Trombulak (1986; i.e., as the mass of both testes/expected mass, where expected mass=$0.031\times$body mass$^{0.77}$). The differences in the relative testes sizes between species were analyzed by one-way analysis of variance (ANOVA) and post hoc Tukey tests in STATISTICA 6.0 (StatSoft, Inc. 2001).

Paternity analysis

DNA was extracted from embryos and tails of females after proteinase K digestion using Tissue and Blood DNA extraction kit (Qiagen). Previous tests identified that only embryos larger than 2 mm can be used: this is because smaller embryos are often contaminated with the mother's tissues as shown by genetic analysis. The genotypes of each mother and her embryos were determined using eight highly variable microsatellite loci originally described by Makova et al. (1998) for *A. agrarius* and *A. sylvaticus*. Different subsets of these loci were used for particular *Apodemus* species since some loci did not amplify in some species or have low levels of polymorphism (Table 1). Microsatellite loci were amplified in a multiplex polymerase chain reaction (PCR) in an Eppendorf ep thermal cycler using the Multiplex PCR Kit (Qiagen) according to manufacturer's instructions. One primer of each primer pair was labeled with a fluorochrome (either FAM, NED, or HEX). Two microliters of PCR product were mixed with 0.3 μl of ROX500 Size Standard (Applied Biosystems) and 12 μl of formamide and loaded on an ABI 3130 Genetic Analyzer (Applied Biosystems) for separation and detection. DNA fragments were quantified and analyzed using GeneMapper® v.3.7 software (Applied Biosystems).

For each fetus, paternal alleles at each locus were determined by comparing each mother's genotype with that of her offspring (Baker et al. 1999). Multiple paternity (determined on the basis of multilocus genotypes) was assumed if the minimal number of paternal alleles at a locus within one litter was greater than two.

Table 1 Numbers of alleles at microsatellite loci in four species of the genus *Apodemus*

Locus	Label	*A. uralensis* (46)	*A. flavicollis* (25)	*A. sylvaticus* (22)	*A. agrarius* (34)
GCATD7S	FAM	4	12	8	13
GTTC4A	HEX	6	3	2	–
GACAE12A	HEX	11	12	2	21
GACAD1A	NED	3	14	9	2
TNF	FAM	14	10	13	6
CAA2A	HEX	17	11	8	8
GTTA1A	HEX	–	2	–	6
GTTD8S	FAM	–	2	–	–
Total exclusionary power (%)		99.3	99.9	99.4	99.7

The number of female genotypes used for calculation of the total exclusionary power in the presence of the mother's genotype in the program Cervus 2.0 are shown in parentheses.

– Loci that could not be amplified as a result of technical difficulties or were excluded due to monomorphism

The suitability of the set of microsatellite loci used for each species was assessed as the total exclusionary power in the presence of the mother's genotype computed by the program Cervus 2.0 (Marshall et al. 1998). Multilocus genotypes of all females of a particular species were used as input data for calculations. Exclusion probabilities were used to quantify the probability that multiple paternity occurred and was simply undetected due to a paucity of marker polymorphism.

Statistical analysis

For each female, body length (measured in millimeter from the muzzle to the anus), season (number of days from the beginning of the year to the capture date), and number of embryos (hereafter referred to as 'litter size') were recorded. Abundance (number of individuals of a particular species captured per 100 trap nights) was also calculated. We used a generalized linear model approach (GLM; binomial error, logit link function) to evaluate the effect of several predictors and their two-way interactions on the occurrence of multiple paternity for all four species of *Apodemus*. In the basic analysis, paternity status of each litter (fathered either by one or more than one male) was a binary dependent variable, species was categorical predictor, and population abundance, female body size, and litter size were used as the continuous explanatory variables, respectively. Separate analyses were then performed for each species. Due to the insufficient sample size available for *A. sylvaticus* and *A. flavicollis*, interaction terms were evaluated only in models concerning *A. uralensis* and *A. agrarius*. Best models were chosen using backward elimination of nonsignificant terms, starting with two-way interactions whenever these were included in the full model (Crawley 2002). The significance of a particular term adjusted for the effects of other terms was based on the change in deviance between the full and reduced models, distributed as χ^2 with degrees of freedom (*df*) equal to the difference in the degrees of freedom between the models with and without the term in question. F statistics instead of χ^2 statistics were applied when over- or underdispersion was detected ($\varphi \ll 1$ or $\varphi \gg 1$). This was particularly true in an additional analysis where the number of fathers instead of binary presence/absence of multiple paternity was the dependent variable. In this analysis, GLM with quasi-Poisson distribution was applied to the data (Faraway 2005). Minimum adequate models (MAMs), i.e., models with all effects significant (Crawley 2002), are presented. A posteriori simplification of multilevel categorical predictors involved in MAMs was applied where necessary (Crawley 2002). Analyses were performed using S-Plus 6.0 (Mathsoft 2001) and R 2.5.1 statistical packages.

Results

Multiple paternity and relative testis size differences between species

We successfully amplified and scored six to eight polymorphic microsatellite loci per species and the results of microsatellite typing were highly reproducible. The combined power of the set of loci to exclude a randomly selected unrelated candidate father from the parentage of an arbitrary offspring, given the genotype of the offspring and of a mother, was higher than 99% in all four species (Table 1), i.e., the probability of an undetected case of multiple paternity was very low. Most embryos received at least one of the mother's alleles, so the identification of paternal alleles was straightforward. The only exceptions were two out of five offspring of one *A. agrarius* female homozygous at the GACAE12A locus; the lack of a maternal allele in these two embryos can probably be explained by a null allele in mother's genotype.

MMM was detected in all analyzed *Apodemus* species (Table 2). In *A. uralensis* and *A. flavicollis*, up to two males sired a litter, while three fathers per litter were found in two *A. sylvaticus* females (9.1% of analyzed litters) and seven *A. agrarius* females (20.6% of analyzed litters). There were clear differences in the proportion of multiply sired litters between particular species (Table 2). The least promiscuous species is apparently *A. uralensis* (only 43.5% of litters were sired by multiple fathers), while the most promiscuous species is *A. sylvaticus* (68.2% multiple sired litters). However, in early spring, the occurrence of promiscuous mating may be biased by low numbers of accessible males as a consequence of high winter mortality; in fact, when the comparison between species is restricted to litters obtained after mid-May, when young males born that year are usually present and reproductively active in the population (i.e., the opportunity for multiple mating is high), the most promiscuous species became *A. agrarius* (69.2%; Table 2).

Relative testes size differed significantly between species (one-way ANOVA, $F_{3,1295}=594.8$, $p<0.001$). The smallest relative testes sizes were observed in *A. uralensis* while the relative largest testes occurred in *A. agrarius* (Table 2); all interspecies comparisons were significant (post hoc Tukey honestly significantly different tests, $p<0.001$). Even if the number of species is low, linear regression analysis revealed that there is strong relationship between the mean relative testes size and the proportion of multiple sired litters after mid-May ($R^2=0.836$, slope=20.05±4.96[SE], $F_{1,2}=16.32$, $p=0.056$).

Table 2 Interspecific variation of multiple-male mating and mean relative testes size (±standard deviation) of four *Apodemus* species

Species	*N* litters	Minimum number of sires			% MMM	Relative testes size	*N* testes
		1	2	3			
A. uralensis	46 (40)	26 (22)	20 (18)	0 (0)	43.5 (45.0)	1.3±0.24	393
A. flavicollis	25 (16)	10 (7)	15 (9)	0 (0)	60.0 (56.3)	2.0±0.49	444
A. sylvaticus	22 (16)	7 (5)	13 (9)	2 (2)	68.2 (68.8)	2.2±0.52	76
A. agrarius	34 (26)	14 (8)	13 (11)	7 (7)	58.8 (69.2)	2.6±0.54	386

The values for pregnant females captured after May 15 are in parentheses (see text for details). *Minimum number of sires*—number of litters with minimum number of sires (1, 2, or 3) estimated by comparison of maternal and embryonic multi-locus genotypes; % MMM—percentage of litters sired by more than one male

The correlates of MMM levels

When data from all species were pooled for analysis, the probability of occurrence of multiple paternity was independent of abundance, body size, litter size, season, and species (all $p>0.10$). Similarly, no two-way interactions were significant except the interactions between species and season and between species and abundance (chi=12.91, $df=3$, $p=0.0048$ and chi=13.90, $df=3$, $p=0.003$, respectively). The MAM for the whole dataset included these two interaction terms and their components (partial effect of season—chi=2.03, $df=1$, $p=0.15$; partial effect of abundance—chi=0.74, $df=1$, $p=0.38$; partial effect of species—chi=4.74, $df=3$, $p=0.19$), and was significant (chi=25.77, $df=11$, $p=0.007$). The results suggest that the effect of abundance and season on the occurrence of multiple paternity varies across the species of *Apodemus* (Fig. 1). When we repeated analysis with the number of sires as a dependent variable, the results remained similar; however, the species effect also became significant ($F_{3,116}=3.71$, $p=0.012$) and the MAM also included litter size ($F_{1,116}=8.93$, $p=0.003$; slope=0.082±0.026 SE). The significant difference among species in the number of males siring a litter was largely due to *A. uralensis*, as revealed by a posteriori simplification of factor levels (lumping all the remaining species together into one factor level did not change the explanatory power of the MAM).

Subsequent analyses confirmed different effects of abundance and season on the occurrence of multiple paternity in particular species of *Apodemus* (Table 3). The proportion of litters sired by more than one male slightly decreased with season in *A. uralensis* but strongly increased in *A. agrarius* and remained constant in *A. flavicollis* and *A. sylvaticus* (Fig. 1). Similarly, higher abundance was associated with increased occurrence of multiple paternity in *A. uralensis* (Table 3). No significant effect of abundance was detectable in the remaining species, although a positive trend is evident in *A. agrarius* and *A. sylvaticus* (Table 3; Fig. 1). Litter size was only important as a predictor of multiple paternity in *A flavicollis* (Table 3), whereas body size was unrelated to MMM in all species of *Apodemus* evaluated.

Discussion

The idea that females may benefit from mating with several males has stimulated extensive research and MMM has already been reported in more than 130 mammalian species (Wolff and Macdonald 2004). Substantial fitness benefits of multiple mating have been detected on several levels. For example, in house mice (*Mus musculus domesticus*; Whitten 1958) and in field mice (*A. sylvaticus*; Stopka and Macdonald 1998), the males stimulate the onset and the length of estrus as well as the whole estrous cycle. This effect has been shown to be even stronger in *A. sylvaticus* if males are consecutively changed behind wire mesh that divides a male from a female (Stopka and Macdonald 1998). This suggests that the period of female receptivity is prolonged and more frequent in response to MMM. In fact, the coevolution of MMM with prolonged estrus may be adaptive as it assures successful fertilization with fresh sperm (Dewsbury and Baumgardner 1981). Furthermore, MMM may be responsible for higher litter sizes (Hoogland 1998; Kraaijeveld-Smit et al. 2002), increased genetic diversity of a litter (Madsen et al. 1992; Tregenza and Wedell 2000), and increased probability of finding genetically complementary gametes (Mays and Hill 2004; Stockley 2003). Wolff and Macdonald (2004) also proposed that MMM obscures paternity, thereby deterring infanticide by males.

Genetic analysis of paternity within a single litter using multiple microsatellite loci is now relatively cheap and available to most laboratories. However, although the genetic typing of embryos can provide unequivocal evidence for mating with more than one male during a single estrous cycle, such data may still be an underestimate of true MMM, since sperm competition may result in the female's ova being fertilized by sperm from only a subset

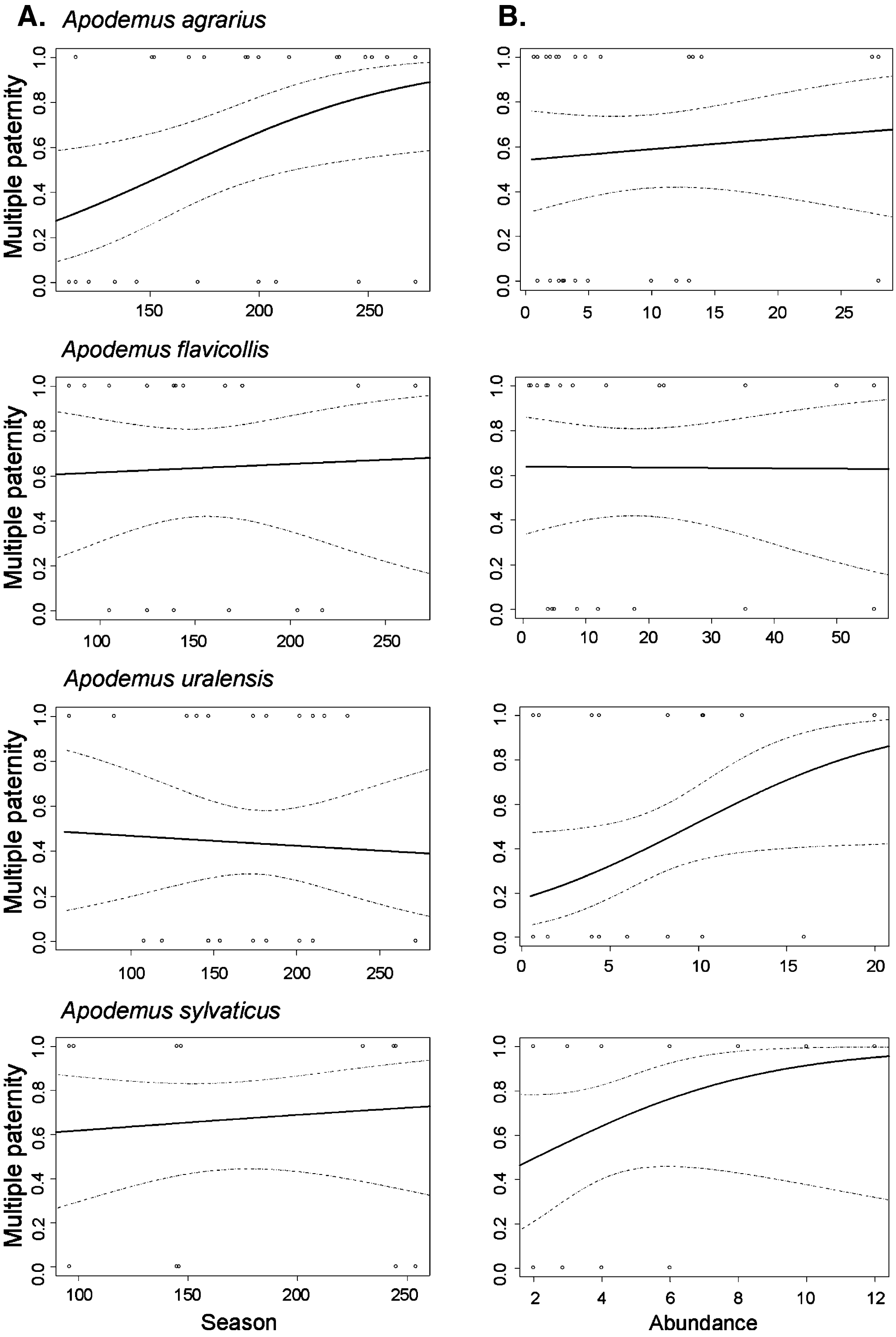

Fig. 1 Predicted fraction of litters sired by more than one male (multiple paternity, *left axis*) as a function of **a** season (number of days from the beginning of the year to capture date) and **b** local population abundance in four *Apodemus* species. *Dotted lines* are 95% confidence limits. Estimations are based on logistic regression models with slopes and intercepts for particular terms not adjusted for other terms

of all male mates. In this study, the proportion of multiple sired litters of four *Apodemus* species in the period with high opportunity for multiple mating (after May 15) varied between 45% (*A. uralensis*) and 69.2% (*A. agrarius*; Table 2). Such high MMM values may be common among species with sperm competition, which would also imply a high proportion of undetected MMMs (Dean et al. 2006). However, even if true levels of MMM remain unknown, our results indicate that multiple mating in a single estrous cycle is very frequent in all four species of the genus *Apodemus* analyzed in this study.

Direct evidence for MMM based on genetic or behavioral data in natural populations of other muroid rodents (subfamily Murinae) except the *Apodemus* mice is only fragmentary. Multiple mating is evidently more frequent in the genus *Apodemus* than in natural populations of the

Table 3 Results of logistic regression (GLM, logit link function) evaluating separately the effects of season, litter size, abundance, and body mass on the occurrence of multiple paternity for four species of *Apodemus*

Species	MAM	Term	Estimate	SE	Chi	*df*	*p*
A. uralensis	Season+Abundance				12.08	2	0.0024
		Intercept	2.693	1.936			
		Season	−0.037	0.017	7.10	1	0.007
		Abundance	0.464	0.195	12.01	1	0.0005
A. flavicollis	Litter size				6.15	1	0.013
		Intercept	−5.529	2.949			
		Litter size	1.191	0.573	6.15	1	0.013
A. agrarius	Season				6.43	1	0.011
		Intercept	−2.885	1.412			
		Season	0.018	0.007	6.43	1	0.011

Two-way interaction terms were only considered for *A. uralensis* and *A. agrarius* (insufficient sample size available for *A. sylvaticus* and *A. flavicollis*). The minimal adequate model (i.e., the model with all terms significant at $\alpha<0.10$) is shown for each species. Values of significance for particular terms are based on type III sum of squares (adjusted for effects of other terms in a particular model). The null model was best supported by *A. sylvaticus* (α level for all terms >0.10; not shown).

house mouse (*Mus domesticus*), where only 33 of 143 litters (23%) were found to be multiple sired (Dean et al. 2006). The comparison of both genera is interesting also in a broader context of sperm competition; for example, field mice have falciform sperm with an apical hook that individual sperm use to attach to other sperm after ejaculation, forming long trains of sperm that swim significantly faster than individual sperm (Moore et al. 2002; Johnson et al. 2007). These clustered sperm also possess an increased fertilizing capacity due to a premature acrosome reaction (see experiments by Inoue et al. 2003), which is more elevated in the genus *Apodemus* than in *Mus* (Johnson et al. 2007). It is likely that premature acrosome reactions in *Apodemus* have had a profound effect upon the evolution of fertility assurance and can also be seen as a male counter strategy to female infidelity. To our knowledge, there are no published data on the occurrence and frequency of multiple paternity in natural populations of other rodents of the subfamily Murinae, but some preliminary results indicate that multiple mating may be frequent in the whole group (e.g., *Mastomys natalensis*—J. Kennis, unpublished Master's thesis).

To a human observer, the species of *Apodemus* lack obvious sexual dimorphism. Therefore, we hypothesized that a promiscuous mating system (in the genetic sense) would be the prevailing reproductive strategy across the genus. A study by Baker et al. (1999) reported multiple paternity in eight of ten litters in *A. agrarius* and in three of six litters in *A. sylvaticus*. Further study by Bartmann and Gerlach (2001) indicated MMM in 29 out of 34 litters produced by laboratory-bred females *A. sylvaticus* housed in experimental outdoor enclosures. Recently, Booth et al. (2007) examined litters of 13 females of the same species and identified multiple paternity in seven of them. Paternity in other *Apodemus* species has been much less studied and with the exception of the Baker's et al. (1999) study of *A. agrarius*, there is only one paper describing relatively low level of promiscuity in *A. uralensis* where eight of 24 analyzed litters were sired by more than one male (Bryja and Stopka 2005), and one paper describing multiple paternity in three of ten litters of *A. flavicollis* (Gryczyńska-Siemiątkowska et al. 2008). Here, we confirmed promiscuous mating in all of these species in much higher numbers of females. Behavioral data from wild populations are now necessary to determine the details of the social and genetic mating systems in *Apodemus* species.

Among members of the genus *Apodemus*, *A. uralensis* is exceptional in that the level of promiscuity is low (Bryja and Stopka 2005; this study): compared to other species, male *A. uralensis* have significantly smaller relative testis size (this study; Table 2) and are rarely aggressive towards each other (Suchomelová and Frynta 2000; Stopka and Graciasová 2001). They also rarely exchange allogrooming for sex as do males with females of *A. sylvaticus* (Stopka and Macdonald 1999) but instead exchange allogrooming more often for allogrooming and with individuals of either sex (Stopka and Graciasová 2001). Together, these results suggest that in this species (unlike *A. sylvaticus*), sex is not a commodity which may be bartered by females for fitness benefits with several males. Unfortunately, the ecology of *A. uralensis* in natural habitats is the least known of the *Apodemus* species; however, relatively small eyes and ears suggest that this species is most highly adapted to subterranean life, where the frequency of interaction with other males is probably more limited and the opportunity for promiscuity decreases, as in other fossorial species (e.g., Salvioni 1988; Faulkes et al. 1997). A predominantly fossorial lifestyle may also explain why this species displays a lower level of intermale aggression than the remaining *Apodemus* species at the beginning of reproductive season (Simeonovska-Nikolova 2007; Suchomelová and Frynta 2000).

An effect of abundance on the levels of multiple paternity has been demonstrated at both the inter- and intraspecific level in several animal taxa (Moble and Jones 2007; Stutchbury 1998; Bennett and Owens 2002; Griffith et al. 2002); however, little data is available for mammals (see Dean et al. 2006 for a rare exception). Hence, this paper provides one of the first analyses of the effect of environmental correlates on mating system variation in natural populations of rodents. Our results indicate that in three out of four *Apodemus* species (*A. agrarius*, *A. uralensis*, *A. sylvaticus*), multiple paternity increased visibly with increasing abundance (Fig. 1). Although this pattern was only significant in *A. uralensis* (Table 3), it is highly probable that high abundance could promote MMM in rodents in several ways. First, the encounter rate might increase with abundance, thus promoting the mean number of potential males a female meets during any one estrus cycle. Secondly, during periods of high abundance, females may try to avoid increased risk of infanticide by copulating with more than one male (Wolff and Macdonald 2004). Moreover, low levels of female promiscuity during phases of low abundance may be caused by the more effective monopolization of females by reproductively active males. To evaluate the relative importance of these mechanisms, precise data on individual spatial activity and its seasonal dynamics are needed: at present, this data is only available for *A. sylvaticus*. In fact, the populations of this species are organized into individual territories whose structure is often seasonally dynamic. For example, during low population abundance in early spring, male–female bonds may be established and monogamy is believed to be the typical mating pattern (Randolph 1977; Montgomery and Gurnell 1985). Instead, during the breeding season (and with increasing population abundance), males enlarge their home ranges which then may overlap with each other and with those of several females, encouraging the establishment of a polygynous/promiscuous mating system in the late summer and autumn (Brown 1969; Wolton and Flowerdew 1985; Tew and Macdonald 1994). Detailed data are missing for the other *Apodemus* species; however, some preliminary indications show that spatial organization is similar in *A. agrarius* and *A. flavicollis* (Zejda and Pelikán 1969; Mazurkiewicz and Rajska-Jurgiel 1998; Wolton and Flowerdew 1985; Vukicevic-Radic et al. 2006), although no data on spatial structure exists for *A. uralensis* thus far.

Multiple paternity was significantly associated with larger litter size in *A. flavicollis*. This correlation is unlikely to be simply due to a higher probability of detecting multiple paternity in larger litters since we found no evidence for an association between litter size and multiple paternity in other species in this study. Our finding is especially relevant to a comparative study by Stockley (2003) who demonstrated that early reproductive failure is significantly higher in species where females only mate with single males (probably due to genetic incompatibility) and that females of these species compensate for this failure by increasing ova production. Such mating behavior (i.e., usually monandrous females and polygynous males) may by typical of *A. flavicollis* for the following reasons: first, morphological sexual dimorphism and male aggression in this species are the most elevated of the different species of the genus *Apodemus* (Frynta et al. 1995), suggesting that a single male has the capacity to monopolize several females. Secondly, *A. flavicollis* has the lowest mean litter sizes among other *Apodemus* species, with the highest variation (Pelikán 1966), and the highest embryonic resorption rate (i.e. highest early reproductive failure; Pelikán 1967). Thirdly, the proportion of multiple sired litters is lower in *A. flavicollis* than in typical promiscuous species *A. sylvaticus* and *A. agrarius* (56% in this study, only 30% in Gryczyńska-Siemiątkowska et al. 2008). We assume that females of *A. flavicollis* usually mate with the only dominant male and in accordance with Stockley (2003), they increase ova production to ensure fertilization success. Occasional mating with extrapair males (observed at least in 30–50% of females) could lead to higher litter size due by averting genetic incompatibility. There are no available data on the extent of genetic incompatibility in natural outbred populations of *Apodemus* species; however, several potential sources of lethal genetic incompatibilities have been suggested (see references in Stockley 2003). The study of relations between individual genotypes, genetic incompatibility, and promiscuity of females is obviously very challenging task for future research, where the species of the genus *Apodemus* may be a suitable model group.

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