Socio-genetic structure of mound-building mice, *Mus spicilegus*, in autumn and early spring

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Mus spicilegus has become a popular biological model species in the last decade because of the evolutionary interest of its behavioural particularities (the building of mounds, bi-parental care and monogamous mating system). The genetic structure of *M. spicilegus* populations should reflect those life-history traits. Although many studies have reported on mound-building mouse populations in the field or in a semi-natural enclosure, only one used hyper variable genetic markers to assess parentage and social structure. In the present study conducted in Hungary, we analysed individuals from seven highly populous mounds in autumn with the set of loci used in a previous study. Our results confirmed that mounds are inhabited by juveniles from several parental units, but revealed population differences. In a capture–recapture field session in spring, we assessed genetic relationships between individuals after dispersal from the mounds, the other key moment of the life cycle of this species. The results indicate that the social structure at this moment reflects a transition phase between the large over-wintering groups and the monogamous pairs described later in the year. Social bonds forged during the long winter cohabitation may have lasting effects on social and genetic structure of this species. This scenario is discussed in respect of the available literature on this species as well as other species. © 2008 The Linnean Society of London, Biological Journal of the Linnean Society, 2008, 93, 689–699.

**ADDITIONAL KEYWORDS:** microsatellite loci – monogamy – relatedness – spatial organization.

**INTRODUCTION**

The cost and benefits of group living have been well documented in the literature (McGuire, Getz & Oli, 2002). Benefits include enhanced thermoregulation (as in marmots; Ortmann & Heldmaier, 2000) and care of young (communal nursing in house mice; König, 1994; Hayes, 2000) or improved detection and defence against predators (Jarman, 1974; Yaber & Herrera, 1994). These benefits can result from direct interactions or from the building of structures (nest, burrow, hibernacula) that will increase the efficiency of the above mentioned functions.

Cooperation between group members increases individual fitness, but can also increase inclusive fitness provided that group members are related. Social behaviours have therefore been linked with kinship between group members, and genetic relatedness is often seen as the key factor explaining the evolution of sociality (Hamilton, 1964a, b). Recent genetic studies have confirmed that, for most social species, individuals living in group are closely related and constitute family groups (voles: Ishibashi et al., 1998; Asian elephants: Fernando & Lande, 2000; common marmoset: Nievergelt et al., 2000). Genetic structure within these family groups is affected by life-history traits. One of the more important traits is differential dispersal. Although the dispersing sex is male in most polygynous mammals (Greenwood, 1980; Dobson, 1982), female offspring tend to be philopatric and to remain in their natal group (Liber & Von Schantz, 1985). The ensuing social organization is then based on a matrilinеal structure.
The mound-building mouse (Mus spicilegus Petényi 1882), an outdoor species from southern-eastern Europe, exhibits spectacular behavioural particularities related to drastic changes in social organization throughout the year. First, this species build complex mounds to encompass winter (Muntyanu, 1990; Muraru, 1981; Muntianu, 1990). This behaviour is unique among mice species (Orsini et al., 1983; F. Bonhomme, pers. comm.). These mounds are built in late summer/early autumn by accumulation of seeds and other vegetal materials covered with earth. Adults do not live on through the winter. Juveniles born in late summer only spend the cold period in nest chambers within the mounds, in large groups often encompassing several litters, without reproducing (Gouat, Feron & Demouron, 2003a). These authors have shown that the inhibition of the reproduction observed in winter is caused by social effects and not by environmental conditions of winter (low temperature, day length) when food is given ad libitum. In spring, now aged 6 months old, mice leave the mound and disperse (Orsini et al., 1983; Duryadi, 1993; Milishnikov, Rafiev & Muntianu, 1998). Because of the death of adults before winter, there are two distinct cohorts of reproducing animals in Mus spicilegus: 'long life' animals, born the previous year, and which experienced long winter cohabitation within mounds, and 'short life' individuals, born from first spring reproduction, which reproduce in summer. Little is known about the social organization of mice immediately after leaving the mounds in spring. In a study conducted right after dispersal from the mounds, Gouat, Katona & Poteaux (2003b) described a female biased sex ratio and clusters of two to three females. Milishnikov et al. (1998) reported a sex ratio at equilibrium before April, becoming biased in favour of females during the breeding season, and which probably reflects male mortality due to differential dispersal. The consequence of male dispersal and female philopatry is that related females are expected to be found all in the same vicinity. This type of social structure deriving from female philopatry has been reported in species presenting winter aggregations, such as certain microtine rodents (Madison, 1984; Saitoh, 1989). In mound-building mice, we expected females to establish their territories in the vicinity of the mound that they left at the end of winter. Genetic relatedness between neighbouring females is therefore expected to reflect the genetic structure within mounds and this philopatry.

The second striking feature of mound-building mouse is the probable existence of a monogamous mating system, a rare trait in mammals. Indeed, a stable pair bond between partners has been demonstrated both in the laboratory and in semi-natural enclosures (Patris & Baudoin, 1998; Dobson & Baudoin, 2002; Simeonovska-Nikolova, 2003; Baudoin et al., 2005). Other species of the Mus genus are commonly considered as polygamous–polygynous species, but monogamy in Mus spicilegus is further supported by two arguments: the first being bi-parental care (Patris & Baudoin, 2000), with an efficient implication of the male in parental care (Féron et al., 2007). The second one is high intolerance of females compared to polygynous mice species (Patris et al., 2002) and a lower reproductive success in polygynous females (Gouat & Feron, 2005). Upon forming a couple with a partner that is likely to be the only partner in their lifetime, mound-building mice should exhibit careful mate choice. One of the most important parameters in mate choice is inbreeding avoidance because inbred reproduction is known to decrease fitness (Meagher, Penn & Potts, 2000). Mus spicilegus mice should therefore avoid mating with close kin and instead choose a partner that will maintain the genetic diversity of offspring, with dis-assortative mating being attributed to genes belonging to the major histocompatibility complex (Potts, Manning & Wake, 1991; Penn & Potts, 1999), to major urinary proteins (Hurst et al., 2001), or to the comparison of the overall phenotypic similarity (Todrank & Heth, 2003). Whatever the mechanism, mating between genetically dissimilar individuals can be confirmed through the comparison of values of relatedness between males and females within mounds and between reproductive partners in spring.

In both winter and the breeding phase of the life cycle, genetic relatedness is thus a crucial factor to consider. Knowledge of actual relatedness between group members within mounds can provide valuable information about parentage and life-history traits. The genetic structure of over-wintering groups may reflect past associations between parents. Those associations result from patterns of dispersal and socio-sexual choices. The population structure of this species has been assessed with protein (Milishnikov et al., 1998) and microsatellite loci, but only the latter genetic markers were variable enough to analyse parentage (Garza et al., 1997). The present study conducted in Bulgaria assessed relatedness between individuals using a set of microsatellite data: Garza et al. (1997) showed that individuals in a mound are the product of multiple parentage. Offspring were nevertheless more related within a mound than between mounds, suggesting that congregation of mice at the time of mound-building is kin-biased.

In the present study, we sampled a population of mound-building mice in Hungary where this species is abundant (Bihari, 2004) at two key-moments of their life cycle: first, individuals from several mounds excavated during autumn were analysed with the set of loci used in the precedent study (Garza et al., 1997).
1997). Our objective was to analyse the genetic structure in mounds from a Hungarian population and therefore to test the relevance of the scenario proposed by Garza et al. (1997). Second, we realized a capture–mark–recapture study in spring to understand the social structure of this species, after dispersal from the mounds. The gathered genetic data were compared with the autumn data to provide a suitable scenario of the particular life cycle of this species. We were especially interested in understanding the shift from winter group-living to monogamous reproductive units in summer.

MATERIAL AND METHODS

Capture in October 1999

The repartition area of mound-building mouse is located in Eastern Europe, extending east–west from the banks of the Caspian Sea to south-eastern Austria and south–north from the shores of the black Sea to northern Ukraine (Orsini et al., 1983; Sage, Atchley & Capanna, 1993; Sokolov, Kotenkova & Michaelenko, 1998). Although this species is occasionally observed in their original steppe grassland habitat, these mice are generally found in agricultural areas (Unterholzner, Willenig & Bauer, 2000). Unlike the other mouse species present in this area, *M. spicilegus* is never found in human buildings, even in winter, because they build mounds to encompass the cold season.

Mice were caught in an agricultural area (mainly composed of vineyards) in the Gyöngyös region (Hungary), at the beginning of October 1999. This period corresponds to a peak in mound building (Muntyanu, 1990). Mounds were found at three sites, separated each by 3, 5 and up to 8 km, respectively. The position of each site was determined by GPS (site 1: 47°44.359′N, 19°53.814′E; site 2: 47°45.164′N, 19°53.423′E; site 3: 47°42.447′N, 20°03.327′E).

A total of five mounds per site were measured before excavation (*N* = 15). A metal sheet (1 × 6 m) was placed around the mound to prevent mice from escaping. The excavation continued until the nest-chamber was found. Mice were found in ten of the 15 explored mounds (2/5 on site 1, 4/5 on site 2, and 4/5 on site 3). All captured animals were sexed and weighted (Pesola spring-scales, precision 0.5 g), and a piece of ear was cut and stored in 95% alcohol for genetic analyses.

Trapping session in April 2000

Dramatic flooding events occurred in this area during winter 1999 (K. Katona, pers. observ.). In April 2000, no individual was caught during a 2-day trapping session in the precedent studied fields, in spite of the presence of mounds. A site in the vicinity of the precedent ones (site 4: 47°44.133′N, 19°58.204′E), and located on the top of a small hill, was chosen and a 5-day trapping session was performed in April. This site encompassed an old and unploughed sunflower field (100 × 493 m) surrounded by vineyards. A square grid of 100 traps (single door and single capture trap, 16 × 5 × 5 cm, baited with mice pellets UAR type AO4) was disposed (traps every 10 m, trap lines every 10 m), partly in the sunflower field (five lines of ten traps) and partly in a young vineyard (five lines of ten traps); for more details on traps and capture-recapture techniques, see Gouat et al. (2003b). The trapping zone encompassed 26 mouse mounds. The trapping session lasted from 24–28 April 2000 and traps were checked twice a night. Traps were closed during the day.

Captured animals were sexed, weighted (Pesola spring-scales, precision 0.5 g), and marked individually for identification by cutting a small piece of the ear (three positions on each ear), which was also used for genetic analysis. The reproductive state of females (gestation or lactation) was checked according to Gouat et al. (2003a).

After measurements and identification in the field, animals were released at their site of capture.

Genetic analyses

Both *M. spicilegus* and *Mus musculus* are present in this area and may be captured in the same field. As field discrimination of these two species is difficult because of similarities in their morphology, genetic identification was performed on all individuals, using the microsatellite locus Musmcka which has been shown to be fixed for alternative alleles in *M. spicilegus* and *M. musculus* (Duryadi, 1993). To perform genotyping and relatedness analyses, we used microsatellite loci known to be variable in this species (Garza et al., 1997): four loci located on the X chromosome (DX3, DX5, DX22, and DX23) and four autosomal loci (D1Mit28, D11Mit150, D15Mit11, and D17Mit20, Table 1).

Total DNA was extracted by Qiam minikit (Qiagen) from a piece of ear. Polymerase chain reaction (PCR) amplification was performed according to a standard protocol (Dallas et al., 1995). PCR products were electrophorezed on denaturing gel and polymorphism revealed after autoradiography. All the captured mice were identified as *M. spicilegus*.

Genetic diversity was measured as the mean number of alleles per locus (*A*), and observed (*H*<sub>e</sub>) and expected (*H*<sub>o</sub>) heterozygosities (Nei, 1978) using the software GENETIX, version 3.07 (Belkhir et al., 1996–1997). The inbreeding coefficients (*F*<sub>IS</sub>) were estimated using the locality as the reference popula-
tion with the F-statistics (SmallF) of Weir & Cocke-
ham (1984) using GENETIX. Standard errors were
obtained by jackknifing over loci. The departure from
the null hypothesis (random mating for F_{IS} and F_{IT})
was tested using 10 000 permutations as imple-
mented in the GENETIX program. Exact tests for
Hardy–Weinberg proportions were tested by using
10 000 randomizations of alleles within samples with
the same software.

Tests for genetic differentiation among localities in
October 1999 and between samples of 1999 and 2000
were performed by estimating F_{ST} using the compari-
on among groups of samples option of GENETIX,
with two-sided P-values being obtained after 10 000
permutations.

We used the ‘allele counting method’ to determine
from individual genotypes the minimal number of
parents contributing to the young in a mound. This
simple method allowed determination of the paternal
and maternal contribution, taking advantages of both
autosomal and X-linked loci genotypes. For example,
for the X-linked loci, one parental pair alone can
account for no more than three alleles (because males
received only one copy of X from their mother). Con-
versely, more than two alleles in juvenile males of a
mound indicated a contribution of at least two differ-
et females; for details of this method, see Garza
et al. (1997). We determined the number of parents as
the minimum number required to account for the
genetic diversity observed. Estimation of parentage is
therefore a possible under-estimation of the actual
number of parents. However, the number of juveniles
captured provides a good way to determine whether
this hypothesis is the more parsimonious because
the usual number of young per litter is known in
this species, as observed in laboratory (7.72 ± 0.20,
N = 18), which corresponds to a parental unit (Gouat
& Féron, 2005).

Parentage analysis was performed for the only
adult found, using multilocus genotypes and the
program PARENTE (Cercueil, Bellemain & Manel,
2002). This program calculates the probability of suc-
cessfully allocating an individual offspring to its
parents.

Average mound relatedness was estimated from
individuals using the computer program Relatedness,
version 5.0.1 (Queller & Goodnight, 1989). For these
estimations, mounds were weighted equally and stan-
dard errors were obtained by jack-knifing over mound
or sites. Student t-tests were performed to compare
inbreeding coefficients with the reference value of
zero and relatedness estimates to the theoretical
value of r = 0.5 (Sokal & Rohlf, 1995).

Comparisons of R-values between pairs were con-
ducted through permutation tests with the series
used as a stratum (StatXact-3; Cytel Software Corpo-
ration). Permutation tests provide the exact probability (two-sided $P$-value) of the occurrence of such distributions, or more extreme distributions, than the observed distribution.

Effective population size ($N_e$) was computed from the genetic data following the SMM model (Ohta & Kimura, 1973). Dallas (1992) used one of these loci (D11Mit 150) to estimate the average mutation rate for laboratory strains of mice ($M. musculus$), obtaining a mean value of $\mu = 2.95 \times 10^{-4}$ that is also used in the present study. Estimates of $N_e$ from X-linked loci were based only on genetic data obtained from females.

**RESULTS**

**MICE FROM EXCAVATED MOUNDS (OCTOBER 1999)**

Mice were found in ten of the 15 explored mounds (2/5 on site 1, 4/5 on site 2, and 4/5 on site 3). A total of 83 individuals were caught, with a significant male-biased sex ratio of 1.59 (51 males and 32 females, $\chi^2 = 4.34$, d.f. = 1, $P < 0.05$). The number of animals per mound was in the range 6–23 (mean ± SE = 11.43 ± 2.4, $N = 7$) with the exception of three mounds with only one individual. Only one adult female (20 g) was found in the sample and was excluded from analyses of parentage. The remaining 82 individuals were all juveniles, according to genital morphology and body mass. The mean body mass was 10.27 ± 1.05 g, with males being significantly heavier than females (10.47 ± 1.04 and 9.94 ± 0.98, respectively, $t = 2.29$, $P = 0.025$). From the narrow range of body mass (7–13 g), we can assume that all these individuals belonged to the same cohort.

Polymorphism varied among the 11 microsatellites surveyed, in the range 2–13 alleles, and 3–25 genotypes per locus (Table 1). Expected heterozygosity was in the range 0.22–0.8, with a value higher than 0.6 for seven loci. At the population level (three sites mixed), the inbreeding coefficient was not different from zero ($F_{IS} = -0.018 \pm 0.061$) indicating that random mating appears to occur at the locality level. However, the mean $F_{ST}$ value is rather high ($F_{ST} = 0.086 \pm 0.003$, $P < 0.001$) indicating microstructure due to differences in allele frequencies among localities.

Parentage could only be assessed in the seven mounds where at least two individuals were found. We compared individual genotypes for all loci to determine the number of possible parents by mound (Table 2). We found that only two mounds presented parentage compatible with a single couple of parents, as we observed less than three alleles for loci based on the X chromosome and only four (or less) alleles for autosomal loci. Of course, these numbers are also compatible with more than two parents, but the

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<th>Site</th>
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<th>$N_f$</th>
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</table>

Table 2. Distribution of alleles at height microsatellite loci between seven mounds.
number of pups fitted the normal litter size, comforting the single couple hypothesis. For two other mounds (\(N = 6\) and 10), at least three parents would contribute to juveniles, of which two were females. For all other mounds (Table 2), there were at least five parents, corresponding to at least two parental pairs, which is compatible with the number of offspring captured in these mounds (\(N = 14, 16,\) and 23, respectively).

For the female adult found with 14 juveniles in a mound, the software PARENTE assigned five young to this female as their mother (0.8 < \(P < 0.98\)) and excluded four young because of at least one genotypic incompatibility. For the other young, their genotypes were compatible with the female’s one but the probability was lower.

Average relatedness coefficient for all individuals (Table 3) was slightly positive (except for X-linked loci for females). Average relatedness coefficients between individuals within a mound (Table 3) were 0.262 ± 0.101 for autosomal loci and 0.334 ± 0.138 for X-linked loci. Among mounds, values were higher for X-linked loci (in the range 0.206–0.526) than for autosomal loci (0.145–0.458). Male relatedness values were always higher than those of females.

In some mounds, the number of animals found was very high, compatible with three or four litters. Because all individuals are assumed to belong to the same cohort, these numbers can only be due to a high number of contributing parents. As a consequence, a negative relationship between number of animals per mound and within-mound \(R\)-values is expected if parents are not related. Within-mound \(R\)-values were variable and the regressions are presented in Figure 1. The relationships (Fig. 1) were not significant either in autosomal loci (\(N = 7, P = 0.13\)) or in X-linked loci (\(P = 0.25\)).

**Table 3.** Relatedness coefficients for autosomal and X chromosomes

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<th>X-linked</th>
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<td></td>
<td>All individuals</td>
<td>Males</td>
<td>Females</td>
<td>All individuals</td>
</tr>
<tr>
<td>Total sample</td>
<td>0.037 ± 0.016</td>
<td>0.038 ± 0.02</td>
<td>0.038 ± 0.03</td>
<td>0.043 ± 0.03</td>
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<td>Within mound:</td>
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<tr>
<td>all data</td>
<td>0.262 ± 0.101</td>
<td>0.317 ± 0.128</td>
<td>0.211 ± 0.165</td>
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<td>Multiparentage</td>
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<td>0.266 ± 0.114</td>
<td>0.299 ± 0.43</td>
<td>0.258 ± 0.058</td>
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**Figure 1.** Regressions of individuals within-mound relatedness values on number of animals in a mound. A, data from the four autosomal loci. B, data from the four X-linked loci .

5-day session of trapping (Table 4). With only ten males out of 43 individuals, the sex ratio was significantly biased in favour of females (3.3; \(\chi^2 = 12.3,\) d.f. = 1, \(P < 0.001\)). On the 5-day trapping session, some individuals of both sexes were captured in the same trap (see ‘Spatial association of individuals’ in Table 4), sometimes repeatedly: we found 11 cases where two or more females were captured together in the same trap.

In the present study, we report the genetic data so that it could be compared with the autumnal social structure of this species. Inbreeding coefficient values for April 2000 sample was not significantly different.
from zero ($F_{IS} = 0.048 \pm 0.01; t = 0.94, P = 0.45$). Polymorphism observed among these 43 individuals was of the same magnitude as in mice from mounds in autumn, except for locus DX3 which was almost fixed (Table 1). The two samples did not differ by their allelic richness (4.29 and 5.25, respectively, $P = 0.68$), observed heterozygosity ($P = 0.76$), gene diversity ($P = 0.97$), or inbreeding coefficient ($F_{IS}$) ($P = 0.76$). Average relatedness coefficient for all individuals was close to zero ($R_T = 0.031 \pm 0.048, N = 43$) and only marginally positive for females only ($R_f = 0.049 \pm 0.058, N = 33$).

Most of the females were trapped with another animal: 18% were paired with a male, 55% were grouped with one or two females, and 27% were solitary (Table 4). Females caught in the same trap were significantly more related than any other pair of females ($N = 22$, two-sided $P$-value, $P = 0.0105$). Moreover, females in pairs or trios present a value of relatedness similar to the one found in winter mounds ($N = 16$, two-sided $P$-value = 0.93).

Conversely, males associated with one or two females in spring were no more related to them than to any other female trapped in the area ($N = 8$, $P = 0.62$). We compared $R$-values of pairs in April ($N = 8$) with the mean relatedness of males with females in the same mound ($N = 48$) (Fig. 2). The test was significant ($P = 0.04$) and suggested that males were less related to their associated female(s) in spring than the females with whom they shared a mound in winter.

From $F_{ST}$ data of October and April, the effective population size ($N_e$) ranged from 5557 to 7567 for the four autosomal loci and from 2879 to 3914 individuals for the four X-linked loci. These values were lower than those of Garza et al. (1997), who obtained $N_e$ values of 38 048 for automal loci and 23 102 for the same X-linked loci.

**DISCUSSION**

In the present study, we observed multiple parentages in five out of seven mounds, with three to five
parents minimum contributing to the next generation (allele counting method). Relatedness coefficients were high and compatible with full-sib to cousin relationships depending on the mound considered. Some mounds were very populous, but the regressions between the number of animals and within-mound $R$-values were not significant for both types of loci. These results suggest that parents contributing to juveniles in a mound are related. This is consistent with the pattern of relationships observed within mounds by Garza et al. (1997) in Bulgaria. However, our results differed significantly from the ones of Garza et al. (1997) with respect to some parameters.

We reported a sex ratio significantly biased in favour of males (1.59) whereas they observed a sex ratio at equilibrium. The number of individuals observed per mound was significantly higher ($t = 3.09, P < 0.001$) than in Garza et al. (1997): the fact we sampled less ($N = 7$ versus 28), but more populous (11.43 versus 5.65 individuals per mound) mounds may have influenced the genetic diversity. As a consequence, we obtained a lower population effective size ($N_e$). Moreover, $F_{ST}$ values indicate a subdivision between fields. Although the general pattern of organization within mounds is confirmed, the population structure of *M. spicilegus* appears to be geographically more variable than that previously documented.

According to their body mass, individuals within a mound belonged to the same age class, although not always to the same litter (Garza et al., 1997; present study). As adults die before winter, only young of the last litter over-winter without reproducing (Gouat et al., 2003a). This mouse appears thus to be a strict seasonal breeder, compared to the closely related species *M. musculus* where females can remain reproductively active in winter according to their body conditions (caloric intake; Bronson, 1979).

In spring, it has been reported that mice leave the mounds to dig burrows of simple design where they overwinter, but in Garza et al. (1997) the fact we sampled less ($N = 7$ versus 28), but more populous (11.43 versus 5.65 individuals per mound) mounds may have influenced the genetic diversity. As a consequence, we obtained a lower population effective size ($N_e$). Moreover, $F_{ST}$ values indicate a subdivision between fields. Although the general pattern of organization within mounds is confirmed, the population structure of *M. spicilegus* appears to be geographically more variable than that previously documented.

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In spring, it has been reported that mice leave the mounds to dig burrows of simple design where they spend the entire spring–summer period (Sokolov et al., 1998). However, our data of spatial organization suggest that the pattern of social structure is not so simple over the breeding season. First, in April, we found a sex ratio significantly biased in favour of females. The previous autumn, and in the same local population, we observed the opposite, with a male bias into the mounds. Numerous males thus disappeared in or before spring, certainly due to dispersal, to predation, or to a lack of dispersing males from adjacent field, because these populations disappeared because of dramatic flooding events during winter 1999. Differential dispersal is confirmed by the comparison of male–female pair relatedness in autumn (mound) and in spring (field traps): in spring, males were genetically dissimilar and thus did not come from the same mound than their associated females. We therefore can conclude that males of *M. spicilegus* disperse in spring, which allows inbreeding avoidance. A similar conclusion is obtained from micro-satellite loci analysis of mice from mound (our data; Garza et al., 1997). This differential tendency to disperse between males and females is also supported by an open field behaviour study conducted by Simeonovska-Nikolova (2000) who found that males explored their environment more actively than females.

Once they are out of the mounds, mound-building mice appear to be sedentary and to occupy a small home range (Gouat et al., 2003b). In our capture–mark–recapture study, we considered individuals captured in the same trap as being socially associated. The first important point to note is that more than half the trapped females were associated with at least one female (55%). Some pairs were identified as full-sisters and the mean relatedness between associated females was rather high, similar to the mean relatedness found in winter mounds. We think that spatially associated females, and especially groups of kin-related females, originated from the same mound and remained together after over-wintering. Spatial organization based on clusters of related females is also known in other rodent species (Ishibashi et al., 1998; but see Madison & McShea, 1987). However, a detailed analysis of kinship among paired females revealed great variability, ranging from values compatible with full-sisters to negative values for some other pairs. Even if these latter females were not related, they may be familiar if they shared the same mound during winter. Indeed, all individuals in a mound do not belong to the same litter and we found some animals to be less related than cousins ($R < 0.1$). This species is known to be aggressive towards unfamiliar individuals, both males and females (Patris & Baudoin, 1998; Patris et al., 2002). Mice are more tolerant with familiar individuals (Patris et al., 2002) and familiarity plays a major role in tolerance (Bekoff, 1981). This social tolerance between females (related or not) could result from a social bond developed during the time spent in the mound (4–6 months according to the winter season (Gouat & Féron, 2005). Cluster of females in spring would then reflect familiarity rather than kinship, although individuals within mounds are often related.

The biased sex ratio in spring may influence the observed socio-spatial associations in another way. Males are assumed to be mainly distributed as a function of the spatial and temporal pattern of availability of females (Ims, 1987; Ostfeld, 1990). In the present study, males were trapped in low density, sometimes paired with one or several females. Although the monogamous system in this species has been ascertained by experiments conducted in the
laboratory (Patris & Baudoin, 1998, 2000; Patris et al., 2002), in outdoor enclosures (Dobson & Baudoin, 2002; Baudoin et al., 2005), and in open field studies (Suchomelova, Munclinger & Frinta, 1998; Simeonovska-Nikolova, 2003), only 18% of the females were found to be paired with a male. The number of monogamous pairs is probably higher: some solitary females were pregnant (Gouat et al., 2003b), which may indicate that we were unable to capture the male. However the spacing pattern of other animals rather acts in favour of facultative polygyny as the mating system during this period of the biological cycle (Gouat et al., 2003b). This was confirmed by Simeonovska-Nikolova (2007) in a field study conducted over three periods of the breeding season.

Moreover, although monopolization of several females, due to their spacing pattern, is assumed to increase the fitness of males, the reproductive success of females is likely to suffer from this biased sex ratio. Indeed Gouat & Féron (2005) compared the reproductive success of pairs of sister females and single females mated with unrelated males under laboratory conditions. They reported that, even if there was no agonistic behaviour between individuals, the polygynous situation had a strong negative effect on the reproductive success of females (lower average number of young per litter, higher interval between two successive litters). Their reproductive interest to stay grouping in the field would be linked to: (i) a better defence of the litter, but no communal nesting was ever reported in this species or (ii) an adaptation to a temporary limited factor (i.e. the low number of available males), with the possibility that spatial females association ceased when sexual activity began.

The results of the present study appear to contradict the observations of Simeonovska-Nikolova & Gerasimov (2000), who observed an exclusive home range for each female (approximately 232.4 m$^2 \pm$ 20.5), supporting the idea of a monogamous system in the field (Komers & Brotherton, 1997). Two alternative hypotheses may explain this discrepancy between the present study and the study by Simeonovska-Nikolova & Gerasimov (2000) is that it was conducted earlier in the year (April instead of summer). The observed associations are then the expression of the transition between winter and summer.

According to Garza et al. (1997), mound building appears to be kin based, and the evolution of mound building and communal nesting in this species would then be partly due to kin selection. It is actually impossible to decipher what is due to kin selection and what is a mere by-product of female philopatry. The female kin clusters observed during the breeding season lead to a high probability that juveniles building the same mound will be related (especially through their mothers). Furthermore, even if territoriality is more expressed in summer because of the shorter cohabitation period prior to mating, familiar related females should be more tolerant with each other, or may establish neighbouring territories, which promotes preferential grouping between maternally related litters.

Sokolov, Kotenкова & Ljalukchina (1990) showed that this species is not as flexible as the commensal species (the house mouse and its wild eastern form). However, *M. spicilegus* appears to exhibit a mating system that is not restricted to monogamy. Our data confirmed a facultative polygyny in the wild, as first proposed by Gouat & Féron (2005) and also found in Bulgaria by Simeonovska-Nikolova (2007). As micro-tine rodents, *M. spicilegus* may present a changing social structure over the breeding season and a rather flexible mating strategy, depending here on the males’ density as the limiting factor. This needs further investigation in the field, especially in summer, to confirm the proposed scenario of the life cycle of this species.

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