Short report

Multiple paternity in free-living root voles (Microtus oeconomus)

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1. Introduction

Behavioural ecologists have long been interested in mating systems and variance of reproductive success. Highly variable molecular markers such as microsatellites are now used to reassess mating systems from the genetic point of view. Multiple paternity of litters, resulting from insemination of a female by at least two males, has been reported in rodents (e.g., Goossens et al., 1998; Bartmann and Gerlach, 2001; Dean et al., 2006). A number of genetic investigations have yielded mating system classifications diverging from earlier ones based on behavioural data. For example, the prairie vole (Microtus ochrogaster) was considered to be a monogamous species with reproductive exclusivity, territoriality, behavioural monogamy and biparental care (Getz et al., 1981). Molecular analyses, however, revealed multiple paternity in 56% of wild prairie vole litters (Solomon et al., 2004).

Microtine rodents have been reported to have modes of mating systems ranging from genetic monogamy (M. pinitorum, Marfori et al., 1997) to genetic promiscuity in most vole species (e.g., Microtus pennsylvanicus, Berteaux et al., 1999; Microtus arvalis, Fink et al., 2006). In the root vole (Microtus oeconomus), both promiscuous groups (Tast, 1966) and monogamous pairs (Viitala, 1994) have been observed. However, the basic modes of spatial organisation in root vole populations are female territoriality and overlapping male home ranges (Gliwicz, 1997). According to the author, each female territory is overlapped by several male ranges, and larger males have access to more territories of females. Such a spatial structure of the population suggests promiscuity rather than monogamy as the prevailing mating system. Here we investigated pattern of mating system by genotyping 21 females and their 111 offspring (5.28 ± 0.27 S.E. pups per female), we found evidence for multiple paternity in 38% of the litters sired by two or three males. Paternity was not significantly skewed away from the null expectation of equal proportions of offspring sired in any of the multiple-sired litters, and the most successful male fathered between 40% and 60% of the pups in a litter. The results indicate that promiscuity is a common mode of reproduction, consistent with the previous classification of the mating system based on the spatial structure of the root vole population.

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2. Materials and methods

2.1. Sampling and microsatellite analysis

The study was done in the Biebrza valley in an open sedge bog near Gugny settlement (NE Poland, 53°22′N, 22°35′E) in 2004–2006. We used the catch-mark-release (CMR) method, with wooden live-traps placed in a 7 × 11 grid pattern at 10 m intervals. The traps were checked twice a day. A total of 21 pregnant females were caught in two breeding seasons: 2004 (n = 10) and 2005 (n = 11). The females were taken to the laboratory and housed separately in their own cages (46 cm × 29 cm × 22 cm) with wood shavings and hay as bedding and with food and water ad libitum until they weaned the young (21 litters). Mothers and their offspring were housed for 5 weeks; then samples of 20–30 hair bulbs for DNA analyses were plucked from all of them, and the voles were released in the field at the females’ catch sites.

Total genomic DNA was extracted using the Genomic Mini Kit (A&A Biotechnology). All individuals were genotyped at 10
microsatellite loci (Table 1). Forward primers were labelled with fluorescent dye (Applied Biosystems): Ma35-FAM, Ma54-PET, Ma66-VIC, Ma88-NED and MSMM6-PET in set 1; and MAG6-NED, MSMM2-FAM, MSMM3-FAM, MSMM5-VIC and MSMM7-NED in set 2. Amplification of each set was carried out in a 5-μL reaction volume using Qiagen Multiplex PCR Master Mix under standard multiplex PCR conditions (Qiagen) with 26 cycles. PCR products were separated using ABI3130 DNA sequencer, and microsatellite alleles were analysed using GENEMAPPER4.0 software (Applied Biosystems). The same microsatellite markers were also assayed in 110 individuals caught in the population in autumn 2006; this was done in order to estimate allele frequencies, the frequency of null alleles of the entire data set (altogether 131 field-collected voles and 111 lab-born offspring) and the exclusion probabilities of offspring genotypes with the known maternal genotype. This method may underestimate the actual number of fathers because of the low frequency of null alleles (Table 1) which could generate high frequencies of false paternity exclusions (Dakin and Avise, 2004).

Exclusion probabilities for the first parent and for the second parent with first parent assigned were high for each locus and for all loci combined. We assessed the ability to detect single, double or triple mating by simulations in GERUDsim2.0. In five independent runs of 1000 iterations and using five variable loci with the highest exclusion probabilities (Ma35, Ma54, Ma88, MAG6 and MSMM7; Table 1) and with 5 or 6 pups as litter size, one sire was detected in 100% of the simulations, two in 99.4–99.8%, and three in 60.2–89.9% of them. Therefore it seems unlikely that a mistake was made in classifying any multiple paternity cases as single paternity or in assessing the number of sires in a litter.

The proportion of paternal genotypes correctly reconstructed depends on the number of offspring genotyped, and the use of additional polymorphic loci does not markedly increase the probability because it adds more paternal genotypes that must be reconstructed correctly (Jones, 2005).

3. Results and discussion

Paternity analysis and genotyping of mothers and their litters clearly indicated that the promiscuity is frequent in the root vole. Multiple paternity was detected in 8 of 21 litters (38%) both by manual comparison of mother and offspring genotypes and using GERUD software (Table 2) in two breeding seasons. Half of the multiple-sired litters were sired by two males, and the other half by three males. Male reproductive success varied 5.62 ± 0.38 S.E. pups, a nonsignificant difference from the average of 5.08 ± 0.37 S.E. pups found in all other litters (Mann–Whitney test, U = 33.0, P > 0.05). The effective number of fathers (mean value 2.39 ± 0.11 S.E.) did not differ from the minimum number of fathers (2.50 ± 0.19 S.E.) in multiple-sired litters (Mann–Whitney test, U = 20.0, P > 0.05). The estimated frequencies are fairly consistent with the findings of previous studies in other voles: 22% multiple-sired litters in Myodes glareolus (Ratkiewicz and Borkowska, 2000), 50% in M. arvalis (Borkowska and Ratkiewicz, unpublished) and 56% in M. ochrogaster (Solomon et al., 2004).

The microsatellite set used in our study provided considerable power to detect multiple paternity in the root vole litters. The number of alleles present at each locus ranged between 7 and 22, with a low frequency of null alleles (Table 1) which could generate high frequencies of false paternity exclusions (Dakin and Avise, 2004).

Exclusion probabilities for the first parent and for the second parent with first parent assigned were high for each locus and for all loci combined. We assessed the ability to detect single, double or triple mating by simulations in GERUDsim2.0. In five independent runs of 1000 iterations and using five variable loci with the highest exclusion probabilities (Ma35, Ma54, Ma88, MAG6 and MSMM7; Table 1) and with 5 or 6 pups as litter size, one sire was detected in 100% of the simulations, two in 99.4–99.8%, and three in 60.2–89.9% of them. Therefore it seems unlikely that a mistake was made in classifying any multiple paternity cases as single paternity or in assessing the number of sires in a litter.

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For this reason we set the parameters in GERUDsim2.0 at one father (with 100% of the offspring sired), two and three fathers with equal proportions of offspring sired, and two loci (Ma88 and MAG6). The probabilities of correctly reconstructing the paternal genotypes were 84.7–90.9% for one sire, 26.0–34.0% for two sires, and 6.7–10.1% for three sires, with litters of 5 or 6 pups. Because the root vole litter is relatively small, the efficacy of genotypic reconstruction is low, especially for multiple mating. However, the proportion of offspring sired by each male in a particular litter was the same despite the number and polymorphism of the loci used.

In Microtine rodents, the spacing system of sexually receptive females is the most important factor determining the spatial distribution and mating strategy of males (Ims, 1987). Root vole males try both to control female and to copulate with another ones to increase their reproductive success. Males could monopolise females in their oestrus period as we found for the 62% of females single-mated in the population. In vole populations it is difficult to monopolise females when the local density of males is high (Ishibashi and Saitoh, 2008) and females actively seek fertilisation by several males (Berteaux et al., 1999). Thus, another strategy of males for increasing their reproductive success is to increase their number of mates, that is, to gain access to already mated females. Here we

Table 1

Allele number (A), frequency of null allele (Fnull) and exclusion probability of the locus for the first parent (ExcP1) and for the second parent assigned (ExcP2) in the population studied, for 10 microsatellite loci used to infer parentage in root vole litters.

<table>
<thead>
<tr>
<th>Locus</th>
<th>A</th>
<th>Fnull</th>
<th>ExcP1</th>
<th>ExcP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ma35a</td>
<td>15</td>
<td>0.001</td>
<td>0.592</td>
<td>0.746</td>
</tr>
<tr>
<td>Ma34a</td>
<td>17</td>
<td>0.022</td>
<td>0.610</td>
<td>0.759</td>
</tr>
<tr>
<td>Ma66a</td>
<td>15</td>
<td>0.008</td>
<td>0.575</td>
<td>0.731</td>
</tr>
<tr>
<td>Ma88a</td>
<td>22</td>
<td>0.001</td>
<td>0.687</td>
<td>0.815</td>
</tr>
<tr>
<td>MAG6a</td>
<td>17</td>
<td>0.016</td>
<td>0.636</td>
<td>0.778</td>
</tr>
<tr>
<td>MSMM2a</td>
<td>15</td>
<td>0.001</td>
<td>0.541</td>
<td>0.704</td>
</tr>
<tr>
<td>MSMM3a</td>
<td>11</td>
<td>0.022</td>
<td>0.505</td>
<td>0.675</td>
</tr>
<tr>
<td>MSMM5a</td>
<td>9</td>
<td>0.026</td>
<td>0.373</td>
<td>0.557</td>
</tr>
<tr>
<td>MSMM6a</td>
<td>7</td>
<td>0.019</td>
<td>0.404</td>
<td>0.586</td>
</tr>
<tr>
<td>MSMM7a</td>
<td>14</td>
<td>0.002</td>
<td>0.622</td>
<td>0.767</td>
</tr>
<tr>
<td>Total</td>
<td>14.2</td>
<td>0.999</td>
<td>0.999</td>
<td></td>
</tr>
</tbody>
</table>
found that paternity in the root vole was not significantly skewed away from the null expectation of equal proportions of offspring sired in any of the multiple-sired litters (Table 2), and the distribution of reproductive success across all sires conformed to a Poisson distribution ($\chi^2 = 2.59, P > 0.05$). Although there were significant differences in number of pups sired between the most successful male (2.75 ± 0.25 S.E.) and the other males (1.92 ± 0.29 S.E.) in multiple-sired litters (Wilcoxon test, $Z = 2.37, P < 0.05$), the most successful male sired only 40–60% of the pups in a litter. 

Mating with an already mated female appears to be advantageous for root vole males even if they mate second. Equal contributions to litters by several males have rarely been noted in rodents (Ratkiewicz and Borkowska, 2000), because in a competitive mating situation the effectiveness of a given insemination depends on the interaction of mating order, time interval since the previous mating, and time of insemination relative to ovulation (Huck et al., 1989). The absence of a reproductive skew among root vole males siring offspring in a multiple litter may result in changes of their copulatory behaviour in response to the risk of sperm competition, leading to, for example, a higher frequency of repeated ejaculations (Stockey and Preston, 2004).

### Acknowledgement

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### Table 2

<table>
<thead>
<tr>
<th>Litter</th>
<th>$N_0$</th>
<th>$N_a$</th>
<th>Min. no. of fathers</th>
<th>Effective no. of fathers</th>
<th>$N_t$</th>
<th>$N_s$</th>
<th>Paternity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Allele count</td>
<td>GERUD</td>
<td></td>
<td></td>
<td>Proportion</td>
</tr>
<tr>
<td>L5</td>
<td>5</td>
<td>4 (1)</td>
<td>2</td>
<td>2</td>
<td>1.92</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>L7</td>
<td>6</td>
<td>4 (3)</td>
<td>2</td>
<td>3</td>
<td>3.00</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>L8</td>
<td>8</td>
<td>4 (3)</td>
<td>2</td>
<td>3</td>
<td>2.67</td>
<td>50</td>
<td>49</td>
</tr>
<tr>
<td>L13</td>
<td>5</td>
<td>3 (6)</td>
<td>2</td>
<td>2</td>
<td>1.92</td>
<td>34</td>
<td>30</td>
</tr>
<tr>
<td>L14</td>
<td>5</td>
<td>3 (6)</td>
<td>2</td>
<td>2</td>
<td>1.92</td>
<td>50</td>
<td>28</td>
</tr>
<tr>
<td>L16</td>
<td>5</td>
<td>4 (2)</td>
<td>2</td>
<td>2</td>
<td>1.92</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>L18</td>
<td>6</td>
<td>4 (4)</td>
<td>2</td>
<td>3</td>
<td>3.00</td>
<td>50</td>
<td>45</td>
</tr>
<tr>
<td>L21</td>
<td>5</td>
<td>5 (3)</td>
<td>3</td>
<td>3</td>
<td>2.78</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

$N_0$: number of offspring; $N_a$: maximum number of parental alleles, and in parentheses the number of loci with the greatest number of paternal alleles; $N_t$: total number of GERUD solutions; $N_s$: number of solutions that returned the same paternal contribution ratio.

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