## Sex-related spatial structure and effective population size in the common vole, *Microtus arvalis*, as revealed by mtDNA analysis

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The sex-related spatial genetic structure of a free-living population of the common vole (*Microtus arvalis*) was assessed using sequence and haplotype frequency data of the complete mitochondrial *cytb* gene in three seasons. Six haplotypes were resolved, three of them (A, B and C) were shared among seasons and sexes. The remaining three singletons did not match any of the local females. Pairwise tests did not show significant differences in haplotype frequencies between seasons. However, we observed such differences between the sexes. The aggregation index calculated for haplotype B showed a clumped female spatial distribution and revealed two clusters of the matrilineal lineage in the population. The female effective population size ( $N_{\rm ef}$ ) estimated from shifts of haplotype frequencies between seasonal samples was quite small and the  $N_{\rm ef}/N_{\rm f}$  ratio ranged from 0.18 to 0.35. We suggest that both factors, low  $N_{\rm ef}$  and female philopatry may be responsible for considerable inter-population differentiation previously reported in this abundant vole species.

## Introduction

One of the most ubiquitous phenomena in mammalian populations is the formation and maintenance of discrete groups of individuals. Because such groups often comprise non-random subsets of individuals from the population, they have received much attention in both behavioural ecology and population genetics. In microtine rodents, like in most mammals, female philopatry and male-biased dispersal are generally the rule (Greenwood 1980). Additionally, female relatives locate in close proximity to each other and form kin clusters in vole population, whereas no such clusters are apparent in males (Ishibashi *et al.* 1997). Therefore, spatial structure of the population is based on groups consisting of matrilines defined here as individuals descending from the same ancestral female. Variation in numbers and/or spatial range within and between matrilineal lineages affects demographic processes of the entire population (Johannesen *et al.* 2000).

Our study species, the common vole, *Microtus arvalis*, is a small rodent, with a distribution range stretching from central Russia to the Atlantic coast of France and Spain (Zima 1999). The study of Fink et al. (2004) identified five European mtDNA lineages in the common vole and their occurrence was attributed to the existence of multiple refugia during the last glacial period. The species usually occupies open cultivated agricultural land, grazed pastures and meadows and it is a serious pest of agriculture during peak years. The common vole is considered a social (communal) species (Hayes 2000). It lives in colonies occupying large underground burrow systems and corresponding systems of runways above ground. Colony consists of 2-3 females, usually one male and their offspring, which represents a polygynous mating system (Boyce & Boyce 1988a). Ecological studies have shown that females are probably related and a new colony is formed by a mother and her daughters, and frequently by sisters (Boyce & Boyce 1988b). However, as yet there are no studies that have explored the reality of spatial and social structures in natural populations of the common vole using molecular markers.

Detailed analyses of spatial genetic structure within mammalian populations have mostly been carried out in species with well-known social systems and employed a simple Mantel tests to correlate genetic and geographic distances of sampled individuals (Ishibashi et al. 1997) or performed well established technique of a spatial autocorrelation analysis that can detect a pattern in the frequency distribution of alleles (Peakall et al. 2003). In our empirical study we employed for the first time a novel procedure based on Miller's (2005) statistical concept of an aggregation. Miller (2005) adopted a modification of the aggregation index of Clark and Evans (1954) to test for nonrandom patterns of genetic diversity across a landscape. We used this method to detect and characterize patterns of a spatial structure of mtDNA haplotypes in a free-living population of the common vole, Microtus arvalis, one of the most common small mammals in Europe.

A fine-scale genetic substructure and nonrandom spatial distribution of individuals with respect to their genotype leads to a different perspective on how genetic variation is partitioned in natural populations (Sugg *et al.* 1996). Understanding dynamics of genetic changes within a population over time provides means to estimate a genetic effective population size  $(N_{\rm o})$ . The effective size of a population is the size of an ideal population that would be affected by genetic drift at the same rate as the actual population (Wright 1931). An ideal population exhibits random matings and has no substructure due to philopatry, mating system and kin clustering, but real populations usually have an internal genetic substructure via the spatial distribution of related individuals. Hence, a social structure or dispersal influence  $N_{a}$  directly by decreasing population size and a population growth rate, or indirectly by increasing reproductive skew (Anthony & Blumstein 2000). Genetic methods of estimating  $N_{e}$  are becoming more widely used. One of them is a temporal approach based on a rationale that when genetic drift is the only cause for allele frequency change over time,  $N_{a}$ can be estimated from empirical observation of temporal change in allele frequencies (Waples 1989). The temporal method has been used to estimate effective population sizes in a number of species, using both allozymes, microsatellites and mitochondrial DNA haplotypes (e.g., Laikre et al. 1998, Johnson et al. 2004).

In the present study we (i) examined patterns of population genetic structure of the common vole (*Microtus arvalis*) using sequence variation in the complete mitochondrial *cytb* gene. The mtDNA is known to show a maternal inheritance and hence we were able to (ii) estimate maternal lineages and their spatial distribution within a population, and (iii) the female component of the effective population size ( $N_{ef}$ ) by quantifying the variance of shifts in mtDNA haplotype frequencies between seasonal samples.

### Material and methods

#### Sampling

The study was conducted on an abandon field near Białystok (NE Poland). The catch–mark– release method (CMR) was applied. Wooden live-traps baited with oats and carrots were placed at 256 trap sites, which were distributed in a  $16 \times 16$  grid pattern at 5-m intervals. A total of 115 common voles were taken during three trapping sessions: in summer (July 2003), autumn (October–November 2003) and spring (April– May 2004) (Table 1). Traps were checked twice a day. At first capture, animals were marked by plantar micro-tattooing (Leclercq & Rozenfeld 2001), sexed and weighted. In addition, samples of individuals were sexed by successful

May 2004) (Table 1). Traps were checked twice a day. At first capture, animals were marked by plantar micro-tattooing (Leclercq & Rozenfeld 2001), sexed and weighted. In addition, samples of individuals were sexed by successful amplification of the *Sry* gene in males (Bryja & Konecny 2003). Hairs with bulbs (15–20) were taken for the molecular study and all individuals were released in the same place where they were caught. Voles recaptured in the following seasons were classified to the season when they were captured the first time. For each vole of either sex, the arithmetic centre of the locations at which it was trapped in a given season was calculated as an indicator of individual location.

# Sequence analysis of the cytochrome *b* gene

Total genomic DNA was extracted from hair bulbs using the Genomic Mini kit (Aabiot). The complete mitochondrial *cytb* gene was amplified in a single reaction using the *Microtus*-specific primers L14727-SP and H-ISO-SP (Jaarola & Searle 2002). All PCR amplifications were as described by Fink *et al.* (2004) but with 50  $\mu$ l reaction volumes containing 1.75 units of Taq DNA polymerase (Fermentas). The PCR primers L14727-SP and L15162Marv (Jaarola & Searle 2002) were used for sequencing. Direct sequencing was performed using the BigDye<sup>™</sup> Terminator Cycle Sequencing Ready Reaction Kit ver. 3.1 (Applied Biosystems). The sequencing reaction conditions were following those presented by Fink et al. (2004) and the products were separated with an ABI PRISM 3100 capillary automated sequencer.

Sequences were edited and aligned using BIOEDIT 7.0.1 (Hall 1999) and were revised manually. The number of transitions and transversions, haplotype (h) and nucleotide diversity ( $\pi$ ) were calculated using ARLEQUIN 2.0 (Schneider *et al.* 2000). An exact test of sample differentiation based on haplotype frequencies was also performed to reveal differences between seasonal samples and males and females (Raymond & Rousset 1995).

#### Spatial genetic structure analysis

We used a novel procedure included in the AIS computer software (Miller 2005) to detect patterns of spatial genetic structure. We calculated an allele-specific aggregation index  $(R_i)$  for allele i (haplotype) for the entire dataset, females and males and seasons. The significance of  $R_i$  and  $R_{iAVE}$  (arithmetic mean of all individual  $R_i$  values) was evaluated through the use of a randomization procedure where individuals and genotypes were randomly redistributed among individual locations.  $R_i = 1$  if sampled individuals are randomly distributed across the study area. In contrast,  $R_i < 1$  when samples show a clumped (aggregated) spatial distribution, and  $R_i > 1$  when samples display a tendency towards a uniform spatial distribution (Miller 2005).

#### Female effective population size estimates

We used a temporal method to estimate genetic effective population size  $(N_{\rm ef})$  of the common vole by quantifying the standardised variance of shifts in mitochondrial DNA haplotype frequencies (*F*) across three seasonal samples (summer

**Table 1.** Mitochondrial DNA haplotype (A–F) frequencies from three seasonal samples of the common vole population. Number of individuals possessing a given haplotype are in parentheses.

	А	В	С	D	E	F
Summer (38) Autumn (55)	0.737 (28) 0.909 (50)	0.211 (8) 0.073 (4)	0.026 (1)	0.026 (1)	0.000 0.018 (1)	0.000
Spring (22)	0.818 (18)	0.046 (1)	0.091 (2)	0.000	0.000	0.046 (1)
Overall (115)	0.835 (96)	0.112 (13)	0.026 (3)	0.009 (1)	0.009 (1)	0.009 (1)

2003, autumn 2003 and spring 2004). Because mtDNA is maternally inherited, only the female component of the effective population size was estimated. *F* was calculated using the following formula:

$$F = \frac{1}{K-1} \sum_{i=1}^{K} \frac{(x_i - y_i)^2}{(x_i + y_i)/2}$$
(1)

where K is the number of haplotypes,  $x_i$  and  $y_i$  are frequencies of haplotype *i* at the first and the next sampling period (season), respectively (Pollak 1983). The mean F was calculated over two pairwise comparisons of adjacent seasons and then used to estimate  $N_{\rm ef}$  (Nei & Tajima 1981) as follows:

$$N_{\rm ef} = \frac{t}{2(F - 0.5S_0 - 0.5S_t)}$$
(2)

where *t* is the number of generations that have elapsed between time points,  $S_0$  is the sample size at the first time point, and  $S_t$  is the sample size at the second time point. The common vole is short-lived mammal with an average life expectancy of 2–3 months in nature and three generations per year (Ryszkowski *et al.* 1973). Thus, because our sampling periods were separated by 4–5 months, t = 1.95% confidence intervals (CI) for  $N_e$  was calculated as described by Waples (1989).

#### Census female population size estimate

Census population size (N) was calculated using the programme JOLLY (Pollock *et al.* 1990). As an input we used a data record with a capturehistory format. We estimated population size using the standard time-dependent Jolly-Seber model (Model A) with both death and immigration (Pollock *et al.* 1990). The value of census female population size ( $N_f$ ) was based on the estimate of an average number of individuals per season using JOLLY and the assumption that the proportion of females is about 60% in midsummer (Bryja *et al.* 2005).

#### Results

#### mtDNA haplotype variation within and among seasons

Six polymorphic sites were observed from comparison among 115 sequences of the complete mitochondrial cytb gene (1140 bp) obtained in the common vole population in three seasons. All nucleotide substitutions detected were transitions. From a combination of substitutions, six haplotypes (A-F types, GenBank accession nos. EU439454–EU439459) were identified (Table 1). All haplotypes belong to the eastern mtDNA evolutionary lineage (Fink et al. 2004). The consensus sequence (haplotype A) was the most common haplotype in all three seasonal samples and in both sexes in the study area (Table 1). All other haplotypes could be derived from the consensus sequence by one, two or three nucleotide substitutions. The number of haplotypes was similar in different seasonal samples and ranged from 3 to 4. Haplotype diversity (h) varied from 0.1710 to 0.4225 in seasons with an overall value of  $0.2920 \pm 0.0523$  (Table 2). Nucleotide diversity  $(\pi)$  values in the consecutive seasons (summer, autumn and spring) were 0.08%, 0.03% and 0.05%, respectively, with an overall value of 0.05%. We noted significant differences in

**Table 2.** Number of mtDNA haplotypes ( $N_{H}$ ) and singletons ( $N_{S}$ ), and haplotype diversity (h) in three seasonal samples of the common vole population. M = males, F = females. Sample sizes are shown in parentheses as a ratio of M to F.

	N <sub>H</sub>			N <sub>s</sub>			h		
	М	F	All	М	F	All	М	F	All
Summer (17:21)	3	3	4	1	1	2	0.2279	0.5286	0.4225
Autumn (32:23)	3	2	3	1	0	1	0.1230	0.2372	0.1710
Spring (16:6)	3	2	4	2	0	2	0.3417	0.3333	0.3333
Overall (65:50)	6	3	6	3	1	3	0.2043	0.3812	0.2920

haplotype frequencies among three seasonal samples (Fisher's exact test for the entire data set: P < 0.05) and an almost five-fold decrease in haplotype B frequency occurred from summer to spring (Table 1) in the population studied. However, pairwise tests showed significant differences in haplotype frequencies only between summer and autumn samples (Fisher's exact test: P < 0.05), but not after the Bonferroni correction.

# Spatial differences in the distribution of mtDNA haplotypes between sexes

A different number of haplotypes and singletons were observed in the two sexes. The analysis of the entire *cytb* gene in 65 males in the population studied revealed the existence of 6 haplotypes, while in 50 females only 3 haplotypes were found. Sixty-two males had the same three haplotypes (A, B or C) as the local females. However, the remaining three males had distinct haplotypes (D, E or F) which did not match any of the local females (Table 2 and Fig. 1). Furthermore, we found significant differences in haplotype frequencies between sexes (Fisher's exact test for the entire data set: P < 0.01). Mean haplotype diversity (h) did not differ between the sexes and the corresponding values were  $0.2043 \pm 0.0668$  (95%) CI = 0.234-0.790 and  $0.3812 \pm 0.0684$  (95%) CI = 0.213 - 0.786) in males and females, respectively. Detailed h value estimates for the two sexes in seasonal samples are given in Table 2.

The most frequent haplotype A appearing in 58 males and 38 females was randomly distributed in space in both sexes and studied seasons ( $R_j$  values ranged from 0.95 to 1.52, P > 0.05; Fig. 1). In contrast, the  $R_j$  index calculated for haplotype B showed a clumped spatial distribution for females ( $R_j = 0.71$ , P < 0.01) with two clusters of females sharing haplotype B in the study area (Fig. 1). The aggregation index for haplotype B was not calculated for males as it appeared only twice.

# Effective and census female population sizes

We estimated seasonal shifts in frequencies of all



**Fig. 1.** Spatial distribution of the common vole (**A**) females and (**B**) males and their *cytb* mtDNA haplotypes. Location of individuals are the arithmetic centre of the locations at which a vole was trapped in the first trapping season. Female kin clusters are encircled. Units are metres.

mtDNA haplotypes. The standardised variance in shifts of haplotype frequency (*F*) was 0.0775 and 0.0605 for summer–autumn and autumn–spring comparisons, respectively. The estimated values of  $N_{\rm ef}$  and their confidence intervals were fairly low and similar:  $N_{\rm ef} = 9$  (95% CI = 3.2–75.4)

and  $N_{\rm ef} = 17$  (95% CI = 6.2–145.5) from shifts in haplotype frequency between summer and autumn and between autumn and spring, respectively. The Jolly-Seber estimate of population size was on average 82 individuals per season. Since the proportion of females is about 60% in mid-summer (Bryja *et al.* 2005), our calculated value of census female population size ( $N_{\rm f}$ ) was 49 per season.

### Discussion

In this study, we investigated and quantified patterns of spatial structuring and the effective size of the common vole population. In this species, most reproductive females live in exclusive groups of 2 to 6 and share a large burrow system (Boyce & Boyce 1988b). Such female kin structure is thought to be the basic social structure of several Microtus species (Boonstra et al. 1987) and it was also well demonstrated in Clethrionomys rufocanus (Ishibashi et al. 1997). Females of the common vole were more sociable than males and after a brief period of intolerance dyads of females started to build their burrows in response to social nesting (Dobly & Rozenfeld 2000). However, these authors did not find any differences in burrowing patterns between dyads of kin and unrelated females, so they conclude that groups of females may be constituted by related or neighbouring unrelated individuals. In contrast to direct laboratory observations (Dobly & Rozenfeld 2000), our genetic analysis supported the hypothesis that the formation of female kin clusters in a natural common vole population is due to natal philopatry. Females possessing haplotype B were present in the population in three study seasons and they were caught in two separated places in the study area (Fig. 1A). Such a spatial pattern of haplotype B could be formed only when daughters stayed near their mother and nest in the same burrow system in following seasons.

The comparison of genetic markers with different modes of inheritance (microsatellites and mtDNA) demonstrated a strongly male-biased gene flow in the common vole at the large geographical scale (Hamilton *et al.* 2005). It was shown that females are extremely philopatric and that the migration rate of males is 20 times higher than that of females. On the other hand, fine-scale analyses provided no evidence for a strong male-bias dispersal in this species (Schweizer et al. 2007). Although the immigration rate of males (12.9%) was not significantly higher than that of females (8.1%), genetic assignment analyses identified more males as being immigrants than females (Schweizer et al. 2007). Lower mean haplotype diversity in males than in females due to singletons appearance in males (haplotype D, E and F) obtained in our study also suggests male-biased dispersal in the common vole. Mitochondrial DNA markers show maternal inheritance and that rare haplotypes, carried into the population by immigrant males, did not pass to the next generation, so it was not possible to detect successful reproduction after dispersal (e.g. effective male dispersal). Ecological observations also showed short-range natal dispersal of *M. arvalis* females (Boyce & Boyce 1988a). We detected only one female with the rare haplotype C in summer. This haplotype appeared in the population once again in the following spring in two males. Thus, all breeding females come from the local population (Boyce & Boyce 1988b). It seems that territorial behaviour could prevent the establishment of immigrant females in groups and the introgression of new mtDNA haplotypes into the population.

We obtained an estimate of the effective population size from the observed haplotype frequency changes. As we used mtDNA markers, the effective size estimate refers only to the females of the population. It seems that the effective number of females estimated for this common vole population is quite small  $(N_{ef})$ ranged from 9 to 17, respectively in the seasons compared). However, the values are not much lower than the estimated female census size  $(N_{c})$ of 49. The ratios of  $N_{\rm ef}/N_{\rm f}$  for the common vole population ranged from 0.18 to 0.35. Theoretical studies suggest that the ratio of effective population size to census size should range between 0.25 and 0.75 (Nunney & Elam 1994). However, Frankham (1995) suggested that  $N_{/N}$  may be substantially smaller because they did not take into account fluctuations in population size. Nunney (1996) reported two reasons why estimates of  $N_{a}/N$  would be small. The estimates of

 $N_{\rm o}$ , N or both may be inaccurate or high variances in family size are required to generate low  $N_{a}/N$ ratios. Another factor that would result in lower estimates of N/N is unequal sex ratios accounting for a 36% reduction (Frankham 1995). In the common vole population, sex ratios varied seasonally (Bryja et al. 2005) and fluctuations in the population size were noted (Adamczewska-Andrzejewska & Nabagło 1977); a low  $N_{\rm e}/N$  ratio may, therefore, be expected. It seems that fairly low  $N_{\rm ef}$  and female philopatry may be two important factors responsible for considerable inter-population differentiation previously reported (Ratkiewicz & Borkowska 2006, Schweizer et al. 2007) in this common and abundant vole species.

To conclude, this study illustrates the usefulness of genetic analysis and an aggregation index to detect and characterize patterns of a spatial population structure. The results indicate that social groupings create genetic structure within a population of the common vole. We showed, using a mtDNA marker and earlier ecological data, that a sex-related spatial structure was attributable to female philopatry and malebiased dispersal in the common vole.

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