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Seasonal changes of population genetic structure and relatedness in the bank vole *Clethrionomys glareolus*: An analysis of age cohorts

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The genetic consequences of age structure were investigated in five populations of the bank vole, *Clethrionomys glareolus*, using 18 variable allozyme loci. Temporal samples and age cohorts were analysed to detect seasonal differences in population genetic structure and relatedness between individuals. Changes in allele frequencies between seasons were found in one population. While over-wintered cohorts were genetically homogenous in all study populations, there were within year differences in allele frequencies among cohorts in two of the populations. Pairwise relatedness changed throughout the year and differed between sexes in some populations. The N_e/N ratio was high, presumably due to the promiscuous mating system in *C. glareolus*. Lack of correlation between genetic distance matrices from different seasons revealed that the pattern of interpopulation genetic structuring was not stable over time. The tendency for higher genetic differentiation among populations in autumn than in spring, as well as large differences between F_{IS} and F_{ST} in autumn indicated lower dispersal rate at the end of the breeding season than in the spring.

Introduction

The field of population genetics has traditionally focused on levels of genetic variation (and inbreeding) in populations as well as on average degree of genetic differentiation between populations (e.g., Stewart *et al*. 1999). It is also often implicitly assumed that the observed genetic patterns are stable over time. Studies that examine temporal changes in genetic structure and diversity over time are rare, and most detailed studies in this realm focus on invertebrate species with short generation times (e.g., Lessios *et al*. 1994 and references therein). A large body of literature exists about short and long-term dynamics of gene flow and temporal stability of genetic population structure in fish (e.g., Lundy *et al*. 2000, Heath *et al*. 2002). However, only a few studies have specifically been designed to assess temporal stability of population structure in mammals. Gaines *et al*. (1978) tried to demonstrate genetic changes over population cycles in various microtine species. Queney *et al*. (2000) used temporal changes in allele frequency to estimate effective population size (N_e) and putative genetic bottlenecks in the wild rabbit. Here, we present

empirical investigation of seasonal changes in population genetic structure of the bank vole (*Clethrionomys glareolus* Schreber, 1780).

The bank vole is a common microtine rodent that is distributed over most of Europe, except for some marginal southern and northern areas (Mitchell-Jones *et al*. 1999: pp. 214–215). The life history and ecology of the species have been thoroughly investigated (*see* Petrusewicz 1983 and Bujalska & Hansson 2000 for reviews). Annual reproductive season of bank voles lasts about six months (April–September), and they bear 3–4 litters during this time. The age differences among breeding animals are usually less than one year, and rarely, as much as 18 months (Gliwicz 1983). The age structure of the bank vole populations changes completely during these six months: in spring, there are only over-wintered individuals in populations, while in autumn the populations are made up of individuals born during the current year (Gliwicz 1983). Furthermore, spring-born young mature early and breed in the year of their birth, whereas late-summer and autumn-born animals remain immature and breed for the first time after they have over-wintered (Gliwicz *et al*. 1968). Hence, patterns of genetic relatedness in the population may change with time. Although relatedness between individuals in the bank vole populations is not known, the presence of female kin clusters has been reported in several experimental populations (Lambin & Krebs 1991, Mappes *et al*. 1995). In the bank vole, as in other *Clethrionomys* species, young females must acquire exclusive home ranges before they maturate (Bujalska 1970). Thus, the most common dispersers in this species are young voles born early in the spring (K1 cohort). They disperse from habitats densely populated by over-wintered animals into vacant habitats. Individuals born later in the season (K2 and K3 cohorts) do not emigrate from their natal habitats (Gliwicz 1993). However, adult females of *C. glareolus*, once established in breeding territories, are only rarely observed to disperse. Mature males are relatively mobile but breeding dispersal of males seems to be uncommon (Gliwicz & Ims 2000).

Differences in allele frequencies between spring and autumn samples of the bank vole have already been reported (e.g., Fedyk & Gębczyński 1980, Borkowska 1999), but the reasons for these differences remain unclear. Part of the reason for this is that observed seasonal changes in genetic structure were not analysed with respect to dynamics of age cohorts and relatedness between individuals. To this end, the aims of this study were: (1) to determine the genetic structure and effective size of the bank vole populations with respect to genetic differences between seasons as well as age cohorts; (2) to asses whether the degree of genetic relatedness among individuals varies between seasons, sexes, and age cohorts. In addition to this, we also (3) examined whether there was any evidence for genetic divergence among the bank vole populations to vary between seasons. The results are discussed in the context of the bank vole breeding tactics and dispersal patterns.

Table 1. Genetic diversity of *C. glareolus* samples from different seasons and populations. *N*, number of individuals; *A*, mean number of alleles per locus; H_E, mean expected heterozygosity calculated over 18 polymorphic loci; F_{IS}, inbreeding coefficient. Abbreviations of the populations are in parentheses.

Population	Sample	*N*	*A*	H_E	F_{IS}
Suprasl (SUP)	Spring 1996	31	2.06	0.167	–0.018
	Autumn 1996	38	2.06	0.166	0.034
Szelagowka (SZE)	Spring 1996	29	2.11	0.178	–0.085
	Autumn 1996	41	1.89	0.181	0.031
Białystok (BIA)	Autumn 1996	71	2.11	0.162	0.063
	Spring 1997	44	1.83	0.172	0.119
Przewalanka (PRZ)	Autumn 1996	41	2.06	0.177	0.099
	Spring 1997	31	2.06	0.191	0.002
Zednia (ZED)	Autumn 1996	39	1.78	0.148	0.042
	Spring 1997	26	1.83	0.162	0.008

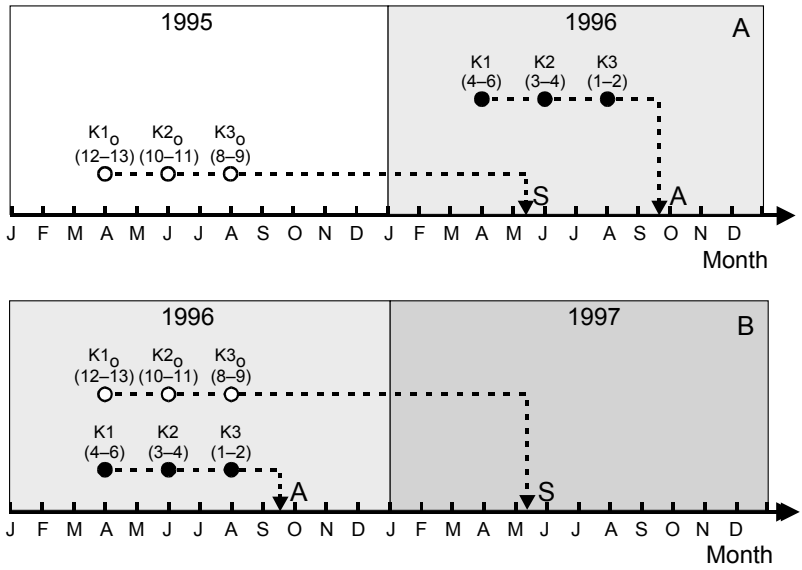

Fig. 1. Month of birth of individuals from age cohorts in five bank vole populations: SUP and SZE (A) and BIA, PRZ and ZED (B). $K1_o$, $K2_o$ and $K3_o$, cohorts of over-wintered voles caught in May; K1, K2 and K3, cohorts of the year caught in October; S, spring collection; A, autumn collection. Animals' age in months is given in parentheses.

Material and methods

Populations and age cohorts

Five populations of the bank vole were sampled in the vicinity of Białystok (NE Poland 23°07´E, 53°18´N) in 1996–1997, the distance between sampling sites ranging from 10 to 50 km (Table 1). A total of 390 animals were caught in live-traps during two seasons: spring and autumn. In two populations (SUP and SZE) voles were caught in May and October 1996, while in the other three populations (BIA, PRZ and ZED) animals were collected in October 1996 and May 1997.

The age of voles was estimated by measuring the length of M1 tooth roots as explained in Pucek and Zejda (1968). In the group of over-wintered voles collected in spring, there were individuals, which originated from all three cohorts (K1, K2 and K3) of the previous year. The over-wintered cohorts were caught and defined by their age in May 1996 (Fig. 1A) and May 1997 (Fig. 1B). They were named $K1_o$, $K2_o$, and $K3_o$. $K1_o$: 12–13 months old, $K2_o$: 10–11 months old and $K3_o$: 8–9 months old. Animals caught in autumn were divided into three cohorts, i.e. groups of individuals of similar age born at different times during the reproductive season. Three cohorts: K1, K2 and K3 were defined by their age in October 1996. K1: 5–6 months old (produced by over-wintered voles in April–May), K2: 2–4 months old (produced by over-wintered or K1 voles), and K3: 1–2 months old (produced by over-wintered, K1 or K2 voles, Fig. 1).

Electrophoretic analysis

Samples of blood plasma, kidney, liver and salivary gland were taken from each vole and stored at –80 °C until used. Starch gel, cellulose acetate plate and agar gel electrophoresis was performed according to Nielsen (1977) and Murphy *et al.* (1996). Thirty-seven isozyme loci were scored for each individual in all samples. Eighteen loci were found to be polymorphic, as defined by the presence of more than one allele: *Aat-1* and *Aat-2* (EC 2.6.1.1), *Acy* (EC 3.5.1.14), *Amy1-2* (EC 3.2.1.1), *Cat* (EC 1.11.1.6), *Dia* (EC 1.6.2.2), *EstB3* and *EstD* (EC 3.1.1.1), *Idh-2* (EC 1.1.1.42), *Ldh-1* and *Ldh-2* (EC 1.1.1.23), *Me-2* (EC 1.1.1.40), *Mpi* (EC 5.3.1.8), *Pep-2* (EC 3.4.11), *Pgd* (EC 1.1.1.44), *Pgm-1*, *Pgm-2* and *Pgm-3* (EC 2.7.5.1). The remaining 19 loci were monomorphic in all the populations studied and they were excluded from further statistical analysis (the list of monomorphic loci is available from the authors upon request).

Genetic variability and seasonal stability of population structure

The temporal variation of genetic diversity was quantified in terms of heterozygosity, number of alleles per locus and allele frequencies observed in the two temporal samples (spring and autumn) of a given population. Values for expected heterozygosity (H_E), allele frequencies and the number of alleles per locus (A) and inbreeding coefficient (F_{IS}) were calculated for 18 polymorphic loci using GENEPOP 3.3 (Raymond & Rousset 1995) and FSTAT 2.9.3 (Goudet 2001). We tested departures from Hardy-Weinberg and linkage equilibrium between pairs of loci in each seasonal sample with GENEPOP. We also analysed changes in allele frequencies between cohorts throughout reproductive period (SUP and SZE) and after winter (BIA, PRZ and ZED populations) using Fisher's exact tests as implemented in GENEPOP. Differences in H_E estimates and number of alleles per locus between temporal samples of each population were tested with a nonparametric Wilcoxon test. We used the program BOTTLENECK (Piry *et al*. 1999) to test for a recent reduction of effective population size under the assumptions of the infinite allele model (IAM). We estimated the effective population size (N_e) and 95% confidence intervals of N_e using temporal changes in allele frequencies (Wang 2001) with the help of the program MLNE (Wang & Whitlock 2003). We calculated N_e for SUP and SZE populations where over-wintered voles were sampled in spring and voles from the next generation were caught in autumn. In the other three populations (BIA, PRZ and ZED), the calculation of N_e was not possible as two temporal samples consisted of voles from the same generation. We also reported estimates of the N_e/N ratio, where N is the number of adults (breeding individuals, e.g. over-wintered voles and K1, and K2 cohorts).

Genetic differentiation of populations was estimated using F_{ST} index (Weir & Cockerham 1984) among all five populations in spring and autumn, and between temporal samples of a given population. F_{ST} estimates were tested for significant departure from zero by bootstrapping over loci using FSTAT. The statistical significance of between-season differentiation was tested with Fisher's exact test for allele frequency distributions (FSTAT). To investigate seasonal changes in population structure, a Mantel's (1967) test as implemented in FSTAT was used to study relationships between matrices of Rogers' genetic distances among the bank vole populations in various seasons.

Relatedness assessment

Estimates of relatedness between individuals within two seasonal samples (spring and autumn) of each population, and seasonal cohorts ($K1_o$, $K2_o$ and $K3_o$ in spring and K1, K2 and K3 in autumn) and within males and females, were obtained using a computer program KINSHIP 1.1.2 (Goodnight *et al*. 1997). The pairwise relatedness coefficient (r) is calculated between any two individuals by comparing the shared alleles of these individuals with the allele frequencies in the whole population. This assumes that the average relatedness within the population as a whole is 0 and r therefore varies between –1 and 1 (Queller & Goodnight 1989). Standard errors and 95% confidence intervals for the pairwise relatedness estimates were calculated and r values were judged to differ significantly from zero if their 95% CIs failed to overlap this value. Mann-Whitney and the Kruskal-Wallis tests were used to test the statistical significance of the differences between the mean pairwise relatedness estimates of individuals, males and females within seasonal groups, as well as among cohorts. For multiple tests, wherever significance testing occurred, the sequential Bonferroni correction was employed (Rice 1989).

Results

Genetic diversity within populations

No significant deviation from Hardy-Weinberg expectations (HWE) was observed within the bank vole populations. Both exact probability testing and permutation testing of F_{IS} values failed to detect a significant deviation from HWE after a sequential Bonferroni correction.

The number of linkage disequilibria considered significant after sequential Bonferroni correction was 0–2 departures. Thus, linkage disequilibrium does not appear to be a common feature for *C. glareolus* either in spring or in autumn. Similar levels of genetic variability were observed in seasonal samples of the five populations (Table 1). The total number of alleles, mean number of alleles per locus and heterozygosity values were similar between temporal samples at the same location (Table 1). No significant differences (Wilcoxon test: $P > 0.05$) in H_E and number of alleles per locus were observed between spring and autumn samples. Tests for genetic signatures of population bottleneck revealed no evidence for a recent bottleneck in any of the temporal samples from the five bank vole populations ($P > 0.05$). We have only one generation separating spring and autumn samples, $T = 1$ in the SUP and SZE populations. The maximum likelihood effective size was equal to 48.4 (95% CI: 20–1058) and 64.1 (95% CI: 27–40 000) in SUP and SZE, respectively. The ratio of N_e/N was estimated as 97% in the SUP and 126% in the SZE vole population.

Significant differences were observed in allele frequency distribution between seasonal samples in one of the five populations studied. A spring sample from the SUP population differed from the subsequent autumn sample and F_{ST} value reached 0.020 (Fisher's exact test: $P < 0.01$; Table 2). The BIA population also showed differentiation between seasonal samples: $F_{ST} = 0.015$ (Fisher's exact test: $P < 0.05$), but not after Bonferroni correction (Table 2). Analysis of genetic variation among the seasonal cohorts revealed that $K1_o$, $K2_o$ and $K3_o$ cohorts (overwintered individuals) were genetically homogenous in all the populations studied (Fisher's exact test: $P > 0.05$). On the other hand, there were statistically significant differences in allele frequencies among cohorts within the same year (K1 *vs.* K2 *vs.* K3) in the BIA population (Fisher's exact test: $P < 0.01$ over all the loci) and in the PRZ population (Fisher's exact test: $P < 0.001$ at *EstB3* locus only). We noted a significant decrease in the frequency of the most common allele during the reproductive period (from K1 to K3) in the BIA (Fisher's exact test: $P < 0.01$ at *Pgm-3* locus), and the PRZ popula-

Table 2. Pairwise F_{ST} estimates and their 95% confidence intervals (CI) among five of the bank vole populations in spring and autumn, and between the two periods for each sample of voles originating from the same population.

Population	F_{ST} (95% CI)			
	Spring 1996 *vs.* Autumn 1996			
SUP	0.020*	(0.006 to 0.041)		
SZE	0.008	(–0.001 to 0.018)		
	Autumn 1996 *vs.* Spring 1997			
BIA	0.015	(–0.005 to 0.033)		
PRZ	0.004	(–0.008 to 0.028)		
ZED	0.005	(–0.008 to 0.028)		
	Spring		Autumn	
BIA *vs.* PRZ	0.015*	(–0.002 to 0.038)	0.010*	(0.002 to 0.021)
BIA *vs.* ZED	0.028*	(0.005 to 0.046)	0.018*	(0.009 to 0.029)
BIA *vs.* SUP	0.019*	(–0.002 to 0.043)	0.046*	(0.010 to 0.083)
BIA *vs.* SZE	0.007	(–0.007 to 0.020)	0.021*	(0.002 to 0.037)
PRZ *vs.* ZED	0.007	(–0.010 to 0.034)	0.016*	(0.004 to 0.043)
PRZ *vs.* SUP	0.017	(–0.002 to 0.023)	0.037*	(0.012 to 0.066)
PRZ *vs.* SZE	0.016*	(0.001 to 0.033)	0.027*	(0.006 to 0.048)
ZED *vs.* SUP	0.001	(–0.012 to 0.008)	0.015	(0.005 to 0.028)
ZED *vs.* SZE	0.008	(–0.003 to 0.025)	0.027*	(0.011 to 0.055)
SUP *vs.* SZE	0.001	(–0.009 to 0.004)	0.050*	(0.022 to 0.071)

* Significant ($P < 0.05$) differences after adjustments for multiple tests with sequential Bonferroni correction.

tion (Fisher's exact test: $P < 0.001$ at *EstB3* locus). In populations SUP and SZE, where voles were caught twice during the same reproductive period, we found no genetic differences between over-wintered voles ($K1_o + K2_o + K3_o$) and the K1 and K2 cohorts (Fisher's exact test: $P > 0.05$). Only the K3 cohort differed in allele frequencies from over-wintered animals in the SUP population (Fisher's exact test: $P < 0.01$ over all the loci). In two populations, where voles were caught before and after winter, the K1 cohort differed from $K1_o$ (PRZ: $P < 0.01$ at *EstB3* and *Me-2* loci; ZED: $P < 0.01$ at *EstD* locus) and for these loci significant increase in frequency of the most common allele was observed.

Relatedness between individuals in seasons and age cohorts

The estimates of average relatedness between a pair of voles were relatively low in both seasons and ranged from –0.041 to –0.016 in spring and from –0.026 to 0.063 in autumn. In spring samples all but one relatedness estimates were not significantly different from zero, while in autumn there were both significant negative and positive values of *r* coefficient (Table 3). Negative relatedness values indicate that individuals were less related than would be expected for a pair taken at random from a randomly mating population in spring. The same was observed when the data were divided according to two sexes (Table 3). The differences in pairwise relatedness were significant between two seasonal samples in the SUP population (Mann-Whitney test: $P < 0.001$). The same relationship was found for males in this population (Mann-Whitney test: $P < 0.001$). The pairwise relatedness estimates for the two sexes differed significantly in the SUP and SZE populations in autumn (Mann-Whitney test: $P < 0.05$, Table 3).

The $K1_o$, $K2_o$ and $K3_o$ cohorts differed among each other in mean pairwise relatedness in the SUP population in spring, while in autumn such differences were found in the other two populations (BIA and PRZ, Table 4). Two out of 14 values of the pairwise relatedness estimates cal-

Table 3. Mean (± SE) and 95% confidence intervals (CI) for estimates of pairwise relatedness (*r*) in populations, males and females in two seasons; *N*, sample size.

Population	Spring 1996		Autumn 1996	
	r ± SE (*N*)	95% CI	*r* ± SE (*N*)	95% CI
SUP	–0.037 ± 0.019 (31)	–0.073 to 0.001	0.063 ± 0.016* (38)	0.032 to 0.093
Males	–0.034 ± 0.025 (23)	–0.084 to 0.015	0.205 ± 0.030* (18)	0.145 to 0.265
Females	–0.005 ± 0.073 (8)	–0.156 to 0.145	–0.057 ± 0.032 (20)	–0.120 to 0.006
SZE	–0.040 ± 0.020* (29)	–0.079 to –0.002	–0.026 ± 0.014 (41)	–0.052 to 0.001
Males	–0.025 ± 0.029 (20)	–0.082 to 0.033	0.017 ± 0.023 (25)	–0.029 to 0.063
Females	–0.034 ± 0.062 (9)	–0.160 to 0.091	–0.078 ± 0.031* (16)	–0.139 to –0.017
	Autumn 1996		Spring 1997	
	r ± SE (*N*)	95% CI	*r* ± SE (*N*)	95% CI
BIA	–0.025 ± 0.008* (70)	–0.041 to –0.009	–0.020 ± 0.015 (44)	–0.049 to 0.010
Males	–0.060 ± 0.018 (32)	–0.095 to –0.025	0.004 ± 0.024 (26)	–0.043 to 0.051
Females	–0.005 ± 0.015 (38)	–0.035 to 0.024	–0.094 ± 0.040* (18)	–0.172 to –0.016
PRZ	0.040 ± 0.013* (41)	0.015 to 0.065	–0.016 ± 0.017 (31)	–0.049 to 0.018
Males	0.010 ± 0.028 (18)	–0.046 to 0.066	–0.022 ± 0.031 (17)	–0.083 to 0.040
Females	0.070 ± 0.024* (23)	0.023 to 0.118	–0.022 ± 0.038 (14)	–0.098 to 0.055
ZED	–0.016 ± 0.014 (39)	–0.044 to 0.011	–0.041 ± 0.022 (26)	–0.085 to 0.003
Males	–0.048 ± 0.029 (19)	–0.101 to 0.014	0.009 ± 0.034 (16)	–0.060 to 0.077
Females	0.001 ± 0.027 (20)	–0.053 to 0.055	–0.089 ± 0.065 (10)	–0.220 to 0.042

* $P < 0.05$.

culated for over-wintered cohorts, and 5 out 15 values for cohorts caught in autumn, were positive (Table 4). Positive *r* values might indicate that individuals from autumn cohorts were more closely related than would be expected for a randomly mating population.

Seasonal differentiation among populations

The pattern of interpopulation genetic differentiation was not similar for the two seasonal periods. There was a low, albeit significant, genetic differentiation among vole populations in spring (F_{ST} = 0.012; 95% CI: 0.003–0.023; $P < 0.01$). The value of F_{ST} was twice as high in autumn than in spring (F_{ST} = 0.027; 95% CI: 0.018–0.034; $P < 0.001$), but the difference in genetic differentiation among populations between seasons was not significant ($P > 0.05$ after 10 000 permutations). Pairwise F_{ST} values in spring ranged from 0.001 to 0.028 and corresponding probabilities indicated population differentiation in 4 out of 10 pairs (Fisher's exact test: $P < 0.01$). In autumn, we found significant subdivision between 9 out of 10 population pairs and F_{ST} values ranged from 0.010 to 0.050 (Fisher's exact test: $P < 0.01$, Table 2). The low correlation of two Rogers' distance matrices from different seasons revealed that genetic differentiation among the bank vole populations in spring was not similar to interpopulation differentiation in autumn (Mantel test: $r = 0.06$, $Z = 0.01$, $P > 0.05$). In spring, the BIA population was the most divergent from the other three populations (PRZ, ZED and SUP), while in autumn the SUP population was the most divergent from BIA, PRZ and SZE populations (Table 2).

Discussion

The first objective of this study was to determine whether genetic variation within populations might reflect the existence of genetic differences between seasons and age cohorts. We found differences in allele frequencies between spring and autumn samples from only one population (SUP). Thus, seasonal differences were not general, but

Table 4. Mean (± SE) and 95% confidence intervals (in parentheses) for estimates of pairwise relatedness (*r*) in age cohorts (K1, K2 and K3) in spring and autumn; *P* = probability from Kruskal-Wallis testing differences among cohorts. Sample sizes are shown as ratio of K1 to K2 and K3.

Population	Sample size	Age cohorts			*P*
		$K1_o$	$K2_o$	$K3_o$	
Spring					
SUP	6:13:12	0.272 ± 0.068* (0.125 to 0.419)	0.075 ± 0.044 (–0.013 to 0.164)	–0.162 ± 0.048* (–0.258 to –0.066)	0.0001#
SZE	12:15:2	–0.104 ± 0.051* (–0.206 to –0.003)	–0.027 ± 0.038 (–0.101 to 0.048)	–	0.2730
BIA	6:18:20	–0.078 ± 0.187 (–0.560 to 0.404)	0.046 ± 0.034 (–0.021 to 0.114)	–0.086 ± 0.034* (–0.154 to –0.018)	0.0580
PRZ	6:14:11	–0.170 ± 0.115 (–0.430 to 0.089)	–0.035 ± 0.039 (–0.113 to 0.043)	0.118 ± 0.041* (0.035 to 0.201)	0.0331
ZED	7:13:6	–0.047 ± 0.085 (–0.224 to 0.129)	–0.087 ± 0.050 (–0.186 to 0.011)	–0.017 ± 0.086 (–0.200 to 0.167)	0.7751
Autumn		K1	K2	K3	
SUP	7:12:19	0.010 ± 0.074 (–0.144 to 0.163)	0.134 ± 0.052* (0.030 to 0.239)	0.136 ± 0.032* (0.074 to 0.198)	0.3693
SZE	13:9:19	–0.054 ± 0.043 (–0.141 to 0.032)	–0.116 ± 0.076 (–0.270 to 0.039)	0.005 ± 0.027 (–0.049 to 0.058)	0.3206
BIA	33:20:18	–0.094 ± 0.019* (–0.131 to –0.056)	0.070 ± 0.030* (0.012 to 0.129)	0.084 ± 0.027* (0.030 to 0.138)	0.0001#
PRZ	16:12:13	0.145 ± 0.033* (0.080 to 0.210)	–0.050 ± 0.043 (–0.136 to 0.036)	0.071 ± 0.039 (–0.006 to 0.149)	0.0036#
ZED	13:16:10	0.065 ± 0.038 (–0.010 to 0.140)	–0.066 ± 0.039 (–0.144 to 0.012)	–0.051 ± 0.059 (–0.171 to 0.068)	0.0553

* $P < 0.05$, # $P < 0.05$ after sequential Bonferroni correction

they could occur in the bank vole populations, especially in unstable environments. The SUP population occupied alder woodland on a small depression that was deluged with rain in early spring of 1996. The spring inundation might result in die-off of over-wintered animals and an increase in the proportion of younger cohorts (or migrants) among breeding voles. Therefore, we observed differences in allele frequencies between seasons and over-wintered individuals and K3 cohort, that were not observed in the stable SZE population.

The cohorts of over-wintered individuals were genetically homogenous in all of the study populations. However, there were allele frequency differences among cohorts within the same year in two populations. The decrease in the frequency of the most common allele in some loci during the reproductive period could be a result of promiscuous mating system with multiple paternity (Ratkiewicz & Borkowska 2000). The consequence of such a mating system is an increase in the genetic diversity of offspring and the number of heterozygotes in the whole population (Sugg & Chesser 1994). Thus, during reproductive period, the frequencies of common alleles decrease while rare alleles increase in their frequency. Opposite, an increase in frequency of the most common allele for some loci after winter could be explained by the loss of individuals possessing rare alleles. The rare alleles lost on a local scale could be replaced by common alleles, introduced in early spring by immigrants.

The bank vole populations studied do not seem to have experienced a reduction of effective population size due to a recent bottleneck. The likelihood estimate of N_e from temporal changes of allele frequencies was high, and the N_e/N ratio was close or even exceeded one. The lack of large fluctuations in the bank vole population dynamics in central Europe (Hansson *et al*. 2000) and mating system with multiple paternity (Ratkiewicz & Borkowska 2000) increase effective population sizes and lower the effects of genetic drift. Thus, other factors, such as different migration patterns in spring and autumn might be responsible for temporal changes in genetic structure of vole populations.

The second aim of our study concerned relatedness between individuals in different seasons, age cohorts and sexes. Degree of relatedness can vary between seasons as we found in the SUP population. Ritland (2000) notified two processes that might promote variation of relatedness: philopatry in stable environments, and founder events in stochastic environments. In the bank vole population females that start the breeding season have been thought to be 'founders' of a new generation. *Clethrionomys* females show rigid territoriality: only females with a territory gain breeding status (Bujalska 1991). Thus, in spring, female aggressiveness peaks when they take up exclusive home ranges, and very few dispersing females are able to establish themselves in breeding populations (Mappes *et al*. 1995). In summer, food resources become more abundant than in early spring (the environment becomes more stable), so females born in spring and summer are philopatric and often breed close to their mother and sisters (Lambin & Krebs 1991). Thus, at the end of the breeding season vole populations consists of related females and their offspring, and consequently, as we showed in the SUP population, the average degree of pairwise relatedness is higher in autumn than in spring.

Ideally, individuals sampled for the estimation of population genetic structure should belong to the same generation (or the same cohort for organisms with overlapping generations), because allele frequencies vary not only over space, but also over time. In our study, we did not find a clear pattern of seasonal differentiation among five populations of *C. glareolus*, but we noted seasonal changes in allele frequencies and relatedness between individuals in one population. Lack of correlation between the two genetic distance matrices from spring and autumn showed that the pattern of interpopulation genetic structuring was not stable over time. Nevertheless, our results empirically confirmed the assumption that timing of sampling could indeed affect the level of population structuring (Basset *et al*. 2001). Basset *et al*. (2001) found that F_{ST} values were always higher when sampling was carried out before than after dispersal, this difference being more pronounced with high migration rates. In our study, there was a tendency for higher F_{ST} values among populations in autumn ($F_{ST} = 0.027$) than in spring ($F_{ST} = 0.012$). The variability in dispersal rates is

known to influence genetic variance. Low dispersal amplifies the differences between F_{IS} and F_{ST} (Basset *et al*. 2001). As expected, greater differences between F_{IS} and F_{ST} occurred in autumn (F_{IS} = 0.058; F_{ST} = 0.027) than in spring (F_{IS} = 0.011; F_{ST} = 0.012). Thus, we conclude that over-wintered bank voles disperse effectively in early spring before the reproductive period started. Gliwicz (1988) found that the dispersal rates of *C. glareolus* individuals vary significantly with seasons, being the lowest in summer and early autumn. The low dispersal rate in the middle and at the end of the breeding season probably results in restricted gene flow, leading to differentiation of the populations in autumn. Dispersal in early spring may cause dilution of philopatric maternal relatives aggregated closely during winter. Hence, there was a tendency for a lower genetic differentiation among populations and lower average pairwise relatedness values in spring than in autumn.

To conclude, this study illustrates the usefulness of genetic analysis performed on seasonal samples and age cohorts, not only to infer changes in interpopulation genetic differentiation, but also to assess the complexity of population structure throughout the year. We have shown that temporal variation should be a major consideration in interpreting population genetic structure. Hopefully, temporal genetic studies will become more common in mammal populations, and patterns of genetic changes over time within and among populations will emerge to aid in the critical evaluation of various models in genetics and ecology.

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