Genetic variation in a fluctuating population of the yellow-necked mouse *Apodemus flavicollis*

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Protein polymorphism in 767 yellow-necked mice *Apodemus flavicollis* (Melchior, 1834), was studied in spring and autumn during five years in the Białowieża National Park, Eastern Poland. Two peaks and two declines of population number were observed, and population density varied from 3 to 63 inds/ha. Twenty three loci of proteins were studied, 4 of them were polymorphic; and mean heterozygosity of the studied population was $H = 0.057$. The greatest variability was observed in the transferrin locus and 4 alleles (Trf^a, Trf^b, Trf^c, and Trf^d) were described. The frequency of the Trf^a allele and Trf^a/Trf^a homozygotes was negatively correlated with population density in autumn, whereas the frequency of the Trf^c allele and Trf^a/Trf^c heterozygotes was positively correlated with population density in autumn. The frequency of heterozygotes was higher than the frequency of homozygotes during each spring. Significant excess of Trf^a/Trf^c heterozygotes, compared to the values expected from the Hardy-Weinberg ratio, was observed in autumn 1983 and spring 1984, 1986, and 1987. Natural selection may be an important force in maintenance of the transferrin polymorphism in *A. flavicollis*. It is suggested that heterozygotes survive better than homozygotes during winter and at high population densities. It is also supposed that non-selective forces influence on the *Trf* allele frequency changes in the studied population of *A. flavicollis*.

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Introduction

The use of electrophoresis to identify the allozymic variation showed that plant and animal populations are genetically highly variable (Frydenberg and Simonsen 1973, Nevo 1978, 1983). Electrophoretically variable proteins were often used as markers in studies concerning the factors involved in maintenance of genetic variation in populations. Natural selection, genetic drift, mutations, and migrations may change a gene pool. Since they usually act simultaneously in different combinations and intensities, there are many difficulties in interpretation of genetic variation in population. Population number changes may also influence the genetic structure of population.

Periodic fluctuations in population density were commonly observed in rodents, especially voles *Microtinae*. Many hypotheses have been proposed to explain the

causes of population cycles (see e.g. Taitt and Krebs 1985). Chitty (1960, 1967) suggested that demographic changes are maintained by natural selection acting on genetic structure of population. In many studies, the frequency of alleles and genotypes correlated with population density in various rodents: *Microtus* (Semeonoff and Robertson 1968, Tamarin and Krebs 1969, Gaines and Krebs 1971, LeDuc and Krebs 1975, Gaines *et al.* 1978, Kohn and Tamarin 1978, Dobrowolska 1983, Gulyaeva 1983), *Clethrionomys* (Fedyk and Gębczyński 1980, Mihok *et al.* 1983, Hall and Semeonoff 1985, Kuryshev and Chernyavsky 1988), and *Apodemus* (Leigh Brown 1977). In spite of the correlations between genetic changes and density it was very difficult to distinguish the cause and effect of this phenomenon. Most of these studies were carried out in permanently reproducing vole populations, therefore, the changes of allele frequency associated with season and age structure of population were perhaps mixed with allele changes correlated with population fluctuations.

The yellow-necked mouse *Apodemus flavicollis* (Melchior, 1834) is characterized by fluctuations of population density. In woodlands of Central Europe, this species shows population dynamics patterns characterized by predominant seasonal variations and irregular multiannual fluctuations in numbers (review in Flowerdew 1985). Seasonal and multiannual fluctuations in population number were described by various authors in *A. flavicollis* from the Białowieża National Park (Adamczewska 1961, Grodziński *et al.* 1966, Wójcik and Wołk 1985, Wołk and Kozłowski 1989). Pucek *et al.* (1993) found that the extraordinarily high population numbers (outbreaks) in *A. flavicollis* occurred in 1977, 1983 and 1990 – one year after the mast (synchronous seed crop of oak, hornbeam, and maple) and was followed by rapid decline of numbers (crashes). All other years were characterized by intermediate abundance of mice. The analysis of cyclicity indices for autumn abundances showed, that intermediate years were characterized by very low values, typical of stable populations and outbreak-crash years were characterized by high cyclicity indices, typical of cyclic populations.

In the present study I investigated whether there are any correlations between seasonal and multiannual fluctuations in numbers and the genetic structure of *A. flavicollis* population. Thus, the specific aims of the present study were as follows: (1) description of protein variation in *A. flavicollis*; (2) analysis of changes of allele and genotype frequency of transferrin in relation to different seasons, population density, age, and body mass of animals; (3) an attempt to explain the factors involved in maintenance of transferrin polymorphism in *A. flavicollis* population.

Material and methods

Trapping of mice and age determination

A total of 767 yellow-necked mice was collected from autumn 1983 to spring 1988 in the Białowieża National Park in the mature deciduous forest (Querco-Carpinetum) composed of oak *Quercus robur*, linden *Tilia cordata*, and hornbeam *Carpinus betulus*, with admixtures of spruce *Picea abies*

and few other tree species. Two hundred and eighty-eight live traps were distributed at 20 m intervals in a 12 × 12 grid of 5.76 ha (in 144 double stations). Mice were collected during 5 days twice a year: in spring (May) and autumn (September-October). In spring (April) 1988 trapping was conducted according to a different scheme. During 4 weeks, the catch-mark-release method was applied, and a small sample of blood from a tail of each mouse was collected into heparinized capillary for electrophoresis. Each mouse was marked by toe clipping at the first capture. At the end of May, a total of 19 from 27 marked mice were captured and brought to the laboratory.

Population density index (see Table 1) was calculated in two ways: (1) according to Zippin's formula based on maximum likelihood method (Southwood 1966), and (2) a total number of captured mice in following seasons divided by 5.76 ha of the trapping area. The index calculated by the second method was used to correlate population density and the frequency of transferrin alleles.

Captured mice were killed with chloroform in the laboratory. Body weight, sex, and reproductive condition were recorded. Skulls were removed for age determination.

Some pregnant females captured during spring seasons were housed singly in breeding cages till parturition. The females and their laboratory-born offspring were killed after parturition, and tissues were collected for electrophoresis. Blood samples only were collected from new-born mice.

Age of mice was determined on the basis of teeth growth and wear (Adamczewska-Andrzejewska 1967). Two age groups were distinguished: young (< 3 months) and adults (≥ 3 months).

Electrophoresis

Blood, liver and kidney were used for screening all proteins. Blood was collected from heart and prepared for electrophoresis according to the method described by Selander *et al.* (1971). Tissues were frozen at −20°C until use. Horizontal electrophoresis was performed with gels composed of 13% starch hydrolyzed in the laboratory. The following proteins systems were screened (abbreviation, E.C. number and tissue used are given in parentheses: H-hemolysate, K-kidney, L-liver, P-plasma): albumin (ALB, P), alcohol dehydrogenase (ADH, E.C. 1.1.1.1, L), esterases (ES, E.C. 3.1.1.1, H, P), glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.49, L), glucose-phosphate isomerase (GPI, E.C. 5.3.1.9, K), glutamate-oxaloacetate transaminase (GOT, E.C. 2.6.1.1, L), α-glycerophosphate dehydrogenase (GPD, E.C. 1.1.1.8, K), hemoglobin (HB, H), indophenol oxidase (IPO, E.C. 1.15.1.1, K), lactate dehydrogenase (LDH, E.C. 1.1.1.27, K), leucine aminopeptidase (LAP, P), malate dehydrogenase (MDH, E.C. 1.1.1.37, K), malic enzyme (ME, E.C. 1.1.1.40, K), mannose phosphate isomerase (MPI, E.C. 5.3.1.8, K), phosphoglucomutase (PGM, E.C. 2.7.5.1, K), phosphogluconate dehydrogenase (PGD, E.C. 1.1.1.44, K), sorbitol dehydrogenase (SDH, E.C. 1.1.1.14, L) transferrin (TRF, P).

Buffers and staining procedures were used according to Selander *et al.* (1971), Harris and Hopkinson (1976) – MPI, and Tomaszewska-Guszkiewicz *et a*l. (1971) – TRF.

Transferrin was stained in two ways: (1) gels were placed in 2% amido black 10B solution and washed with ethanol : water : acetic acid (5:5:1); (2) gels were placed in Nitroso R salt solution (Mueller *et al.* 1962) and washed with ethanol : water : acetic acid (5:5:1).

The genetic interpretation of electrophoretic patterns was based on analysis of bands on gels representative of homozygotes and heterozygotes. The information given by Harris and Hopkinson (1976) was also used for analysis of the structure of studied proteins. Presumptive alleles were designated alphabetically by their relative mobility, the allele variant migrating further anodically denoted as *A*.

Heterozygosity (*H*) was estimated from electromorphic genotypes according to the method given by Nei (1978), and averaged across loci to estimate the percentage of loci heterozygous per individual per population.

Statistical analysis

Genetic differentiation between samples and deviations of *Trf* genotype frequencies from Hardy-Weinberg proportions in samples from following years were tested using Wright's (1965)

F-statistics modified by Nei (1977). Significance of genotype excess and deficiency was tested using χ^2-test:

$$\chi^2 = NF^2_{IS}$$

where N is the total sample size (Li 1955). Significance of allele frequency differences between populations was tested for each locus by the χ^2-test:

$$\chi^2 = 2NF_{ST}(k\text{-}1)$$

with $(k\text{-}1)(s\text{-}1)$ degrees of freedom, where N is the total sample size, k is the number of alleles for the locus, and s is the number of populations (Workman and Niswander 1970).

Deviations from sex ratio 1:1, were tested by G-test for goodness of fit (Sokal and Rohlf 1981). Significance of allele and genotype frequency differences between samples collected in following years and seasons, and between samples of different age classes was tested using G-test of independence (Sokal and Rohlf 1981).

Relationships between body weight and genotype of transferrin among samples with two, one, and without the most common allele (Trf^a) were tested by analysis of variance ANOVA using Statistical Graphics System STATGRAPHICS (version 5, 1991). Significance of differences in body weight between males and females, and between different age groups in successive seasons was tested using Student t-test.

The transformation $arcsinp$, where p is the frequency of alleles or genotypes, was used for testing correlation between population density and allele and genotype frequencies.

Results

Population density vs sex and age structure

The number of captured mice varied in various seasons from 15 individuals in spring 1985 to 304 individuals in autumn 1983, and population density from 2.9 inds/ha to 62.8 inds/ha, respectively (Table 1). Population density was rather stable during spring seasons (about 3–5 inds/ha) except the one in spring 1986 (13

Table 1. Number of trapped A. *flavicollis*, estimated population density and number of mice used for electrophoretical study. A – autumn, S – spring. n.e. – not estimated, * – calculated from total number of mice removed from the study plot during 5 days of trapping, ** – density index estimated only for four days trapping, due to trap damages caused by wild boars, *** – 8 blood samples were collected from living animals in the field.

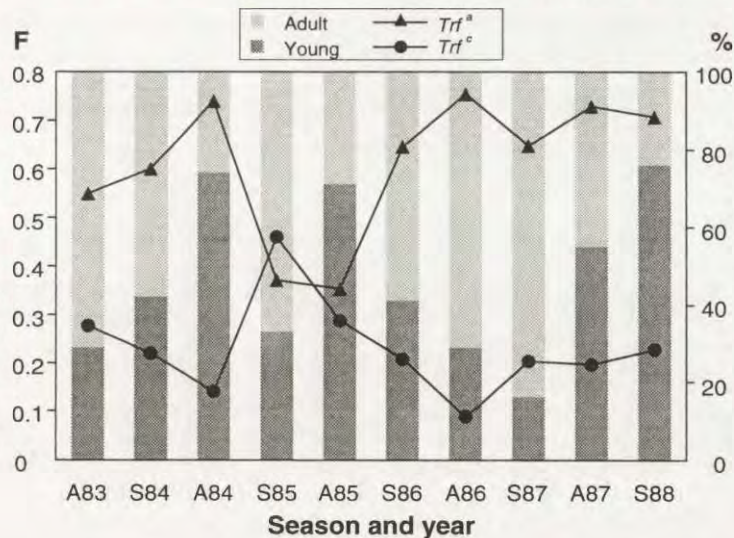| Year | 1983 | 1984 | | 1985 | | 1986 | | 1987 | | 1988 |
Season	A	S	A	S	A	S	A	S	A	S
n trapped mice	304	25	42	15	214	65	60	19	110	47
Zippin's density index, n/ha	62.8	4.7	7.3	2.9	n.e.	13.0	11.8	4.0	22.5**	9.6
Density index*, n/ha	52.8	4.3	7.5	2.6	37.1	11.3	10.4	3.3	19.1	8.2
Samples used for electrophoresis	184	25	38	15	201	65	57	27***	109	46

Fig. 1. Changes in frequency of Trf^a (triangles) and Trf^c (dots) alleles and percentage of adult and young individuals in the population of yellow-necked mouse (shaded bars). A – autumn, S – spring.

inds/ha). High fluctuations were observed during autumn seasons, with low density in 1984 and 1986, medium in 1987, and high in 1983 and 1985 (Table 1).

Sex ratio was closed to 1:1 in all study series, except the one from spring 1986, when significant excess of males was observed ($G = 4.5$, $p < 0.05$). Age structure of studied population varied in seasons and years (Fig. 1), and depended on the beginning and duration of the breeding period.

Genetic variation and heterozygosity

Among 23 loci studied 19 were monomorphic (*Alb, Adh, G6pd, Gpi, Got1, Got2, Gpd, Ipo, Ldh1, Ldh2, Lap, Mdh1, Mdh2, Me1, Me2, Mpi, Pgm1, Pgm3, Sdh*) and 4 polymorphic ones (*Pgd, Pgm2, Es1, Trf*). The frequency of polymorphic loci was $P = 0.17$. The mean heterozygosity calculated for 23 loci in a sample of 42 individuals from spring 1988 was $H = 0.057$. In the case of three variable systems observed on gels (HB and two ES systems), it was not possible to determine the genetic mechanism of inheritance (Table 2).

Polymorphic loci

Phosphogluconate dehydrogenase was coded by one locus (*Pgd*). Three bands were observed, and the commonest one showed intermediate mobility towards the anode. Fast migrating and slow migrating bands occurred mainly in heterozygous state.

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Table 2. The allele frequencies and heterozygosites (H) for each locus of studied proteins. ? – unscorable.

Protein	Locus	Allele	n inds	Allele frequencies	Heterozygosity (H)
ALB	Alb	a	767	1	0
ADH	Adh	a	42	1	0
ES	EsH	?	245	–	–
	Es0	?	723	–	–
	Es1	a	723	0.95	
		b		0.05	0.086
G6PD	G6pd	a	42	1	0
GPI	Gpi	a	42	1	0
GOT	Got1	a	42	1	0
	Got2	a	42	1	0
GPD	Gpd	a	42	1	0
HB	Hb	?	187	–	–
IPO	Ipo	a	42	1	0
LDH	Ldh1	a	99	1	0
	Ldh2	a	99	1	0
LAP	Lap	a	42	1	0
MDH	Mdh1	a	85	1	0
	Mdh2	a	85	1	0
ME	Me1	a	99	1	0
	Me2	a	99	1	0
MPI	Mpi	a	42	1	0
PGM	Pgm1	a	42	1	0
	Pgm2	a	42	0.31	
		b		0.69	0.381
	Pgm3	a	42	1	0
PGD	Pgd	a	42	0.07	
		b		0.85	
		c		0.08	0.262
SDH	Sdh	a	42	1	0
TRF	Trf	a	767	0.61	
		b		0.14	
		c		0.24	
		d		0.01	0.515

Phosphoglucomutase was polymorphic and three phenotypes were observed in *Pgm2* locus: a fast migrating anodal band, a slow migrating band, and a two-banded phenotype. These phenotypes suggest that the isoenzyme is a monomer.

Hemoglobin (Hb) migrated towards the anode. A wide band was observed in all studied mice and a narrow additional faster migrating band was found in some individuals. It was not possible to decide whether this variation had a genetic base, and the enzyme was not included in the calculation of population heterozygosity.

A large number of esterase isoenzymes were found in erythrocytes and plasma of *A. flavicollis* those made visible by three stains, based on the substrates α-naphtyl acetate, α-naphtyl butyrate, and α-naphtyl propionate. Only three esterases migrating anodally were scored: the one in erythrocytes migrating faster than hemoglobin and two ones, migrating fast, in plasma. The locus *Es1* migrating fast in plasma (stained using α-naphtyl acetate) showed clear variation. Three phenotypes were found in this locus and the enzyme was believed to be a monomer. Homozygotes were represented by a one-banded phenotype and heterozygotes by a two-banded phenotype. In the case of two other polymorphic loci, *EsH* and *EsO*, it was not possible to determine the genetic base of this variation.

Transferrin (Trf) was a monomer, which migrated towards the anode. The analysis of transferrin phenotypes of 7 females and their 34 offsprings showed that young had at least one allele common with their mother. It was found that the transferrin patterns in *A. flavicollis* were controlled by four alleles at an autosomal locus: Trf^a, Trf^b, Trf^c, and Trf^d. A one-banded phenotype in homozygotes and a two-banded phenotype in heterozygotes were observed.

No differences were found in the transferrin patterns and genotype frequency in 24 pregnant females and other studied females captured in spring seasons ($G = 6.19$, df = 7, $p > 0.3$). Also, no differences were found in transferrin phenotypes of mice whose blood was sampled two times during 4 weeks in spring 1987. These results suggest that physiological conditions of animals did not influence the transferrin variation in *A. flavicollis*.

Transferrin polymorphism

The frequency of transferrin alleles in the whole sample of mice was estimated: $Trf^a = 0.61$, $Trf^b = 0.14$, $Trf^c = 0.24$, and $Trf^d = 0.01$. Significant differences of Trf allele frequency between samples from all seasons were found ($F_{ST} = 0.0445$, $\chi^2 = 204.97$, df = 27, $p < 0.001$). No differences were found in Trf genotype and allele frequencies between sexes, and between young and adult mice in the whole material and in samples from consecutive seasons.

The frequency of transferrin alleles varied in samples from different seasons (Table 3). Trf^a was the most common allele, except spring 1985, when the frequency of Trf^c allele was higher than the frequency of Trf^a. The frequency of Trf^d was very low and this allele was only found in heterozygous state. The frequency of Trf^a and Trf^c alleles was not significantly correlated with the frequency of young and adult mice (Fig. 1).

Significant differences in the frequency of Trf^a/Trf^a homozygotes and Trf^a/Trf^c heterozygotes in various samples were found ($G = 27.5$, df = 9, $p < 0.01$). The

Table 3. Homozygote, heterozygote, and allele frequencies of transferrin in A. *flavicollis* from Białowieża National Park. A – autumn, S – spring.

Year	1983	1984		1985		1986		1987		1988
Season	A	S	A	S	A	S	A	S	A	S
n	184	25	38	15	201	65	57	27	109	46
Trf^a	0.549	0.600	0.740	0.370	0.535	0.646	0.754	0.648	0.729	0.707
Trf^b	0.169	0.120	0.070	0.170	0.172	0.115	0.158	0.148	0.060	0.065
Trf^c	0.277	0.220	0.140	0.460	0.288	0.208	0.088	0.204	0.197	0.228
Trf^d	0.005	0.060	0.050	0.000	0.005	0.031	0.000	0.000	0.014	0.000
Homozygotes	0.40	0.28	0.60	0.33	0.43	0.37	0.61	0.48	0.60	0.46
Heterozygotes	0.60	0.72	0.40	0.67	0.57	0.63	0.39	0.52	0.40	0.54

Table 4. F_{IS} statistics for homozygotes and heterozygotes in *Trf* locus indicated differences between observed and expected values according to the Hardy-Weinberg equilibrium. Genotypes *AD* and *BD* are not included. A – autumn, S – spring. [a] – $p < 0.05$, [b] – $p < 0.02$, [c] – $p < 0.01$, [d] – $p < 0.001$.

| Genotype	Year 1983	1984		1985		1986		1987		1988
	Season A	S	A	S	A	S	A	S	A	S
	n 184	25	38	15	201	65	57	27	109	46
AA	0.0119	−0.3333	0.0261	−0.0153	0.0094	−0.3448[c]	0.0562	−0.0548	0.0498	−0.2092
AB	0.3264[d]	−0.1111	−0.0160	−0.0599	0.1079	−0.4496[d]	0.1164	0.4207[a]	−0.0487	0.0539
AC	−0.1793[b]	−0.5151[b]	0.2380	0.0208	−0.0332	−0.2595[a]	−0.0576	−0.4009[a]	0.0737	−0.2812
BB	0.1449[a]	−0.1364	−0.0753	−0.2048	0.1416[a]	−0.1299	0.0761	0.4137[a]	−0.0638	−0.0695
BC	−0.1609[a]	0.2424	−0.3426[a]	−0.2788	0.1965[c]	0.6784[d]	−0.2618[a]	0.3866[a]	0.2238[b]	−0.4668[c]
CC	−0.1660[a]	−0.2820	0.2743	−0.0467	0.0322	0.0758	−0.0965	−0.2563	0.1026	−0.2953[a]
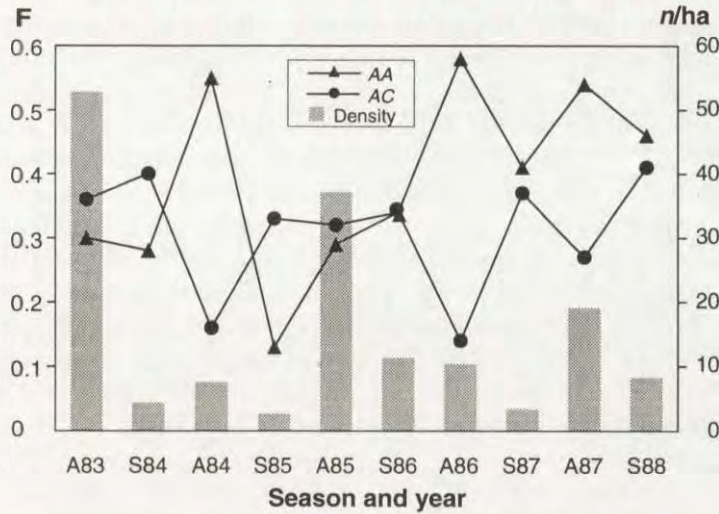
Fig. 2. Changes in frequency of Trf^a/Trf^a (triangles) and Trf^a/Trf^c (dots) genotypes and numbers of yellow-necked mouse (shaded bars). A – autumn, S – spring.

frequency of heterozygotes was higher than the frequency of homozygotes during spring of each year and in autumn 1983 and 1985, whereas a reverse proportion was observed during autumn 1984, 1986, and 1987 (Table 3).

The F_{IS}-statistics (a measure of deviations of genotype frequencies from Hardy--Weinberg proportions) varied in various samples (Table 4). Significant excess of Trf^a/Trf^c heterozygotes was observed in autumn 1983 and in spring 1984, 1986, and 1987. Deficiency of Trf^a/Trf^a homozygotes was observed during each spring, but only the value from spring 1986 was statistically significant. In the cases of other transferrin genotypes, significant deviations from Hardy-Weinberg proportions existed in some seasons but no clear directions of these differences were seen.

A clear frequency variation of the most common Trf genotypes was observed (Fig. 2). The frequency of Trf^a/Trf^a homozygotes increased from spring to autumn,

Table 5. Correlation coefficients between Trf allele frequency and population density, and between Trf^a/Trf^a homozygote and Trf^a/Trf^c heterozygote frequencies and population density of A. flavicollis. $* - p < 0.05$, $** - p = 0.05$.

Season	n	Trf^a	Trf^b	Trf^c
Spring	5	0.585	−0.647	−0.494
Autumn	5	−0.927*	0.606	0.874**
		Trf^a/Trf^a	Trf^a/Trf^c	
Autumn	5	−0.929*	0.924*	

while the frequency of Trf^a/Trf^c heterozygotes declined. Significant differences between frequencies of these genotypes were found in 1984 ($G = 6.04$, df = 1, $p < 0.02$) and in 1986 ($G = 8.9$, df = 1, $p < 0.01$). There were significant differences between frequencies of the Trf^a/Trf^a and Trf^a/Trf^c genotypes in autumn 1984, 1986, and 1987 ($G = 29.43$, df = 4, $p < 0.001$). In these seasons, the frequency of Trf^a/Trf^a was 2-3 times higher than the frequency of Trf^a/Trf^c.

A strong negative correlation between the frequency of Trf^a allele, and a strong positive correlation between the frequency of Trf^c allele and population density were found in autumn. Also, there were significant correlations between the frequency of Trf^a/Trf^a homozygotes and the population density in autumn as well as the frequency of Trf^a/Trf^c heterozygotes and the autumn density of mice (Table 5). No significant correlation was found between the frequency of Trf allele and genotypes and population density of A. flavicollis in spring.

No significant differences between body weight of homozygous (Trf^a/Trf^a) and heterozygous individuals (Trf^a/Trf^b, Trf^a/Trf^c) in age and sex groups from various seasons were found. Body weight was not associated with transferrin genotypes in age and sex groups of the whole sample. Body weight of the Trf^a/Trf^a homozygotes, heterozygotes with the Trf^a allele, and other Trf genotypes without the Trf^a allele did not differ statistically.

Discussion

The mean heterozygosity ($H = 0.057$) and the frequency of polymorphic loci ($P = 0.17$) in A. flavicollis population from Białowieża Forest was similar to those found in populations from different parts of the species range (Table 6).

Among various proteins, transferrin showed a clear variation in the studied population of A. flavicollis. Transferrin polymorphism was also described in various populations of this species (see Table 6), as well as, in different Apodemus species: A. agrarius (Dobrowolska and Chabros 1978), A. giliam (Moriwaki et al. 1971), A. sylvaticus (Gemmeke 1980), and A. penninsulae (Pavlenko et al. 1984).

Transferrin was studied in many other rodent species, e.g.: Microtus pennsylvanicus, M. ochrogaster, M. townsendii (Maurer 1967, Tamarin and Krebs 1969, Gaines and Krebs 1971, Gaines et al. 1971), Clethrionomys rutilus, C. gapperi (Canham and Cameron 1972), Peromyscus polionotus (Griswold and Dawson 1971) Peromyscus maniculatus (Dewsbury 1985), Mus musculus (Ashton and Dennis 1970). All these results suggest that transferrin variation is genetically controlled.

Nevertheless, some doubts have been raised about the reliability of transferrin electromorphs as genetic markers. Results of some studies suggested that the animal's physiological condition and environmental factors may influence transferrin variation. Gulyaeva and Olenev (1979) found that staining intensity of minor transferrin band changed during the reproductive cycle in Clethrionomys glareolus females and it was the strongest during pregnancy. They suggested that this

Table 6. Polymorphic proteins (1% level) in various studied populations of A. flavicollis. [1] Csaikl et al. (1980), [2] Gemmeke (1980), [3] Nascetti and Filippucci (1984), [4] Gębczyński et al. (1986), [5] Gill et al. (1987), [6] Filippucci et al. (1989), [7] Mezhzherin (1990), [8] Britton-Davidian et al. (1991), [9] Filippucci (1992), [10] Hartl et al. (1992), [11] present study. H – mean heterozygosity (for details see references), M – monomorphic, P – polymorphic, ? – unscorable, * – only two individuals examined; A, D, PL – Austria, Germany, Poland.

Protein	Austria[1,9]	Germany[2]	Italy[3,9]	Denmark[4]	Yugoslavia[5,9]	Israel[6,9]	Ukraine[7]	France[8]	A,D,PL[10]	Greece[9]	Sweden[9]	Poland[11]
H	–, 0.082	–	0.014, 0.020-0.064	0.059	0.056	0.045, 0.053-0.074	0.036-0.043	0.040	–	0.036-0.068	0.094	0.057
Loci	26, 28	11	28-35	36	21, 28	28-36	29	20	36	28	28	23
n	2-23, 12	23	69-83	36-40	11, 5	52-53	59	2-19	8	8	5	42-767
ADA	P	–	P	M	M	P	–	–	M	P	M	–
AMY	–	–	–	P	–	–	–	P	–	–	–	–
ES	P	–	P	P	P	P	P	–	–	P	P	P
G6PD	M	–	M	M	M	M	M	–	M	M	M	M
GPI	P	–	P	P	P	M	–	P	P	P	P	M
GOT	–	M	P	M	P	M	–	M	–	P	P	M
GPD	–	M	P	M	M	P	M	P	M	M	M	M
HB	M	–	–	M	P	–	M	M	–	–	–	?
HK	–	M	M	M	P	P	–	–	M	M	M	–
IDH	?	P	P	M	M	M	M	M	P	M	M	M
LDH	M	M	P	M	M	M	P	M	M	P	M	M
ME (MOD)	M	P	P	M	P	P	P	P	M	P	P	M
MPI	–	–	P	M	M	P	–	–	M	M	M	M
DIA	–	–	–	M	–	–	–	–	P	–	–	–
PEP	–	–	–	P	–	–	–	–	P	–	–	–
PGM	–	M	P	P	M	P	M	M	M	M	P	P
PGD	P	P	P	P	M	P	P	P	P	P	P	P
IPO (SOD)	M	M	M	–	M	M	P	M	M	M	M	M
TRF	M*	–	–	P	–	–	P	–	–	–	–	P

phenomenon was caused by higher iron saturation of transferrin in blood of pregnant females. However, Mihok *et al.* (1983) did not support the relative iron saturation of transferrin in various individuals of *C. gapperi*. McGovern and Tracy (1981) observed the changes in the mobility of transferrin electromorphs in field caught *M. ochrogaster* exposed to different temperature regimes in the laboratory. They questioned the suitability of transferrin as a marker in population studies of microtine rodents. In contrary, Mihok and Ewing (1983) studied transferrin in the same individuals in different period of their lives, and found the complete reliability of transferrin electromorphs in *M. pennsylvanicus*. It is also noteworthy that Gulyaeva and Olenev's (1979) and McGovern and Tracy's (1981) results are not consistent with extensive mating data on the transferrin system in *Clethrionomys*, *Microtus*, *Peromyscus* and *Mus* species (see e.g. Maurer 1967, Tamarin and Krebs 1969, Gaines and Krebs 1971, Gaines *et al.* 1971, Canham and Cameron 1972, Griswold and Dawson 1971, Dewsbury 1985, and Ashton and Dennis 1971).

In my study, no differences were found in transferrin electromorphs of *A. flavicollis* females in different phases of the reproductive period, and all genotypes found in this population occurred both in females and males. The analysis of transferrin electromorphs in females and their offspring suggested that transferrin variation is genetically controlled. Thus, I believe that an individual physiological condition does not affect transferrin electromorphs, and this protein can be used as genetic marker in population study.

Transferrin is capable of inhibiting or arresting the growth of a variety of iron--dependent microorganisms, many of which are pathogens (Schade and Caroline 1944, Kochan *et al.* 1969, cited after Allendorf and Leary 1986). It was also found that heterozygous transferrin of pigeons *Columba livia* and ring-necked pheasants *Phasianus colchicus* had a greater inhibitory effect on the growth of iron-dependent microorganisms than either of the homozygous transferrins (Frelinger 1972, Lucotte and Kaminski 1976, cited after Ferguson 1980). It is suggested that the greater ability of heterozygous eggs to resist microbial infection is at least partially responsible for the maintenance of transferrin polymorphism in birds (Frelinger 1972, cited after Ferguson 1980).

It seems, therefore, that also the *Trf* heterozygous individuals of *A. flavicollis* have greater ability than the homozygous ones to inhibit the growth of iron--dependent microorganisms. Possibly, this ability is responsible for better survival of heterozygotes in winter and in high population density. Excess of Trf^a/Trf^c heterozygotes in three spring samples of *A. flavicollis* and in high population number may be explained by a heterozygote advantage in winter and early spring and in high population density. The heterozygote advantage may also explain a high heterozygosity in *Trf* locus during spring seasons and the maintenance of the transferrin polymorphism in *A. flavicollis*. Similar results have been obtained by Mihok *et al.* (1983) in *Clethrionomys gapperi* and by Pemberton *et al.* (1988) in red deer *Cervus elaphus*.

The direction and intensity of natural selection on proteins of different species have been studied. Tamarin and Krebs (1969) found that usually the Trf^E/Trf^E homozygotes in *M. ochrogaster* had a better survival than the other transferrin genotypes. Certain *Trf* and *Lap* genotypes were favored during the increase phase of a population cycle of *M. ochrogaster*, while others were favored in the decline phase (Gaines and Krebs 1971). Significant differences in breeding activity, growth rates, and mean body weights among different *Trf* and *Lap* genotypes were found (Gaines *et al.* 1978). Birdsall (1974) suggested that deviation of genotypic frequencies from Hardy-Weinberg ratio may be interpreted as evidence for natural selection acting on the *Trf* and *Lap* loci in fluctuating populations of *M. pennsylvanicus*. Different patterns of deviations in the proportion of genotypes at the two loci during the density cycle were found. There was a significant excess of *Trf* heterozygous males in the decline phase, whereas a significant excess of *Lap* heterozygous males was recorded in the increase phase (Birdsall 1974).

Although natural selection may play an important role in maintaining polymorphism, non-selective forces (e.g. genetic drift and migration) may also influence the changes of allele frequency in populations. Gaines and Whittam (1980) found that the intensity of selection was not strong enough to counteract the effects of non-selective forces, such as genetic drift, in fluctuating *M. ochrogaster* population.

The studied *A. flavicollis* population from Białowieża was characterized by a high rate of winter mortality, and population density during spring was usually low. Therefore, a genetic drift may affect genotype frequency changes in the population (bottleneck). This probably occurred in spring 1985, when the frequency of Trf^c allele increased significantly, whereas in other seasons, the Trf^a allele was the most common one. Results obtained from epigenetic variation study showed that the sample from spring 1985 was significant different from the other samples in this *A. flavicollis* population (M. D. Sikorski and A. M. Wójcik, unpubl.).

The regularly observed bottlenecks in population density should result in a decline of allele number during periods of low densities. This was not observed in the studied *A. flavicollis* population. Moreover, the *Trf* frequency changes from autumn to spring were not random, but a similar change pattern was found in consecutive years. Thus, it may be suggested that the genetic drift was not a main force causing the *Trf* frequency changes in *A. flavicollis*.

Mating system may also affect the changes in genetic structure of population. It is suggested that *A. flavicollis* is characterized by a typical polygamy (Gliwicz 1988). Mature males perhaps maintain individual territories that are actively defended against other males. Several females may live on a male territory, and non-territorial males have a little chance to mate with them. Thus, genotypes of territorial males may have an important effect on genetic structure of population. Considerabley high frequency of certain allele in breeding (territorial) males should affect an increase of homozygosity in the next generation. This might have occurred in the studied population of *A. flavicollis* from Białowieża Forest in the

case of the Trf^a allele; the increase of Trf^a/Trf^a homozygote frequency from spring to autumn was observed in each year (Fig. 2).

Smith et al. (1975, 1978) and Garten (1976) found a positive correlation between mean heterozygosity, and population density in Peromyscus polionotus. Similar results have been obtained in populations of C. glareolus (Leitner and Hartl 1988) and P. maniculatus (Baccus and Wolff 1989). Smith et al. (1975) suggested that in low population densities inbreeding decreased heterozygosity, while in high densities, there was an increased dispersal and the population became relatively more outbred, leading to increased heterozygosity. A positive correlation between the heterozygote frequency in Trf locus and the population density in A. flavicollis was found in this study as predicted Smith et al. (1975).

Chitty (1967) proposed a genetic-behavioral hypothesis to explain multiannual density cycles in microtine rodents. The major principle of this hypothesis is that demographic changes are mediated by natural selection operating on the genetic structure of the population. According to Chitty's (1967) hypothesis allele frequency changes should be closely associated with density.

Several studies in different Microtus species confirmed that correlations between allele frequency changes in certain loci and density were consistent with Chitty's (1967) hypothesis, but it was difficult to distinguish cause and effect (Tamarin and Krebs 1969, Gaines and Krebs 1971, LeDuc and Krebs 1975, Gaines et al. 1978, Kohn and Tamarin 1978). Genetic changes may cause demographic changes or they may be an effect of density changes. Computer simulations carried out by Charlesworth and Giesel (1972) showed that a correlation between allele frequency and population density could be produced by a change in the rate of population growth. These results suggested that fluctuations in allele frequency could be a side effect of population cycles (see Charlesworth 1980, Gaines 1985). Gaines (1985) discussed evidences supporting Charlesworth and Giesel's (1972) contention that changes in allele frequency are effects of density changes. He summarized that, in spite of evidences from a number of studies confirming close correlations between allele frequency changes and density in Microtus populations, it is an error to interpret these association as support for Chitty's (1967) hypothesis. Strong correlations between the Trf allele frequency changes and population density was also found in A. flavicollis in the present study, but this does not seem to support Chitty's (1967) hypothesis. Theoretical and field studies (mainly in Microtus species) suggest that allele frequency changes at a single locus were effects of demographic changes of population rather than vice versa (see also Gaines 1985).

In summary, there are strong evidences that Trf allele frequency changes are closely associated with population density changes in a number of species of the genus Microtus, Clethrionomys and Apodemus. Natural selection may be an important force in maintenance of the transferrin polymorphism in small rodents. The excess of Trf heterozygotes in spring samples of A. flavicollis, compared to the expected number based on Hardy-Weinberg equilibrium, suggested that a

heterozygote advantage during winter and early-spring seasons is an important factor responsible for transferrin variation. Population density fluctuations, social structure, and mating system may also modify gene frequency changes. Future investigations concerning the physiological significance of transferrin and other protein polymorphisms will work towards our understanding of mechanisms responsible for the maintenance of genetic variation in natural populations. Finally, as Gaines (1985) proposed: "in the future, attempts to relate genetic structure to population regulation must focus on those traits deemed ecologically relevant".

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