

Correlation between Level of Chromosomal Aberrations and Demographic Parameters

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Abstract—The correlation between the level of chromosomal abnormalities and demographic parameters has been studied using individuals from a local wildlife population of the bank vole (*Clethrionomys glareolus*) from a Middle Ural locality in the southern taiga subzone (57°15' N, 58°44' E). Variations in the rate of structural chromosomal aberrations, gaps, and changes in the number of chromosomes in the bone marrow cells of the bank vole has been examined using routine cytogenetic methods. The effect of demographic parameters, i.e., population density, age, sex, and reproductive status, has been estimated using log-linear analysis. It has been shown that the share of individuals with an elevated rate of cells that carry structural chromosomal abnormalities and gaps decreases with increases the population size. This pattern agrees with the standpoint that a lower rate of mutations in somatic cells enhances survival of organisms. This pattern can have established as a consequence of natural selection, which induces the rearrangement of the genetic structure in the population or as a byproduct of other processes in the population that accompanies the changes in its size. A high population density leads to an increase in the share of individuals that display an elevated rate of cells with abnormal chromosome numbers among the yearling voles. A similar effect may result from an asymmetric interference competition between adults and yearling individuals. Our results demonstrate that the proportion of animals that display an elevated rate of somatic cells with structural chromosomal abnormalities and aberrant chromosome numbers considerably increases with age, which agrees with both the theoretical concepts and experimental data. Neither sex nor involvement in reproduction has any significant effect on the level of cytogenetic instability.

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INTRODUCTION

The errors in correcting abnormalities in DNA molecules used on various repair mechanisms lead to the emergence of chromosomal aberrations in living cells (Griffin and Thacker, 2004; Pfeiffer et al., 2004). It is known that the initial DNA structure is restored under genetic control (Thacker, 1999; Symington, 2002). Mutations in the genes that control repair processes may lead to an increase in the rate of chromosomal abnormalities in cells of various tissues, as well as to a delay in the growth of individuals and a decrease in their viability at a particular developmental stage (Difilippantonio et al., 2000; Gao et al., 2000; Nijnik et al., 2007). On the other hand, the survival rate as a characteristic of viability regularly changes during population cycles (Krebs and Myers, 1974). This suggests a correlation between the intensity of mutation processes and population density. The clarification and understanding of this correlation will enhance the insight into the mechanisms underlying the population dynamics of living organisms. The previous studies failed to provide a clear understanding of the pattern of this correlation (Dmitriev et al., 1997; Gileva et al., 2006).

The current view on the cyclic pattern of population dynamics is based on the understanding that this phenomenon is influenced by a multitude of factors, in particular food abundance and accessibility, weather conditions, predation, social interactions, and diseases (Krebs, 2009). An essential issue is the potential interaction between these factors. Our results suggest that the intensity of the mutation process depends on the size of the population.

MATERIALS AND METHODS

The object of this work was the wildlife population of the bank vole (*Clethrionomys glareolus*) from a Middle Ural locality in the southern taiga subzone (57°15' N, 58°44' E) with only a global technogenic pollution. The animals were trapped in the second half of July over 10 years (1999–2008). The population size was estimated according to the number of catches per 100 trap-days during the first 2 days of trapping. In a narrow sense, this is a relative population size and in a broad sense, it is characteristic of the population abundance of animals. In this work, the terms “population density” and “population size” are used in the broad sense as synonyms of animal abundance. The

animal age was estimated according to the alveolar shape and the root index of the second upper tooth (Olenev, 2009). The values of interannual abundance (D) varied from 2.5 to 69.0 individuals per 100 trapping days. The data for individual years were grouped into three categories of population size (D1–3), namely, low (below ten individuals per 100 trapping days), medium (10–30 individuals), and high (over 30 individuals). Three complete cycles of population dynamics, including 2-, 3-, and 4-year cycles, have been observed. Each year of medium abundance was followed by a year with a high population density. One peak was observed during the first observation year; the fourth took place after 3 years of low population size. The share of overwintered individuals in population increases with the population density from 15.2% at a low density through 18.3% at a medium density to 25.4% at a high density.

The rates of structural chromosomal aberrations (SCAs) and gaps (Gs), as well as the total rate of aneuploid and polyploid cells (numerical chromosome aberrations, NCAs), were used as characteristics of mutation intensity. The preparations of metaphase chromosomes were made of rodent bone marrow and stained with azure–eosin to examine 25–50 or more cells per each animal. Round metaphase plates with densely arranged chromosomes with or without (when possible) a minimal number of overlapping chromosomes, which did not prevent them from being counted and their state from being assessed, were selected for analysis. The chromosomes were counted in each metaphase cell, and the presence of SCAs and Gs were taken into account. Solitary fragments account for about 80% of SCAs; chromosomal translocations, including Robertsonian translocations, and paired fragments account for 7–8%; and chromatid translocations and multiple abnormalities account for 2–3%. Achromatic gaps are interruptions in the staining of chromatids. The true breaks differ from gaps based on the following criteria: shift relative to the chromatid axis and/or presence of a gap exceeding the chromatid width. It is still unclear whether gaps emerge as a result of chromosome breaks similar to true chromosomal aberrations. Nonetheless, some data suggest a similarity between the mechanisms inducing true breaks and gaps (Harvey et al., 1997), as well as the similarity between their rates in comparisons between and within populations (Gileva, 2002). It is considered necessary to include gaps in the analysis of chromosomal aberrations and to analyze their rates separately (Paz-y-Mino et al., 2002). Among the gaps, 94% are single, 4% are paired, and 3% are multiple. When considering aneuploidy, a special attention was paid to hypoploid cells (with the chromosome number below the diploid one, a normal number for the bone marrow). The fact is that chromosomes may be lost while making the preparations; correspondingly, only metaphases with ideally round shapes and fairly dense chromosome arrangements were taken

into account in the case of hypoploidy. Presumably, this approach has led to a certain underestimation of the rate of hypoploid cells; however, since the principles used in selecting the cells for analysis were constant, we believe that the degree of underestimation was approximately the same for all samples. The hyperploid cells (the cells with the chromosome number exceeding the diploid set) can be identified with sufficient reliability. For statistical analysis, the rates of aneuploid and polyploid cells were considered together, since the cytogenetic mechanism that leads to both types of NCAs are partially similar. The aneuploid cells accounted for 53% of NCA cells and polyploid cells accounted for 47%. The data on the rate of cells that carry chromosomal aberrations were used in the initial (SCA, NCA, and G) and categorized forms, i.e., SCA3 and G3 (1, 1% and less; 2, >1% to ≤4%; and 3, >4%); NCA3 (1, ≤1%; 2, >1% to ≤3%; and 3, >3%); and SCA2, NCA2, and G2 (1, ≤1% and 2, >1%). We have analyzed the data for 461 individuals (99 overwintered and 362 yearling animals). The annual values of vole abundance, the rates of the cells with different chromosomal abnormalities, sample sizes, and total numbers of examined cells are listed in Table 1.

The reproductive status of voles was estimated according to the state of their mating system. The pregnant and parous females with placental spots and/or embryos in their uterus and the males with a weight of their testicles exceeding 150 mg, developed epididymides, and pronounced spermatogenesis were regarded as reproducing (sexually mature) individuals. The data were statistically processed using the Statistica 6 software package (log-linear analysis, χ^2 -test, Spearman's rank correlation coefficient (R_s), and multiple regression analysis).

RESULTS

Initially, the effects of sex, involvement in reproduction, and population size represented in a categorized form (D1–3) on the level of chromosomal aberrations were assessed using the data for yearling animals and log-linear analysis. For SCAs, the effects of sex (for partial correlations, $\chi^2 < 1.8$, $df = 1-2$, $p > 0.40$) and reproductive status ($\chi^2 < 2.1$, $df = 1-2$, $p > 0.35$) were statistically insignificant, whereas the partial ($\chi^2 = 13.0$, $df = 2$, $p < 0.01$ and $\chi^2 = 14.2$, $df = 4$, $p < 0.01$) and marginal ($\chi^2 = 17.3$, $df = 2$, $p < 0.001$ and $\chi^2 = 18.5$, $df = 4$, $p < 0.001$) correlations with population size were statistically significant. With an increase in population size from a low level through medium to a high level, the rate of the individuals carrying over 1% of aberrations decreased from 54 to 39%, then further to 28% ($R_s = -0.19$, $n = 362$, $p < 0.001$). All effects were statistically insignificant for individuals that displayed over 4% of SCAs. For NCA2, both the partial and marginal correlations were statistically insignificant ($\chi^2 < 2.8$, $df = 1-2$, $p > 0.24$). For NCA3, the effect of population size was statistically significant

Table 1. Interannual variation in the population abundance of animals, sample sizes, and rates of chromosomal abnormalities

Year	Index of abundance (individuals/100 trap-days)	Number of animals	Number of cells	Mean cell rate (%)		
				with structural chromosomal aberrations	aneuploid and polyploid	with gaps
1999	54.0	69	2225	0.54	1.08	1.45
2000	13.1	60	2025	1.42	0.78	2.25
2001	37.2	81	2275	2.45	1.10	3.19
2002	6.8	39	1350	2.44	0.59	2.46
2003	17.5	33	1500	2.69	0.73	4.25
2004	69.0	62	2100	1.00	1.71	1.74
2005	2.5	13	1100	1.31	0.18	2.54
2006	9.2	28	1850	1.46	0.46	1.43
2007	5.7	32	1600	2.19	1.00	2.06
2008	50.0	44	2728	0.84	0.70	0.94

(for partial correlation, $\chi^2 = 13.7$, $df = 4$, $p < 0.01$ and for marginal correlation, $\chi^2 = 16.3$, $df = 4$, $p < 0.01$). With an increase in vole abundance, the proportion of individuals that displayed more than 3% of NCAs increased from 4% at a low population density through 9% at a medium density to 20% at a high density ($R_S = 0.20$, $N = 362$, $p < 0.001$). For G3 and G2, the effect of population size was statistically significant ($\chi^2 > 17.2$, $df = 4$, $p < 0.002$ and $\chi^2 > 11.2$, $df = 2$, $p < 0.01$, respectively), as was the interaction of the G rate with the reproductive status and population size ($\chi^2 > 12.1$, $df = 4$, $p < 0.02$ and $\chi^2 > 8.8$, $df = 2$, $p < 0.02$). For nonreproducing yearling voles, the rate of the individuals that displayed more than 1% of gaps was lower (0.42) in the case of high density than in other periods of the population-dynamics cycle (0.73, $\chi^2 = 23.4$, $df = 1$, $R_S = -0.29$, $N = 279$, $p < 0.001$). For sexually mature yearling individuals, analogous differences were statistically insignificant ($p > 0.50$).

At the next stage, we performed a log-linear estimation of the effects of sex and population size with three gradations on the level of chromosomal aberrations in yearling and overwintered voles. For SCAs, the effect of sex was still statistically insignificant ($\chi^2 < 2.4$, $df = 2$, $p > 0.30$), unlike a statistically significant effect of age ($\chi^2 > 11.6$, $df = 1$, $p < 0.001$) and population size ($\chi^2 > 21.2$, $df = 4$, $p < 0.001$). The share of the animals with more than 1% of SCAs increased with age from 0.37 in yearling individuals to 0.57 in overwintered voles ($R_S = 0.16$, $n = 461$, $p < 0.001$) and decreased with growth in the population size from 0.59 at low population sizes to 0.45 at medium sizes and to 0.32 at high ($R_S = -0.22$, $n = 461$, $p < 0.001$) population sizes. The share of the individuals displaying >1% of NCAs changed dependent only on the age from 0.21 for yearling animals to 0.39 for overwintered ones ($\chi^2 > 12.7$, $df = 1$, $p < 0.001$). Note that for the individuals with

the NCA rates exceeding 3%, the effects of population density ($\chi^2 > 7.3$, $df = 2$, $p < 0.03$) and interaction between the density and age ($\chi^2 > 9.7$, $df = 2$, $p < 0.01$) were statistically significant. For the overwintered voles, the share of the individuals displaying more than 3% of aneuploid and polyploid cells insignificantly changed with an increase in the population abundance expressed in three gradations ($\chi^2 = 1.9$, $df = 2$, $p = 0.39$, $R_S = -0.12$, $n = 99$, $p = 0.23$). However, when using interannual abundance values (D), this negative correlation became statistically significant ($R_S = -0.23$, $n = 99$, $p < 0.03$, $\chi^2 = 18.1$, $df = 9$, $p < 0.04$). Similar changes have been observed in yearling individuals; they were positively directed and determined the variation in mean population values, since young individuals are prevalent in all phases of the population cycle. Thus, without taking into account the animal age, the share of the individuals with an NCA rate over 3% changed with an increase in population size from 6% at low population sizes to 12% at medium sizes to 18% at high population sizes. For gaps (G3 and G2), only the effect of population size (for partial correlation, $\chi^2 = 19.5$, $df = 4$, $p < 0.001$ and for marginal, $\chi^2 = 18.4$, $df = 4$, $p = 0.001$) was statistically significant. For overwintered voles, the differences in the rate of gaps associated with the levels of population size are statistically insignificant ($p > 0.20$). When using the interannual abundance values (D), the negative correlation for the share of individuals with over 3% of abnormalities was statistically significant ($R_S = -0.25$, $N = 99$, $p < 0.02$, $\chi^2 = 31.6$, $df = 9$, $p < 0.001$). Consequently, the overwintered and young animals display similar directions of the correlations with the levels of population size.

We have observed statistically significant correlations of the vole abundance index (D) with the sizes of animal samples ($R_S = 0.33$, $n = 461$, $p < 0.001$) as well

Table 2. Effects of various factors on proportion of individuals with increased rate of cells carrying structural chromosomal abnormalities (SCA3)

Factor	Parameter of regression equation	<i>p</i>
Constant	1.28 ± 0.14	4 × 10 ⁻¹⁹
Annual abundance values	-0.008 ± 0.02	3 × 10 ⁻⁷
Vole age	0.21 ± 0.07	0.002
Number of cells per capita	0.002 ± 0.01	0.16
Size of annual samples	0.002 ± 0.02	0.28

Table 3. Effects of various factors on proportion of individuals with increased rate of cells carrying numerical chromosomal abnormalities (NCA3) in yearling voles

Factor	Parameter of regression equation	<i>p</i>
Constant	0.93 ± 0.21	2 × 10 ⁻⁵
Phase abundance values (D1–2)	0.20 ± 0.07	0.009
Number of cells per capita	0.002 ± 0.02	0.19

as with the number of cells examined per capita ($R_S = -0.27$, $n = 461$, $p < 0.001$). This suggests that the found correlation between the share of individuals with an increased SCA rate and the population size is an artifact. We used multiple regression analysis to verify this hypothesis; the results are listed in Table 2. The partial correlation between the population size and the share of individuals displaying an elevated rate of cells with SCAs is statistically significant ($p < 0.001$) and amounts to -0.24 . The share of animals with abnormalities increases in the group of overwintered animals compared to yearling animals. The effects of the sample size and the number of examined cells per individual are statistically insignificant.

The effects of several factors on the share of individuals with increased rate of the cells that display NCAs were estimated in the group of yearling voles (Table 3). Here, the relative population size was represented in a categorized form with two gradations: 1, low and medium population size and 2, high population size (D1–2). In this case, only its effect is statistically significant. The partial correlation with the share of individuals displaying NCAs is 0.14 ($p < 0.01$). After the pairwise exclusion of variables, the model retained one independent variable, the effect of which was statistically insignificant. Thus, the correlation found between the level of cytogenetic instability in somatic cells of the bank vole and the indices of abundance expressed as a relative population size is an actual phenomenon, rather than an artifact.

DISCUSSION

The total size of the studied population significantly depends on the number of overwintered animals ($R_S = 0.95$, $n = 10$, $p < 0.001$). Most likely, the SCA and G rates in yearling individuals is, to a considerable degree, determined by the corresponding rates in the parental generation, i.e., overwintered animals ($R_S = 0.43$, $n = 99$, $p < 0.001$ and $R_S = 0.32$, $n = 99$, $p < 0.01$, respectively). As for the increase in the share of individuals with NCAs among the young voles at a high population size, this is the effect of density, which can influence the level of chromosomal aberrations via asymmetric interference competition between adults and yearling individuals. The possible influence of the waste products of adult individuals on an increase in the rate of cytogenetic abnormalities in somatic cells and gametes of youngsters has been demonstrated under laboratory conditions (Skorova et al., 1986; Daev et al., 1995). Presumably, animals born in the year of peak population size will display an increased level of aneuploid and polyploid cells during their entire lives, which may lead to a subsequent decrease in their viability and drop in population size.

According to our results, the rate of chromosomal aberrations increases with age, which agrees with the theoretical concepts and experimental data (Morley, 1995; Charlesworth and Hughes, 1996; Paashuis-Lew and Heddle, 1998). As is known, the rate of spontaneous mutations in somatic cells increases from conception to senility (Wojda and Witt, 2003). The emerging mutations may affect the genes responsible for the control of mutation process (Morris, 2002), as well as lead to the accumulation of highly efficient mutator alleles and an increase in the rate of chromosomal abnormalities in cells of various tissues.

Thus, we have demonstrated the changes in the level of chromosomal instability associated with the population size dynamics. For SCAs, this is a decrease with an increase in population size. This result agrees with the concept that a lower mutation rate in somatic cells is favorable for an increase in the survival rate of organisms. The mechanisms leading to establishment of this pattern may be based on natural selection, which leads to the remodeling of the genetic structure of the population. Alternatively, these changes may be a byproduct of other processes in the population that accompany the changes in its size. In young bank voles, a parameter of genomic instability, the proportion of individuals with an increased rate of cells that carry NCAs increases with increasing population density. The density-dependent mechanisms of the intrapopulation regulation may underlie this effect. Our results favor the presence of both positive and negative correlations between the indices of population abundance and the average level of genomic instability in somatic cells of living organisms. Neither sex nor involvement in reproduction has any statistically significant effect on the level of cytogenetic instability.

The proportion of animals with an elevated rate of the cells that carry structural and numerical chromosomal aberrations significantly increases with age.

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