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Does biotope diversity promote an increase of genetic variation in the bank vole population?

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An electrophoretic study of the variation at 40 protein loci in the bank vole *Clethrionomys glareolus* (Schreber, 1780) was performed in spring 1994–1996 and in autumn 1994 and 1995. A total of 153 individuals from deciduous forest and 122 from coniferous forest subpopulations were collected. During the whole time of the study in spring the coefficient of trappability was 4.1% in deciduous forest and 3.0% in coniferous forest; in autumn 9.3% and 7.6%, respectively. In spring there were no significant differences in allele frequencies between the subpopulations studied. The samples from deciduous and coniferous forests were genetically similar. However, in autumn these differences were statistically significant. Although the level of heterozygosity in both, neighbouring subpopulations is similar, its changes in consecutive seasons have different values. From the calculations performed, based on the level of genetic differentiation between subpopulations (F_{st}), it can be concluded that the level of gene flow between the voles from two biotopes in spring is greater than in autumn. We hypothesise that the essence of this phenomenon lies in a non-random fraction of migrants between the biotopes studied. The data presented in the study indicate that the genetic structure in two neighbouring subpopulations of the bank vole undergoes different processes.

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Introduction

In animals, both the increase and decrease of natural population ranges are associated with formation of local populations (called subpopulations or demes). Such subpopulations differ in their time of persistence. In rodents, with the bank vole *Clethrionomys glareolus* (Schreber, 1780) as a good example – because of its well known ecology, the process of rising and declining of populations continues in two different time rhythms. The first is annual, associated with seasonal breeding persisting intensively in spring and early summer, but practically nonexistent in other seasons of the year. On average, this results in a four-fold increase of vole numbers at the beginning of autumn in comparison with the end of winter (Pucek *et al.* 1993). The other rhythm of population size changes is of multiannual character. An increase takes place after a period of moderate population numbers and lasts 4 to 7 years. The population size, calculated as a

mean for the whole year, may differ in consecutive years as much as 17 times. Also, the number of voles in autumn may be even 61 times higher in comparison with autumn in years of low population size (Pucek *et al.* 1993). The fluctuations of population size not only cause the increase of vole population density in biotopes of permanent presence (so-called optimal), but they also cause an increase of local ranges in suboptimal biotopes (Gliwicz 1991). New subpopulations are formed in areas which were not settled at all by voles in years of low vole numbers, or the population density in such parts of species range was lower than in optimal biotopes. This raises a question whether the voles which disperse and increase the number of individuals in poorely inhabited areas or even colonise the new places are a random fraction from the mother population, or the migrants are the individuals with the characters differing from the average. This has a great influence on the genetic structure of the newly arising subpopulations. It is also said (Patton and Smith 1990, Whitlock 1992) that genetic characters of the groups of individuals considerably depend on spatial localisation of populations in relation to each other. Geographically close populations are genetically more similar than populations isolated by a large distance. This does not determine the character of genetic changes caused by fluctuations in population number resulting from both spatial ("dense" versus "diluted" populations) and seasonal changes in the population size. The existence of fluctuations in the number of individuals in two neighbouring subpopulations can be considered a particularly convenient experiment. There are no available data concerning such situations. There is either no theoretical agreement whether colonisation, disappearing and recolonisation processes increase (Wright 1977) or, conversely, decrease (Slatkin 1987) genetic differences among populations. These opposite opinions are softened by other theoretical considerations on this subject. For example, Wade and McCauley (1988) try to clarify Wright's and Slatkin's opinions showing that increased gene flow between adjacent populations may decrease genetic differences only in particular cases. However, in general gene flow increases genetic differentiation between subpopulations. Moreover, Whitlock and McCauley (1990) emphasised that the mode of colonisation is a significant factor, which establishes genetic differences and similarities between the subpopulations, as the colonisation may be (a) random or (b) selective inflow of individuals. However, there are only few empirical data concerning genetic properties of subpopulations. One study showed only the existence of certain differences in particular populations, without considering dynamics of the process in invertebrates (McCauley and Eanes 1987, Rank 1992). Thus the purpose of this study was to analyse data about genetic structure of the neighbouring bank vole subpopulations in different seasons and consecutive years.

We assumed that this would allow us to: (a) Estimate the dynamics of genetic processes influencing the structure of population in spring and autumn. (b) Determine whether the resident population in deciduous forest (optimal biotope) differs from the subpopulation inhabiting coniferous forest (suboptimal biotope) undergoing a higher fluctuation in number. (c) Answer the possible question whether,

during a dramatic change in vole numbers, the genetic structure of the population changes considerably.

Material and methods

A total of 294 bank voles *Clethrionomys glareolus* were live-trapped in the Solnicki Forest, near Białystok (NE Poland) in three consecutive years (1994–1996) during spring and autumn (Table 1). The trapping sites were located in two neighbouring biotopes: deciduous and mixed coniferous forest. After killing animals, blood, liver, salivary gland and kidney tissue were taken for electrophoresis. Then, vole sex was determined and eye lens were taken to estimate the age of the individuals. When a total dry mass of eye lens was equal to or higher than 6 mg in bank voles trapped in spring they were considered overwintered individuals (Kozakiewicz 1976). Blood was centrifuged at 12 000 rpm for 3 minutes to separate serum and blood cells; other tissues were homogenised in 0.01 M phosphate buffer (pH = 7.5) and centrifuged at 12 000 rpm for 20 minutes. A total of 40 protein loci were studied in each specimen: alcohol dehydrogenase (*Adh*), α and β glycerol-3 phosphate dehydrogenase (α*Gpd-1*, β*Gpd-1,* β*Gpd-2*), sorbitol dehydrogenase (*Sdh*), lactate dehydrogenase (*Ldh-1, Ldh-2*), malate dehydrogenase (*Mdh-1 Mdh-2*), malic enzyme (*Me-1*), isocitrate dehydrogenase (*Idh-1, Idh-2*), 6-phosphogluconate dehydrogenase (*Pgd*), xanthine dehydrogenase (*Xdh*), NADH-diaforase (*Dia*), catalase (*Cat*), superoxide dismutase (*Sod-1, Sod-2, Sod-3*), aspartat-aminotransferase (*Aat-1, Aat-2*), phosphoglucomutase (*Pgm-1, Pgm-2, Pgm-3*), esterase (*Est-B3, Es-D*), α-amylase (*Amy-1, Amy-2, Amy-3, Amy-4*), peptidase (*Pep-1, Pep-2*), aminoacylase (*Acy*), aldolase (*Ald-1, Ald-2*), aconitase (*Acon-1, Acon-2*), glucose phosphate isomerase (*Gpi*), albumin (*Alb*), and protein-1 (*Prot-1*).

For the most of enzymes horizontal starch gel electrophoresis was performed according to Selander *et al.* (1971), Harris and Hopkinson (1976) and Quavi and Kit (1980). Only for aconitase-1, -2, phosphoglucomutase-1, -2, -3 and diaphorase cellulose acetate plates (Helena Labs. Texas) were used with staining methods described by Searle (1985). A locus was considered polymorphic if more than one allele was detected. The alleles at polymorphic loci were designated alphabetically with increasing anodal migration of the corresponding allozymes.

For the genetic data analysis a computer program, Biosys-1, of Swofford and Selander (1989) was used. The differences in allele frequencies between samples were tested using χ^2-test for homogeneity. Deviations from Hardy-Weinberg equilibrium in subpopulations were tested using χ^2 goodness-of-fit test. The value of observed average heterozygosity in each studied group were not normally distributed (tested by χ^2-test at $p = 0.05$). Therefore, Kruskal-Wallis one-way analysis by ranks test (Zar 1984) was performed to test the differences in observed average heterozygosity among populations. To estimate the theoretical number of migrants (N_m) entering a given population the equation $N_m = [(1/F_{st}) - 1/4]$ was employed (Wright 1978). F_{st} was estimated as a mean across polymorphic loci.

Results

The characteristics of deciduous and coniferous subpopulations of the bank vole

The data concerning captures of bank voles (Table 1) show that there is clearly visible seasonal variation in trappability. The coefficient of trappability in spring ranges from 1.7 to 4.7%. In autumn it ranges from 5.5 to 10.4% . It can be also said that in general the bank vole subpopulation from coniferous forest is characterised by lower trappability in comparison with the subpopulation from a deciduous forest. Despite this the trappability value was nearly equal in both subpopulations in autumn 1995, while in autumn 1994 and spring 1996 it was

Table 1. The number of collected bank voles (n) in three consecutive years 1994–1996 in spring and autumn, in two neighbouring populations: from deciduous and coniferous forests. M – males, F – females, *TI* – trappability coefficient.

Forest type	Season and year		n	M	F	*TI*
Deciduous	Spring	1994	31	18	13	4.6
	Autumn	1994	29	9	20	10.4
	Spring	1995	29	23	6	4.7
	Autumn	1995	31	10	21	8.3
	Spring	1996	33	19	14	3.5
Coniferous	Spring	1994	28	16	12	3.5
	Autumn	1994	25	10	15	5.5
	Spring	1995	11	6	5	3.0
	Autumn	1995	46	23	23	8.8
	Spring	1996	12	7	5	1.7

twice as big as in coniferous forest. On the other hand, during three consecutive years of study the coefficient of trappability in spring was from 1.3 to 2 times lower in coniferous forest than in deciduous forest. The variation of trappability coefficient value allows us to assume that the density of voles in the coniferous forest was more variable than in the deciduous forest, especially in autumn. It can also be concluded that in four out of five seasons during which the study was conducted the number of bank voles in the deciduous forest subpopulation was higher than in the coniferous forest. These differences are more evident in spring during three years of study. From the analysis of trappability coefficient value in autumn 1994 it can be concluded that the number of voles in the deciduous forest subpopulation was twice as high as in the coniferous forest subpopulation.

Differences in allele frequencies between the bank vole subpopulations

The following 28 loci were found to be monomorphic with the same allele in all the individuals studied: *Aat-1*, *Acon-1*, *Acon-2*, *Adh*, *Alb*, *Ald-1*, *Ald-2*, *Amy-1*, *Amy-4*, *Cat*, *αGpd-1*, *βGpd-1*, *βGpd-2*, *Gpi*, *Idh-1*, *Idh-2*, *Ldh-1*, *Me-1*, *Mdh-1*, *Mdh-2*, *Pep-1*, *Pgm-1*, *Prot-1*, *Sod-1*, *Sod-2*, *Sod-3*, *Sdh* and *Xdh*. The other 12 loci were polymorphic with more than one allele in at least one individual: *Aat-2*, *Acy-1*, *Amy-2*, *Amy-3*, *Dia*, *Es-D*, *Es-B3*, *Ldh-2*, *Pep-2*, *Pgd*, *Pgm-2* and *Pgm-3*. Allele frequencies for the polymorpic loci are given in the Appendix.

The comparison of allele frequencies in deciduous and coniferous forest subpopulations shows that the frequencies change seasonally (Table 2 and Appendix). In spring, during three years of the study no significant differences in allele frequencies between the deciduous and coniferous forest subpopulations were found. On the other hand, in autumn the opposite situation was observed. Both, in 1994 and 1995 the differences in allele frequencies between the bank vole sub-

populations are statistically significant (Table 2). It can be concluded that in spring, after a severe winter reduction in the number of voles (compare also *TI* in Table 1), selection pressure makes the studied populations genetically similar. No significant deviations from Hardy--Weinberg proportions were found except Sal-2 locus ($p = 0.012$) in deciduous forest subpopulation in spring 1996 and Acy locus ($p = 0.006$) in autumn 1994.

Table 2. χ^2-test for homogeneity in allele frequencies at polymorphic loci between bank voles inhabiting deciduous and coniferous biotopes. [a] significant difference ($p = 0.044$) only for peptidase-2 locus.

Season and year		p
Spring	1994	0.84
Spring	1995	0.081
Spring	1996	0.23[a]
Autumn	1994	0.00002
Autumn	1995	0.0003

The level of heterozygosity in deciduous and coniferous forest populations of the bank vole

The winter mortality in the bank voles studied changes their composition within the population (Table 3). It results in a different level of heterozygosity in spring compared with autumn. Observed average heterozygosity (H_o) differs among populations ($H = 37.72$, $p < 0001$). The level of H_o changes differently in both subpopulations (Table 3). The data presented in Table 3 show that average heterozygosity in both subpopulations is similar in autumn, as the observed heterozygosity (H_o) ranges from 6.3 to 6.5%. If only the polymorphic loci are included (12 out of 40 studied), the average heterozygosity also varies slightly (from 21.5 to 23 %). This shows that in autumn both subpopulations become similar in respect to this coefficient (H_o). However, in spring the picture is more

Table 3. The level of average heterozygosity (in per cent) in the bank voles from the coniferous and deciduous forest subpopulations, in consecutive seasons and years. n – number of individuals studied. H_o – observed average heterozygosity, H_e – expected average heterozygosity.

Forest type	Season and year		n	All loci		Polymorphic loci
				H_o	H_e	H_o
Deciduous	Spring	1994	20	4.3	5.4	15.8
	Autumn	1994	30	6.3	6.2	23.0
	Spring	1995	25	5.9	6.3	21.4
	Autumn	1995	30	6.5	6.7	23.5
	Spring	1996	32	7.3	7.5	26.6
Coniferous	Spring	1994	27	5.0	5.5	17.6
	Autumn	1994	26	6.2	6.1	21.5
	Spring	1995	7	8.9	7.9	32.5
	Autumn	1995	46	6.3	6.4	22.8
	Spring	1996	12	6.1	6.8	22.1

complicated. During two years (1994 and 1995) H_o value was higher in the coniferous forest vole subpopulation than in the deciduous forest subpopulation. However, in spring 1996 this tendency was reversed. The value of H_o ranged from 4.3 to 8.9%. If only the polymorphic loci are included the variation in H_o ranges from 15.8 to 32.5%. Thus, the observed heterozygosity in both subpopulations differs more in spring than in autumn and it also undergoes greater springtime fluctuation than in autumn.

Discussion

It has been found that in spring the average heterozygosity of bank voles inhabiting deciduous biotopes in Białowieża Primeval Forest is 4.2% and it is higher than in autumn as it becomes 3.2% in residents and 3.5% in migrants (Fedyk and Gębczyński 1980). These seasonal changes are explained by the authors by the better survival of heterozygous individuals during the winter. Although we found changes of average heterozygosity in two bank vole subpopulations from Solnicki Forest in the consecutive seasons, they cannot be undoubtedly assigned to higher mortality of homozygous individuals in winter. In some cases the average heterozygosity was lower in spring than in the previous autumn (deciduous forest: autumn 1994 – spring 1995), and remained unchanged (coniferous forest: autumn 1995 – spring 1996). In other cases (deciduous forest: autumn 1995 – spring 1996, coniferous forest: autumn 1994 – spring 1995) a similar process of seasonal changes of H_o value in the bank vole populations took place (Fedyk and Gębczyński 1980). We believe that the explanation is not due to weather conditions since in the case of the subpopulations observed, after the same winter (1994/95) this value (H_o) in the deciduous forest was reduced, while in coniferous forest subpopulation it clearly increased, in comparison with autumn 1994. After the next winter (1995/96) in the deciduous forest subpopulation the average heterozygosity H_o was greater in spring while it did not change in the coniferous forest subpopulation. These changes in H_o value are not associated with the amount of winter reduction of voles, or our trappability coefficient results. The strong reduction in number of individuals in the population during winter does not always result in the increase of the H_o value. Therefore, the earlier hypothesis of Smith *et al.* (1978) suggesting differential winter mortality of homozygous and heterozygous individuals of rodents, and confirmed in the bank vole populations from deciduous biotopes of Białowieża Primeval Forest (Fedyk and Gębczyński 1980) was only partly supported here. Despite the need of the further attempts to answer the question whether a winter season is characterised by unequal survival of homozygous and heterozygous rodents, the geographical differences in genetic structure of the bank vole populations should be taken to a consideration. It has been shown that the bank vole population on Jutland Pennisula consists almost only of homozygotes, where H_o = 0.6% (Gębczyński *et al.* 1986). In 10

populations in Austria H_o ranges from 2.8 to 8.5% (Leitner and Hartl 1988) and in 5 populations in southern and eastern Poland this value ranges from 3.9 to 6.3% (Gębczyński *et al.* 1993).

An explanation is needed for the fact that in the present study no significant differences in allele frequencies between two subpopulations were found in spring, while in autumn these differences were highly statistically significant. This surprising fact may be explained in three different ways. First, it may be due to small sample sizes in spring 1995 and 1996. The second possible hypothesis assumes that migration in autumn does not exist or only takes place within each biotope. This allows an independent genetic differentiation within the studied subpopulations. Thus the changes in genetic structure during a peak of bank vole number are different even in neighbouring subpopulations. The other hypothesis relies on the assumption that migration in autumn takes place and allows the exchange of the individuals between the subpopulations. However, the migrants do not represent a random sample from a population. This fraction of the individuals in the subpopulations, which shows a tendency to disperse, belongs to a different genetic category than those which remain close to their place of birth. It seems – according to the previous data concerning ecology of the bank vole (Petrusewicz 1983, Gliwicz 1991) – that the third hypothesis is more probable. The calculation of the number of migrants (N_m) between two subpopulations (Wright 1978) seems also to confirm the third hypothesis. Slatkin and Barton (1989) showed that, under the assumption that individuals migrate at random and mutation rate is negligible, N_m calculated using F_{st} is a quite reliable estimate of gene flow between populations which are at Hardy-Weinberg equilibrium. In the case of our study the level of gene flow found between deciduous and coniferous forests subpopulations is different (Table 4). The data presented in Table 4 show the theoretical number of individuals exchanged between the subpopulations in different seasons under study, which would result in the observed genetic differentiation between subpopulations. It can be assumed from these calculations that exchange of the individuals between the subpopulations seems to be lower in autumn than in spring. However, N_m changes considerably in consecutive years.

Table 4. The genetic differentiation F_{st} and the level of gene flow as the number of migrants (N_m) between the bank vole subpopulations from deciduous and coniferous forests in the different seasons in years 1994–1996.

Season and year		F_{st}	N_m
Spring	1994	0.007	35
Spring	1995	0.029	8
Spring	1996	0.019	13
Autumn	1994	0.046	5
Autumn	1995	0.019	13

It should also be mentioned here that genetic differentiation between subpopulations (F_{st}) found in our study is relatively small, as it is always lower than 0.05 (Wright 1978). If we assume that average N_m in spring during three consecutive years of the study and in autumn 1994 and 1995 is genuine, despite considerable variation, it could be concluded that gene flow was greater in spring than in autumn. Such a conclusion is inconsistent with our understanding of bank vole ecology. The bank vole is characterised by moderate ability to migrate. Migration may occur during any time of the year, but it is most intense in autumn every year when number of voles is the greatest (Petrusewicz 1983). Males migrate more often than females, younger individuals than older ones (Wolton and Flowerdew 1985, Gliwicz 1991). Thus, the phenomenon could be explained by the hypothesis of a non-random fraction of migrants between deciduous and coniferous forests in autumn. The main direction of migration remains an open question. Recently Kierus (1996) postulated (contrary to former assumptions) that voles may migrate from coniferous to deciduous forests. The additional argument showing different processes forming the genetic structure in two vole populations is, we suspect, the genetic differentiation (F_{st}) coefficient and N_m values calculated for the consecutive bank vole generations (Table 5). F_{st} and N_m between generations are, on average similar in the two subpopulations studied. However, in the coniferous forest subpopulation these values are nearly the same in four out of five generations. N_m ranges from 10 to 12 individuals, and only in spring N_m was 25. On the other hand the value of this coefficient ranges from 7 to 62 (Table 5). It may indicate a great, probably selective migration, affecting the subpopulation from deciduous forest. This could eventually support an assumption of migrating individuals from coniferous to deciduous forest.

Data presented here allow us to conclude that: (a) changes of genetic structure in the bank vole populations are different in spring and autumn, (b) the subpopulation inhabiting an optimal biotope for a given species is under different selection pressures compared with that from a sub-optimal biotope. In our opinion this conclusion is an important empirical corroboration of the suggestions proposed by

Table 5. The genetic differentiation (F_{st}) and the level of gene flow as the number of migrants (N_m) between consecutive generations in bank vole subpopulations inhabiting deciduous and coniferoud forests. ad – overwintered voles, juv – voles born in a given year.

Generation and season	Deciduous forest		Coniferous forest	
	F_{st}	N_m	F_{st}	N_m
Spring 1994 ad – spring 1994 juv	0.009	28	0.010	25
Spring 1994 juv – autumn 1994 juv	0.004	62	0.025	10
Autumn 1995 juv – spring 1995 ad	0.017	14	0.021	12
Spring 1995 ad – autumn 1995 juv	0.036	7	0.023	11
Autumn 1995 juv – spring 1996 ad	0.024	10	0.024	10

the theoreticians, suggesting that widely distributed species, inhabiting different biotopes, should be characterised by high genetic differentiation (Wright 1977, Lande 1988).

During the time of our study no data were collected concerning genetic processes in the bank vole population experiencing dramatic changes in number. The answer to this question demands further studies.

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Appendix. Allele frequencies in the bank vole subpopulations during 1994–1996 in Solnicki Forest. sp – spring, au – autumn, ad – overwintered voles, juv – voles born in a given year, dec – deciduous forest, con – coniferous forest, *n* – number of individuals.

Locus	sp'94 ad dec	sp'94 ad con	sp'94 juv dec	sp'94 juv con	au'94 juv dec	au'94 juv con	sp'95 ad dec	sp'95 ad con	au'95 juv dec	au'95 juv con	sp'95 ad dec	sp'96 ad con
Ldh-2	*n*= 19	28	11	6	30	26	25	7	30	46	32	12
a	1.00	1.00	1.00	1.00	0.98	0.94	0.98	0.86	1.00	0.92	0.98	0.96
b	0.00	0.00	0.00	0.00	0.02	0.06	0.02	0.14	0.00	0.08	0.02	0.04
Pgd	*n*= 19	28	11	6	30	26	25	7	30	46	32	12
a	1.00	0.98	1.00	1.00	1.00	1.00	1.00	0.93	0.92	0.99	1.00	1.00
b	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.07	0.08	0.01	0.00	0.00
Aat-2	*n*= 20	28	11	6	30	26	25	6	30	46	32	12
a	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.04	0.06	0.00
b	1.00	1.00	1.00	1.00	1.00	1.00	0.92	1.00	1.00	0.96	0.94	1.00
Est-D	*n*= 19	24	10	6	30	25	25	7	29	46	32	12
a	0.82	0.9	0.85	0.83	0.85	0.82	0.76	0.79	0.85	0.91	0.84	0.88
b	0.18	0.1	0.15	0.17	0.15	0.18	0.24	0.21	0.16	0.09	0.16	0.12
Es-B3	*n*= 19	24	11	6	30	26	25	7	29	45	32	12
a	0.87	0.85	0.91	0.75	0.83	0.65	0.66	0.43	0.90	0.73	0.67	0.87
b	0.13	0.15	0.09	0.25	0.17	0.33	0.34	0.57	0.10	0.27	0.33	0.13
c	0.00	0.00	0.00	0.00	0.00	0.002	0.00	0.00	0.00	0.00	0.00	0.00
Acy-1	*n*= 19	28	11	6	30	26	25	7	29	46	32	12
a	0.97	1.00	1.00	1.00	0.97	0.98	0.98	0.93	0.98	0.96	0.89	0.96
b	0.03	0.00	0.00	0.00	0.03	0.02	0.02	0.07	0.02	0.04	0.11	0.04
Pep-2	*n*= 19	24	11	6	30	26	25	7	29	46	32	12
a	0.11	0.12	0.05	0.17	0.22	0.02	0.10	0.07	0.10	0.08	0.06	0.21
b	0.89	0.83	0.95	0.83	0.78	0.98	0.90	0.93	0.90	0.91	0.94	0.73
c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
Amy-2	*n*= 18	24	11	6	30	24	25	7	30	44	26	10
b	0.972	0.958	1.00	0.917	0.933	0.896	0.980	0.857	0.833	0.85	0.67	0.65
c	0.028	0.042	0.00	0.083	0.067	0.104	0.020	0.143	0.167	0.15	0.33	0.35
Amy-3	*n*= 19	25	11	6	30	25	25	7	30	44	26	10
a	1.00	0.980	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
b	0.00	0.020	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pgm-2	*n*= 19	24	11	6	30	26	25	7	27	46	32	12
a	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.01	0.00	0.00
b	0.92	0.93	0.91	1.00	0.85	0.96	0.88	1.00	0.83	0.98	0.86	0.96
c	0.08	0.07	0.09	0.00	0.15	0.02	0.12	0.00	0.15	0.01	0.14	0.04
Pgm-3	*n*= 19	23	11	6	30	25	25	7	28	46	30	12
b	0.18	0.22	0.09	0.33	0.20	0.18	0.08	0.14	0.23	0.11	0.08	0.08
c	0.16	0.26	0.18	0.33	0.22	0.16	0.14	0.21	0.14	0.30	0.10	0.17
d	0.24	0.24	0.41	0.17	0.15	0.40	0.30	0.29	0.29	0.24	0.33	0.21
e	0.42	0.26	0.32	0.17	0.43	0.26	0.48	0.36	0.32	0.35	0.49	0.54
f	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00
Dia	*n*= 19	24	11	6	30	26	25	7	28	45	32	12
a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
b	0.579	0.604	0.636	0.667	0.733	0.423	0.760	0.571	0.500	0.61	0.61	0.50
c	0.421	0.396	0.364	0.333	0.267	0.577	0.240	0.429	0.500	0.38	0.39	0.50