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Spontaneous and induced activation of genes affecting the phenotypic expression of glucose 6-phosphate dehydrogenase in *Daphnia pulex*

3. Occurrence frequencies of the alternative electrophoretic variants of G6PD in a natural population

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Summary. Evidence has previously been presented on the occurrence spontaneous variations in the electrophoretic mobility (EM) of glucose-6-phosphate dehydrogenase (G6PD E.C. 1.1.1.49) in laboratory clones of parthenogenetically reproducing *Daphnia* (Ruvinsky et al. 1983). The present study is concerned with a natural population of *Daphnia* living under the extreme conditions of shallow, dessicating pond. The number of individuals having the slow (S) variant of the EM of G6PD increased sharply during their 1.5 month life span. This increase is suggested to result from alternational variability related to activation of latent genetic material.

Key words: *Daphnia* – Natural populations – Spontaneous variation – G6PD

Introduction

Spontaneous variations in the electrophoretic mobility (EM) of glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.49) have been observed in laboratory clones of parthenogenetically reproducing *Daphnia:* females having G6PD with a normal EM produced offspring having the slow migrating fraction of the enzyme, and vice versa (Ruvinsky et al. 1983 a).

Clones both variable and stable with respect to this character were identified. The occurrence frequencies of *Daphnia* with this electrophoretically modified variant attained $1-5\times10^{-1}$ in the variable clones. Intraclonal selection of *Daphnia* with one of the alternative electrophoretic variants of G6PD was efficient. Upon analysis for the possible causes of this phenomenon the spontaneous activation of genes (or gene groups) was suggested, whose expression produces a de novo synthesis of a polypeptide capable of modifying the structure of native G6PD (Ruvinsky et al. 1983 b).

The results obtained stimulated interest in the occurrence of spontaneous variability in the EM of G6PD in natural populations of *Daphnia pulex* and in its implications. Analysis of the adaptive significance of any character of the causes promoting its establishment and maintenance of its polymorphism would be inadequate without the use of various markers. For this reason, we utilized an extremely polymorphic system of esterases controlled by three closely linked genes in *Daphnia pulex*. Each of these genes is represented in the *Daphnia* population by several allelic variants (Ruvinsky and Lobkov 1981).

Material and methods

A *D. pulex* population contained by dessicating pond in the vicinity of Novosibirsk (Cherbuzy) was studied. In this population, individuals from over-wintering sexual eggs start to appear at the beginning of May. These parthenogenetically reproducing individuals rapidly increase in number to the end of May and the beginning of June. Parthenogenetic reproduction decelerates with deteriorating conditions – the drying up of the ponds – when males are seen and reproduction ceases to be exclusively parthenogenetic and alternates with the bisexual form. This gives rise to ephippial eggs, and such eggs can be activated the next spring. This life cycle is characteristic of rapidly drying ponds, but not of stable ones in which parthenogenesis proceeds continuously from May to November under the conditions of Novosibirsk.

We took advantage of this specificity of the *Daphnia* population to analyze how its genetic composition changes throughout 1981–1982 and also how the EM of G6PD alters during the life span from emergence (the beginning of May) to death (the middle of June). The changes observed were recorded every 10–15 days.

Daphnia individuals were collected in ponds, placed in vessels containing pond water and transported to the laboratory in thermos flasks. This standard procedure provided conditions least differing from the natural habitat of *Daphnia*.

The EM of G6PD was analysed in the patterns yielded by 6 samples from the Cherbuzy population in 1981–1982; and the esterase patterns obtained from 4 samples of this population were analysed in 1982. The EM of G6PD and, concomitantly, esterase patterns were described for each individual from samples No. 3 and No. 4 in 1982.

Results

A characterization of the genotypic composition of the Daphnia population from the Cherbuzy pond

In 1982, we studied the genotype composition of the population of Cherbuzy. For this purpose we examined the esterase patterns of 364 *Daphnia* from 4 random samples obtained from the beginning of May to mid-June. Figure 1 presents a survey of the diverse esterase genotypes and their occurrence frequencies in the individuals studied. The total number of genotypes distinguished is 95. Only a small portion of this total number is represented by more than 10 individuals and many genotypes are rarely encountered: 23 types only twice and 49 only once.

It appeared of interest to observe how the genotypic composition and occurrence frequencies of genotypes change in consecutive samples (1982). Figure 2 presents these changes with time. Thirty-two esterase genotypes were identified in the first sample; of these, 10 were not encountered in subsequent samples; of the remaining 22 genotypes, 15 were present up to the fourth sample. Forty-one esterase genotypes were identified in the second sample, and, of these, 26 have not been hitherto encountered. The same number of genotypes were detected in the third sample, 22 of them were "new". The 24 genotypes present in the third sample were, however, absent from the next sample. The fourth sample was found to contain 43 esterase genotypes, of which 28 had been found in the preceding samples and 15 were "new". Thus, the genetic composition of the Cherbuzy population is not only complex and diverse, it also undergoes changes that become particularly obvious when compared with typically "discontinuous" populations (3, 4).

The occurrence frequencies of the S-variant of G6PD in consecutive samples of Daphnia

The first evidence of the high occurrence frequencies of *Daphnia* with the S-variant of G6PD EM in the Cherbuzy population was obtained in 1981. Of 40 individuals randomly sampled from the pond the 27–28th of May 1981, 7 exhibited the S of G6PD EM

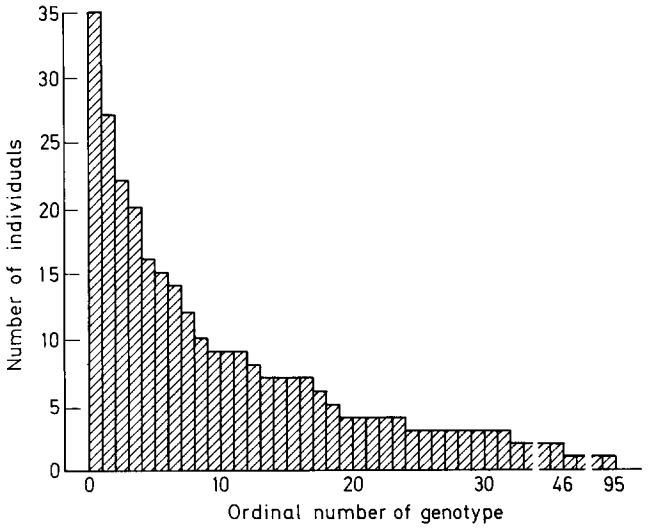

Fig. 1. Distribution of the occurrence frequencies of 95 esterase genotypes detected in *Daphnia* sampled from the Cherbuzy population

Fig. 2. Changes in the genotype composition of the Cherbuzy population in 4 consecutive samples (1982): *a* sample 1 (May 3–7): 82 *Daphnia* examined, 32 esterase genotypes identified; *b* sample 2 (May 17–21): 88 *Daphnia* examined, 41 esterase genotypes identified; *c* sample 3 (May 31–June 3): 92 *Daphnia* examined, 41 esterase genotypes identified); *d* sample 4 (June 15–18): 102 *Daphnia* examined, 43 esterase genotypes identified. ▩ esterase genotypes first detected in sample 1; ▨ in sample 2; ▧ in sample 3; □ in sample 4

(17.5%). After 7 days, 12 carriers of the S of G6PD EM were found among the 24 individuals reexamined. Consequently, in a short span of time the frequency of individuals with the S variant increased significantly from 17.5%–50% ($F_{\varphi}=8.05$; $P>0.99$). Studies were discontinued because there were no more survivors in the dried pond.

In 1982, promptly after the appearance of adult individuals, we resumed analysis of the occurrence of

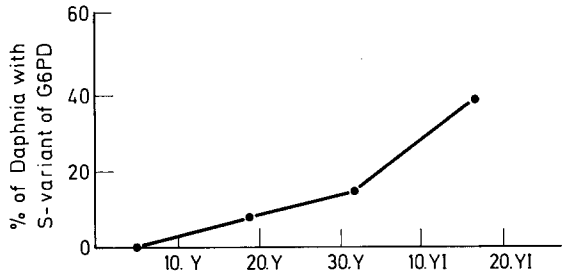

Fig. 3. Changes in the occurrence frequencies of *Daphnia* with the S-variant of the EM of G6PD in consecutive samples from the Cherbuzy population (*points* indicate consecutive samples)

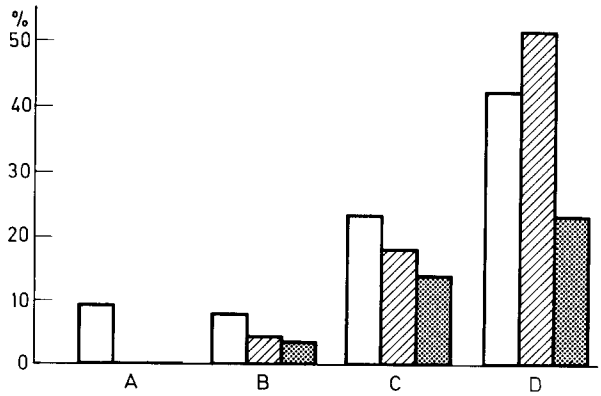

Fig. 4. Comparison of four contrasting *Daphnia* clones. □ occurrence frequency of the S-variant of the EM of G6PD; ▨ %of males in a crowded culture; ▩ % of ephippial females in a crowded culture. Clones compared: *A, B, C, D*

the S of G6PD EM in the population. The results are shown in Fig. 3. The EM of G6PD was studied in 367 animals; random samples were examined at four time points corresponding to the beginning, intermediate and final time units of their life cycles. There is a clear-cut increase in the frequency of *Daphnia* with the S of G6PD EM. The difference between samples 1 and 3 ($F_{\varphi}=22.94$; $P>0.99$), as well as between samples 3 and 4 ($F_{\varphi}=14.75$; $P>0.99$) are statistically significant in this regard.

The question was raised whether the increase in the S frequency in the population examined may be result of prevailing reproduction of certain clones among those studied. This question appeared quite reasonable in the light of data indicating that the genotype structure and, consequently, the clonal composition of the population examined has been continuously changing with time.

There is no doubt that the clones within the population reproduce differentially. If this differential reproduction were the main cause of increase in the number of carriers with the S-variant of G6PD, three consequences would follow: (1) *Daphnia* from clones absent from sample 1, in which carriers of the S occurred, would be the major contributors to the increased number of individuals with the S in the last samples; (2) *Daphnia* from clones most frequently occurring in sample 1 would not carry the S in samples 3 and 4; (3) the number of clones characteristically encountered in sample 1 would be much reduced in samples 3 and 4 because of the increasing proportion of clones not occurring in sample 1. However, analysis of genotype frequencies in the population examined led to conclusions completely opposite to expectations. It was found that the proportion of *Daphnia* with 12 of the most frequently occurring genotypes was 50% in all the samples, including sample 3 (47 of 92 individuals, 52%) and sample 4 (57 of 102 individuals, 56%), which meant that their proportion changed very slightly. Meanwhile the frequency of the S increased to 11% (5 of 47 *Daphnia*) and to 42% in sample 4 (24 of 57 *Daphnia*). Therefore, it may be asserted that during the period of observation there was no decrease in the proportion of clones most frequently occurring in sample 1 and there was a sharp increase in the number of carriers of the S-variant of G6PD. In is noteworthy that *Daphnia* newly appearing in sample 4 were not numerous (15 of 102, 15%) and only 4 of them had the S-variant of G6PD. This excludes a selective reproduction of *Daphnia* having the S-variant of G6PD as the main cause of the sharp increase in the frequency of the S-variant of G6PD in the Cherbuzy population. The observation made becomes explicable when assuming that deteriorating conditions may produce changes associated with a decrease in the EM of G6PD like those observed earlier in glucose-treated *Daphnia* (Ruvinsky et al. 1983). This effect is particularly striking in a dying population no longer capable of withstanding maximum environmental stresses.

Relationship between the reproduction characters of a clone and the occurrence frequencies of the S-variant of G6PD

In the course of these studies we distinguished several clones differing in such important reproductive characters as the occurrence frequencies of males, and ephippially and parthenogenetically reproducing females in crowded conditions (Ruvinsky et al. 1985). The frequency of the S of G6PD was studied in some of these clones. It can be seen there is a positive correlation between the occurrence frequencies of males and individuals with the S of G6PD in a clone, especially with reference to the clones with low percentages of males where the occurrence frequencies of the S is low and vice versa. From the data of Fig. 4 it also follows that in those clones with low percentages of the S, the

percentage of ephippial females is low and, therefore, that of parthenogenetic ones is high. In contrast, in clones where *Daphnia* with the S occur frequently, the percentage of females reproducing by parthenogenesis is sharply decreased, while that of bisexually reproducing ephippial females is increased. It should be noted that one the 4 clones, Ch-26, was derived from the Cherbuzy population (Fig. 4), and it is the one exceptional in high frequency of spontaneous passages from the normal to the slow variant N → S and exhibiting strong proclivity to high frequency transitions from parthenogenetic to bisexual reproduction and, hence, to giving rise to males.

Discussion

There are some important aspects in which the *Daphnia* population studied herein differs from those previously described. Its salient feature is that it exists only 5–6 weeks annually. The duration of its existence is limited by its temporary habitat of melting snow. The conditions are extremely harsh compared to those in which other populations live. In Cherbuzy, *Daphnia* remarkably manage in such a short time to reproduce actively through 3–4 parthenogenetic generations and then to take recourse to bisexual reproduction culminating in the laying of latent ephippial eggs.

Under these specific conditions, the particular population studied, presumably like others, develop adaptive responses ensuring survival. One characteristic in which this population differs strongly from those previously described is its extremely high level of polymorphism; another characteristic is its drastic change in genotype composition occurring during a short time interval (Fig. 2). One finds much slower changes in the genotype frequency of *Daphnia* populations living in stable ponds (Young 1979; Ruvinsky and Lobkov 1981). The rapidity of alteration of the genetic composition in the Cherbuzy population is unique. After a very brief period, 50% of the genotypes encountered in the first samples were no longer present in the fourth. And, the last but not least important characteristic of the Cherbuzy population is the high occurrence frequencies of *Daphnia* with the slow-migrating fraction of G6PD which attains maximum values when the pond is almost dried up (Fig. 3). In previous assays of the EM of G6PD in natural populations, *Daphnia* with altered EM were rarely, if at all, observed (Hebert and Crease 1980; Ruvinsky et al. 1983 a).

What may be the adaptive significance of these characteristics, and are they interrelated? The high level of polymorphism and, hence, interclonal polymorphism of populations of Cherbuzy type, appear to be the direct consequences of bisexual reproduction stimulated by specifically extreme conditions. Bisexual reproduction demands the ample production of males which is determined by the stressing ecological situation (Banta and Brown 1929) and provided during the ontogenesis of *Daphnia* by the reorganization of the functional activity of the genome. In this connection, one may think that the intraclonal variability of the EM of G6PD observed earlier and particularly frequent in the Cherbuzy population serves to mark the genetically determined ability of the clones to adaptation in fluctuating environmental conditions than to confer some adaptive advantage.

Transition from parthenogenetic to bisexual reproduction, as well as the appearance of *Daphnia* with the S-variant of G6PD, seem to be the consequences of this activation of latent genetic material (Ruvinsky et al. 1983, 1978). A demonstration of common mechanisms underlying the two phenomena would provide clues to the understanding of why they occur together in some of the clones. The recognized adaptive value of bisexual reproduction in *Daphnia* populations, especially in rapidly dessicating ponds, is beyond doubt. Adaptive advantages are conferred upon those clones with increased susceptibility to unfavourably changing conditions, such as crowding, food scarcity, temperature-fall in ponds. These clones produce males – ephippial females in advance – to resort to bisexuality when critical situations arise.

In general terms, Schmalhausen (1949) has emphasized the importance of the emergence of di- and polymorphism in provision of populational high adaptivity to sharply fluctuating conditions. He had indicated that stabilizing form of selection promotes the formation and evolutionary fixation of such di- and polymorphic systems. With reference to the phenomena we observed in *Daphnia,* di- or polymorphism seems to be based not only on the diversity of allelic variants, but also on latent genes in reserve. These genes have the crucial ability of passing from inactive to active state and vice versa, and thus to comply with stressing environmental conditions. This alternational variability may be a factor of population adaptation. Clone ability for alternational variations is genetically determined (Ruvinsky et al. 1983 a), and intraspecific polymorphism with respect to this character is observed. However, alternational variability is a property providing survival in extreme conditions and it is subjected to natural selection, and, therefore, not consistently observed in all the clones.

The sensitivity threshold to stressing conditions in some of the *Daphnia* clones is so high that they either do not produce males or in very small number, if ever. Being uncapable of becoming bisexual, these clones are eliminated under the pressure of drastically changing

conditions. Only clones that are capable of destabilizing ontogenesis, to pass over to production of males and consequent bisexual reproduction contribute to the gene pool of subsequent generations. This replenishes the hereditary diversity of the progeny to come, thereby presumably ensuring their survival in infavourable conditions and their renewed flourish in favourable ones.

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