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Esterase Genes in *Daphnia pulex*: Linked Inheritance and Genotypic Distribution in Natural Populations

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Summary. Esterase patterns were examined in three populations of *Daphnia pulex*. The total number of bands showing esterase activity was 17. Three major genes Est-1, Est-2, Est-3 controlling esterase synthesis were identified and genetically studied. These genes were found to be located in the same linkage group. It was shown that two or three homologous chromosomes differing in sets of esterase alleles predominantly occur in the populations considered.

Key words: *Daphnia pulex* – Esterases – Genes cluster – genotype frequences – Disequilibrium linkage

Introduction

Cladocera, a parthenogenetically reproducing genera in which both sperm production and sexual eggs occur has long been regarded as a favourable organism with which to study genetical problems of development (Weismann 1877; Banta 1939; Philipchenko 1920; Ruvinsky et al. 1978). One of the representatives of this order is *Daphnia pulex*, which alternates ameiotic parthenogenesis with bisexual reproduction.

It is difficult to take advantage or morphological characters for experimental purposes because their changes are, as a rule, incompatible with normal reproduction and life under demanding environmental conditions. However, electrophoresis has made it possible to examine enzyme patterns in *Daphnidae* (Hebert and Ward 1972). As a result, variability in their biochemical characters was established, and certain clones were marked with biochemical characters.

Materials and Methods

This paper presents the results of the genetical analysis of esterases and describes their polymorphism in natural populations of *Daphnia pulex*. These populations were taken from small ponds near Novosibirsk. *Daphnia* clones were maintained in the laboratory, cultured in bottles and fed with *Chlorella*.

For electrophoretic analysis, individuals were homogenized in 20 μl of a solution containing 40% saccharose and 1% Triton X-100 in the presence of bromphenol blue. Electrophoresis was performed in 7.5% PAG according to Davis (1964). A flat glass chamber was used for vertical electrophoresis without a spacer gel. The procedure took 4 hr. (voltage 200-250 V, current intensity 25-50 mA). A gel staining medium of 10 mg α-naphtyl acetate, 10 mg α-naphtyl propionate and 20 mg β-naphtyl acetate was used. This was dissolved in 2 ml acetone, 100 ml of 0.1 M phosphate buffer, pH 7.4 and 30 mg Fast Blue RR salt.

Results

The *Daphnia pulex* populations studied were very polymorphic for esterase patterns (Fig. 1). Some individuals showed a four-banded esterase pattern while others exibited a pattern consisting of eight or even nine bands. The esterase composition was consistently reproduced from one experiment to another in the same parthenogenetic clone. Staining with a mixture of substrates demonstrated a particular substrate specificity: some zones, which will be further referred to as EST-1, EST-2, EST-3,

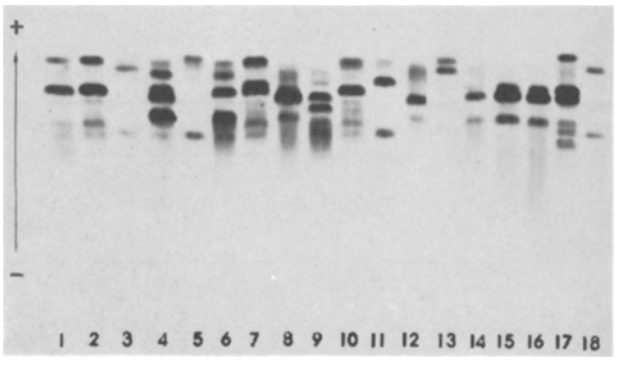

Fig. 1. An esterase pattern obtained from *Daphnia pulex* clones

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Phenotype	Bands	Populations KZ	Z	NZ	Number of bands	Esterase type
EST-4	a	—	—		2	?
	b	—	—	—		
EST-3	a	—	—	—	3	Carboxyl esterase
	b	—	—	—		
	c	—	—	—		
EST-2	a		—	—	6	Carboxyl esterase
	b	—	—	—		
	c	—	—	—		
	d		—	—		
	e	—				
	f	—	—	—		
EST-1	a		—	—	3	Acetyl esterase
	b	—	—	—		
	c	—	—	—		
EST-X	a	—	—	—	3	?
	b	—	—	—		
	c	—				

Fig. 2. A scheme suggested for esterase patterns obtained in three studied *Daphnia pulex* populations

stained dark red; this indicated a high affinity for β-naphtyl acetate. The remaining zones stained black. In a series of experiments, esterase activity was inhibited with different inhibitors (10^{-4}M eserinsulfate, 10^{-5}M E-600, 10^{-3}M p-chlormercuribenzoate Na, 10^{-2}M $CuSO_4$). The slow migrating esterase, EST-1, was tentatively designated as acetyl esterase, and EST-2, EST-3 as carboxyl esterase (Augustinsson 1958) (Fig. 2).

The determination of the number of genes controlling the appearance of the different esterases was of importance. Intraclonal crosses were performed for this purpose. Figure 3 (left) shows the phenotypes of the esterases of clone BG-1 and those of the intraclonal F_1 hybrids. The first channel from the left presents the phenotype of clone BG-1, consisting of three pairs of bands. Judging by their staining and position, they correspond to EST-1, EST-2, EST-3. The F_1 offspring obtained from intraclonal

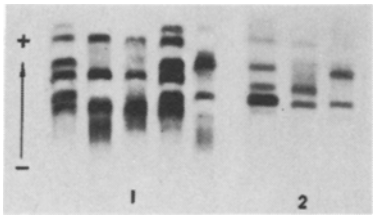

Fig. 3. An esterase pattern yielded by clone BG-1 and clone 13 and their offsprings by intraclonal crosses

crosses consisted of three phenotypic classes. The esterase pattern of phenotypic class I was identical to parental esterase (EST-$1^{b,c}$, EST-$2^{c,e}$, EST-$3^{b,c}$), as shown in Figure 3(1), (the fourth channel from the left). That of phenotypic class II, was represented by three bands, each zone displaying one band. Of interest here is that each band represented a slow migrating variant of the parental esterase (EST-1^{c}, EST-2^{e}, EST-3^{c}) Figure 3(1), (the second and third channels from the left). Those of phenotypic class III, were composed of the fast variants of parental esterases (EST-1^{b}, EST-2^{c}, EST-3^{b}), Figure 3(1) – the fifth channel from the left. The ratio of these phenotypic classes in the F_1 hybrids did not deviate from the monohybrid (Table 1). It is noteworthy that one offspring from this cross had an unusual set of esterase fractions (EST-$1^{b,c}$, EST-2^{c} and EST-3^{b}), i.e., two bands in zone EST-1 and a band in zones EST-2 and EST-3.

Taken together, the results of inter se mating of clone BG-1, the data indicating population variability of esterases in *D. pulex* and finally the results of experiments with inhibition of enzyme activity (demonstrating affinity for different substrates), allowed us to assume that there are three major esterase genes located in the same linkage group. The appearance of individuals with phenotypes EST-$1^{b,c}$, EST-2^{c} and EST-3^{b} may be regarded as a result of a recombinational event.

To test this hypothesis, intraclonal crosses (13 × 13) were performed. The progeny phenotypes of esterases are shown on the right of Figure 3(2). The esterase pattern of paternal clone 13 was composed of two bands in the zone of EST-2 activity and one band in each of the other zones. Their phenotypes may be designated as EST-1^{b}, EST-$2^{c,f}$, EST-3^{b}. Three phenotypic classes appeared

Table 1. Results of genetic analysis of esterases in *Daphnia pulex*

Cross	Parental genotypes	Offspring genotypes	χ^2	P
BG-1 × BG-1	Est-$1^{b,c}$ Est-$2^{c,e}$ Est-$3^{b,c}$	8 Est-$1^{b,b}$ Est-$2^{c,c}$ Est-$3^{b,b}$ 19 Est-$1^{b,c}$ Est-$2^{c,e}$ Est-$3^{b,c}$ 4 Est-$1^{c,c}$ Est-$2^{e,e}$ Est-$3^{c,c}$	1.61	$p < 0.95$
N13 × N13	Est-$1^{b,b}$ Est-$2^{c,f}$ Est-$3^{b,b}$	14 Est-$1^{b,b}$ Est-$2^{c,c}$ Est-$3^{b,b}$ 14 Est-$1^{b,b}$ Est-$2^{c,f}$ Est-$3^{b,b}$ 7 Est-$1^{b,b}$ Est-$2^{f,f}$ Est-$3^{b,b}$	4.62	$p < 0.95$

Table 2. Frequencies of the different esterase genotypes found in *Daphnia pulex* populations studied[a]

Populations	Z, spring 1978				NZ, spring 1979				Z, autumn 1979				KZ, spring 1978					
Genotypes	Gz^1	Gz^2	Gz^3	Rare, recombinants	Gz^1	Gz^2	Gz^3	Rare, recombinants	Gz^1	Gz^2	Gz^3	Rare, recombinants	Gk^1	Gk^2	Gk^3	Gk^4	Gk^5	Gk^6	Rare, recombinants
Genotype frequencies %	9.4	48.2	30.6	4.7 7.1	18.4	39.8	15.3	16.3 10.2	3.6	41.1	35.7	15.3 14.3	22.5	39.3	7.9	7.9	11.2	2.2	4.5 4.5
Phenotypes																			
Total number	85				98				56				89						

Designations: Gz^1 – Est-$1^{b,b}$ Est-$2^{b,b}$ Gk^1 – Est-$1^{b,b}$ Est-$2^{f,f}$ Est-$3^{b,b}$ Gk^4 – Est-$1^{b,c}$ Est-$2^{e,f}$ Est-$3^{b,c}$
Gz^2 – Est-$1^{a,b}$ Est-$2^{c,b}$ Gk^2 – Est-$1^{b,b}$ Est-$2^{c,f}$ Est-$3^{b,b}$ Gk^5 – Est-$1^{b,c}$ Est-$2^{c,e}$ Est-$3^{b,c}$
Gz^3 – Est-$1^{a,a}$ Est-$2^{c,c}$ Gk^3 – Est-$1^{b,b}$ Est-$2^{c,c}$ Est-$3^{b,b}$ Gk^6 – Est-$1^{c,c}$ Est-$2^{e,e}$ Est-$3^{c,c}$

[a] The electrophoretic mobility of EST-1^a is nearly identical to that of EST-2^f.

in the F_1: one class was identical to parental esterase and the other two differed in EST-2 and had either fast (c) or slow (f) variants. The ratio of offsprings with different phenotypes did not deviate from that expected in a monohybrid cross (Table 1). From the results of this cross it was concluded that clone 13 is heterozygous for EST-2 and homozygous for EST-1, EST-3. Thus, based on hybridological data, it was inferred that there are three major genes (Est-1, Est-2, and Est-3) located in the same linkage group and responsible for the appearance of the corresponding esterases.

The fastest esterase was designated as EST-4. However, it was not always detected and its genetic control is unclear. The slowest 2-3 esterases bands were called EST-X because their electrophoretic expression was unstable.

A scheme for *D. pulex* esterase patterns and genetic control is given in Fig. 2. The populations differ in esterase composition. These differences are most distinct with respect to genotype frequencies in the populations (Table 2). While allele 'b' of Est-1 was widely distributed in all the populations, its allele 'a' occurred in Z and NZ and allele 'c' in the KZ and NZ populations. Of the 6 alleles identified at locus Est-2, only allele 'c' was frequently encountered in all the populations. Alleles 'a' and 'd' were observed in the Z and NZ populations and allele 'e' was found in KZ population only. Allele 'b' was frequent in the Z and NZ populations, but rare in KZ populations; the reverse was observed for allele 'f'. At the Est-3 locus, allele 'b' was the most frequent (the other alleles appeared rarely, if at all).

As a result of analysis of genotype frequencies in the populations (Table 2), it was established that the majority of individuals in the Z and NZ populations have, as a rule, three esterase genotypes[a], with Gz^1 and Gz^3 genotypes being homozygous for Est-1 and Est-2, and Gz^2 heterozygous for these genes. From the genotypic formula for homozygotes and heterozygotes, it may be expected that two chromosomes, Est-1^b Est-2^b and Est-1^a Est-2^c, would be the most frequent. The data obtained confirm this expectation. Alternatively, it may be assumed that a high number of heterozygotes have chromosomes with another set of alleles, Est-1^b Est-2^c and/or Est-1^a Est-2^b. Accepting this assumption, many homozygotes for such chromosomes should arise in the panmictic crosses; however, this was not the case. Furthermore, the genotype frequencies calculated by Hardy-Weinberg's formula did not differ significantly from those observed. This conformance demonstrates that the populations studied were sufficiently large and panmictic when bisexually reproducing. An exception was the Z population, which was examined

[a] In Z and NZ populations the electrophoretic expression of Est-3 is weaker and, for this reason, variants of this esterase were excluded from further analysis

repeatedly in 1979 after an obligatory cycle of bisexual reproduction and a long period of predominantly parthenogenetic reproduction.

Comparisons of genotype frequencies in Z population in 1978 and 1979 show a good fit (Table 2). The samples examined were not very large and the time of year differed, nevertheless the frequencies of genotypes and specific chromosomes remained quite stable. In addition to the three major genotypes in the Z and NZ populations, rare genotypes also occur. These genotypes may be assigned to two groups: the first includes individuals which have rare alleles for Est-2-a,d,f, and the second includes those having the commonly observed alleles in unusual combinations – Est-$1^{a,a}$ Est-$2^{b,c}$; Est-$1^{b,b}$ Est-$2^{b,c}$; Est-$1^{a,b}$ Est^{b,b}; Est-$1^{a,b}$ Est-$2^{c,c}$, presumably resulting from crossing over. More than half of *Daphnia* with rare genotypes were recombinants.

The esterase patterns obtained from the K-Z population were similar, but more complex. Six main genotypic groups were distinguished. They possibly arose as a result of the combination of three major homologous chromosomes widespread in this population: Est-1^{b} Est-2^{f} Est-3^{b}; Est-1^{b} Est-2^{c} Est-3^{b}; Est-1^{c} Est-2^{e} Est-3^{c}. As shown in Table 2, individuals bearing chromosome Est-1^{b} Est-2^{f} Est-3^{b} are the most frequent and those bearing chromosome Est-1^{c} Est-2^{e} Est-3^{c} are rare.

Among animals with rare genotypes, 5 *Daphnia* with an unusual esterase set were identified. Parthenogenetic clones were obtained from these individuals. The esterase pattern of clone NZ-10, for example, had bands showing EST-1 and EST-3 activities, but no band corresponding to EST-2 activity. Cases of nonexpression of EST-1 and EST-3 were also observed (Fig. 1). The reason for this non-expression is not quite clear.

There was another unclear observation. The esterase patterns yielded by individuals from the same parthenogenetic clone were, as a rule, identical. However, the expression on EST-X was variable within some clones. As seen in Figure 4, *Daphnia* from clone BG-4 have either both the slow and fast esterases, or one of them.

The esterases identified in *Daphnidae* can be used as an additional character in taxonomic investigations (Fig. 5).

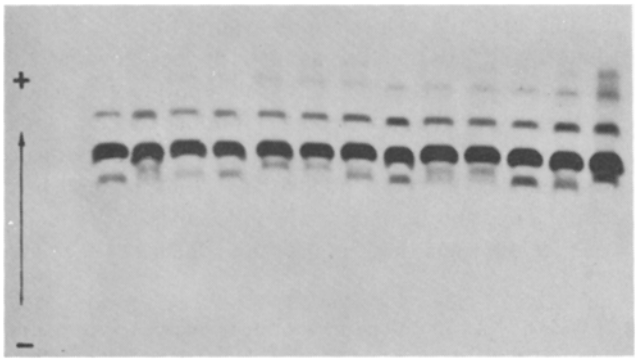

Fig. 4. Variability for EST-X individuals from clone BG-4

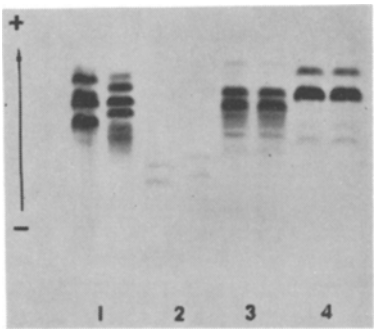

Fig. 5. A comparison of the esterase patterns obtained from different *Daphnidae*: *1 D. pulex*; *2 D. magna*; *3, 4 D. longispina*

Discussion

Seventeen bands esterase activity for high polymorphism for this biochemical character in the *Daphnia* populations were examined. Our unpublished data indicate that *Daphnia pulex* is also polymorphic for other enzymes. *Daphnidae* has been thought to be more monomorphic than other species studied (Hebert 1978). However, the number of biochemical markers used in previous studies were too small to reveal the true range of polymorphism. Recent data demonstrate that *Daphnia* are, indeed, polymorphic (Hebert and Crease; 1980).

Genetic analysis allowed us to establish three major loci, Est-1, Est-2, Est-3, controlling the synthesis of esterases which are located in the same linkage group. This point deserves consideration. The location of esterase genes in the same linkage group suggests that these genes have resulted from tandem duplication (Ohno 1970). This appears plausible because the esterases, which the genes code for, are similar in electrophoretic mobility and other biochemical properties.

Clusters of esterase genes have been described in many animals assigned to different taxons (Green 1975; Ogita and Kazaky 1965). We observed such clusters in *Crustacea*. It seems unlikely that esterase clusters of this kind could have been inherited by such distant classes of animals from a common ancestor. If they arose as a result of convergence, one has to concede that similar genetic processes providing the optimum organization of cellular biochemical systems underlied the origin of an esterase gene cluster, and this appears more probable.

The observations made are seemingly contradictory: the number of esterase alleles at the loci studied is large, yet the number of homologous chromosomes widely-spread throughout the populations is very small. The data obtained indicate that recombinational events do occur in *Daphnia* when they reproduce bisexually with the involvement of, at least, that chromosome in which the esterase genes are located. The observations can be reconciled when one recalls that chromosomes with a particu-

lar allelic set confer adaptive advantages to its' bearers. As a result, definite alleles of different esterase genes happen to be linked, in spite of proceeding recombination. Thus there arises a situation similar to the one described in the literature as disequilibrium linkage. It is quite possible, that under some conditions, coadapted gene complexes may have higher adaptive value than recombinant chromosomes or chromosomes with rare alleles. The latter seem to be used in emergencies, and they never exceed very low concentrations. Further critical evaluation of experimental data is needed.

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Literature

Augustinsson, K.B. (1958): Electrophoretic separation and classification of blood plasma esterases. Nature 181, 1786-1789

Banta, A.M. (1939): Studies on the physiology, genetics, and evolution of some *Cladocera*. Washington, D.C.: Publ. Carnegie Inst. of Washington

Davis, B.J. (1964): Disc electrophoresis. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. 121, 404-427

Green, M.C. (1975): The laboratory mouse, *Mus musculus*. In: Handbook of Genetics, Vol. 4. New-York-London: Acad. Press

Hebert, P.D.H. (1978): The population biology of *Daphnia*. Biol. Rev. 53, 387-426

Hebert, P.D.H.; Crease, T.J. (1980): Clonal coexistence in *Daphnia pulex* (Leydig): another planktonic paradox. Science 207, 1363-1365

Hebert, P.D.H.; Ward, R.D. (1972): Inheritance during parthenogenesis in *Daphnia magna*. Genetics 71, 639-642

Ogita, Z.; Kazaki, I. (1965): Genetic control of multiple esterases. In: *Musca domestica*. Jpn. J. Genet. 40, 1-14

Ohno, S. (1970): Evolution by gene duplication. Berlin-Heidelberg-New -York: Springer-Verlag

Philipchenko, Yu.A. (1920): Topics on variability. Variability in young and adult *Cladocera*. Proceed. Petrograd. Natur. Soc. 51, 1-17

Ruvinsky, A.O.; Cellarius, S.F.; Cellarius, Yu.G. (1978): The possible role of genome activity changes in the sex determination of *Daphnia pulex*. Theor. Appl. Genet. 52, 269-271

Weismann, A. (1877): Beiträge zur Naturgeschichte der Daphnoiden. Z. Wissenschaftl. Zool., 28, 93-254

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