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The search for hidden enzymatic variation in the aphid *Macrosiphum rosae* (L.)

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Summary. Using two different buffer systems and up to 6 different electrophoretic polyacrylamide gel concentrations, hidden enzymatic variability was investigated in samples of the rose aphid *Macrosiphum rosae*. MDH1, PGM1, SDH, EST and LAP were found to be polymorphic. No additional variation was observed by changing the test conditions compared to those of earlier investigations using starch gel electrophoresis.

Key words: Apids – Allozyme variation – Hidden polymorphism

Introduction

During the last two decades the main interest of population geneticists has focused on genetic variability at the enzyme level. The results of numerous investigations generally revealed a much higher allozyme variability than had been expected from the selection theory (Ayala 1976; Nevo et al. 1984), while on the contrary, they strongly supported the theory of neutrality (Nei 1975). It could be shown that differences exist with respect to the enzyme variation between species within a genus, and between the genera, without a generally valid correlation to distinct biological factors. In the meantime the interest of population geneticists shifted from the importance of allozyme variability in the evolution of the species per se, to various other topics. This does, however, not imply that enzyme systems are entirely devoid of interest. For investigations of population structures and the characterization of species and their relationships enzymes are still significant as markers for the identification of geno- and phenotypes respectively.

With these aims in mind, populations of different aphid species have recently been investigated. Publications discussing this field of research have been reviewed by Young (1983). Although comparatively few publications exist on aphids, it could be shown that many species of the family *Aphididae* exhibit a relatively low degree of polymorphism, i.e. the percentage of polymorphic loci is low (May and Holbrook 1978; Wool et al. 1978; Suomalainen et al. 1980; Tomiuk and Wöhrmann 1980, 1981, 1983; Tomiuk et al. 1979; Wöhrmann et al. 1978), although there are species, for example *Sitobion avenae*, which are highly polymorphic (Loxdale et al. 1985a). The degree of allozyme variability is different at each locus and the electrophoretic patterns are typical for the respective enzyme systems investigated. The rose aphid, *Macrosiphum rosae*, is polymorphic at three enzyme loci – PGM1, MDH1, SDH – out of 29 and their quaternary structure is monomeric, dimeric and tetrameric, respectively (Tomiuk and Wöhrmann 1983). In the past, the polymorphic esterase loci were disregarded in our population genetic studies. The reason for this was the existence of several genetically independent isoenzymes. An unequivocal interpretation of the observed electrophoretic patterns is not possible without undertaking crossing experiments. If the degree of heterozygosity H is accepted as a measure of the genetic variability, we calculated $H = 0.032$ in the case of *M. rosae*, and from 16 more species, a mean value $H = 0.010$ was produced (Tomiuk and Wöhrmann 1983). These are very low values compared to species from other families (cf. Nei and Graur 1984; Graur 1985). For *Drosophila* and other invertebrates, a heterozygosity degree of $H = 0.12$, and for vertebrates $H = 0.06$, was found (Ayala 1982).

The electrophoretic methods used detect only some of the existing variability since the separation of proteins depends on their quaternary structure as well as the charge on the molecular surface. Each exchange of an amino acid does not necessarily result in a change of the electric charge of the protein. Nei (1975) estimated that only about 30% of the protein variants could be detected by electrophoresis. Hidden genetic variability, which is not based on obvious differences in the electric charge, was subsequently found following the introduction of sequential electrophoresis, heat and urea

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denaturation, and peptide mapping. However, the greatest proportion of protein variation has been detected using the standard methods (Ayala 1982). Moreover, additional variability was generally only detected at such loci already known as polymorphic. Loxdale et al. (1985b) consequently assumed that in aphids more variability could be found if appropriate methods were used. The authors spoke of a high degree of polymorphism in *Sitobion avenae* for three peptide isozymes and 6-phosphogluconate dehydrogenase, by varing the running time and the gel concentration. They presumed that the degree of polymorphism described by Tomiuk and Wöhrmann (1983) could also be increased by suitable electrophoretic methods.

In this paper, we discuss our results on *M. rosae* using starch gel and polyacrylamide gel electrophoresis, respectively.

Materials and methods

Aphids

Samples of the rose aphid *Macrosiphum rosae* (L.) were taken from different European populations and from different host plants (Table 1). Clones were reared in the laboratory under constant environment conditions (21 °C, 17 h day length, 60% air humidity). All electrophoretic methods used in these investigations were applied to species of the same clone.

Electrophoresis

In our recent works, aphids were investigated by means of starch gels (Tomiuk and Wöhrmann 1980, 1983). In the present investigations, polyacrylamide slab gels (Havanna System, Desaga Heidelberg, FRG) were used. The tris-glycine buffer system, pH 8.9 (modified system 1; Allen and Maurer 1974) and the tris-veronal buffer system, pH 7.5 (modified system 6; Allen and Maurer 1974) were applied. The monomer concentration (T) was varied from 5.25 to 9% in the experiments. The proportion of bisacrylamide (cross linkages, C) relative to the total amount of monomers was 4%. The electrophoresis was performed at 4 °C for about 3.5 h (6 W/gel constant, 120 V). For optimal sample staining, up to 6 aphids per clone were electrophoresed. The staining procedures were carried out as detailed by Shaw and Prasad (1970), Allen and Maurer (1974) or Blaich (1978).

Regression

The relationship between enzyme mobility and monomer concentration was calculated using the formula $R_m = M_0 \exp(K_r T)/U_t$ (Chrambach and Rodbard 1971), where R_m is the relative mobility, M_0 the free mobility, U_t the absolute mobility of the gradient in front of the separation phase and K_r the retardation coefficient ($K_R = K_r/2.303$). T represents the percentage of monomers in the separation gels. A linear relationship exists between log R_m and T.

Results

The number of clones investigated in the different disc-systems is listed in Table 2. No more variants are found in the case of the monomeric PGM1 and the dimeric MDH1, respectively, in comparison to starch gel electrophoresis. Using polyacrylamide gels, however, additional bands were observed in all MDH phenotypes which could be explained by the different stages of an enzyme-coenzyme complex. Such a mechanism is already known in the case of ADH by Jacobson et al. (1972) and Schwartz and Sofer (1976). Moreover, in the case of PGM, a second monomorphic locus (PGM2) was detected. SDH exhibits two phenotypes which are the homozygotes of a di-allelic system. Using starch gels, all three expected genotypes could be shown, with the heterozygotes displaying a five banded pattern which suggests a tetrameric structure for the enzyme (Tomiuk and Wöhrmann 1983). Due to the suboptimal separation and staining of the esterases in starch gels, no information about the genetic basis of the polymorphic loci could be given in the past (Tomiuk and Wöhrmann 1983). On the contrary, the separation in acrylamide gels yields a multiplicity of clear bands (Fig. 1), whereby 4 classes of isozymes may be distinguished. The classes are designated with EST1 to EST4 in order of their electrophoretic mobility. EST1 is

Table 1. Number of clones (*n*), their origin and host plants

n	Origin	Host
65	Tübingen[a]	Rosa
2	Mössingen[a]	Rosa
6	Stuttgart[a]	Rosa
15	Pfalz[a]	Rosa
4	Jugoslavia	Rosa
3	Turkey	Rosa
8	Tübingen[a]	Knautia
3	Bremen[a]	Knautia
6	Austria	Knautia

[a] Location in FRG

Table 2. Number of clones (*n*) investigated in different disc-systems. The observed polymorphism (+, –) of 11 enzyme systems investigated in starch and in polyacrylamide gels, is given.

Enzyme	Disc 1	Disc 6	Polymorph in	
	n	*n*	Polyacryl-amide	Starch
MDH1	79	31	+	+
MDH2	79	31	–	–
PGM1	90	32	+	+
PGM2	90	32	–	(–)
SDH	37	–	+	+
EST	112	32	+	(+)
LAP	85	35	+	0
ME	81	–	–	–
ACPH	90	32	–	–
KAT	20	–	–	–
PGI	20	–	–	–

0 = not investigated; (±) = no clear indentification

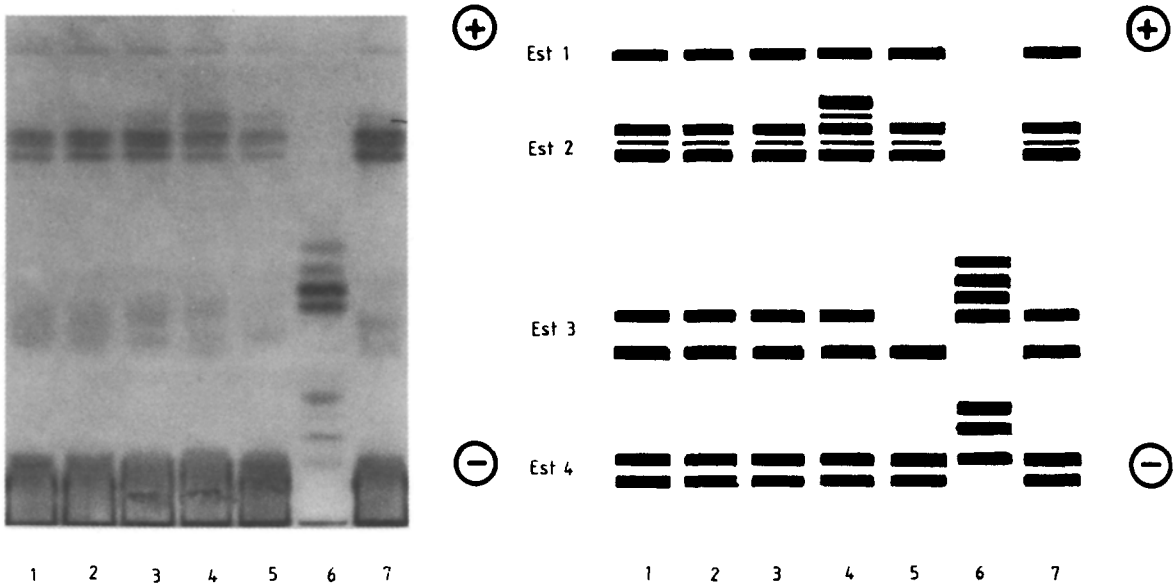

Fig. 1. Esterase patterns of aphids in polyacrylamide slab gels (Disc 1; T=7.5, C=4) and their schematic representation. No. 6 is the pattern of an endoparasited aphid

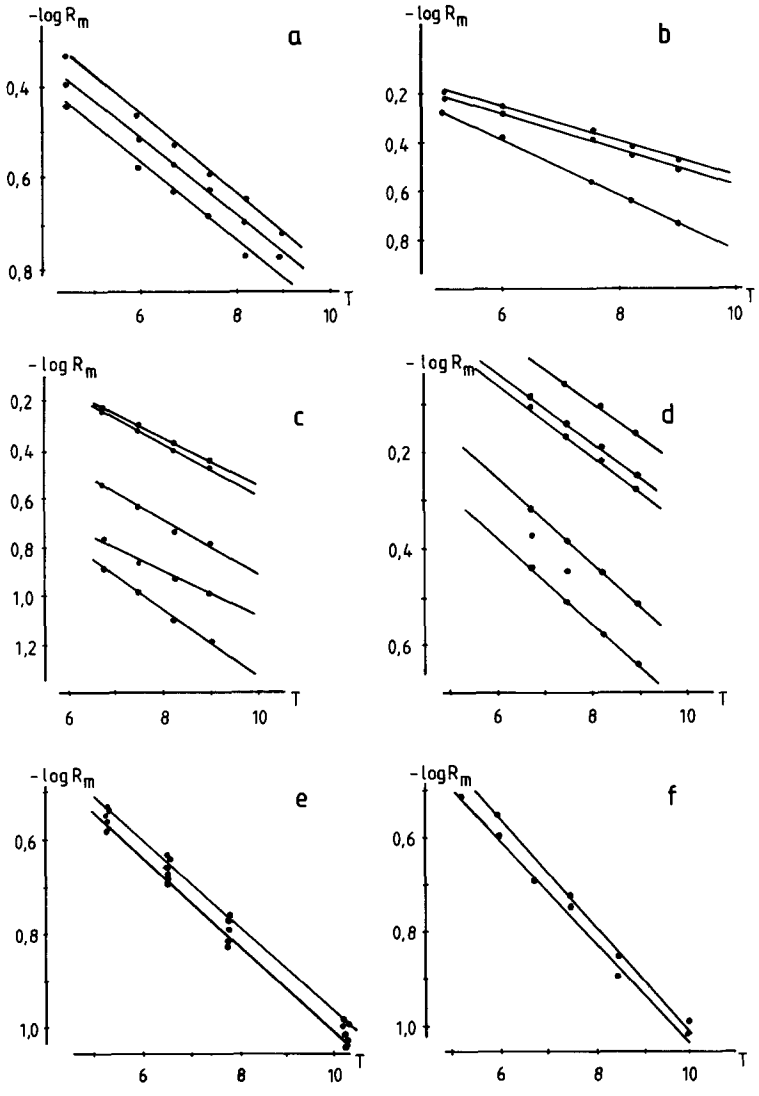

Fig. 2a–f. Correlations between the mobility (–log R_m) and the monomer concentration in the separation gel (T). The retardation coefficient is given for each enzyme system in descending order. **a** Pattern of the MDH1 heterozygote (Disc 1, C=4, K_R: 0.075±0.005, 0.077±0.014, 0.075±0.003). **b** Pattern of the PGM1 heterozygote m/f (Disc 1, C=4, K_R: 0.072±0.003, 0.071±0.004) and the PGM2 band (0.111±0.003). **c** Different bands of the LAP (Disc 1, C=4, K_R: 0.098±0.010, 0.104±0.005, 0.114±0.002, 0.096±0.008, 0.137±0.011). **d** Pattern of the EST-systems (Disc 1, C=4). EST1 (0.075), EST2 (0.072±0.003, 0.075±0.003), EST3 (0.087±0.008, 0.091±0.006). **e** SDH patterns (Disc 1, C=2.65, K_R: 0.088±0.004, 0.088±0.004). **f** SDH patterns (Disc 1, C=4, K_R: 0.108±0.003, 0.114±0.002)

single banded and corresponds to the pattern found in starch gel electrophoresis. The structure of EST2 may be likewise interpreted. Two classes occur which can be separated presupposing two loci under the regime of two alleles. EST3 patterns differ in number and intensity of bands. EST4 is not polymorphic and all clones exhibit two bands (Weber 1980).

Two different phenotypes are observed in the case of LAP, one of which was only found on a single occasion in a sample of 85 clones. Both variants are considered to be the homozygotes of a locus with two alleles. All other enzymes (MDH2, ME, ACPH, CAT, PGI) do not vary. Prior to this, they had also not exhibited any polymorphism using starch gels.

In Fig. 2 the relationship between electrophoretic mobility ($-\log R_m$) and monomer concentration (T) is shown. Figure 2a shows the heterozygote phenotype of MDH1, Fig. 2b the PGM heterozygote. Fig. 2c shows the LAP and Fig. 2d the EST patterns. Finally, in Fig. 2e and f the SDH patterns, dependent on two proportions of cross linkage, are demonstrated. It is of importance that (1) all gel concentrations yield a separation of the bands, (2) the regression lines are not parallel to each other in some cases. This proves the possible interaction of enzyme mobility and gel concentration.

Discussion

Loxdale et al. (1985b) employed one-dimensional polyacrylamide gel electrophoresis in their research. This technique is very suitable as it is possible to extend the molecular sieving effects of the gels in a wider range. This is not possible to the same extent using starch gels (Brewer 1970). Furthermore, the transparency of polyacrylamide gels permits the detection of even weakly stained bands, whilst the use of discontinuous systems improves the separation of the proteins. Different pore diameters and pH-values in the spacer and the separation gel result in the concentration of the sample before it is separated (Allen and Maurer 1974). In the above mentioned investigations, two different disc systems and at least four monomer concentrations were used to detect a possible hidden genetic variability. However, additional variability was not found in the systems (MDH1, PGM1, SDH) already known as polymorphic. Only in the case of esterases and leucine amino peptidases did the clear separation of isoenzymes facilitate the distinction of different phenotypes. Yet again, no additional variation was found by varying the experimental conditions.

This contrasts with Loxdale et al.'s (1985b) investigations where they found much greater variability in *S. avenae* by varying electrophoretic methods. Loxdale et al. (1985b) investigated two enzyme systems, PEP and PGD. A remarkable hidden variability was detected by varying running time and gel concentration (5% and 6%). The variation of the running time might, however, be less significant because the optimal time should be ascertained in preliminary experiments. Thus, the gel concentration remains the only test parameter. Moreover, for each clone, genetically identical individuals should be tested under the different conditions used while independent samples were investigated by Loxdale et al. (1985b). It is thus more likely that the new variants described are rare alleles within the population rather than variants detected by the methodical variations. Furthermore, the different alleles described can also be identical since the relative mobility between alleles can be changed by different test conditions. This becomes apparent if the change of the enzyme mobility dependent on the gel concentration is considered in the cases of PGM (Fig. 2b), LAP (Fig. 2c), and SDH (Fig. 2f). For example, using identical genotypes from one clone in the PGM system, different relative mobilities are found for the monomer concentrations T=6 and T=9. Therefore, the existence of three alleles might be erroneously concluded if the test conditions are not comparable.

The PEP system investigated by Loxdale et al. is highly complex. The difficulties and the peculiarity of these samples lie in the fact that double heterozygotes occur for isoenzymes in connection with the very low observed degree of heterozygosity at the single loci and, thus, the lack of heterozygotes at only one locus. We conclude that the interpretation of such a complex enzyme system is only possible on the basis of crossing experiments. But due to the cyclic parthenogenesis experienced by many aphid species, genetic experiments are difficult to carry out. The sexual generation as well as the resulting parthenogenetic populations in spring are induced by environmental factors (i.e. the day length, temperature). Up-to-date crossing experiments are not possible all year round in the laboratory. Therefore, enzyme patterns can only be explained by analogy to those in *Drosophila* and other species.

A general question – whether it is useful to use such enzyme systems as markers in population biology investigations – may be raised. Rhomberg et al. (1985) and Singh and Rhomberg (1984) investigated *M. rosae* and *Aphis pomi,* respectively. In both investigations frequency changes of esterase phenotypes were discussed although the genetic basis of these has not unequivocally been solved. Moreover, Rhomberg et al. (1985) interpreted two banded MDH phenotypes as heterozygotes assuming a monomeric structure of the protein. This has already been refuted by Tomiuk and Wöhrmann (1981). We rather suppose that parasited species were included in the samples. Both factors can lead to an overestimation of the average degree of heterozygosity and to incorrect genetic models.

However, such difficulties support the assertion that such complex systems should be rejected in the investigations on aphids. On the basis of the above investigations, we assume that the estimated degrees of

heterozygosity ($H < 0.04$) are unbiased. We still do not rule out the possibility of detecting and additional variability by means of more improved methods but where different species and their degree of heterozygosity are compared, the same methods have to be applied to all organisms under observation.

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