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Electrophoretic study of enzymes from cereal aphid populations

4. Detection of hidden genetic variation within populations of the grain aphid *Sitobion avenae* (F.) (Hemiptera: Aphididae)

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Summary. A study of variation in three peptidases (PEP-3 to -5) in a parthenogenetic *S. avenae* field population at Rothamsted using serial one-dimensional polyacrylamide gel electrophoresis (involving changes of gel concentration and electrophoretic run-time) increased the overall number of "allozymes" (mobility variants) detected from 10 under standard conditions (6% gels, 2 h run-time) to 22, as well as revealing putative heterozygous banding patterns under some test conditions. However, an examination of another enzyme, 6-phosphogluconate dehydrogenase (6-PGD) in a sample collected at Rothamsted the following year failed, using a combination of serial methods (changes of gel concentration) and isoelectric focusing, to increase the total number of 6-PGD bands separated (seven, none of which appeared to be allelic in origin). Nevertheless, some major bands were split into several bands, whilst other infrequent bands were either gained or lost. The findings are briefly discussed.

Key words: Parthenogenetic aphid populations – Allozymes – Polymorphic loci – Hidden genetic variation

Introduction

Using electrophoretically-separated enzyme markers, studies on populations of nineteen aphid species have shown the majority of these to be largely or completely genetically invariant, including species which regularly annually overwinter in the egg stage following sexual reproduction (May and Holbrook 1978; Wool et al. 1978; Suomalainen et al. 1980; Tomiuk and Wöhrmann 1980, 1983).

Exceptions include American field populations of *Macrosiphum euphorbiae* (May and Holbrook 1978), German field populations of *Acyrthosiphon pisum* and *Wahlgreniella nervata* (Tomiuk and Wöhrmann 1980) and British and Spanish field populations of *Sitobion avenae* (the grain aphid, a major pest of cereals in Europe) (Loxdale et al. 1985). The latter species showed a high percentage polymorphism in two successive years (64 and 19%), a low average heterozygosity (ca. 2%) in both years, as well as clear spatial and temporal population differences in allozyme frequency at some polymorphic loci.

It is now well established that variation of electrophoretic technique (e.g. changes of run-time, buffer, pH ('serial electrophoresis'), urea and heat denaturation, isoelectric focusing, two-dimensional electrophoresis etc) can reveal substantial additional enzyme/protein variation in sexually reproducing organisms such as *Drosophila* and mammals (Coyne 1976; Cochrane and Richmond 1979; Ramshaw et al. 1979; Jones 1980; Aquadro and Avise 1982). In contrast, however, there have been relatively very few studies to examine the amount of "hidden genetic variation" in parthenogenetic organisms.

We report here on variation in three peptidases (PEP-3 to -5) (EC 3.4.11) and 6-phosphogluconate dehydrogenase (6-PGD) (EC 1.1.1.44) in asexual *S. avenae* field populations at Rothamsted using, respectively, either serial electrophoresis (gel concentration and run-time changes) or serial electrophoresis (gel concentration changes only) together with isoelectric focusing.

Materials, methods and enzyme banding patterns

A dark brown *S. avenae* clone derived from a single parthenogenetic female and maintained on wheat seedlings (cv. 'Cappelle') under greenhouse conditions (14–16 h day length, 20–30 °C) was used as the standard clone throughout the study.

All field populations examined were sampled from wheat at Rothamsted, aphids used for the peptidase study during June–July 1982 from several cultivars in an area of approximately 150 ha, whilst those used for the 6-PGD study were collected during July 1983 from a single 2 ha field (cv. 'Avalon').

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Table 1. Allele frequencies at three peptidase loci in samples of *S. avenae* electrophoresed under standard and test conditions

Gel concentration		6%		5%	
Run-time		2 h	3.5 h	2 h	3.5 h
Condition		(i) (Standard)	(ii)	(iii)	(iv)
Enzyme locus	"Allele"	$n=82$	$n=92$	$n=96$	$n=84$
PEP-3	0.82		0.005		
	0.84				0.006
	0.94	0.024	0.022	0.010	
	0.96		0.022	0.052	0.012
	1.00	0.939	0.897	0.896	0.970
	1.02		0.043		0.012
	1.04	0.024		0.031	
	Absent	0.013	0.011	0.011	–
	H	–	0.011	–	0.012
PEP-4	0.92	0.049		0.020	
	0.94			0.010	0.012
	0.96	0.073	0.076	0.266	0.131
	0.98		0.158		0.131
	1.00	0.878	0.717	0.688	0.726
	1.02		0.033		
	1.04		0.016	0.016	
	Absent	–	–	–	–
	H	–	0.011	0.033	–
PEP-5	0.79				0.012
	0.94	0.110	0.033	0.146	0.107
	0.96	0.085	0.152		0.095
	0.98		0.087	0.188	0.036
	1.00	0.780	0.679	0.640	0.750
	1.02		0.033		
	1.04	0.025	0.011	0.021	
	1.06		0.005	0.005	
	Absent	–	–	–	–
	H[a]	–	0.011	0.021	–

[a] H = frequency of heterozygotes, n = sample size

For each enzyme, aphids run under the different conditions were all collected at the same time and represented subsamples of the main sample. Methods of sampling, sample transport, extraction of aphids from host plants and storage prior to electrophoretic testing are described by Loxdale et al. (1985).

Sample preparation and the (vertical) one-dimensional slab polyacrylamide techniques, staining and fixing systems used for PEP and 6-PGD are detailed by Loxdale et al. (1983). Electrophoresis of peptidases was performed using a discontinuous tris/barbitone, pH 7.45; tris/HCl buffer system; pH 7.8, and four experimental regimes: 6% gels, 2 h (standard conditions) and 2.5 h run-time; 5% gels, 2 and 3.5 run-time (all at constant 150 V). Electrophoresis of 6-PGD was performed using a continuous tris/citrate buffer system, pH 6.8 and two experimental regimes: 6% gels, 5 h run-time (standard conditions) and 5% gels, 5 h run-time (constant 100 V).

Isoelectric focusing gel mixture (pH range 3–10) was prepared according to the Bio-Rad (Caxton Way, Watford, Herts, U.K.) literature and cast on 100×125×1 mm glass plates using a thin (0.8 mm) layer capillary casting system (Bio-Rad), enough to make four gels. These were then run on horizontal equipment (Bio-Rad, Model 1415) at 250 V, 4 W constant power for 3 h with 1 M NaOH- and 1 M H_3PO_4-soaked cathodal and anodal wicks, respectively.

The mobility of all sample electrophoretic variants (from cathodal gel origin) was expressed relative to that (1.0) of an appropriate standard band in the reference strain run on every gel. Differences in peptidase allele frequency between standard and experimental conditions were tested for significance using the genetic chi-square contingency test of Workman and Niswander (1970), a test which takes into account differences in sample size.

Previously, using standard techniques, PEP-3 was found to be monomorphic within most of 13 British and Spanish populations surveyed and had a maximum of four "allozymes" ($PEP^{0.8,\ 0.86,\ 0.95,\ 1.0}$), whilst PEP-4 and -5 were polymorphic (frequency of commonest allele $\leqslant 0.95$) both with four "allozymes" ($PEP^{0.96,\ 1.0,\ 1.04,\ 1.08\ \mathrm{and}\ 0.95,\ 1.0,\ 1.05\ \mathrm{and}\ 1.11}$, respectively) (Loxdale et al. 1985). These peptidases have different substrate

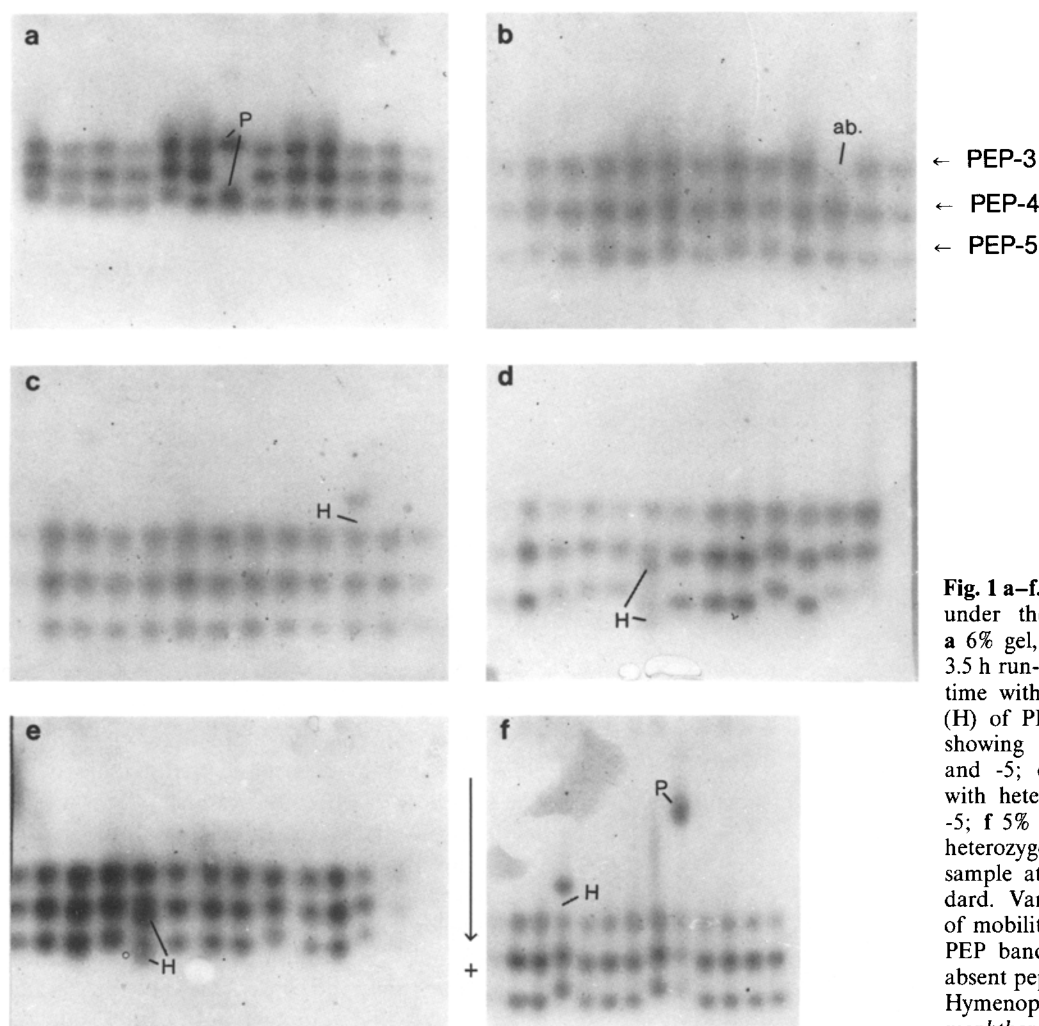

Fig. 1 a–f. Peptidase separation under the following conditions: **a** 6% gel, 2 h run-time; **b** 6% gel, 3.5 h run-time; **c** 6% gel, 3.5 h run-time with (putative) heterozygote (H) of PEP-3; **d** same as **c**, but showing heterozygotes of PEP-4 and -5; **e** 5% gel, 2 h run-time with heterozygotes of PEP-4 and -5; **f** 5% gel, 3.5 h run-time with heterozygote of PEP-3. First sample at *left* on each gel = standard. Variants recorded as ratio of mobility of respective standard PEP bands (-3, -4 and -5). ab = absent peptidase (-3) on gel b; P = Hymenopterous parasite or *Entomophthoralean* fungal peptidase

specificities (either partial or complete) and hence were earlier assigned to separate loci, two of which (PEP-4 and -5) appear to be genetically associated since their mobilities vary in concert (Loxdale et al. 1983, and unpublished data). Since putative heterozygotes at each locus are double banded compared to the single bands of homozygotes, peptidases-3 to -5 are apparently monomers. 6-PGD was found to be polymorphic in British clones in 1979 with four "allozymes" (6-PGD$^{0.87,\ 0.93,\ 1.0 \text{ and } 1.15}$), but monomorphic in a similar survey conducted the following year. Certain clones showed double bands instead of the usual single band, but it is unclear if these were heterozygotes; other clones had additional faster bands. Thus, the genetic control of 6-PGD in *S. avenae* is not yet fully resolved and may involve more than one locus (Loxdale et al. 1983).

Results

The results for peptidases are shown in Table 1 and Fig. 1 a–f. Under standard conditions (6% gels, 2 h run-time), all three PEP loci were polymorphic with either three or four electrophormorphs (regarded here as alleles, although sexual crosses would be required to confirm this). Some of these were readily identifiable with alleles found previously (e.g. PEP-3$^{1.00}$, PEP-4$^{0.96,\ 1.00}$, PEP-5$^{1.00}$), whilst others were most probably synonymous (e.g. PEP-3$^{0.94-0.95}$, PEP-5$^{0.94 \text{ and } 0.96-0.95}$ and PEP-5$^{1.04-1.05}$), alleles of similar mobility such as PEP-5$^{0.94 \text{ and } 0.96}$ being combined in the earlier surveys to increase scoring reliability of PEP bands.

The most common alleles at each locus occurred at frequencies of 0.94, 0.88, and 0.78 for PEP-3, -4 and -5, respectively; all other variants were much less frequent and no heterozygotes were detected.

When run time was increased to 3.5 h (Fig. 1 b), frequencies of the most common alleles of PEP-3, -4 and -5 declined to 0.90, 0.72 and 0.68, respectively, whilst the number of variants resolved increased by seven (from 3–5, 3–5 and 4–7, respectively) including a low frequency of double banded patterns, presumed to be heterozygotes (Fig. 1 c, d).

Table 2. χ^2 analysis of peptidase data

Enzyme locus	Conditions	χ^2	d.f.	Probability $P (=0.05)$	+ Significant; – Non-significant
					difference from condition (i)
PEP-3	(ii)	16.1		< 0.05	+
	(iii)	9.9	6	> 0.05	–
	(iv)	13.1		< 0.05	+
	(ii)	45.8		< 0.001	+
PEP-4	(iii)	29.2	6	< 0.001	+
	(iv)	36.8		< 0.001	+
	(ii)	33.2		< 0.001	+
PEP-5	(iii)	51.4	7	< 0.001	+
	(iv)	12.3		> 0.05	–

Table 3. Frequencies of 6-PGD isoenzymes separated on 6% and 5% gels

Ratio	Band	6% gel	5% gel
		$n=117$	$n=139$
0.48	1	0.008	0.007
0.64	2	0.008	–
0.80	3	0.008	0.014
0.95	4	–	0.568
1.00	5	0.957	0.986
1.08	6	–	0.863
1.18	7	1.00	0.856
1.27	8	0.060	–
1.36	9	0.103	–
2.23	10	–	0.036

A gel concentration of 5% and a 2 h run-time gave mobilities and resolution of peptidases similar to standard conditions (Fig. 1e). However, frequencies of the most common alleles were again reduced compared to standard values (i.e. to 0.90, 0.69 and 0.64 for PEP-4, -4 and -5, respectively) and the combined number of alleles at all three loci increased by four (from 3–4, 3–5 and 4–5, respectively). Unlike the 3.5 h run on 6% gels, no heterozygotes were identified for PEP-3, yet heterozygote frequencies increased slightly from 0.011 to 0.033 and 0.021 for PEP-4 and -5.

Extending run-time for 5% gels to 3.5 h improved the separation of peptidases (Fig. 1f), although the frequencies of the commonest alleles of all three loci increased to 0.97, 0.73 and 0.75 for peptidases -3, -4 and -5. The number of additional alleles increased by only three, i.e. from 3–4 for PEP-3 and -4 and 4–5 for PEP-5 and no heterozygotes were found.

Thus overall, the three experimental treatments increased the total number of alleles detected at PEP-3 to -5 by approximately two fold – from 10–22 alleles, the increase often being associated with a reduction in frequency of the standard alleles, whilst "unique" alleles (e.g. PEP-$3^{0.82}$ and PEP-$4^{1.02}$: condition ii; PEP-$5^{0.79}$: condition iv) and heterozygotes were observed only under some test conditions.

The detection of some of these rare variants probably reflects chance effects of sampling rather than the ability of the experimental techniques to reveal hidden variants. However, since seven of the nine sets of allele frequencies revealed by altering experimental conditions were significantly different from 'standard' results at the 5% level (Table 2), the regimes undoubtedly did resolve hidden variation, though none was obviously superior in this respect.

The running and staining of 6-PGD under standard conditions (6% gel, 5 h run-time) gave similar banding patterns to those observed previously (Loxdale et al. 1983), with one darkly staining band (band 7) always present, a slower band (band 5) also present at high frequency (0.96), and several much less common bands which, when present, were respectively invariant in terms of mobility (Table 3). No heterozygotes were detected under the two experimental conditions used; the main slow band (5) was previously thought to be allelic to band 7, but the high frequency of the former in the present study makes this unlikely. The sporadic occurrence of certain other bands is difficult to explain; it may be due to variation in technique, to invading organisms (Hymenopterous parasitoids, fungi or symbiotes), or to switching on and off of genes coding for the bands in different individuals in response to biotic factors: Esterase-2 and hexokinase-3 show similar variable activity in *S. avenae* (Loxdale et al. 1983) as do certain esterases in the Peach-potato aphid *Myzus persicae* (A. L. Devonshire, personal communication; Wool et al. 1978).

With 5% gels and the same run-time (5 h), 6-PGD isoenzymes showed improved resolution and some bands apparently split into two (band 5 to 4 and 5; band 7 to 6 and 7) without affecting the total number of bands

Table 4. Frequencies of 6-PGD isoenzymes separated by isoelectric focusing between pH 3–10

Ratio	Band	Frequency
	$n=122$	
0.80	1	0.582
0.96	2	0.475
1.00	3	1.00
1.03	4	0.016

Table 5. Number of peptidase alleles found under standard conditions and following serial electrophoresis

Locus	Standard conditions	All conditions
PEP-3	3	7
PEP-4	3	7
PEP-5	4	8

(seven) detected (Table 3). The loss of some rare bands upon changing gel conditions and the appearance of new rare variants probably resulted from random sampling effects, although it is unclear why band 9, which previously occurred at a frequency of around 0.1 (10%) under standard conditions, disappeared under test conditions.

Isoelectric focusing resolved only four 6-PGD bands compared to the seven using conventional electrophoresis (Table 4). Bands separated on IEF gels cannot always be compared directly with those found using conventional electrophoresis since they separate protein molecules according to different properties (i.e. predominantly charge in the former and charge/net mass in the latter), although this can be done easily by using 2-D runs. Based on frequency (i.e. 1.0), IEF band 3 and PAGE band 7 are probably synonymous.

Discussion

These results show that for *S. avenae,* an aphid species considered to be predominantly parthenogenetic in Britain (Hand 1982) and which displays mainly homozygous genetic variation at most polymorphic loci studied (Loxdale et al. 1985), serial electrophoresis of three polymorphic peptidase bands using just two variable criteria (gel concentration and run-time) substantially increased the number of alleles detected (Tables 1 and 5) as well as increasing the number of putative heterozygotes under some conditions (Table 1).

The observed increase in numbers of alleles at these loci may be compared with work on parthenogenetically reproducing clones of *Myzus persicae* by Wool et al. (1978) who demonstrated an increase in the number of electromorphs of certain esterases (EST-1 to -3) using serial pH/buffer changes (although another 29 enzyme bands remained completely invariant even when gel concentration and buffer were altered) and also, many studies on *Drosophila* enzymes involving serial methods (e.g. Coyne 1976; Singh et al. 1976).

Serial electrophoresis can thus reveal additional genetic variation at certain loci in asexual as well as sexually reproducing insects and it may be that with other aphid species presently shown to be largely or completely monomorphic at many enzyme loci using a single electrophoretic method (see for example Tomiuk and Wöhrmann 1983), a similar approach will reveal much greater variability.

In contrast, with the enzyme 6-PGD from *S. avenae* which appeared to be nationally polymorphic one year (1979) but nationally monomorphic the next (1980) as well as monomorphic in the single Rothamsted population examined in 1983, serial electrophoresis and isoelectric focusing produced only a separation of some invariant 6-PGD bands into several bands rather than revealing clear electromorphs of probable allelic origin. The monomorphism presently detected for 6-PGD can be directly compared with the rather persistent invariability of certain other enzymes (e.g. octanol dehydrogenase and malic enzyme) in some populations of *Drosophila* spp. subject to one or more serial techniques (e.g. Loukas et al. 1981).

Single substrate enzymes (Group I) which utilize internally derived metabolites are known to be far less variable than multisubstrate enzymes (Group II) utilizing externally derived compounds (Gillespie and Kojima 1968; Johnson 1973). 6-PGD which is involved in the pentose phosphate shunt, a major energy producing pathway, may be classed as a Group I enzyme, whilst peptidases are usually multisubstrate and hence classed as Group II enzymes so that these peptidases separated from *S. avenae* follow the general trend.

According to Johnson (1976), balancing selection may maintain biochemical polymorphisms within natural populations by acting upon "integrated metabolic phenotypes" rather than individual polymorphic loci, thereby buffering "functional integration from environmental perturbation". Group I enzymes are presumed to be subject to greater balancing selective pressure than Group II enzymes. This is because of the tight functional constraints placed on the former, involved, as they are, in the major metabolic pathways, whereas the latter enzymes may vary more in terms of molecular and functional (kinetic) changes since they are peripheral to these pathways (cf. Gillespie and Kojima 1968; Johnson 1976). If this is so, then some enzyme polymorphisms, including "hidden polymorphisms", may be of adaptive importance to aphids, especially to those species multiplying very rapidly in the summer by pathenogenesis, with selection operating

upon genotypes specialised to various ephemeral, heterogeneous ecological situations, for example particular cereal crop varieties (cf. Lowe 1981).

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