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THE GENETICS OF *CEPAEA* ESTERASES
I. *CEPAEA NEMORALIS*

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SUMMARY

Using polyacrylamide disk electrophoresis, at least 23 zones of general esterase activity can be distinguished in extracts of *Cepaea nemoralis* hepatopancreas. Breeding experiments have thrown light on the inheritance of three esterase systems. It has been suggested that the four most cathodally migrating esterase zones may be controlled by four linked loci, forming an esterase supergene. Zone Est. 5 also seems to be part of the same system and might be coded for by another linked locus. At each of these loci three alleles have been postulated, one producing no detectable enzyme, another producing a weak enzyme and the third coding for an active enzyme. Each locus seems to control enzyme activity at one level in the gel. Another esterase system is controlled by three alleles at one locus. The enzyme produced is a dimer, heterozygotes having three zones of activity. Attemps to hybridise " parental " enzymes so as to give a hybrid zone on electrophoresis failed. The third esterase system examined is one of presence and absence of the enzyme. It is controlled by two alleles at one locus with the presence of enzyme being dominant to its absence. There is no linkage detectable between any of the (effective) esterase loci or between the esterase loci and three loci determining the shell colour and banding morphs.

1. INTRODUCTION

POPULATIONS of the polymorphic land snail *Cepaea nemoralis* in calcareous upland districts of Britain exhibit what have been termed " area effects " (Cain and Currey, 1963). These are the occupation of very large areas of varied habitats and terrains by snails showing remarkable constancy in the frequency of shell colour and banding morphs. This is in contrast to the situation in lowland areas where morph frequencies are correlated with the background on which colonies are found (Cain and Sheppard, 1950, 1954; Currey, Arnold and Carter, 1964). Abrupt changes from areas characterised by particular morphs to other areas characterised by completely different morphs can occur over distances of 100 m. or so of seemingly uniform terrain. In an attempt to elucidate the cause or causes of these phenomena some non-visual polymorphisms have been investigated both within and between areas.

Using the powerful technique of polyacrylamide disk electrophoresis the non-specific esterases occurring in the hepatopancreas of these snails can be made amenable to genetic analysis. However, before gene frequencies at particular esterase loci can be calculated, it is obvious that the esterase zones corresponding to individual loci must be identified. This can only be done reliably by a consideration of the results of breeding experiments and it is the

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purpose of this paper to present data relating to the inheritance of three esterase systems.

2. Methods

(i) *Breeding and rearing of snails*

Snails for breeding were collected from the wild as medium-sized juveniles. Storage of sperm for several years makes it essential to set up breeding pairs with known virgins as offspring might otherwise be the result of multiple matings (Murray, 1964). Pairs were placed in large sandwich-boxes, the floors of which were covered with a 3 cm. depth of damp compost. Calcium was always available in the form of natural chalk lumps.

During the summer, breeding and rearing boxes were maintained in an unheated potting shed. In winter, snails were moved to a greenhouse with artificial lighting for 16 hours a day and a temperature which fluctuated between 15° and 25° C. Under these conditions adult snails could be kept in a breeding condition and the young in an actively growing state throughout the year. Using these methods, the generation time can be reduced substantially from 3 to 4 years in the wild (Cain and Currey, 1968) to between 1·5 and 2 years in the laboratory.

(ii) *Extraction and electrophoresis*

Adult snails were killed after they had produced four to five egg masses. Initially, young were killed and extracted when they had reached a shell width of approximately 8·5 mm., but later smaller individuals were processed.

Extracts were made of the hepatopancreas. A weighed portion of gland was transferred to a centrifuge tube and a measured volume of extracting fluid added (0·05 ml. fluid to 10 mg. of gland). Trials with several extracting solutions indicated that the Tris/glycyl-glycine/calcium chloride solution of Manwell and Baker (1968) gave the clearest resolution of esterase zones. Maceration of the gland tissue was achieved with sharp-ended glass rods; more thorough homogenisation was found to be unnecessary. The centrifuge tubes, after standing at room temperature for 5 minutes, were spun down for a further 5 minutes at 3250 × *g* when the supernatant was removed and either used immediately or stored in a deep freeze at −20° C. Electrophoresis was performed at 5° C. in 7·5 per cent. gels, the procedure being essentially the same as that described by Davies (1964).

Gels were preincubated in phosphate buffer *p*H 6·8 (0·2 M) for 5 minutes before being transferred to the stain solution which consisted of:

100 ml. phosphate buffer *p*H 6·8 (0·2 M),
18 mg. α-naphthyl acetate,
18 mg. β-naphthyl acetate,
80 mg. Fast Garnet GBC salt (Gurr).

The substrates were first dissolved in 0·5 ml. acetone before adding to the stain solution. The use of mixed substrates enables esterase zones specific for the α- or β-naphthyl moieties to be distinguished (Johnson *et al.*, 1968). Optimum staining intensities were achieved after incubation for 20 minutes at 25° C. Gels were stored in 30 per cent. v/v ethanol.

(iii) *Source and pairing of snails*

Table 1 lists the pairs of *Cepaea nemoralis* set up during this study with information on the sources from which they came and their colour and

TABLE 1

C. nemoralis—Breeding pairs

Mating code	Parent 1		Parent 2
C	P 00300 (HS3)	×	Y 00000 (HS3)
D	Y 12345 (New.)	×	B 00000 (Pt. A. 14)
E	P 00300 (HS?)	×	Y (123) (45) (HS?)
F	P (12)3(45) (HS3)	×	P 12345 (HS3)
G	Y 12345 (New.)	×	P 00300 (Pt. A. 50)
H	Y 00300 (Pt. A. 50)	×	Y 00000 (New.)
I	P 00300 (New.)	×	Y 12345 (Pt. A. 50)
J	P 12345 (New.)	×	P 12345 (New.)
K	P 12345 (New.)	×	P 00300 (Pt. A. 50)
L	P 12345 (New.)	×	Y 12345 (New.)
M	Y 00000 (New.)	×	P 00300 (New.)
N	Y 12345 (Pt. A. 50)	×	Y 00300 (Pt. A. 50)
O	P (12345) (New.)	×	Y 00300 (New.)
P	Y 00345 (New.)	×	P 12345 (New.)
R	B 00000 (D1)	×	B 00000 (D5)
S	P 10345 (BR2a)	×	Y 12345 (Clwyd)
BG	Y 12345 (BH1)	×	P 00000 (?)

HS3 Heathcote Sewage Works, Warwickshire. Sample site 3. SP 305625.
HS? Heathcote Sewage Works but exact site unknown.
New. Newborough Warren Nature Reserve, Anglesey. SH 431639.
Pt. A. Point of Ayr, Flintshire. Numbers indicate colonies based on Cain (1968). SJ 110850 (approximately).
BR2a Barbary Road 2a, Marlborough Downs, Wiltshire. SU 145765.
BH1 Burderop Hill 1, Marlborough Downs, Wiltshire. SU 157762.
D1, 5 Mating between individuals from Broods D1 and D5.
Clwyd An unnamed site in the Clwyd Range, Flintshire. SJ 193586.

banding phenotypes. As it was necessary to kill snails to determine their esterase phenotypes matings had to be made " blind ", the enzyme typing of the parents being performed after mating and egg laying. The detailed scoring of all individuals assayed in this breeding programme is given in Oxford (1971).

3. ESTERASE, LOCUS AND ALLELE NOMENCLATURE

The relative positions of esterase zones of *C. nemoralis* are shown diagrammatically in fig. 1. Band positions are numbered from cathode to anode, taking into consideration both *C. nemoralis* and *C. hortensis*. Esterases that are homologous in the two species have been assigned the same number. Substrate spectificities are shown by different shadings.

Esterase zones are designated at " Est" , *e.g.* the most cathodal zone is Est. 1, while loci are shown as " *Es* ". Loci are also numbered from cathode to anode, beginning in *C. nemoralis*, for which there is extensive breeding data, and carrying on to *C. hortensis*. Alleles at particular loci are shown as superscripts, the allele coding for the most cathodal zone being 1. Null alleles, *i.e.* alleles that do not produce an active product detectable by the staining method used, are designated at 0.

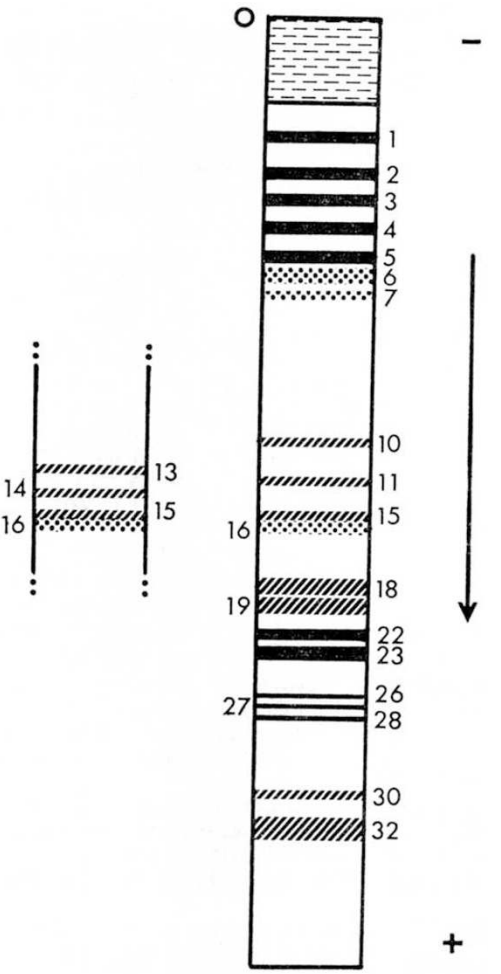

FIG. 1.—A diagram of the commonest esterase zones revealed after electrophoresis of *C. nemoralis* hepatopancreatic extracts. The gels were stained with mixed substrates allowing the simultaneous discrimination of zones specific for one or the other substrate. O = origin. ▒ = red staining zones (hydrolysing β-naphthyl acetate). ▨ = purple staining zones (hydrolysing both substrates). ■ = blue/black staining zones (hydrolysing α-naphthyl acetate).

4. INHERITANCE OF EST. 1 TO 4

In each band position (fig. 1), at least for the snails assayed here, three main conditions prevailed. There could be a very strongly staining zone, a weakly staining zone or no detectable zone at each level in the gel. Individuals were classified as heavy banded or not heavy banded for each band position. For brevity, each phenotype has been called after the position(s) of its heavy band(s), *e.g.* when only Est. 2 is heavy, phenotype = 2, when Est. 1 and Est. 3 are heavy, phenotype = 1-3, and so on. The results from the breeding experiments are shown in table 2. Parents 1 and 2 refer to the numbering of parents in table 1.

Broods of C, E, F and M all derive from matings of the same Est. 1 to 4

TABLE 2

C. nemoralis—Inheritance of Est. 1 to 4

	Parent		Offspring											
Mating	1	2	1	2	3	1-2	1-3	1-4	2-3	2-4	3-4	χ^2	P	N
C	1-3	1-4	14	—	—	—	8	16	—	—	10	3·3333	n.s.	48
D	1-3	2	—	—	—	49	—	—	10	—	—	25·7797	***	59
E	1-3	1-4	8	—	—	—	7	11	—	—	6	1·7500	n.s.	32
F	1-4	1-3	8	—	—	—	17	9	—	—	9	4·9070	n.s.	43
G	1-3	1-3	5	—	7	—	10	—	—	—	—	0·5454	n.s.	22
H	2	1	—	—	—	22	—	—	—	—	—	—	—	22
I	1	3	—	—	—	—	45	—	—	—	—	—	—	45
J	1-3	2-3	—	—	20	4	11	—	9	—	—	12·1818	**	44
K	1-2	2	—	22	—	12	—	—	—	—	—	2·9412	n.s.	34
L	1-3	1	25	—	—	—	29	—	—	—	—	0·2963	n.s.	54
M	1-3	1-4	11	—	—	—	11	9	—	—	12	0·4419	n.s.	43
N	2-3	1	—	—	—	12	14	—	—	—	—	0·1538	n.s.	26
O	1	1-3	15	—	—	—	23	—	—	—	—	1·6842	n.s.	38
P	2-3?	1-2	—	5	—	3	10	—	9	—	—	4·8518	n.s.	27
R	1-2	1-2	6	2	—	9	—	—	—	—	—	1·9412	n.s.	17
S	1	1?	17	—	—	—	—	—	—	—	—	—	—	17
BG	1-4	1-2	6	—	—	5	—	3	—	4	—	1·1111	n.s.	18

n.s. = Not significantly different from the hypothesis discussed for each mating in the text.
*** = Probability less than 0·001.
** = Probability less than 0·01.

phenotypes, viz. 1-3 × 1-4. The results are what would be expected if Est. 1, Est. 3 and Est. 4 are all coded for by alleles at one locus, giving offspring with phenotypes 1, 1-3, 1-4 and 3-4 in a 1 : 1 : 1 : 1 ratio. As there is no heterogeneity in the results from these four crosses (χ^2 for 9 d.f. = 9·5888, $0{\cdot}5 > P > 0{\cdot}3$) the data can be summed to give totals of 41 : 43 : 45 : 37 for 1 : 1-3 : 1-4 : 3-4 respectively, a good fit to a 1 : 1 : 1 : 1 ratio (χ^2 for 3 d.f. = 0·8434, $0{\cdot}9 > P > 0{\cdot}8$).

Broods of G, derived from a mating between two 1-3 phenotypes, showed three phenotypes, 1, 3 and 1-3, in proportions that do not differ significantly from a 1 : 2 : 1 ratio for 1, 1-3 and 3 respectively. This again suggests that heavy zones at Est. 1 and 3 positions are controlled by alleles at a single locus. Mating R, another mating between like genotypes, gave a 1 : 2 : 1 ratio of 1 : 1-2 : 2 in its offspring, indicating allelism of Est. 1 and Est. 2.

Broods of J, offspring of a mating between 1-3 and 2-3 parents, had phenotypes 3, 1-2, 1-3 and 2-3. It would appear from this mating that a heavy zone at the Est. 2 position is controlled by an allele at the same locus as that controlling heavy zones at the other three positions. This is confirmed by mating P. Parent 1 of this pairing died before its esterase phenotype could be scored but the most probable phenotype of this individual was 2-3. The other parent had the phenotype 1-2 and the offspring show four arrangements, 2, 1-2, 1-3 and 2-3, in the ratio 1 : 1 : 1 : 1. If the retrospective typing of the dead parent is correct, then again a heavy zone at the Est. 2 position would seem to be controlled by an allele at the same locus that controls the other three heavy zones.

Broods of H, I and S give no information on allelism. They do show, however, that parents were either both homozygous or one homozygous and one heterozygous for the heavy bands they carried. Parent 2 of mating S died before typing, but as all of the offspring had only Est. 1 heavy, the

retrospective genotype assigned to this individual is almost certainly correct. Broods of D show that heavy zones at positions 1 and 3 are allelic and that parent 2 must have been homozygous for the " heavy " allele producing a zone at position 2. Similarly, broods of N indicate allelism of heavy zones at positions 2 and 3 and show that parent 2 was homozygous for a heavy zone at position 1.

Broods from matings K, L and O give no information on allelism. They show, however, that heavy and non-heavy zones are segregating at position 1 in the case of K and position 3 in the cases of L and O. Parent 2 of matings K and L and parent 1 of mating O are shown to be homozygous for the alleles determining their heavy zones. As matings L and O are of the same type and there is no heterogeneity in the data (χ^2 for 1 d.f. = 0·4153, $0{\cdot}7 > P > 0{\cdot}5$) the results can be pooled to give a ratio of 40 : 52 for 1 : 1-3 respectively, a ratio that does not differ significantly from the expected 1 : 1 (χ^2 for 1 d.f. = 1·5652, $0{\cdot}3 > P > 0{\cdot}2$). Finally, broods of BG, a mating between 1-4 and 1-2 parents, gave the expected 1 : 1 : 1 : 1 ratio of 1 : 1-2 : 1-4 : 2-4.

The offspring of parents D and J differ from all other broods in that their segregation ratios are significantly different from the expected 1 : 1 and 1 : 1 : 1 : 1 ratios, respectively. Among the offspring of mating D there is a vast excess of phenotype 1-2 compared with the phenotype 2-3. This is not due to any intrinsic superiority of the 1-2 type or inferiority of the 2-3 type because other broods segregating for these phenotypes exhibit normal ratios (*e.g.* broods of P). Likewise, there is no indication of why the offspring of J do not fit the expected ratios.

It appears from these results, then, that the heavily staining zones Est. 1, Est. 2, Est. 3 and Est. 4 are produced by alleles at a single locus. This hypothesis cannot, however, be correct. In many cases, when one particular band position is not occupied by a strongly staining zone, a weakly staining zone can be detected. To take an example, if Est. 3 is the only zone to stain strongly it is often possible to see weak bands in Est. 1, 2 and 4 positions. This is an impossible occurrence if all four zones are coded for by alleles at a single locus since, in a diploid organism, only two alleles can be present at any one locus.

This same phenomenon can be seen in material collected in the field. Some individuals have 3, 4 or even 5 strongly staining zones in the positions of Est. 1 to 5. It seems reasonable to include Est. 5 in with this discussion of Est. 1 to 4 since it is in strong linkage disequilibrium with Est. 1 to 4 when field data are analysed and exhibits similar physical and biochemical properties (Oxford, 1973*a*, 1973*b*). There is, as yet, no breeding data available on the inheritance of this zone.

From this evidence it is clear that the allelism shown by the breeding data cannot be true allelism at all. The differential migration of zones in this region of the gel is not caused by the addition of sialic acid residues to a basic molecule (for example by another locus) because incubation of extracts prior to electrophoresis in a neuraminidase solution for 24 hours at 37° C. (Pantalouris and Arnason, 1967) resulted in zones in the same positions and of the same intensities as in the control. Two closely linked loci, one perhaps coding for Est. 1 and 2, the other for Est. 3 and 4, again cannot explain the observations. Parent 2 of mating K is shown to be homozygous for heavy Est. 2 zones, yet a faint zone of Est. 1 could be seen on the gel. Est. 1 and 2

cannot, therefore, be allelic. Similar arguments can be made against any other combination of zones produced by alleles at two loci. After considering all hypotheses, the most parsimonious explanation that can be produced is that Est. 1 to 5 are coded for by alleles at five very closely linked loci forming an esterase supergene.

At each locus there must be a number of alleles. A zone at any one position can be apparently absent, weakly staining or strongly staining. In the field, all gradations between weakly and strongly staining bands occur and the intensity of staining has been shown to depend, to some extent, on the food eaten (Oxford, in preparation). In the laboratory the three conditions described above prevail and are clear cut, the food given to the snails being constant.

The weakly staining zones are not produced by a heterozygote for an allele giving no band and an allele producing a strong band. Parents with heterozygous zones (*e.g.* G) gave gel patterns with intensities equal to those produced by snails with homozygous zones (*e.g.* H). There is, therefore, a dominance situation with " strongly staining bands " dominant to " not-strongly staining bands ". In all probability, measurements of actual enzyme content of gels would show that homozygotes at one locus produce more esterase than heterozygotes at the same locus. On stained gels, however, once a certain intensity of stain has been reached, more stain makes no difference, all zones appearing as " heavily stained ".

There are, therefore, probably at least three alleles at each of the loci coding for Est. 1 to 5. One is a null allele and produces an enzymatically inactive molecule on the substrates used, another produces an enzyme with low activity while the third gives an enzyme with high activity. These are designated as follows:

e.g. Est. 1

Locus: *Es.* 1

Alleles: *Es.* 1^0 *Es.* 1^{1*} *Es.* 1^1

Genotypes:	Phenotypes
Es. $1^{0/0}$	No activity
Es. $1^{0/1*}$	?
Es. $1^{1*/1*}$	Partial activity
Es. $1^{1*/1}$	Apparently full activity
Es. $1^{1/1}$	Full activity

Since there are no positional changes with different genotypes but there are quantitative changes, the allele with low activity has been called *Es.* 1^{1*}. The same sort of allele designation applies to the other loci which are *Es.* 2, *Es.* 3, *Es.* 4 and *Es.* 5, giving rise to bands Est. 2, Est. 3, Est. 4 and Est. 5 respectively.

5. Inheritance of Est. 10, 11, 13, 14 and 15

All of these esterases are coded for by alleles at a single locus and the breeding results demonstrating this are given in table 3. Again, for brevity, phenotypes (in this case equivalent to genotypes as there is no dominance) are designated in a shorthand form. The most cathodally migrating zone in the homozygous condition has been called 10/10 (*i.e.* only Est. 10 is present). The heterozygote for Est. 10 and Est. 15 is called 10/15 and so on.

In heterozygotes a third zone of activity appears that is not present in either homozygote. This is becoming a familiar phenomenon as more and more proteins are analysed by gel electrophoresis. The third band, equidistant between the homozygous bands, can be explained if the enzyme is composed of two polypeptide subunits, *i.e.* the enzyme is a dimer. The enzyme zone of intermediate mobility will be formed of subunits produced by both alleles.

The breeding results (table 3) bear out this dimeric interpretation. The two critical matings were I and P, in which the parents were homozygous for different alleles (showing only single zones of activity). The offspring, all heterozygous, showed the hybrid zone between the two parental zones. Thus a zone of activity appeared in the offspring that was not present in either parent.

TABLE 3

C. nemoralis—Inheritance of Est. 10, 11, 13, 14 and 15

	Parent		Offspring								
Mating	1	2	10/10	10/13	13/13	13/15	15/15	10/15	χ^2	P	N
C	13/15	15/15	—	—	—	24	24	—	0·0	n.s.	48
D	10/15	15/15	—	—	—	—	32	27	0·4237	n.s.	59
E	13/15	13/15	—	—	10	11	11	—	3·1875	n.s.	32
F	15/15	15/15	—	—	—	—	43	—	—	—	43
G	10/15	15/15	—	—	—	—	11	11	0·0	n.s.	22
H	15/15	10/15	—	—	—	—	9	13	0·7273	n.s.	22
I	10/10	15/15	—	—	—	—	—	45	—	—	45
J	10/15	10/15	10	—	—	—	14	20	1·0909	n.s.	44
K	10/15	15/15	—	—	—	—	15	19	0·4706	n.s.	34
L	10/15	10/15	11	—	—	—	13	24	0·1667	n.s.	48
M	10/15	10/15	10	—	—	—	10	23	0·2093	n.s.	43
N	15/15	13/15	—	—	—	13	12	—	0·0400	n.s.	25
O	10/10	10/15	22	—	—	—	—	16	0·9474	n.s.	38
P	10/10?	15/15	—	—	—	—	—	27	—	—	27
R	15/15	15/15	—	—	—	—	17	—	—	—	17
S	15/15	13/15?	—	—	—	8	9	—	0·0588	n.s.	17
BG	15/15	15/15	—	—	—	—	26	—	—	—	26

n.s. = Not significantly different from the hypothesis discussed for each mating in the text.

Matings C, D, G, H, K, N, O and S, all between a homozygote and a heterozygote gave, without exception, offspring with parental genotypes in the ratio 1 : 1. Matings D, G, H and K were all between types 10/15 and 15/15. Heterogeneity between the results is not significant (χ^2 for 3 d.f. = 1·5559, $0{\cdot}7 > P > 0{\cdot}5$) and the pooled data gives a ratio of 67 : 70 for 10/15 : 15/15, a good fit to the expected 1 : 1 (χ^2 for 1 d.f. = 0·0657, $0{\cdot}8 > P > 0{\cdot}7$). Likewise matings C and S were both between genotypes 13/15 and 15/15. Heterogeneity being insignificant (χ^2 for 1 d.f. = 0·0434, $0{\cdot}9 > P > 0{\cdot}8$), the summed data give a ratio of 32 : 33 for 13/15 : 15/15, a very good fit to a 1 : 1 ratio (χ^2 for 1 d.f. = 0·0154, $0{\cdot}95 > P > 0{\cdot}90$).

Matings E, J, L and M between two heterozygotes gave offspring segregating for three genotypes, two homozygous and one heterozygous in the ratio 1 : 1 : 2 respectively. As matings J, L and M were all of the same type, the data can be pooled (heterogeneity χ^2 for 4 d.f. = 0·9261, $0{\cdot}95 > P > 0{\cdot}90$) to give the ratio 31 : 67 : 37 for 10/10 : 10/15 : 15/15 respectively, a good 1 : 2 : 1 ratio (χ^2 for 2 d.f. = 0·5407, $0{\cdot}80 > P > 0{\cdot}70$). All other matings

were between homozygotes 15/15 × 15/15 and gave offspring all of genotype 15/15.

The locus involved in producing Est. 10, 11, 13, 14 and 15 is *Es.* 7, since there are at least two other loci coding for zones Est. 6 and Est. 7, zones that are more cathodal to the ones under discussion here (fig. 1). The situation at the *Es.* 7 locus is, therefore, as follows:

Alleles: *Es.* 7^1 *Es.* 7^2 *Es.* 7^3	
Genotype:	Phenotype: (as in fig. 1)
Es. $7^{1/1}$	Est. 10
Es. $7^{1/3}$	Est. 10, Est. 11, Est. 15
Es. $7^{2/2}$	Est. 13
Es. $7^{2/3}$	Est. 13, Est. 14, Est. 15
Es. $7^{3/3}$	Est. 15

The hybrid *Es.* $7^{1/2}$ has not been observed or the predicted hybrid zone numbered.

Attempts to hybridise these enzymes *in vitro* failed. Mixtures of extracts known to contain only Est. 10 and only Est. 15 were made and subjected to three treatments. In one experiment the mixture was frozen and thawed in the presence of 1 M sodium chloride, a standard technique used for the *in vitro* hybridisation of lactate dehydrogenase isoenzymes (Markert, 1963). In other mixtures the *p*H was lowered to 4·5 overnight before neutralising, a method used by MacIntyre and Dean (1967) to hybridise mixtures of the fast and slow variants of *Drosophila melanogaster* acid phosphatase-1. In a third experiment, the mixture was heated for 30 minutes at 55° C. in an attempt to break the bonds holding the subunits together. In no case was a hybrid zone formed. Control heterozygote extracts survived the treatments with all three zones appearing on subsequent electrophoresis.

6. Inheritance of Est. 16

Table 4 shows the data relating to the genetics of Est. 16. The variation observed at this position is essentially one of presence or absence of an esterase zone. Unfortunately heterozygotes and the homozygotes for presence of a band are indistinguishable from one another, *i.e.* there is dominance. The genetics of this zone must therefore be determined by examining the ratios of offspring with and without the band for each parental pair.

Matings C, J, L, N, O and possibly P (one parent of which was of unknown phenotype) all had one parent with Est. 16, the other without and the offspring had enzyme (+) or no enzyme (−) in equal numbers. Heterogeneity between broods was not significant (χ^2 for 5 d.f. = 4·0498, $0{\cdot}70 > P > 0{\cdot}50$) and the summed results gave a ratio of 111 : 126 for + : −, a good fit to 1 : 1 (χ^2 for 1 d.f. = 0·9494, $0{\cdot}80 > P > 0{\cdot}70$). The parent, in each case, which showed Est. 16 must have been heterozygous for two alleles. The locus in question is *Es.* 8 and the two alleles are *Es.* 8^0 and *Es.* 8^1 for the inactive and the active alleles respectively. The heterozygote is shown as *Es.* $8^{0/1}$.

Matings D, E, H and M were all between individuals possessing an Est. 16 zone. Within the broods, no individual was of the genotype *Es.* $8^{0/0}$.

The matings must, therefore, have been one of two possible types, *Es.* $8^{0/1} \times$ *Es.* $8^{1/1}$ or *Es.* $8^{1/1} \times$ *Es.* $8^{1/1}$.

Parent 2 of mating F, when crossed to a snail of genotype *Es.* $8^{0/0}$ gave offspring all possessing Est. 16 and so this parent must have been of the genotype *Es.* $8^{1/1}$ and the young would all be *Es.* $8^{0/1}$. Matings G, I, BG and S (parent 2 of the latter mating died before typing) were all between individuals of genotypes *Es.* $8^{0/0}$ and gave, as expected, all *Es.* $8^{0/0}$ offspring.

Matings K and R were between snails with Est. 16 but gave in their offspring approximately 25 per cent. of snails of genotype *Es.* $8^{0/0}$ indicating that all parents were heterozygotes *Es.* $8^{0/1}$. In both matings the ratio of young with Est. 16 (+) to those without (−) did not differ significantly from the expected 3 : 1. The summed results gave a ratio of 37 : 14 for + : −,

TABLE 4

C. nemoralis—Inheritance of Est. 16

	Parent		Offspring				
Mating	1	2	+	−	χ^2	P	N
C	−	+	25	23	0·0833	n.s.	48
D	+	+	59	0	—	—	59
E	+	+	32	0	—	—	32
F	−	+	43	0	—	—	43
G	−	−	0	22	—	—	22
H	+	+	22	0	—	—	22
I	−	−	0	45	—	—	45
J	+	−	16	28	3·2727	n.s.	44
K	+	+	24	10	0·3529	n.s.	34
L	−	+	29	25	0·2963	n.s.	54
M	+	+	43	0	—	—	43
N	−	+	13	13	0·0	n.s.	26
O	+	−	17	21	0·4210	n.s.	38
P	− ?	+	11	16	0·9259	n.s.	27
R	+	+	13	4	0·0196	n.s.	17
S	−	− ?	0	17	—	—	17
BG	−	−	0	26	—	—	26

n.s. = Not significantly different from the hypothesis discussed for each mating in the text.
+ = Est. 16 present.
− = Est. 16 absent.

a good fit to a 3 : 1 ratio (χ^2 for 1 d.f. = 0·1634, $0{\cdot}70 > P > 0{\cdot}50$). Heterogeneity between the two broods was not significant (χ^2 for 1 d.f. = 0·2091, $0{\cdot}70 > P > 0{\cdot}50$).

From these results it appears certain that the variation seen at the Est. 16 position on gels of *C. nemoralis* extracts is due to two alleles *Es.* 8^0 and *Es* 8^1 segregating at one locus, *Es.* 8.

7. Inheritance of other esterase zones

In some animals from some populations of *C. nemoralis*, *e.g.* Penmon Point 1 and 2, Anglesey and the one Irish sample analysed, the most anodal heavily staining zone Est. 32 (fig. 1) is absent. In every other population the zone is present in all individuals studied. This variation resembles that of Est. 16 and most probably is produced in the same manner, *i.e.* dimorphism at one locus. If this is the case, the locus involved is *Es.* 15 (there being probably at least seven loci controlling esterase zones with electrophoretic

mobilities between this one and Est. 16) and the alleles *Es.* 15^0, the null allele, and *Es.* 15^1 the active allele. No breeding experiments have yet thrown light on this variation but eight adults from Penmon Point 1 (P_1) have been isolated in the hope that they have been inseminated in the wild and will produce young. Although only one parent will be known it is possible that these broods will provide evidence bearing on the above hypothesis.

8. Discussion

From the breeding data presented here and a consideration of the esterase patterns found in wild populations it has tentatively been suggested that esterase zones Est. 1, Est. 2, Est. 3 and Est. 4 of *C. nemoralis* are coded for by four linked loci. Est. 5 also appears to be coded for by a locus closely linked to those mentioned above, as seen by the linkage disequilibrium found in the wild between the presence of a heavy zone in the Est. 5 position and heavy zones at the other positions (Oxford, in preparation).

If these esterases *are* coded for by a supergene, it is interesting to note that in all of the individuals used in the breeding programme only one allele coding for a heavy zone occurred on each chromosome. Heavy zones therefore segregated as if they were produced by alleles at one locus and linkage could only be inferred rather than directly demonstrated. This is not too surprising when the situation in the field is examined. Maximum likelihood estimates of chromosome frequencies for this supposed supergene in natural populations suggest that in many such populations, frequencies of chromosomes carrying one " heavy " allele can be very high (Oxford, 1971, and in preparation).

Individuals in wild populations exhibiting more than two heavy zones of activity must have at least two " heavy " alleles in coupling and so this condition, at least under certain circumstances, is not deleterious. Indeed, a heavy zone at any of these five band positions is not necessary for the survival of the individual, as was shown by an adult snail from Penmon Point A, Anglesey, which lacked a heavy Est. 1 to 5 zone altogether. If Est. 1 to 5 are produced at separate, linked loci it is remarkable that for each locus allele products differ only with respect to enzyme activity and not with respect to charge.

It is worth pointing out that the Est. 1 to 5 system discussed above is probably the same as that mentioned by Manwell and Baker (1968, 1970). In their book (1970, p. 127) they describe " a multiallelic set of esterases migrating cathodally in the usual buffer systems " but considered " satellite zones " to be a problem in the investigation of these enzymes. In the earlier paper (1968) they briefly mentioned an esterase system with " at least seven alleles " which again probably refers to the Est. 1 to 5 enzymes. Their " satellite " zones were presumably the weakly staining bands produced by alleles such as Es. 1^{1*}.

This example should serve as a warning against " armchair " genetics in which zones on an electrophoretic gel are grouped as being allelic or non-allelic merely by inspection. With multimer systems the inspection of gels can give much information on the genetics of zones but with other systems, *e.g.* most esterases, where heterozygotes do not have readily recognisable patterns of bands, mere gel inspection without breeding data can lead to grossly misleading conclusions.

Many of the matings set up in the breeding programme are capable of showing whether the esterase loci discussed in this paper (*i.e. Es.* 1 to 4, *Es.* 7 and *Es.* 8) are linked *inter se* or to the loci controlling colour/banding of the shell, to the U locus (U^3 converting the 12345 banding pattern to 00300) or to the T locus (T^{345} converting 12345 to 00345). Which matings can give information on particular linkage relationships are shown in table 5.

TABLE 5

The linkage relationships capable of being demonstrated by each breeding pair

	Es. 1 to 4	*Es.* 7	*Es.* 8
Colour/ banding	C, E, F, J, K, L, M, P, R, BG	C, E, H, J, K, L, M	C, J, K, O, P, R
U^3	E, M, O	E, M, N, O	K, N
T^{345}	E, P, BG	E	None
Es. 1 to 4	—	C, D, E, G, J, K, L, M, O	C, J, K, P, R
Es. 7	—	—	J, K, L, N

U = Locus controlling mid-banded condition.
T = Locus suppressing bands 1 and 2 (after Cain, Sheppard and King, 1968).
Letters refer to matings (see table 1).

The matings at each position in the table are capable of showing linkage with the locus (loci) indicated above with the locus (loci) to the left. The full breakdown of the breeding results for these six effective loci is given in Oxford (1971).

The search for linkage between any esterase and any other locus has been in vain. *Es.* 1 to 4, *Es.* 7 and *Es.* 8 are not detectably linked to each other or to the colour/banding or U loci. *Es.* 1 to 4 is not linked to the T locus and *Es.* 7 probably is not either, although data on the latter point are few. Esterases, coupled with the loci controlling the visual morphs, may serve as important chromosomal markers in further studies on the biochemical genetics of *Cepaea nemoralis*. It will be of great interest to determine the positions of loci coding for other esterases (*e.g. Es.* 15) in relation to the loci already examined.

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