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Genetic Control of Esterase Isoenzymes in Rye (*Secale cereale* L.)

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Summary. Using the method of isoelectric focusing in polyacrylamide flat gels rye inbred lines of different origin are genetically analyzed for their esterase patterns. Evidence is found for the existence of at least 10 esterase loci, *Est 1–Est 10,* encoding for up to five different allelic esterases per locus. With the exception of *Est 10,* all loci exhibit null alleles. At the *Est 10* locus, regular patterns of multiple bands adjacent to the realized *Est 10* alleles are observed. The mechanisms causing the multiple bands are still unknown.

Key words: *Secale cereale* L. – Esterase polymorphism – Genetic analysis – Null alleles

Introduction

Electrophoretic methods have found general usage in genetic research during the last two decades. In plant breeding this technique was first used by Schwartz (1960) in detecting allelic esterase isoenzymes at the E_1 locus in maize. Esterases in barley were genetically analyzed by Kahler and Allard (1970) who found three somewhat closely linked loci: EA, EB and EC. A series of closely linked loci has also been observed in tomato (Tanksley and Rick 1980).

The inheritance and linkage relationships of six rye esterases was studied by Johansson (1969), however, only in limited material (steel rye). A first step in setting up a linkage map of isoenzymic markers in rye has recently been carried out by Garcia et al. (1982) who genetically analyzed rye peroxidases in caryopses. They found the embryo and endosperm peroxidases to be controlled by a total of 13 loci with linkage between all 5 endosperm loci.

A further approach in the completion of the linkage map of isoenzyme loci in rye is attempted in this study by analyzing leaf esterase polymorphism. Genetic analysis of single esterase loci is performed in this study while the linkage analysis data will be given in a second paper.

Systematic analysis of esterase polymorphism in rye material of different origin has already been started by Schmidt-Stohn (1979 b). Instead of starch gel electrophoresis the method of isoelectric focusing in polyacrylamide flat gels was used. This allows the separation of numerous isoenzymes with isoelectric points (pI's) from pH 3 to 10 with high resolution in a single gel. These studies, which led to the identification of two rye esterase loci, *Est 1* and *Est 2,* were continued in this work in order to obtain a larger number of genetic markers and to detect their inheritance.

Materials and Methods

With regard to the expected codominant expression of isoenzymic loci, selfed progeny of heterozygous plants were used for genetic and linkage analysis in order to obtain maximal information (Allard 1956). In the genetic analysis of single esterase loci, hypotheses about their genetic control were formulated on the basis of segregation patterns of heterozygous F_1 plants. Hypotheses were at first based on the spatial arrangements of esterase bands in the zymographic zones. Esterases focusing immediately adjacent to one another in potentially heterozygous plants (double-banded) and occurring as single bands in potentially homozygous individuals were supposed to be allelic isoenzymes of a single locus. Esterases only occurring as single bands with medium intensity in potentially heterozygous plants and with high intensity or null expression in the potential homozygotes were appointed to a single locus with an active and a null allele, respectively.

These hypotheses were then tested using inbred generations of heterozygous and homozygous selfed parents.

Selfed parents were used which showed a large number of double bands or, in the case of intensity variations between single-banded types, esterase bands with medium intensity. Thus, as many esterase loci as possible could be studied with equal genetic background under comparable environmental influences and the number of inbred lines necessary for linkage analysis could be minimized.

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From the F_1 generations, plants could be selected which were up to ninefold heterozygous with regard to their esterase genotype. Parents originated from self fertile material from different sources (Wricke 1969; Schmidt-Stohn 1979b). Therefore, it was possible to confirm the results in successive selfed generations. For genetic analysis 65 inbred lines in total were used.

Cultivation of rye seedlings, extraction of leaves and isoelectric focusing are described by Schmidt-Stohn (1979a). Isoelectric focusing was performed in pH 3.5–10 gradients.

Although our own measurements of pI's with a pH-electrode (Fa. Ingold 403–6298) showed good linearity over the whole gradient and were reproducible, the pI's given in Fig. 1 were determined using marker proteins with known pI's (Fa. Pharmacia, pI calibration kit 3–10) in order to obtain a standardization. For pI determination marker proteins and rye extracts were applied to the same gel. This gel had been polymerized on a silanized polyester foil (Hostaphan, Hoechst/Kalle, type BN, 180 µm). Silanization was performed in a 0.4% solution of Polyfix-1000 (Fa. Desaga) in a 1:1 mixture of methanol/H_2O.

After focusing, the foil with the gel was sliced. Slices were stained for marker proteins with Coomassie Blue G-250 (see Instruction Manual of Pharmacia IEP pI calibration kit) and for esterase, respectively, using a modification of the method of Bergman and Maan (1973) with 50 mg α-naphtyl acetate and 100 mg Fast Blue RR dissolved in 3 ml acetone and 97 ml, 0,1 M Tris/HCl buffer, pH 7.

After fixation and destaining the slices were recomposed into the complete gel allowing a determination of the isoelectric points of the rye esterases. Standard error values according to Pharmacia (Instruction Manual pI calibration kit) are ± 0.05 pH units for markers with pI's < 6 and ± 0.08 pH units for markers with pI's between 6 and 9.

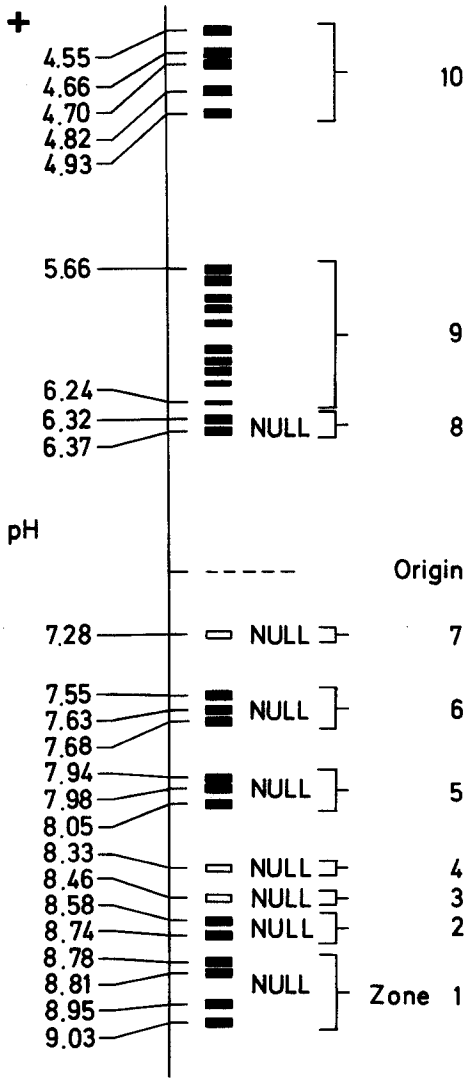

Fig. 1. Schematic representation of the distribution of analyzed rye esterases over the pH gradient after isoelectric focusing. *Zones 3, 4* and *7* include single bands with intensity variation. In all zones except zone *10* null variants have been observed

Results

Characterization of Esterases

Isoelectric focusing of rye esterases reveals a high polymorphism with numerous bands in a zymogram region ranging from about pH 4 to pH 9 (Figs. 1 and 2). Bands can be appointed to 10 zones within the zymogram with zone 9 involving a complex banding pattern not yet completely analyzed. All esterase isoenzymes analyzed in this study behave genetically as monomers. The esterase loci are designated by *'Est'* and a serial number, beginning at the cathode (Hvid and Nielsen 1977). Allelic isoenzymes and the alleles encoding for them are characterized through their isoelectric point (pI) which is added as an index to the locus indication (Schmidt-Stohn 1979b). All pI's are listed in Fig. 1 except the very closely focusing bands of zone 9 where only the isoelectric points of the outer esterases are given.

Genetic Analysis of Single Loci

Esterase Est 1

Bands in zone 1 can be observed at four positions with pI's of 9.03 (8.95), 8.95 (8.85), 8.78 (8.76), and 8.81, the first three of which have already been described by Schmidt-Stohn (1979b) and are listed here with newly determined pI's (the old values are given in brackets).

Est $1_{9.03}$ was not found in the material analyzed in this study and is listed only for the sake of complete description of the *Est 1* locus.

In addition to progeny of double-banded heterozygous genotypes, segregations were analyzed where individuals with null expression at the *Est 1* locus were observed among those showing either band *Est* $1_{8.96}$ or *Est* $1_{8.78}$. These observations suggest the existence of so-called null alleles in the material studied. *Est 1* segregation data are listed in Table 1. Since tests for heterogeneity revealed in no case a significant dif-

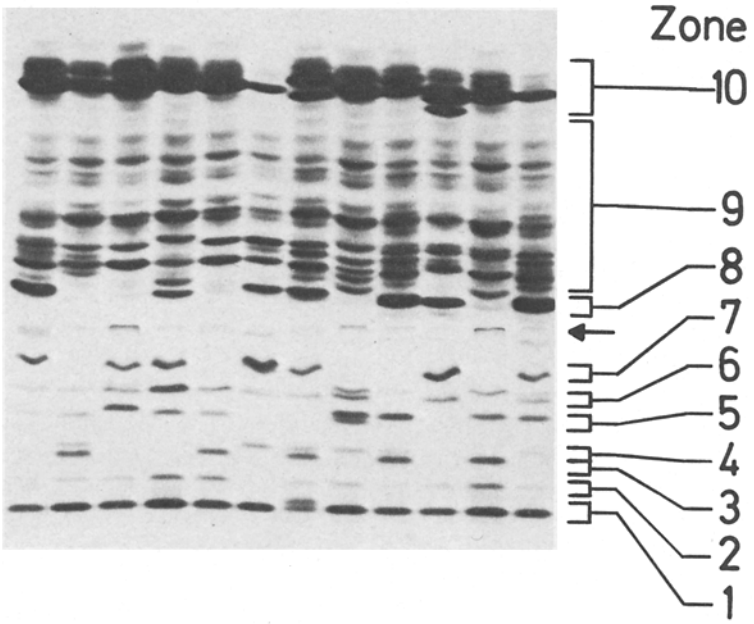

Fig. 2. Rye esterase zymogram after isoelectric focusing in a pH 3–10 gradient. Zones indicated on the right margin correspond to those shown in Fig. 1. The origin is indicated by ←

Table 1. Single locus segregation data for loci *Est 1 – Est 10*

Esterase cross allele A × B	Phenotypes in progeny			χ^2 (found vs expected)	P	Number of lines	Number of plants	Homozygous lines		Lines obtained. Number of plants tested	
	AA	AB	BB					AA	BB	AA	BB
$Est\ 1_{8.95} \times Est\ 1_{8.78}$	18	41	25	1.21	0.50	3	84	1	3	29	61
$Est\ 1_{8.95} \times Est\ 1_{8.81}$	82	149	87	1.42	0.40	6	318	–	–	–	–
$Est\ 1_{8.95} \times Est\ 1_{N}$	584		207	0.52[a]	0.40	9	791	1	1	256	12
	143		69	6.04*	< 0.025	1	212				
$Est\ 1_{8.78} \times Est\ 1_{N}$	192		67	0.06	0.70	2	259	2	1	37	10
$Est\ 2_{8.74} \times Est\ 2_{8.58}$	7	18	15	3.60	0.10	1	40	–	–	–	–
$Est\ 2_{8.58} \times Est\ 2_{N}$	399		123	0.50	0.40	4	522	–	–	–	–
	186		26	17.76*	< 0.001	1	212				
$Est\ 3_{8.46} \times Est\ 3_{N}$	223		67	0.46	0.40	4	290	1	1	7	112
$Est\ 4_{8.33} \times Est\ 4_{N}$	600		204	0.04	0.70	13	804	1	1	256	17
$Est\ 5_{8.05} \times Est\ 5_{7.94}$	61	101	56	1.40	0.40	4	218	–	–	–	–
$Est\ 5_{8.05} \times Est\ 5_{7.98}$	51	137	54	4.31	0.10	4	242	–	–	–	–
$Est\ 5_{7.98} \times Est\ 5_{N}$	355		120	0.006	0.90	8	475	–	–	–	–
$Est\ 5_{7.98} \times Est\ 5_{N}$	849		283	0.00	0.99	11	1132	1	1	16	29
	32		24	8.60*	< 0.01	1	56				
$Est\ 6_{7.63} \times Est\ 6_{7.55}$	101	226	98	1.76	0.30	7	425	2	1	30	5
$Est\ 6_{7.68} \times Est\ 6_{7.55}$	125	239	107	1.48	0.40	8	471	1	1	9	11
	24	15	16	13.69*	< 0.01	1	55				
$Est\ 6_{7.55} \times Est\ 6_{N}$	525		162	0.66	0.30	6	687	–	–	–	–
$Est\ 7_{7.28} \times Est\ 7_{N}$	538		175	0.06	0.70	15	713	1	2	29	37
$Est\ 8_{6.37} \times Est\ 8_{6.32}$	56	110	60	0.30	0.80	4	226	1	1	17	13
$Est\ 8_{6.37} \times Est\ 8_{N}$	863		289	0.001	0.95	10	1152	3	2	35	83
$Est\ 8_{6.32} \times Est\ 8_{N}$	573		189	0.007	0.90	12	762	–	–	–	–
$Est\ 10_{4.93} \times Est\ 10_{4.70}$	177	403	202	2.34	0.30	5	782	–	1	–	12
$Est\ 10_{4.93} \times Est\ 10_{4.66}$	146	267	120	2.54	0.20	12	533	2	2	30	45
	14	10	9	6.64*	< 0.05	1	56				
$Est\ 10_{4.93} \times Est\ 10_{4.55}$	18	31	19	0.56	0.70	2	68	–	–	–	–
$Est\ 10_{4.70} \times Est\ 10_{4.66}$	72	157	64	1.94	0.30	2	293	–	–	–	–
$Est\ 10_{4.66} \times Est\ 10_{4.55}$	29	43	28	1.98	0.30	1	100	–	–	–	–

[a] With Yates' continuity correction
* Significant at $p = 0.05$

ference between the materials, segregation data of different lines and inbred generations are combined. Only lines with significant χ^2 values for segregation are listed separately.

With the exception of one line, the segregation ratios for each line correspond to the 1 : 2 : 1 expectation for a locus with two codominant alleles, each encoding for one esterase band, or to the 3 : 1 expectation for a locus with one active and one null allele, respectively (see χ^2, Table 1).

Heterozygous I_1 and I_2 plants gave segregating I_2 and I_3 lines, respectively, whereas plants supposed to be homozygous only gave non-segregating progeny.

Together with the observations of Schmidt-Stohn (1979 b) the data suggest the existence of four codominant alleles at the *Est 1* locus encoding for different esterase bands. Furthermore, there is evidence for the existence of a null allele.

Esterase Est 2

In addition to alleles *Est* $2_{8.61}$ and *Est* $2_{8.49}$ (with newly determined pI's, assigned as *Est* $2_{8.74}$ and *Est* $2_{8.58}$ in this study; Fig. 1) found by Schmidt-Stohn (1979 b), a segregation of null types with allele *Est* $2_{8.58}$ occurred indicating the existence of a null allele at *Est 2*. Again, there is a significant deviation from expectation (deficiency of null variants) in the same line mentioned above as was the case for the *Est 1* segregation (Table 1). The joint deviation at *Est 1* and *Est 2* is caused by linkage of these two loci as will be shown in a further publication.

Esterase Est 3 and Est 4

Zones 3 and 4 have a single band at pH 8.46 and pH 8.33 or show null expression, respectively (Figs. 1 and 2). The single bands move to relatively closely adjacent positions and often appear together as non-segregating bands or with segregating null types. However, since progeny of genotypes heterozygous in both zones with a null allele comprise all nine different phenotypes of a dihybrid segregation pattern, we conclude that the bands in zone 3 and 4 represent the active alleles of two different loci, *Est 3* and *Est 4*. Null variants are due to null alleles occurring at both loci (Table 1). The significant χ^2 value for the *Est 3* segregation in the line mentioned above hints at possible linkage between *Est 1*, *Est 2* and *Est 3*.

Esterase Est 5 and Est 6

Zones 5 and 6 exhibit in some cases a pattern of closely correlated double and quadruple bands (Fig. 3). In a few cases three-banded types (recombinants) were observed. In other instances segregations of genotypes were found which were heterozygous with double bands in one zone and heterozygous with one band and a null allele in the other. Furthermore, segregations with single bands and null variants in both zones occurred.

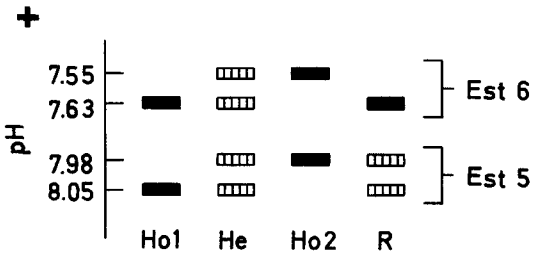

Fig. 3. Segregation of esterase in *zone 5* and *6*. Ho 1 and Ho 2 represent the homozygous and He the heterozygous genotype. The triple-banded phenotype (R) is recombinant. Full bands and hatched bands represent high and medium intensity, respectively

With one exception, segregation data (Table 1) support evidence for the existence of two loci *Est 5* and *Est 6* with alleles *Est* $5_{8.05}$, *Est* $5_{7.98}$, *Est* $5_{7.94}$, *Est* 5_N and *Est* $6_{7.68}$, *Est* $6_{7.63}$, *Est* $6_{7.55}$ and *Est* 6_N, respectively.

Esterase Est 7

Zone 7 encloses a single band focusing at pH 7.28 (Figs. 1 and 2) which either occurs as a non-segregating band or with segregating null variants. All single locus segregation data (Table 1) support the hypothesis that a locus *Est 7* with the alleles *Est* $7_{7.28}$ and *Est* 7_N is responsible for esterase variation in this zone.

Esterase Est 8

In the acid gel region two esterases moving to the closely adjacent positions at pH 6.37 and pH 6.32 were found (Fig. 1). These two bands segregate with each other in a 1 : 2 : 1 ratio in progeny of double-banded plants. In selfed progeny of single-banded individuals with medium band intensity, single bands and null variants occur in a 3 : 1 ratio (Table 1). Both bands and the null variant are therefore taken for alleles of a locus *Est 8*.

It must be noted that in the anodal direction from *Est* $8_{6.32}$ there is a faint band occurring only in the presence of the allele *Est* $8_{6.32}$ (Fig. 4, band 1). Since no recombinants were observed we cannot yet decide whether this band is encoded for by another locus linked with *Est 8* or simply originates from the *Est* $8_{6.32}$ allele by some epigenetical mechanism. For this reason we associate this band with zone 9.

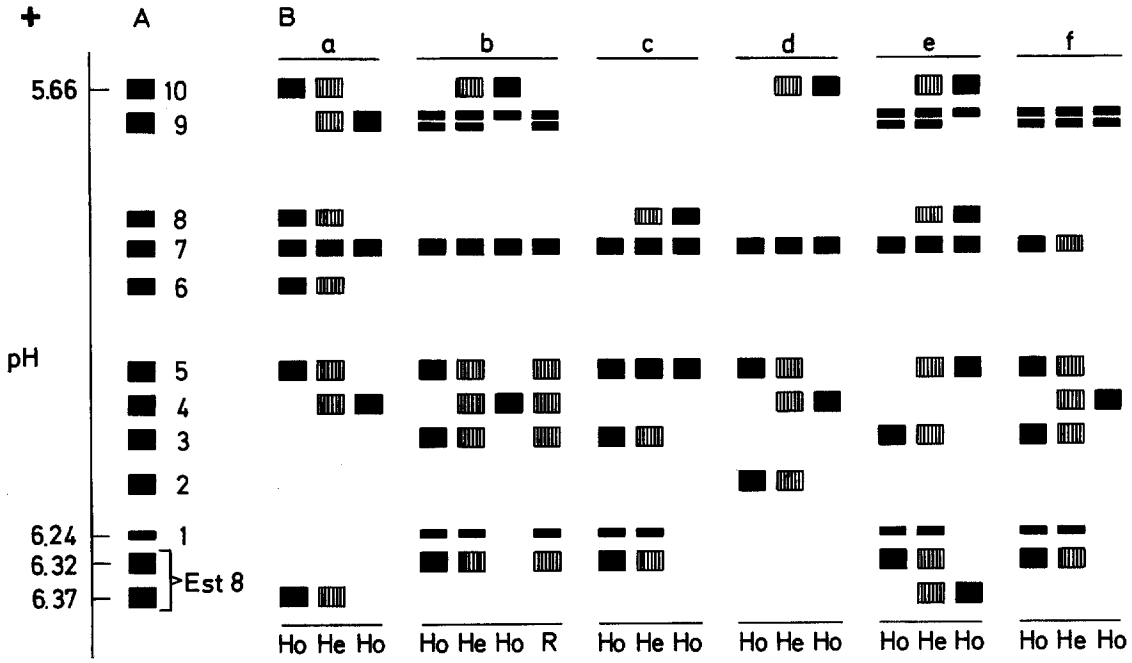

Fig. 4 A and B. Schematic representation of the observed esterase bands in zone 9 (**A**) and their segregation patterns (**B**, *a–f*). Full bands and hatched bands represent high and medium band intensity, respectively. For orientation, the *Est 8* segregation is also given for each of the six patterns. The order of *Est 8* genotypes is Ho 1, He, Ho 2. R is the recombinant type referred to in the text

Esterases of Zone 9

In the region, from pH 6.24 to pH 5.66, a polymorphic pattern of closely adjacent esterases is observed which is not yet completely analyzed because of its complexity (Fig. 2). Segregations of bands in this region, however, exhibit a very close correlation to the simultaneously occurring *Est 8* segregations. For this reason the data for the single segregations in zone 9 are the same as those obtained for *Est 8* in a particular line (Table 1) and thus are not given separately.

In total, 10 bands were observed in zone 9 (Fig. 4 A) which segregate in various patterns within the different inbred lines studied (Fig. 4 B, a–f).

A band only found in a single line focuses at pH 6.0 just above the *Est 8* position (Fig. 4 A, band 2). The combined band 2 segregation data of an I_1 and I_2 progeny line (128 banded: 31 Null, $\chi^2 = 2.28$) are in accordance with the 3 : 1 segregation model. Since the two active alleles of *Est 8* are lacking in this particular line we cannot decide whether band 2 is coded for by a separate locus or by another *Est 8* allele.

An interesting fact which cannot yet be explained is that in pattern b, e and f (Fig. 4 B) there is instead of band 9 a close double band segregating in some cases (pattern b and e) and non-segregating in others (pattern f).

Among a total number of 1,030 individuals analyzed for the six observed segregation patterns we only found one recombinant plant (Fig. 4 B, b). Recombination occurred between the bands 9/10 and the complex of bands 3, 4 and 5. Since the *Est 8* genotype in the recombinant plant cannot clearly be classified (homozygous for an active allele or heterozygous with a null allele) and no progeny of this plant are available for further tests, a recombination between *Est 8* and the middle band complex is not definite. There is no doubt, however, of recombination between bands 9/10 and the complex of bands 3, 4 and 5. These observations suggest that esterases of zone 9 do not originate in a single locus but are encoded by several genetic elements closely linked to each other and to *Est 8*. Esterases of zone 9 thus can be assigned to a compound locus *Est 9*.

Esterase Est 10

Deeply stained bands are found near the anode at a total of five different positions in the material studied (Figs. 1 and 2). With the exception of the pH 4.82 band, progeny of heterozygous double-banded genotypes could be analyzed which segregated in a 1 : 2 : 1 ratio, respectively (Table 1). Significant deviations from the expection only occurred in one line. Selfing of single-banded plants always gave non-segregating single-banded progeny. Thus, four codominant active alleles *Est* $10_{4.93}$, *Est* $10_{4.70}$, *Est* $10_{4.66}$ and *Est* $10_{4.55}$ can be detected at the *Est 10* locus. A fifth allele at pH 4.82

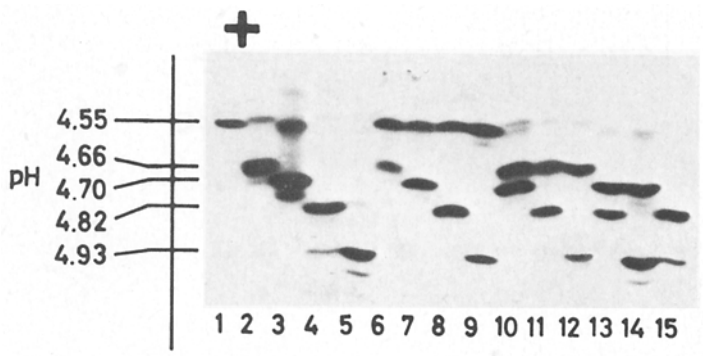

Fig. 5. Genotypes at the *Est 10* locus. *Columns 1–5:* Homozygous genotypes with the alleles *Est* $10_{4.55}$, *Est* $10_{4.66}$, *Est* $10_{4.70}$, *Est* $10_{4.82}$ and *Est* $10_{4.93}$, respectively. *Columns 6–15:* Heterozygous genotypes representing the 10 possible combinations of *Est 10* alleles. Note the faint secondary bands adjacent to the definite *Est 10* alleles

probably exists (Fig. 5) although segregation data are not yet available. To date a null allele has not been found at *Est 10.*

An interesting phenomenon can be observed at the *Est 10* locus. Each of the five alleles is accompanied by a series of secondary bands with lower intensity (Fig. 5). Comparisons of different homozygous and heterozygous genotypes reveal that secondary bands correspond to the five *Est 10* alleles which have been found up to now. These bands of the series focus at the same position as those *Est 10* alleles do, although the latter are actually absent in the particular genotype. Further investigations on this phenomen will be reported in a later study.

Discussion

The results reported above suggest that leaf esterases in rye are controlled by at least 10 loci with codominant alleles.

Although we used the 3 : 1 segregation model for dominance when null alleles were present at a locus, we succeeded in some cases in distinguishing all three phenotypic classes (full intensity: half intensity: null expression) by densitometric analysis of band intensity, thus eliminating the quasi-dominance caused by null alleles. Classification was verified by progeny tests in most cases. However, some methodical problems have to be overcome to ensure a faultless classification.

Garcia et al. (1982) observed an accumulation of null alleles for rye peroxidases. They attributed this to the low specifity of peroxidases which may hinder a selection against null alleles at different loci. To support their hypothesis they point out that a similar abundance of nulls has been observed only for the esterase system (Kahler and Allard 1970, in *Hordeum*) which is also characterized by its unspecificity (Shaw 1969). These suggestions have now also been confirmed for the rye esterases. With the exception of *Est 10* all leaf esterase loci have null alleles.

A remarkable phenomenon are the series of multiple bands at the *Est 10* locus occurring in a similar manner as those found at the E_4 locus in maize (Harris 1966). Harris could exclude possible differences in molecular weight as a reason for the multiple E_4 bands by demonstrating that the relative electrophoretic mobility of the multiple bands in starch gels is not altered by varying gel concentrations. He hypothesizes that conformationally different esterase forms differing in charge may cause multiple band series of the E_4 esterase (Harris 1968). However, since the *Est 10* multiple bands in rye reveal a strict regularity in decreasing intensity from the definite allele in the anodal direction and there is correspondence between multiple bands and all known *Est 10* alleles, this hypothesis is less plausible for the situation in rye.

The correspondence between multiple faint bands and *Est 10* alleles can be observed in the region between the two 'outer' alleles, *Est* $10_{4.93}$ and *Est* $10_{4.55}$. Within this region a deeply staining *Est 10* allele is found at each position where a faint secondary band focuses when the particular allele is absent. Those multiple bands focusing anodally to the *Est* $10_{4.55}$ allele may hint at further *Est 10* alleles which have not yet been detected.

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Book Reviews

Hecht, M. K.; Wallace, B.; Prance, G. T.: Evolutionary Biology. Vol. 14. New York, London: Plenum Press 1982. i–xiii + 445 pp., several figs., several tabs. Hard bound $ 39.50.

This volume 14 of the well-known series "Evolutionary Biology" (edited by M. K. Hecht, B. Wallace, and G. T. Prance) turns out to be in line with the general objectives and standards of these publications: To provide a stimulating and critical forum for extensive review articles, commentaries, original papers concerning essential new results and controversies in evolutionary biology.

Usually these different contributions are given by leading experts from a variety of biological disciplines. But the basic idea of showing the coherence of all the different topics has been always focussed on the conviction that evolution represents a unifying principle in the life sciences. Therefore data from studies of man, animals, plants and microorganisms had been included simultaneously.

The present volume 14 proves to be in accordance with these aspects and requirements. But nevertheless some critical comments must be assigned. But before, we will give some references to the classification and content of the book, which contains a collection of the following six papers: 1. Genetic Relationship and Evolution of Human Races (M. Nei and A. K. Roychoudhury).

The study of the evolution of human races by using measurements of genetic distance between populations requires gene frequency data from many loci. Several years ago Nei and Roychoudhury (1972, 1974) analysed the three major races of man (Caucasoid, Negroid and Mongoloid) applying Nei's new measure of genetic distance. Because in the past five years the amount of gene frequency data has almost doubled the authors have reanalyzed the degree of genetic differentiation among the three major races of man using updated data. Additionally the analysis has been extended to various other races to get some insight into the pattern of racial evolution in man. The major purpose is to understand the genetic relationship and evolution of human races on a global basis. Therefore almost no attention has been paid to the genetic differentiation of local populations within races. The authors are interested in relating the extents of genetic differentiation of representative human races in five major geographic areas on earth.