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A Marker Locus, *Adh-1*, for Resistance to Pea Enation Mosaic Virus in *Pisum sativum*

N. F. Weeden and R. Provvidenti

Linkage between *Adh-1*, the locus specifying the more anodal isozyme of alcohol dehydrogenase, and *En*, the locus controlling resistance to pea enation mosaic virus, was investigated in the garden pea, *Pisum sativum* L. A recombination frequency of 4% was observed between the two loci, indicating that *Adh-1* may be a practical marker for *En*. The use of *Adh-1* in combination with other loci as brackets around *En*, thereby increasing the reliability of an indirect screen, is also discussed.

Isozyme loci have been championed as ideal molecular markers for genes that are difficult or inconvenient to score directly.[8,15] Yet relatively few close linkages between isozyme loci and economically important monogenic traits have been identified. A classic example is the tight linkage between an acid phosphatase locus, *Aps-1*, and the locus *Mi*, controlling nematode resistance in the tomato.[9] Linkage between isozyme loci and self-incompatibility genes have been demonstrated in several species.[3,16,22] *Prx-2* has been shown to be linked to *Ms*, a male sterility gene in the tomato.[17] In the garden pea, an esterase polymorphism can be used to mark *Fw*, the gene conferring resistance to *Fusarium* wilt (race 1),[2] and *Mo* (bean yellow mosaic virus resistance) is tightly linked to *Pgm-p*.[21] One of the major obstacles to using isozyme loci for marking genes is the lack of linkage maps in many crops. Without such a map, one is forced to depend primarily on the chance associations of isozyme variants with the traits being investigated. Hence, most isozyme markers have been identified in species for which a relatively detailed linkage map is available.

Pea enation mosaic virus (PEMV) is an important pathogen in the garden pea, affecting many areas in the United States and western Europe. Resistance to PEMV is controlled by a single dominant gene located at the *En* locus.[12] In segregating populations, homozygous susceptible genotypes can be identified by their characteristic mosaic pattern consisting of hyaline, translucent spots ("windows") and small necrotic flecks, causing foliar reduction and distortion. Pods are affected by ridged overgrowths (enations) and plants are stunted. However, these symptoms can be influenced by environmental conditions. At temperatures above 25°C, systemic symptoms tend to be very mild or absent. Conversely, below 18°C, resistant plants may develop atypical symptoms, consisting of scattered chlorotic flecks.[12] Thus, uncontrollable environmental conditions can lead to misclassification of certain genotypes. This inconvenience could be avoided and breeding for resistance facilitated if a marker gene closely linked to *En* were identified.

It is known that *En* lies between *St* and *Tac* on the long arm of chromosome 3.[1,4,7] Several isozyme loci have been mapped to this region of chromosome 3, including *Acp-3*,[18] *Adh-1*,[19] and *Gal-3*.[19] Of these, *Adh-1* appeared to be the most suitable locus for marking *En* because the ADH phenotypes are more easily distinguished than those of either ACP-3 or GAL-3. Here, we report the results of linkage tests involving three loci on chromosome 2: *Adh-1*, *Lap-1*, and *En*.

Materials and Methods

Two pea lines, A76-46 and B880-221, homozygous for the allele *En*, were selected from the collection of pea lines maintained by Dr. G. A. Marx, Department of Horticultural Sciences, NYSAES, Geneva, New York. Two PEMV-susceptible lines, A683-168 and Alaska, were also supplied by Dr. Marx. Both the susceptible lines were chosen because they possessed the rare "fast" allozyme of ADH-1. The cultivar Bonneville was used as a PEMV-susceptible control. Seed was germinated in petri dishes and transferred to pots containing an artificial soil mix. All plants were grown in greenhouse facilities under natural light. Populations grown in the summer were exposed to higher ambient temperatures (≥25°C) than those grown in the fall (≤20°C).

Two crosses were analyzed for joint segregation of *En* and isozyme loci. An F_2 progeny (52 plants) from the cross Alaska × B880-221 was grown during the summer. This progeny was screened for susceptibility to PEMV as described later and scored for alcohol dehydrogenase (ADH) and leucine aminopeptidase (LAP) phenotypes using root extracts. Seed from 10 selected F_2 plants were germinated in the fall, and the resulting F_3 plants were examined for both PEMV resistance and ADH phenotype.

Sixteen F_2 plants from a second cross,

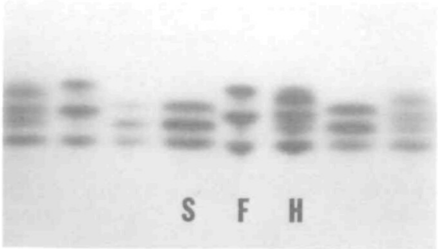

Figure 1. Alcohol dehydrogenase phenotypes in pea root tissue which had been submerged in water overnight. The ADH-1 pattern displays variation resulting from segregation at *Adh-1*. The parental phenotypes are labeled "S" for the common slow ADH-1 allozyme and "F" for the rare fast variant. The hybrid phenotype is labeled "H." Anode is toward the top of figure.

Table 1. Characterization of parental and control lines for *En*, *Adh-1*, and *Lap-1* genotypes

Line	PEMV phenotype: No. resistant	PEMV phenotype: No. susceptible	Adh-1 genotype[a]	Lap-1 genotype[a]
Alaska	0	5	*F/F*	*F/F*
A683-168	0	5	*F/F*	*F/F*
Bonneville	0	25	*S/S*	Not tested
B880-221	5	0	*S/S*	*S/S*
A76-46	5	0	*S/S*	*S/S*

[a] F, allele coding faster migrating allozyme; S, allele coding slower migrating allozyme.

A76-46 × A683-168, were grown during the summer and characterized for PEMV resistance and ADH phenotype using both seed and root extracts. Another 13 plants from this F_2, as well as F_3 progenies from each of the original 16 F_2 plants, were grown in the fall and scored for the same three traits using only root extracts.

To test for resistance to PEMV, plants were inoculated on the first two fully expanded leaves with PEMV. To assure infection in all susceptible genotypes, plants were subsequently reinoculated on the third and fourth leaves. Inoculum was prepared by grinding PEMV-infected leaves of Bonneville with phosphate buffer (K^+) at pH 8.5. Each parental line and the PEMV susceptible cultivar Bonneville were subjected to the identical inoculation sequence.

The ADH-1 phenotype was determined by horizontal starch gel electrophoresis on a pH 8.1 Tris citrate/lithium borate gel system.[10] In both seed and root tissue ADH-1 is produced only under anaerobic conditions.[20] Therefore, to induce production of ADH-1 in seeds, these were placed overnight at the bottom of a 16 × 80 mm test tube filled with distilled water. A small section of one of the cotyledons was removed using a scalpel and macerated with 0.5 ml 0.05 M Tris malate, pH 8.0, containing 7 mM 2-mercaptoethanol, 5% glycerol, 5% soluble polyvinylpyrrolidone (PVP-40), and 0.5% Triton X-100. Root ADH-1 was induced by placing the potted plants overnight in a container of water. Sections of young, healthy root tissue were removed, washed free of adhering vermiculite, and macerated with 0.5 ml of the extraction buffer described previously. The alcohol dehydrogenase assay contained 25 ml 0.1 M Tris HCl, pH 8.0, 0.4 ml 95% ethanol, 0.4 mM NAD, 0.3 mM MTT, and 100 μM PMS. The assay for leucine aminopeptidase was taken from Shaw and Prasad.[11]

Segregation and linkage calculations were performed using the LINKAGE-1 computer program.[14] Homogeneity tests were done by methods given in Sokal and Rohlf.[13]

Results

When the parental lines and control were inoculated with PEMV all plants of the lines Alaska, A683-168, and Bonneville developed typical systemic symptoms. Lines B880-221 and A76-46 reacted with only local lesions on the inoculated leaves but virus failed to move systemically (Table 1). Observed polymorphism in ADH phenotype is shown in Figure 1. The segregation pattern of ADH-1 was the same in both seed and root extracts from plants of the A76-46 × A683-168 F_2. The variation observed in the LAP-1 phenotype in the F_2 populations consisted of the two parental single-banded phenotypes and the double-banded pattern characteristic of the F_1.

In both F_2 populations, the three loci on chromosome 3 exhibited segregation ratios close to those expected for two alleles segregating at a single locus (Table 2). An LAP-1 phenotype was not obtained on several plants, thereby reducing the number of individuals scored for LAP in Table 2. Relatively few seeds (4 to 12) were obtained from each A76-46 × A683-168 F_2 plant grown in the summer, and these were used to determine the *En* phenotype of the parent. The results from the two F_2 populations (summer and fall) of A76-46 × A683-168 proved to be homogeneous and were pooled. Seed set on the Alaska × B880-221 F_2 plants was generally good. Of the F_3 families selected for testing in the fall, four were derived from plants susceptible to PEMV. All 48 progeny in these families displayed the susceptible phenotype. The remaining six F_3 families were derived from resistant parents. Two of these families contained only resistant plants, whereas the other four contained susceptible segregants. All four of these last progenies also were segregating at *Adh-1*. Homogeneity tests showed that the joint segregation data for the four families could be pooled, and this combined data is presented in Table 2.

Joint segregation analysis revealed close linkage (3% to 6%) between *En* and *Adh-1* in all populations in which both were segregating (Table 3). Homogeneity tests indicated that the joint segregation data did not differ significantly for the two crosses, permitting the pooling of the results in Ta-

Table 2. Segregation at *En*, *Adh-1*, and *Lap-1* in the F_2 and F_3 progeny examined

Locus	No. plants with designated phenotype[a]: F/+	H	S/−	Expected ratio	Goodness of fit (*P*)
Alaska × B880-221 (F_2)					
En	37		15	3:1	0.53
Adh-1	14	26	12	1:2:1	0.91
Lap-1	7	25	15	1:2:1	0.29
A76-46 × A683-168 (F_2)					
En[b]	19	—	10	3:1	0.24
Adh-1	11	16	2	1:2:1	0.09
Lap-1	8	14	7	1:2:1	0.95
Alaska × B880-221 (F_3)					
En	45	—	13	3:1	0.64
Adh-1	12	26	20	1:2:1	0.25

[a] +, Dominant phenotype; −, recessive phenotype; F, homozygous fast; H, heterozygous; S, homozygous slow.

[b] Data obtained by testing F_3 generation.

Table 3. Joint segregation of *En* and *Adh-1*

Progeny	No. plants with designated phenotype[a]						Recombinant fraction	SE
	+/F	+/H	+/S	−/F	−/H	−/S		
Alaska × B880-221 (F_2)	0	25	12	14	1	0	3	4
A76-46 × A683-168 (F_2)	1	16	2	10	0	0	3	3
Alaska × B880-221 (F_3)	1	24	20	11	2	0	6	4
Combined data	2	65	34	35	3	0	4	2

[a] +, Dominant phenotype; −, recessive phenotype; F, homozygous fast; H, heterozygous; S, homozygous slow.

ble 3. No linkage was observed between *Lap-1* and either *En* or *Adh-1* (data not shown).

Discussion

The high correlation between segregation at *En* and at *Adh-1* indicates that *Adh-1* can be useful as a marker locus for *En*. The 4% recombination between *Adh-1* and *En* may make *Adh-1* a more reliable predictor of PEMV phenotype than the direct screening process using inoculum. Despite the consistent response of controls during the summer tests, we anticipated that some susceptible plants would have been scored as resistant because of the high environmental temperatures. However, nearly all F_2 plants giving atypical responses generated progenies segregating for PEMV resistance and, thus, must have been heterozygous at *En*. These results, as well as previous experience with direct screening, indicate that 5% to 10% of the plants in a segregating progeny will display atypical symptoms, often resulting in misclassification of these individuals.

The relatively low number of F_3 individuals used to determine the PEMV phenotype of plants from the summer A76-46 × A683-168 F_2 population may have led to the misclassification of one plant. This F_2 plant produced only four F_3 individuals, each of which gave a resistant phenotype. The F_2 plant was therefore classified as homozygous resistant, although there existed a 32% [$(3/4)^4$] chance that the plant was heterozygous. However, our assuming homozygosity did not introduce a significant error into the segregation and linkage analysis. In all other cases, a sufficient number of F_3 plants were tested to show segregation or demonstrate homozygosity at a confidence level of >95%.

Several investigators have documented that in the pea, even when obvious chromosomal rearrangements are disregarded, the recombination frequency between linked loci can differ significantly among crosses.[5,6,18] To test for such variation we used two F_2 familes derived from four parents of diverse genetic background. The *En* allele in the two PEMV resistant parents can be traced back to a common ancestor, but the parental lines possessed very different genotypes. The two susceptible parents were not closely related. Although the number of individuals in the A76-46 × A683-168 F_2 was low, the recombination frequency obtained between *Adh-1* and *En* was very close to that obtained in both the F_2 and F_3 generations of the alternative cross. The lack of linkage between *Lap-1* and either *En* or *Adh-1* is in agreement with the positioning of these latter loci on the opposite side of *St* from *Lap-1*.[4] If either of the two loci were between *St* and *Lap-1*, deviation from random assortment of that locus and *Lap-1* would have been expected because *Lap-1* and *St* have displayed linkage in most crosses examined.[18] The Mendelian segregation ratios obtained at *Lap-1* provides additional evidence that much of chromosome 3 is pairing normally in the crosses examined.

An attractive aspect of using *Adh-1* as a marker locus is the expression of the marker in seed tissue. The simplicity of the sampling procedure would permit many seeds to be analyzed without the need to sacrifice any of the usually limited greenhouse space available to a breeding program. The removal of approximately one-fifth of one cotyledon for extraction does not significantly affect germination or later growth of the seedling. Another advantage in using *Adh-1* is that a rare allele has been identified in cultivated pea germplasm which, when coupled to the resistance gene, would permit identification of the resistant plant in most crosses. The initial obstacle to using *Adh-1* as a marker is the coupling of the rare allele with the resistance gene in a genetic background appropriate for a particular breeding program. At present, we have the *Adh-1* "fast" allele coupled with PEMV resistance in only a few lines derived from recombinants identified in the two F_2 families described.

The reliability of using marker loci as predictors of a phenotype can be increased by using two marker loci, one on each side of the locus of interest.[15] Although we could not determine on which side of *Adh-1* the locus *En* is situated, there are several other loci near *En* and *Adh-1* which might be useful for bracketing *En*. One of these is the morphological mutant, *tac*, which is about 8 map units distal to *En*.[7] Should *En* be located between *Adh-1* and *Tac* the use of both markers in the coupling phase would definitely increase the reliability of the screening process, although all plants would have to be grown in pots or flats until the seedling markers could be scored. Two isozyme loci, *Gal-3* and *Acp-3*, also lie within 10 map units of *Adh-1*.[19] Again, a combination of loci that bracketed *En* might increase predictability; however, both GAL-3 and ACP-3 phenotypes are more difficult to interpret than those of ADH-1. At least initially, it would appear that the *Adh-1-En* linkage is tight enough to permit screening for PEMV resistance on the basis of *Adh-1* genotype alone.

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Pale, an Autosomal Dominant Mutation Affecting Body Pigmentation and Embryogenesis in *Pyrrhocoris apterus* (Heteroptera)

R. Socha

Pale (*Pa*) is the first dominant mutant of *Pyrrhocoris apterus* L. reported. The mutant body coloration, ranging from cream-yellow to yellow-orange, is inherited as an autosomal dominant trait and is detectable at all developmental stages. The homozygotes, *Pa*/*Pa*, die as embryos during postblastokinesis.

The red firebug, *Pyrrhocoris apterus* L., a common palearctic species, belongs to the largest heteropteran section of Pentatomorpha. It is slightly over 1 cm long and is characterized by red and black aposomatic body coloration. In central Europe, it is a monovoltine species that overwinters as an imago. *P. apterus* aggregates at the base of the lime trees, *Tilia cordata* and *T. platyphylos*, whose seeds are the essential component of its food. Simplicity of laboratory breeding makes this firebug a convenient experimental insect.

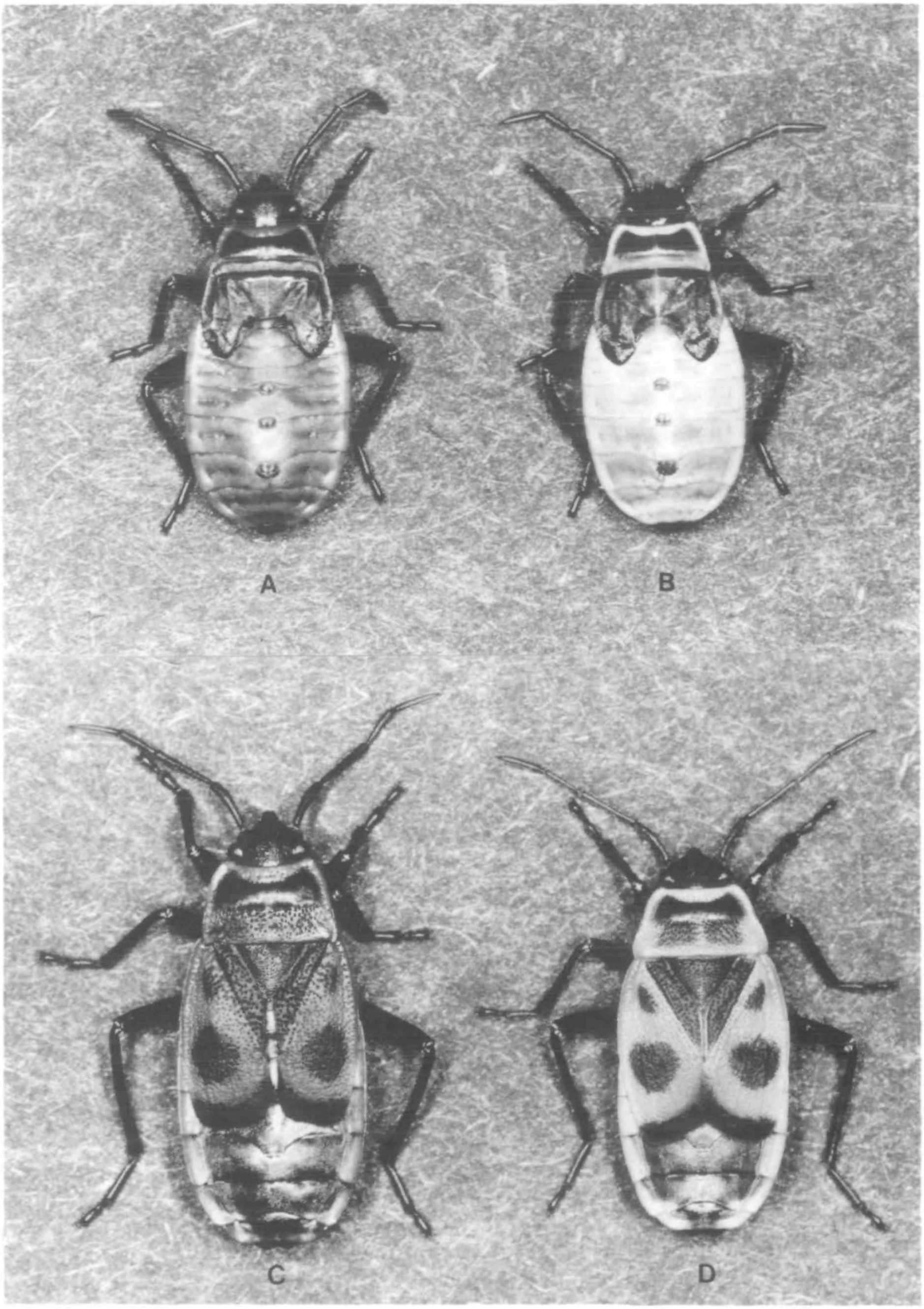

Figure 1. Wild-type and Pale (*Pa*) mutant of *Pyrrhocoris apterus* L. (A, C) Fifth instar larva and adult of the wild-type. (B, D) Fifth instar larva and adult of the *Pa* phenotype.

In 1891, the German zoologist Hermann Henking described chromatin elements from this firebug that he labeled X; this was the first report on sex chromosomes in the history of genetic research.[1] Since then, *P. apterus* has been intensively studied by insect physiologists and endocrinologists, but virtually forgotten by geneticists. The genetics of *P. apterus* were not investigated until 1968, when the first, white-body color mutant was described.[4] This trait, characterized by inhibition of the biosynthesis of red pigment, was shown to be inherited as a single autosomal recessive trait.

In continuing the attempt to expand our knowledge of the genetics of this species, this article describes the inheritance pattern of the first dominant trait, Pale (*Pa*), discovered for this firebug. Besides the morphological and genetic description, a short account of the lethal effects of the *Pa* gene on embryogenesis is presented.