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Inheritance and Linkage Relationships of Nine Isozyme Loci in Wild Radish

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Knowledge of inheritance patterns of genetic markers is important for studies in a variety of fields, but most studies of genetic markers in plants are in managed species or cultivars. We examined inheritance and linkage patterns of nine isozyme loci in a natural population of wild radish (*Raphanus raphanistrum*), a species that is widely used for ecological and evolutionary research. Little evidence for deviations from Mendelian segregation or strong linkage among loci was found. Therefore, these loci are excellent markers for use in a variety of studies. These results are in agreement with a study of seven of the same loci in *R. sativus*, except that evidence for linkage between one pair of these loci was reported in the latter species.

Studies of segregation and linkage of genetic markers provide important basic information about the genome. In addition, genetic markers have become critical tools in ecology and evolution. For example, genetic markers are necessary for studies of male fitness, mating systems, and genetic structure of populations (reviewed in Brown 1990; Snow and Lewis 1993; Weir 1990). Knowledge of inheritance and linkage patterns is crucial to the effective use of genetic markers in such studies (e.g., Clegg 1980; Devlin et al. 1988; Smouse and Meagher 1994).

Studies of segregation and linkage patterns in isozyme markers have been reported for a variety of plant species. Results have shown that most plant isozymes are inherited in a Mendelian fashion, and most studies have reported evidence for linkage among some of the markers analyzed (e.g., Muehlbauer et al. 1989; O'Malley et al. 1979; Salas and Murphy 1995; Vezvaei et al. 1995; Wendel and Parks 1982; but see Arús and Orton 1983; Ellstrand and Devlin 1989; Guries and Ledig 1978).

The majority of plant species for which inheritance of isozymes have been studied are agricultural or sylvicultural species (e.g., Kazan et al. 1993; Knerr and Staub 1992; Rovira et al. 1993; but see Ellstrand and Devlin 1989) that are less useful for ecological or evolutionary work than wild species. Wild radish (*Raphanus raphanistrum*) has become a model system for ecological and evolutionary studies (e.g., Conner and Via 1993; Mazer 1987; Snow 1990; Stanton et al. 1986), but to our knowledge, patterns of inheritance and linkage of isozymes have never been reported in this species. Here we report this information for nine isozyme loci in a natural population of wild radish.

Materials and Methods

R. raphanistrum is a self-incompatible annual weed of disturbed areas and agricultural fields. The parents used in this study were grown from seeds collected from a single large population in New York (for details see Conner and Via 1993). Thirty-eight parental plants were randomly chosen and paired to create 19 families. Flower bud tissue was collected from all parents and from 20 to 40 offspring in each family for a total of 630 offspring.

Standard starch-gel electrophoretic procedures were used (Murphy et al. 1990). Parents and offspring were run on the same gel, and all gels were photographed. A total of 33 loci were surveyed; of these, nine produced consistently interpretable bands and were included in this study. The loci used were esterase (*EST*; EC 3.1.1.-; monomer, four alleles), formate dehydrogenase (*FDH*; EC 1.2.1.2; dimer, two alleles), isocitrate dehydrogenase (*IDH*; EC 1.1.1.42; dimer, three alleles), leucine amino peptidase (*LAP*; EC 3.4.11.1; monomer, six alleles), phosphoglucose isomerase (*PGI*; EC 5.3.1.9; dimer, four alleles), phosphoglucomutase (*PGM*; EC 2.7.5.1; three monomeric loci with three, four, and three alleles, respectively), and triose-phosphate isomerase (*TPI*; EC 5.3.1.1; dimer, three alleles). Two of the buffers used were from Murphy et al. (1990, pp. 123–124): lithium-borate/Tris-citrate (*EST*) and Tris-borate-EDTA I (*LAP, FDH,* and *TPI*). The third buffer was 10 g/L histidine free-base, adjusted to pH 6.0 with citric acid monohydrate (*PGI, PGM,* and *IDH*).

Stain recipes for *IDH, PGI,* and *PGM* were from Murphy et al. (1990). *TPI* was visualized using an overstain of the fructose-biphosphate aldolase recipe in Murphy et al. (1990). For *LAP* we used the recipe in Shaw and Prasad (1970), with the addition of 1 ml N,N-dimethylformamide. The *EST* (calorimetric) and *FDH* stains were from Wendel and Weeden (1989), with the addition of 25 mg EDTA to the *FDH* recipe.

The Linkage-1 program (Suiter et al. 1983) was used for single-factor segregation and linkage analyses. Any genotypes that were impossible given the parents' genotypes were eliminated; only 7 of the 630 offspring had impossible genotypes after rechecking gel photographs. These were probably offspring samples that were not placed in the correct family because of clerical errors.

Results

A total of 171 locus/family combinations were tested for Mendelian segregation; 87% of the isozyme family combinations fit Mendelian expectations after null alleles were assumed at *LAP* (see below). The remaining 13% deviated based on a 5% significance level uncorrected for multiple comparisons, while 6% deviated based on an uncorrected 1% significance level (Table 1). Note that large sample sizes are needed to detect significant deviations in chi-square tests (Mulcahy and Kaplan 1979). Out of the nine loci, all had at least one significant deviation, with *PGM-1* having the most (5 out of the 23). However, based on sequential Bonferroni correction (Rice 1989), only 5 out of 171 isozymes showed significant deviations using a P value of .05; the next most significant deviation had a corrected P value of .16. These five are all from different loci and from four different families (Table 1). Therefore it seems safe to conclude that the nine isozymes studied segregate in a Mendelian fashion, at least in the vast majority of cases.

There are 684 possible linkage tests with

Table 1. Uncorrected *P* values for family/locus combinations showing significant deviations from expected Mendelian ratios

	Locus								
Family	*EST*	*FDH*	*IDH*	*LAP*	*PGI*	*PGM-1*	*PGM-2*	*PGM-3*	*TPI*
A1	0.03	0.03							
A174			0.02						
A244					0.003		0.007		
A299							0.04		
B191									
B242.1						<0.0001	0.01		
B301	0.001							0.04	
B366				0.03	<0.0001			<0.0001	
B9	<0.0001								0.03
C12									
C128						0.04			
C228									
C324									0.02
C328.2			0.03			0.001			
C67									
D107					0.01				
D125						0.02			
D29				<0.0001					
E73						0.009			

Only those that are significant at $P < .0001$ are significant at the 0.05 level after sequential Bonferroni correction.

9 loci and 19 families, but in many of the families both parents were homozygous for one or more loci, so linkage tests involving those locus/family combinations were not possible. Out of the 358 pairwise linkage tests that were possible, only nine were significant using uncorrected *P* values, and these were all between different pairs of loci (Table 2). None of these are significant at $P = .05$ using a sequential Bonferroni correction. Thus, there was little evidence for linkage in our study.

There was evidence for a null allele at *LAP* in 11 of the families. In six families, one parent appeared to be homozygous for an allele not present in the other parent, and a number of the offspring appeared to be homozygous for an allele not present in the first parent. When these "impossible" offspring and the first parent were assumed to be heterozygous for the null, the progeny fit Mendelian expectations. In another four families, the two parents shared one allele, but there were excess homozygotes; again, assuming that one or both parents were heterozygous for the null resulted in Mendelian ratios. The last family had one parent homozygous and the other parent heterozygous for the null allele. This family and two of the other families produced many homozygous null offspring; thus, the two other families were assumed to have both parents heterozygous for the null allele. Null alleles at LAP have been reported in several other plant species [e.g., Guries and Ledig (1978) and references therein].

Table 2. Pairs of loci/family combinations in which there were significant deviations from independent assortment

Locus pair	Family	*P* value	No. of families[a]
EST/FDH	B301	.01	14
FDH/LAP	A299	.03	14
FDH/PGM1	B191	.001	10
FDH/PGM2	C324	.03	14
IDH/PGM2	B242.1	.02	5
LAP/PGI	A174	.0002	11
LAP/PGM1	C128	.009	12
LAP/PGM3	B301	.04	12
PGM2/TPI	C12	.005	10

Note that no pair of loci showed evidence of linkage in more than one family. *P* values shown are uncorrected for multiple comparisons; none of these deviations are significant using a sequential Bonferroni correction.

[a] The total number of families in which that locus pair was tested for linkage.

Discussion

The nine loci studied are clearly excellent markers for use in a variety of studies because they segregate in a Mendelian manner, have a large number of alleles, and are not tightly linked. The lack of linkage found in this study is somewhat surprising. Wild radish has nine chromosomes; for these nine loci to be completely unlinked they would each have to be on a separate chromosome. While this is possible, it is perhaps more likely that some of these loci are loosely linked and that the number of offspring per family in this study was too small to detect these weak linkages.

Most other isozyme linkage studies in plants have reported some evidence of linkage, including some with similar numbers of offspring per family as our study (e.g., Ellstrand and Devlin 1989; Muehlbauer et al. 1989; O'Malley et al. 1979; Wendel and Parks 1982). However, other studies have also reported little evidence for tight linkage, and some of these have had large numbers of offspring per family (e.g., Arús and Orton 1983; Guries and Ledig 1978). Two of the studies cited above were from species closely related to *R. raphanistrum*. No tight linkages were found in *Brassica oleracea*, with an average of almost 300 individuals per family (Arús and Orton 1983), but two strong linkage groups involving six loci were found in *R. sativus* (Ellstrand and Devlin 1989).

Ellstrand and Devlin studied seven of the same loci in *R. sativus* as we did in *R. raphanistrum*. They also found no evidence for linkage among the pairs of loci that we studied with one exception: *PGM2* and *LAP*. These appear to be the same loci in both species (Clegg J, personal communication; Kercher S and Conner J, unpublished data), thus the differences in the results may reflect different arrangements of the loci on the chromosomes between the species. However, the average recombination fraction between these loci was 0.43 in the seven families for which the Linkage-1 program estimated *r*; this is similar to the recombination fraction in *R. sativus* (Ellstrand and Devlin 1989). It is possible that further study of *R. raphanistrum* will provide evidence for weak linkage between *PGM-2* and *LAP*. In five of the 14 families in which this locus pair could be tested, the uncorrected *P* value for the chi-square test was less than 0.15, suggesting that modest increases in family sizes might be adequate to detect linkage in some families.

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Sources for Misclassifying Genealogical Origins in Mixed Hybrid Populations

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Expected genotype character arrays from advanced generation hybrids and backcrosses overlap arrays from parental crosses and first-generation hybrids. Therefore, assignment of hybrid offspring to a genealogical origin solely by genotype inspection is problematic. In several "best case" scenarios of assignment by inspection, where parental taxa display diagnostic alleles and matings are limited to the second generation of hybridization or backcrossing, we demonstrate classification error rates of 87% and 57% for the two and four locus cases, respectively. The error rate decreases with additional loci and more than 13 loci may be required to reduce error rates below a 5% threshold. Departures from "best case" conditions will contribute additional error. These results support use of maximum-likelihood methods which can provide unbiased classification estimates and are more suitable for the examination of complex hybrid mixtures than assignment of genealogical origin by genotype inspection.

Nason and Ellstrand (1993) examined the utility and limitations of using maximum-likelihood procedures for estimating the contribution of various crosses to a hybrid mixture. Their purpose was to explore several sampling variables (number of loci, differences in marker frequency, and number of individuals assayed) in regard to variance and bias on classification error in a theoretical hybrid mixture limited to the second generation of outcrossing. They demonstrated that the potential for incorrectly estimating the contributions of six genealogical cross types (classification error) decreased with (1) an increased number of independently assorting loci, (2) an increase in the frequency differences for alleles between taxa, and (3) an increase in the number of offspring surveyed. The purpose of this communication is to expand on the earlier works of Ellstrand et al. (1987) and Nason and Ellstrand (1993), to illustrate sources of error in a hybrid mixture analysis by investigating the special case of when marker loci are species specific. Although the maximum-likelihood procedure does not require that loci be completely diagnostic, we will demonstrate that even in this best case scenario, the potential for classification error is great if genealogical origin is assigned individually by genotype inspection (Avise and van den Avyle 1984; Campton 1990; Nason and Ellstrand 1993).

The potential for classification error is evident in a number of recent articles that categorize contributing mating types solely by genotype inspection (e.g., Carmichael et al. 1993; Dunham et al. 1994; Todd 1986). In short, the inspection method assigns individuals to a genealogical origin based on their genotypic arrays. The composition of the population is then extrapolated from these assignments. The problem with this method is that genotype arrays of second- (or later) generation crosses overlap with parental and first-generation hybrids. Even where the suite of loci express allelic arrays that do not overlap (i.e., diagnostic), there may be overlap of genotypic distributions of parental, F_1 and F_2 hybrids, and backcrosses. This overlap can prevent the confident allocation of some individuals to genealogical mating type solely by inspection (Epifanio 1992; Nason et al. 1992; Nason and Ellstrand 1993). Furthermore, the classification error resulting from this overlap can be high and, if ignored, can lead to erroneous conclusions about the success and contribution of mating types to an admixture (Nason and Ellstrand 1993). Many of the investigations that use the inspection method acknowledge the risk of misclassifying individuals by genotype, but few adjust their results to account for this error. Obviously, if the error rate is great, inappropriate conclusions may be drawn about the direction, extent, or consequences of hybridization.

A goal of population admixture analysis is to decompose the mixture into contributing cross types (Utter and Ryman 1993). The application of these methods includes determination of human origins (Smouse et al. 1982 and references therein), estimation of fish stocks contributing to mixed-stock harvests (Epifanio et al. 1995; Wood et al. 1987), and estimation of the contribution of distinct populations to restoration programs (Marsden et al. 1989).

Where recipient populations are a simple mixture from well-defined and character-specific sources, analysis is a relatively straightforward task with the mixture's