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Genetics

Effect of polyploidy on phosphoglucose isomerase diversity in *Festuca microstachys*

(hybrid enzymes/fixed heterozygosity/polyploid evolution/electrophoresis)

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ABSTRACT Studies of the inheritance of electrophoretic banding patterns in *Festuca microstachys* support the hypothesis that three closely related loci, one located in each of the three ancestral genomes, code the multiple phosphoglucose isomerase (glucosephosphate isomerase; D-glucose-6-phosphate ketol-isomerase, EC 5.3.1.9) variants found in this hexaploid species. The close relationship among the three loci is indicated by the observation that hybrid enzymes of intercistronic origin form when the loci in different genomes carry alleles coding homodimers with unlike migration rates. Homozygous individuals fixed for different alleles in different genomes produce hybrid enzymes and, when self-fertilized, they breed true for isozyme patterns normally found only in the heterozygotes of diploid species. Biochemical diversity due to this "fixed heterozygosity" is high in *F. microstachys;* although this species is more than 99% self-fertilized, the proportion of individuals with at least one heterodimer exceeded 61% in all of the 16 natural populations studied and it exceeded 92% in 11 of the populations. This great biochemical diversity may contribute to the ability of *F. microstachys* to survive in the wide range of habitats in which it is found over western North America.

It is evident from the fact that more than 30% of flowering plant species are polyploids that this mode of evolution has been a highly successful one in higher plants (1, 2). Following a suggestion by Haldane (3), Barber (4) proposed that the primary reason for the success of polyploid species is that they are more versatile biochemically than diploids. Polyploids presumably possess additional polypeptide variants coded by related genes located in the different parental genomes and there is also the possibility that hybrid forms of multimeric enzymes, which in diploids are found only in heterozygotes, may occur in fixed form and may contribute to selective advantage in polyploids (4–6). However, Zouras (7) has argued that biochemical diversity resulting from association of enzyme subunits coded by different alleles does not lead to superiority in fitness.

In plants, only the wheats (4, 8–11) and *Stephanomeria elata* Nuttall (12) appear to have been studied in detail with respect to the effect of polyploidy on enzyme diversity. The purpose of this paper is to describe the inheritance of electrophoretically detectable variants of phosphoglucose isomerase (PGI; glucosephosphate isomerase; D-glucose-6-phosphate ketol-isomerase, EC 5.3.1.9) in the highly self-pollinating hexaploid [$2N = 42$ (13)] annual grass species *Festuca microstachys* Nuttall. The evidence presented indicates: (*i*) that this species possesses three similar, or perhaps identical, gene loci coding multiple PGI variants, including hybrid enzymes of intercistronic origin; (*ii*) that these loci are the result of triplication of the genetic material through hexaploidy; and (*iii*) that the large number of PGI phenotypes and the extensive polymorphism for PGI found in this highly self-fertilizing species are associated with its polyploid origin.

MATERIALS AND METHODS

F. microstachys is a western North American endemic found from the Pacific Coast to the Rocky Mountains and from Baja California, north to British Columbia. It occurs in a wide variety of habitats but most often is found on dry, rocky, exposed sites from sea level to 6000 ft. (1829.5 m). The materials of the present study were seedlings obtained by germinating seeds harvested from randomly chosen individual plants from 16 natural populations distributed from central California to southern British Columbia. Seeds were harvested from 60 to 106 individuals (average 93) per population and three to six progeny derived from each maternal individual were assayed electrophoretically. In total, about 8000 individuals derived from about 1500 adults were assayed. Details of the horizontal starch gel electrophoretic procedures followed are given elsewhere†. The great majority of these families (>99%) bred true for PGI phenotype. This high level of homozygosity is expected considering that fewer than 1 in 1000 fertilizations results from outcrosses in this species (14). However, some progenies showed variation for PGI phenotype and, to produce the equivalent of F_2 families, we self-pollinated heterozygotes from these progenies. We have not succeeded in making artificial hybrids in this species; consequently, families obtained by selfing heterozygous individuals are the only available materials for studies of inheritance. The segregation data reported in this paper are based on 13 such "F_2 equivalent" families containing about 1100 individuals in total.

RESULTS

The samples taken from the 16 populations studied revealed substantial variability in PGI phenotypes, both within and among populations. Among the banding patterns observed 21 were presumed to represent homozygous genotypes because individuals with these banding patterns produce uniform progeny on selfing. These 21 banding patterns are illustrated in Fig. 1. The number of bands in any one individual ranged from one to six; however, multibanded types were the rule and the frequency of five- or six-banded types was high in all of the 16 populations. In two sites in California, the diploid annual fescue *F. bromoides* Linnaeus [$2N = 14$ (15, 16)] was also sampled. In one of these sites, 93% of the individuals of *F. microstachys* were three-banded and in the other site, 97% were six-banded. However, only two phenotypes, each with an intensely staining single band, were found in the progenies of eight plants of *F. bromoides* from each of these two locations. We propose that this striking difference in number of bands between the two species is directly related to the difference in ploidy level. We also propose that all phenotypes observed, both homozygous and heterozygous, can be explained on the basis of a genetic model incorporating the following five features:

Abbreviation: PGI, phosphoglucose isomerase.

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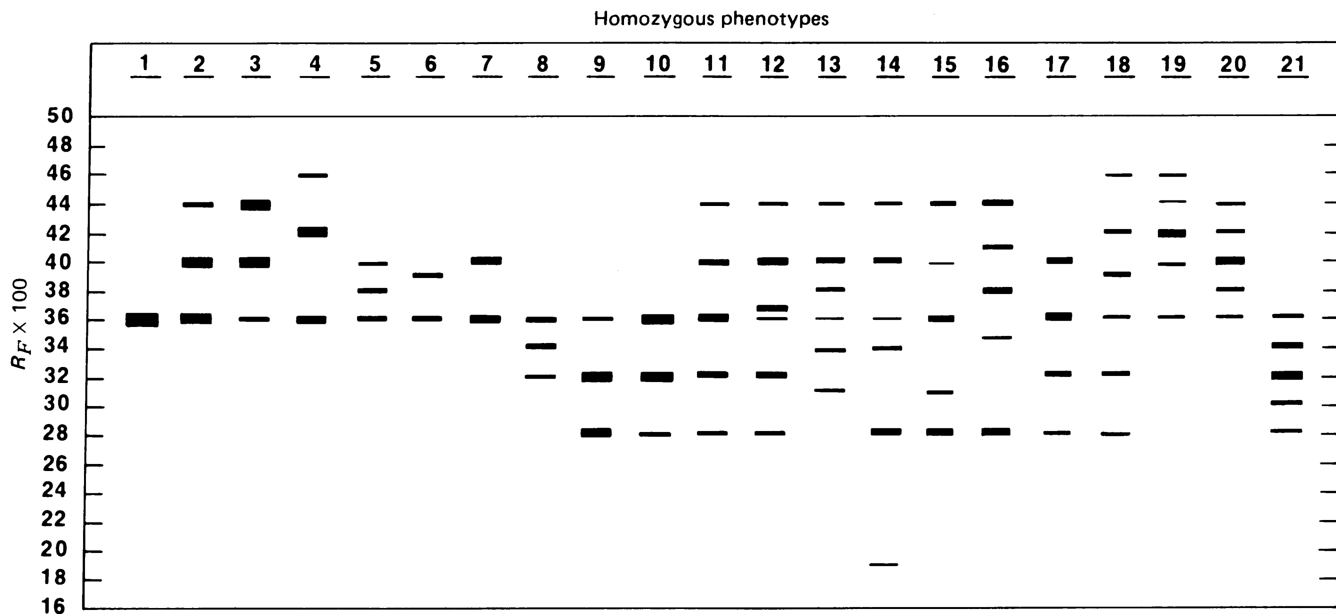

FIG. 1. Banding patterns for 21 homozygous PGI phenotypes.

(*i*) PGI is a dimeric enzyme; (*ii*) migration distances of enzyme bands (isozymes) coded by genes at the three different loci may be identical; (*iii*) migration distances of hybrid enzymes are usually but not always exactly intermediate between homodimers of the constituent polypeptide subunits; (*iv*) band intensities can be predicted if it is assumed (*a*) that all alleles at the three loci involved are equally effective in polypeptide synthesis, (*b*) that dimerization of polypeptide subunits is at random, and (*c*) that dimers are equal in stability and activity; and (*v*) one or more of the assumptions of *iv* above is sometimes not met, leading to occasional failures in prediction of band intensities. None of these features is without precedent and each will be invoked in the discussion to follow. We further propose that *F. microstachys* is a diploidized hexaploid. This proposal, which is based on earlier unpublished cytological observations by one of us (R.W.A.) that this species regularly forms 21 pairs of chromosomes during meiosis, is supported by the diploid type inheritance observed in the present study.

Segregation Patterns. The simplest banding pattern of Fig. 1 is "phenotype 1," which has a single broad and intensely staining band. According to feature *ii* of our model, this phenotype results when all three loci are homozygous for alleles coding enzymes that migrate to position R_F 0.36 [R_F is the migrational distance relative to the distance (8.5 cm) covered by the borate front] on the gel. We will consider all such alleles to be identical and designate them P^{36} because the methods used in this study cannot distinguish among such identically migrating bands. The three-locus genotype that produces phenotype 1 is accordingly represented as $P^{36}P^{36}$, $P^{36}P^{36}$, $P^{36}P^{36}$. Progeny tests support this Mendelian formula because individuals of phenotype 1, on selfing, produced offspring uniformly like their parent.

Families obtained by selfing that segregated for PGI phenotype fell into the six groups illustrated in Figs. 2 and 3. Fig. 2A gives the progeny produced by selfing individuals with phenotype A2. χ^2 for "goodness of fit" of observed numbers of these three phenotypes (A1:A2:A3) to a 1:2:1 ratio is nonsignificant (Table 1). Also, individuals with phenotypes A1 and A3 breed true on selfing. Thus, phenotype A1, as discussed above, is fixed for allele P^{36} at all three loci, phenotype A2 is fixed for allele P^{36} at two loci and heterozygous for alleles P^{36} and P^{28} at the third locus, and phenotype A3 is homozygous for allele 28 at one locus and for allele P^{36} at two loci. We, thus, propose that genotypes for these three phenotypes are: A1—$P^{36}P^{36}$, $P^{36}P^{36}$, $P^{36}P^{36}$; A2—$P^{28}P^{36}$, $P^{36}P^{36}$, $P^{36}P^{36}$; A3—$P^{28}P^{28}$, $P^{36}P^{36}$, $P^{36}P^{36}$.

The basis for assigning the above genotypes is as follows. Dimeric enzymes often form bands of intermediate mobility in heterozygotes and it seems likely that the middle band that occurs in the A2 heterozygote at R_F 0.32 is a heterodimer formed by association of the 0.28 and 0.36 polypeptides, i.e., the polypeptides coded by alleles P^{28} and P^{36}. The bands at R_F 0.28 and R_F 0.36 are then homodimers of the 0.28 and 0.36 polypeptides. Henceforth, allelic designations (e.g., P^{28}) will be in terms of the R_F position of the homodimer (0.28) formed by the polypeptide coded by the allele designated. Also note that all dimers of the 0.36 polypeptides have been labeled as homodimeric regardless of the number of loci involved in their synthesis. The designation heterodimeric will be used only for associations of polypeptides whose homodimers are distin-

Table 1. Observed single-locus segregations for selfed heterozygotes

Family type*	No. of families	Genotype of segregating locus with observed numbers		
		$p^{28}p^{28}$	$p^{28}p^{36}$	$p^{36}p^{36}$
A	2	37	58	29
C	1	13	42	17
DH	2	90	161	97
		$p^{36}p^{36}$	$p^{36}p^{44}$	$p^{44}p^{44}$
B	2	25	63	20
DH	2	89	171	88
		$p^{32}p^{32}$	$p^{32}p^{44}$	$p^{44}p^{44}$
D	2	42	110	49
		$p^{41}Np^{41}N$	$p^{41}Np^{41}$	$p^{41}p^{41}$
E	4	78	142	68

* See *text* and Fig. 2 or Fig. 3. DH refers to doubly heterozygous families. All χ^2 values for deviation and for heterogeneity are nonsignificant.

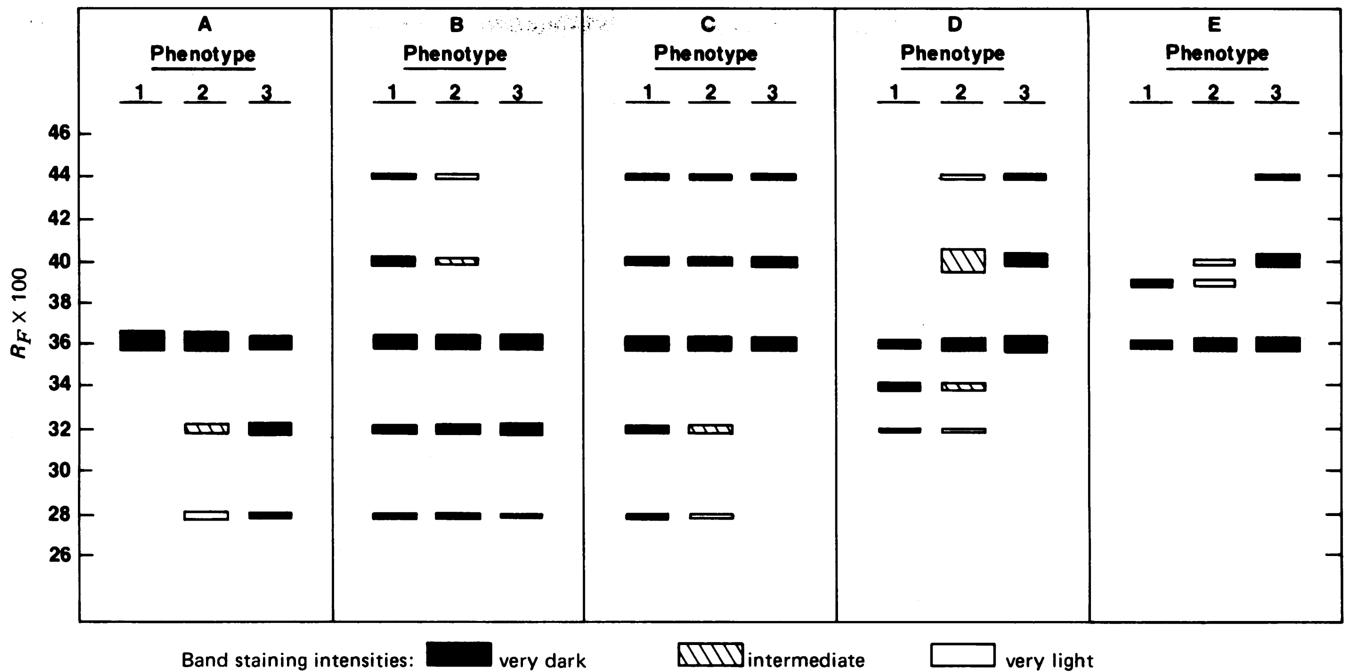

FIG. 2. Banding patterns of the progeny of five different kinds of selfed heterozygotes segregating at single PGI loci. Phenotype 2 in each family is the heterozygote (same as the parent). Proposed genotypes for each heterozygote are: A2—$P^{28}P^{36}$, $P^{36}P^{36}$, $P^{36}P^{36}$; B2—$P^{28}P^{28}$, $P^{36}P^{36}$, $P^{36}P^{44}$; C2—$P^{28}P^{36}$, $P^{36}P^{36}$, $P^{44}P^{44}$; D2—$P^{32}P^{44}$, $P^{36}P^{36}$, $P^{36}P^{36}$; E2—$P^{36}P^{36}$, $P^{36}P^{36}$, $P^{41N}P^{44}$.

guishable by different migration rates. Inability to distinguish alleles of the three loci clearly leads to the possibility of underestimating the total genetic variability present.

The presence of an intermediate band, presumably also a heterodimer, in the homozygous genotype A3 ($P^{28}P^{28}$, $P^{36}P^{36}$, $P^{36}P^{36}$) indicates that association also occurs between subunits coded by different loci. There is ample precedent for such intercistronic pairing resulting from both single-locus and whole-genome duplication (8–11, 17–19). The segregation pattern of family type A indicates that phenotype A3 results from interactions among loci that are homozygous for alleles P^{28} and P^{36}. Relative band intensities indicate that one locus carries alleles that code for homodimers of identical migration 0.36. One way to verify that the third locus is functional is to demonstrate the existence of alleles leading to homodimers with different migration rates at all three loci. The second type of segregating family, discussed next, appears to represent this situation.

The observed numbers of the three phenotypes of family type B (Fig. 2B) give a good fit to the 1:2:1 ratio expected on the basis of single-locus segregation (Table 1). Phenotypes B1 and B3 breed true when self-fertilized. The three bands of B3, which are identical to those of phenotype A3, occur in all individuals of family type B. It is therefore evident that B2 is homozygous at one locus for allele P^{28} and homozygous at another locus for allele P^{36}. However, segregation for the bands

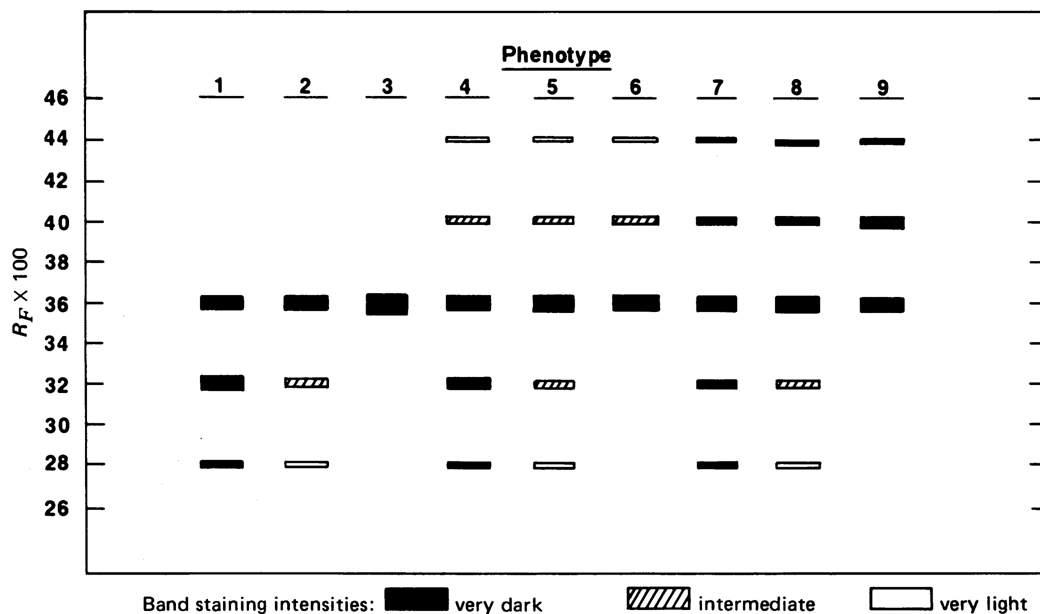

FIG. 3. Banding patterns for the progeny of the selfed double heterozygote $P^{28}P^{36}$, $P^{36}P^{36}$, $P^{36}P^{44}$ (phenotype 5).

at 0.40 and 0.44 establishes that B2 is heterozygous for alleles P^{36} and P^{44}. The genotype of B2 is thus $P^{28}P^{28}$, $P^{36}P^{36}$, $P^{36}P^{44}$ and that of B1 is $P^{28}P^{28}$, $P^{36}P^{36}$, $P^{44}P^{44}$. Thus, the B1 homozygote is fixed for a different allele at each of its three loci. Allele P^{44} codes for the homodimer at the 0.44 position and the heterodimer at position 0.40 is due to both intralocus (B2) and interlocus (B1 and B2) associations of the 0.44 and the 0.36 polypeptides.

The third type of segregating family, obtained by selfing phenotype C2, is illustrated in Fig. 2*C*. This pattern of segregation, which is the inverted equivalent of family type B, is expected if allele P^{44} is homozygous at one locus, P^{36} is homozygous at a second locus, and the third locus is heterozygous for P^{28} and P^{36}, i.e., that the genotype of C2 is $P^{28}P^{36}$, $P^{36}P^{36}$, $P^{44}P^{44}$. χ^2 for "goodness of fit" to the 1:2:1 ratio expected from hypothesis is nonsignificant (Table 1).

The fourth type of segregating family is obtained when phenotype 5 of Fig. 3 is selfed. Phenotype 5 has bands at the same positions as phenotypes B2 and C2, and its progeny array includes all of the nine types of progeny expected for a family segregating for the two pairs of alleles that are heterozygous at single loci in family types B and C. Observed frequencies of these nine phenotypes among 348 progeny gave a good fit to the frequencies expected with segregation of two independent diallelic loci. Thus, phenotype 5 is evidently the double heterozygote $P^{28}P^{36}$, $P^{36}P^{36}$, $P^{36}P^{44}$. Independence (nonlinkage) of the segregating loci is expected because their origin by polyploidy indicates they should be located on different chromosomes.

Fig. 2*D* illustrates the fifth type of segregating family, obtained by selfing phenotype D2. χ^2 for deviation from a 1:2:1 ratio is nonsignificant (Table 1), indicating single-locus segregation. Banding intensities suggest that two loci of phenotype D2 are fixed for allele P^{36} and that the third locus is heterozygous for alleles P^{32} and P^{44}. If the heterodimer between 0.32 and 0.44 were exactly intermediate, this heterodimer should occupy position 0.38 in the heterozygote D2. No band occurs at position 0.38; however, the band at 0.39–0.40 is twice as broad as expected if it were due solely to the heterodimer at 0.40 resulting from association between the 0.36 and 0.44 polypeptides. Thus, the heterodimer between 0.32 and 0.44 apparently occurs at 0.39, indicating that heterodimers do not necessarily occur exactly midway between homodimers (feature *iii*). Some additional deviations from exact intermediacy can be seen in Fig. 1. All of these exceptional cases, like the present one, feature migration of the heterodimers slightly faster than expected.

Staining Intensity. Further evidence concerning the three-locus model and the dimeric nature of PGI in *F. microstachys* comes from observations on the relative staining intensities of the different bands. Consider an individual homozygous at three loci for three different alleles (*a*, *b*, *c*) that code polypeptide subunits A, B, C, respectively. If the conditions of feature *iv* of our model are met, six dimeric enzymes will result (AA, AB, BB, AC, BC, CC), with heterodimers appearing approximately midway between and in twice the amount of homodimers of the constituent polypeptide subunits. Homozygous phenotypes 12, 13, 14, and 18 of Fig. 1 appear to fit this pattern. Five bands are expected to remain when the middle migrating homodimer and heterodimer coincide in migration (e.g., BB and AC where AA and CC are the slowest and fastest migrating homodimers, respectively). These bands are expected to have intensities similar to those seen in homozygous phenotypes B1 and C1 of Fig. 2, and in several of the phenotypes of Fig. 1. Finally, if two of the alleles, say *b* and *c*, code polypeptides whose homodimers migrate to the same band position, the six dimeric molecules are expected to form three distinct bands (BB, BC, CC), (AB, AC), and (AA) with relative intensities of 4:4:1. The observed banding pattern for A3, C3, and D1 (Fig. 2) and for all three-banded phenotypes in Fig. 1 appear to fit this ratio. An identical pattern, also involving three loci and random association of subunits to form active dimeric enzymes, has been described for alcohol dehydrogenase in hexaploid wheat (10).

In five of the 21 phenotypes in Fig. 1 (i.e., 6, 7, 15, 16, and 17) band intensities differ from predictions based on feature *iv* of our model. The heterodimers of phenotypes 15 and 16 stain *less* intensely than the homodimers. Less intense staining heterodimers, which have often been observed in previous studies (9, 12, 17, 18), might be due to incomplete affinity of polypeptide subunits, or to differential regulation or activity of functional enzymes. Phenotypes 6, 7, and 17 have missing bands; this might be due to complete lack of function of a dimeric enzyme, or to complete lack of affinity of subunits. An allele that codes a polypeptide that produces no band is commonly called a "null" allele (N). Fig. 2*E* gives an example of a family segregating for such an allele. This type of family, obtained by selfing phenotype E2, includes progeny types E1, E2, and E3 in the proportions expected with single-locus segregation (Table 1). The banding pattern and band intensities of E3 indicate that its genotype is $P^{36}P^{36}$, $P^{36}P^{36}$, $P^{44}P^{44}$, the same as phenotype C3. The 0.44 homodimer does not, however, Appear in the other homozygote, E1. Thus, the locus in E2 that carries allele P^{44} must be heterozygous, and, because only two of the three bands expected are expressed in the E1 homozygote, the allele that is alternative to allele P^{44} must be a null allele. The expected ratio of band intensities for three banded homozygotes is 4 (0.36 homodimer):4 (heterodimer):1 (null homodimer). The two bands in E1 are about equal in intensity, which suggests that these bands represent the 0.36 homodimer and the heterodimer (intensity ratio 4:4) and that the null homodimer (intensity ratio 1) is missing. If the heterodimer were missing, the intensity ratios of the remaining bands would be expected to be 4:1. Also, even if association of identical polypeptides were complete, so that no heterodimer formed, the null homodimer would be expected to occur at approximately position 0.41, assuming that heterodimers occur midway between the constituent homodimers. We, therefore, designate the postulated null allele as P^{41N}.

The E2 heterozygote, in addition to the missing 0.41N homodimer, lacks two additional bands that might conceivably be expected to occur, namely, the 0.44 homodimer and the heterodimer formed by association of the 0.41N and 0.44 polypeptides. One possible explanation is that the 0.41N polypeptide binds to 0.44 and ties up 0.44 in a nonfunctional hybrid molecule. Another possible explanation is that the low expected intensity of the band of the 0.44 homodimer ($1/16$ of the 0.36 band) causes it to fall below the visibility level in the gel.

DISCUSSION

Analyses of the banding patterns in homozygotes and segregating families support the hypothesis that three loci code PGI variants in *F. microstachys* and that inheritance follows patterns expected if this species is a diploidized hexaploid. The data also support the assumption that PGI is a dimeric enzyme and that the properties of the polypeptides coded by genes at the three loci are very similar. However, genetic segregations were not obtained for all observed phenotypes and hence not all possible variations of the three-locus model could be tested. One untested possibility is that biochemical transformations of nongenetic origin are involved; among the PGI enzymes in

maize one has been shown to undergo transformations in number and intensities of bands when it is subjected to various chemical treatments (20). Such transformations do not seem likely to be involved in band pattern differences observed in this study, for two reasons: (*a*) biochemical assay techniques remained constant throughout the study and (*b*) genetic tests showed that all the bands can be accounted for on the basis of simple Mendelian segregations. Other possibilities involve more extreme cases of differential activity or function, e.g., alleles coding completely inactive polypeptides or polypeptides that do not form heterodimers. However, there is no point in invoking these possibilities because they introduce complexity greater than required to explain the observed patterns.

Homozygous individuals that produce hybrid enzymes through multilocus association of polypeptides are called "fixed heterozygotes" and if selfed, they breed true for enzyme patterns normally only found in heterozygotes of diploid species. Manwell and Baker (6) have pointed out that, even though fixed heterozygosity has the advantage of greater individual biochemical versatility, it can lead to sacrifice in evolutionary flexibility if all individuals of a species are identically homozygous and possess the same fixed "heterozygous" genotype. This appears to be the situation in the tetraploid *Stephanomeria elata* (12). Populations of *F. microstachys*, however, are not rigid in this manner; 13 of the 16 populations sampled were highly polymorphic, containing two or more PGI genotypes at frequencies of 5% or greater.† In addition, the lowest proportion of individuals with one or more hybrid enzymes observed in any population was 61% and in 11 of the populations the proportion exceeded 92%. Thus, extensive "heterozygosity," much greater than that found in most diploid outbreeders, is carried in populations of *F. microstachys* without problems associated with genetic load.

This study is in agreement with others (4, 8–10, 12) in demonstrating the high levels of biochemical diversity that can be present in individuals of polyploid plant species. The results also suggest that neither gene regulation nor other mechanisms counteract the effects of increase in genetic material due to polyploidy, as has been suggested previously (11). On the contrary, this hexaploid grass appears to possess great biochemical versatility that may well be advantageous to survival in varying environments.

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