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Genetic Linkage between Runnering and Phosphoglucoisomerase Allozymes, and Systematic Distortion of Monogenic Segregation Ratios in Diploid Strawberry

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Abstract. **As part of a strawberry *(Fragaria* sp.) genome mapping project, we studied the linkage relationship between runnering and phosphoglucoisomerase PGI-2 allozymes in diploid strawberry. The respective *r* and *Pgi-2* loci were found linked with a recombination frequency of 18.1% ± 1.6%(a map distance of 18.9 ± 1.6 cM). This is the second reported linkage in strawberry. The linkage between runnering and phosphoglucoisomerase allozymes, if conserved at the octoploid level, might provide a means of marker-assisted selection for the nonrunnering and bushy branching growth habits in cultivated strawberry. Severe distortion of monogenic segregation ratios was observed for runnering and PGI-2, and also for an unlinked locus for shikimate dehydrogenase allozymes. Alleles from the perpetual flowering (alpine *F. vesca)* parents were favored in this distortion. This phenomenon should be considered in future genetic studies using crosses between alpine and nonalpine strawberries.**

The strawberry *(Fragaria* sp.) is an excellent subject for genetic and evolutionary studies for many reasons. It is a popular fruit of significant economic importance. The genus has widespread distribution and high genetic diversity in its natural habitats across Asia, Europe, and the Americas (Hancock and Luby, 1993; Luby et al., 1992). There are at least 20 different species and four ploidy levels (Staudt, 1962, 1989). It frequently and spontaneously hybridizes to produce natural hybrids, including interspecific hybrids across ploidy levels (Bringhurst, 1990). The hybrid forms are preserved by vegetative propagation. However, genetic research has been hampered by the genetic complexity stemming from polyploidy, particularly in octoploid cultivated strawberry *(F. ×ananassa* Duch., 2n = 8x = 56). Several authors (Arulsekar and Bringhurst, 1983; Brown and Wareing, 1965; Davis and Pollard, 1991; Williamson et al., 1995) point out that, since there is a similar spectrum of character variation (e.g., seasonal vs. perpetual flowering, runnering vs. nonrunnering) and intercrossability between polyploid and diploid species, the most common diploid species, *F. vesca* L. (2n = 2x = 14), can be used as a model system to study strawberry genes and their linkage relationships.

Only a few morphological traits of Mendelian inheritance have been studied in strawberry (Galletta and Maas, 1990). Of these, two are perpetual flowering (overbearing) and nonrunnering habits associated with the so-called alpine varieties, a group of *F. vesca* of European origin. Brown and Wareing (1965) found that each of these two traits was controlled by a single recessive gene (s/s and *r/r,* respectively). Their results indicated no genetic linkage between flowering and runnering, although the wild type (seasonal flowering, runnering) ceased runner production when flowering. In that study, bushy branching was correlated with nonrunnering in a cross involving the alpine variety 'Bush White', but the genetic basis for this correlation was not established. An *F. vesca* arboreal mutant (with long internodes) was discovered by Staudt (1959), who showed that it was a monogenic recessive mutation *(arb/arb).* In a crossing and backcrossing study involving this mutant *(arb/arb, R/R)* and an alpine variety *(Arb/Arb, r/r),* no runnerless progeny were recovered, suggesting an epistatic effect of the arboreal locus to the runnering locus (Guttridge, 1973).

The genetics of several isozyme systems have been studied in strawberry. These include phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), esterase (EST), leucine aminopeptidase

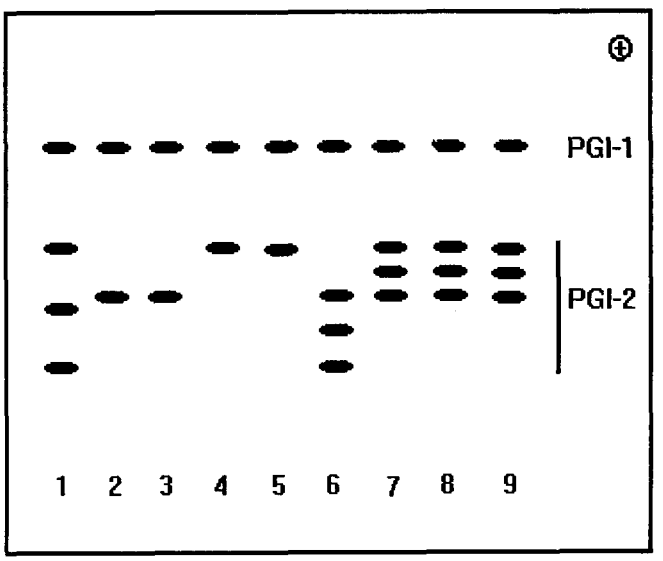

Fig. 1. PGI zymograms of diploid strawberry plants, Lane 1, FRA 364: *Pgi-2^c/Pgi-2^c;* Lanes 2–3, 'Yellow Wonder' and 'Baron Solemacher', respectively: *Pgi-2^a/Pgi-2^a;* Lanes 4-5, WC6 and WC40, respectively: *Pgi-2^c/Pgi-2^c;* Lane 6, F_1 plant GB of FRA 364 x 'Yellow Wonder': *Pgi-2^a/Pgi-2^c;* Lanes 7-9, F, plant S of FRA 364 x 'Yellow Wonder', F, of 'Baron Solemacher' x WC6, and F_1 of 'Baron Solemacher' x WC40, respectively: *Pgi-2^a/Pgi-2^c.*

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(LAP), phosphogluconate dehydrogenase (PGD), and shikimate dehydrogenase (SDH) (Arulsekar and Bringhurst, 1981; Arulsekar et al., 1981; Bringhurst et al., 1981; Nehra et al., 1991; Williamson et al., 1995). PGI is the most well-characterized. The most anodal isozyme on the gel, denoted as PGI-1, is invariant and probably chloroplast-associated. The nucleus-controlled PGI-2 isozyme is slower than PGI-1 on the gel. Three alleles were detected for PGI-2 in California diploid populations, existing in either homozygous or heterozygous genotypes (Arulsekar and Bringhurst, 1981). Symbols *Pgi-2b, Pgi-2c,* and *Pgi-2d* were assigned for the slow, medium, and fast alleles, respectively, according to the nobilities of the allozymes they produced on the gel. The alpine varieties carry a distinctive, slower, fourth allozyme, with the allele designation of *Pgi-2a* (Arulsekar and Bringhurst, 1981). A four-locus genetic model (one for each of the four homologous genomes) was proposed for the PGI-2 allozymes of octoploid cultivated strawberry (Arulsekar et al., 1981). These four loci can be fixed for one allele, producing only one band on the gel, or heterozygous at one or more of the four loci, generating multiple homodimer and heterodimer bands. Alleles *Pgi-2a, Pgi-2b, Pgi-2c,* and *Pgi-2e* (even slower than alpine *Pgi-2a)* were present in the California cultivated strawberry (Arulsekar et al., 1981).

The inheritance of SDH in *F. vesca* was reported recently (Williamson et al., 1995). It is a monomeric enzyme. A single polymorphic band was observed in a survey of several diploid *Fragaria* species (unpublished data). Hybridization experiments suggested that the bands in these species represented allozymes controlled at a single locus, which was designated as *Sdh.*

There had been no quantified linkages reported in any strawberry species until recently it was found that the *Sdh* gene was tightly linked (1. 1 cM) to a fruit color gene *(c)* in *F. vesca* (Williamson et al., 1995). Here, we document a second linkage in the diploid strawberry-in this case, between the runnering gene *(r)* and *Pgi-2.*

Materials and Methods

Fragaria vesca alpine varieties 'Baron Solemacher' and 'Yellow Wonder' (both nonrunnering) were obtained as seed from Johnny's Select Seeds, Albion, Maine, and W. Atlee Burpee and Co., Warminster, Pa., respectively. Diploid accession FRA 364 (runnering) was obtained as a runner clone from the National Clonal Germplasm Repository, Corvallis, Ore. It was listed as *F. vesca,* but we now question this identification, as discussed later. *Fragaria vesca* accessions WC6 and WC40 (both runnering) were collected by Tom Davis, Jim Pollard, and Scott Williamson in the wild from Diamond Peak Trail (Dartmouth College second grant) and Weeks State Park, respectively, in New Hampshire. .

The following crosses were made in the greenhouse at the Univ. of New Hampshire: 'Yellow Wonder' x FRA 364, 'Baron Solemacher' x WC6, and 'Baron Solemacher' x WC40. Four F_2 and two F_3 populations were derived for the linkage analyses of runnering and PGI-2. Because of the presence of heterozygosity in FRA 364, the F_1 population of 'Yellow Wonder' x FRA 364 was segregating for many markers, and the individual F_1 plants were labeled alphabetically. The F_2 families from two particular F_1

Table 1. Joint segregation of runnering and PGI-2 allozymes in diploid strawberry.

Population	Dihybrid phenotypes and frequencies[z]						N	χ^{2y}	*P*	*r* (%)[x]
	R+			R–						
	F(e + f)	H(g + h + i)	S(j + k)	F(1)	H(m)	S(n)				
F_2 GB	6	33	6	29	15	1	90	25.44	<0.01	19.2
F_3 GB-2	6	50	12	38	14	3	123	48.09	<0.01	16.9
F_3 GB-12	6	36	9	28	13	0	91	32.30	<0.01	16.6
	S(e + f)	H(g + h + i)	F(j + k)	S(l)	H(m)	F(n)				
F_2 S	22	87	25	72	28	3	237	71.32	<0.01	18.8
F_2 BS x WC6	6	37	12	33	8	1	97	45.77	<0.01	13.1
F_2 BS x WC40	3	23	4	19	15	2	66	13.55	<0.01	24.8
Pooled data:										
$r = 18.1 \pm 1.6\%$ (18.9 ± 1.6 cM); Homogeneity test: χ^2 (df = 5) = 6.77, $0.30 > P > 0.20$										

[z]R+ and R– denote runnering and nonrunnering, respectively. S, H, and F denote the slow, heterozygous and fast type of the allozymes, respectively, and are population-specific. Allard's (1956) dihybrid genotypic classifications in repulsion phase are given in parentheses. The expected segregation ratio is 3:6:3:l:2:l for (e + f) : (g + h + i) : (j + k) : l : m : n.
[y]Contingency chi-square with df = 2.
[x]Recombination frequency.

Table 2. Alpine allele *(r)* frequency for nonrunnering in diploid strawberry.

Population	Phenotypic frequencies[z]		N	χ^2 (3:1)	*P*	Alpine allele frequency
	R+	*R–*				
F_2 GB	45	45	90	30.00	<0.01	0.71
F_3 GB-2	68	55	123	25.49	<0.01	0.67
F_3 GB-12	51	40	91	17.44	<0.01	0.66
F_2 S	134	103	237	43.07	<0.01	0.66
F_2 BS x WC6	55	42	97	17.32	<0.01	0.66
F_2 BS x WC40	30	36	66	30.73	<0.01	0.74
Mean						0.68

[z]R+ and R– denote runnering and nonrunnering, respectively.

plants, GB and S, provided two segregating populations (referred to as F_2GB and F_2S, respectively). The F3 progenies of F_2 plants number GB-2 and GB-12 accounted for two more segregating populations (referred to as F_3GB-2 and F_3GB-12, respectively). The other two study populations were the F_2 generations of 'Baron Solemacher' x WC6 (F_2BS x WC6) and 'Baron Solemacher' x WC40 (F_2BS X WC40).

Plants were grown in clay pots on the benches of the glasshouse, and observed for about 1 year. They were watered regularly. No artificial light was provided. Pipe steam heating was provided in the winter; the minimum temperatures were at 15.5C at night and 21.0C during the day. Isozyme assays were done for PGI and SDH using young leaf lamina tissues ground in extraction buffer (Bringhurst et al., 1981). Electrophoresis was conducted on gels made of 79% acrylamide plus 2% starch as used for soybean (Bult et al., 1989). PGI and SDH activity staining was as described by Williamson et al. (1995). Contingency chi-square analysis was used to detect linkage. Recombination frequencies were calculated using the LINKAGE- 1 computer program (Suiter et al., 1983). A homogeneity chi-square test was performed according to Allard (1956). We also calculated the frequencies of alpine vs. nonalpine alleles in the six populations. Alpine nonrunnering allele frequency was the square root of the proportion of nonrunnering plants in the population. Alpine allele frequency for the isozymes was the proportion of one-half the number of heterozygotes plus alpine homozygotes in the population.

Results and Discussion

The PGI phenotypes of the crossing parents and F_1 plants used in the analyses are shown in Fig. 1. Three-banded PGI-2 phenotypes were observed in F_1 plants of all crosses (Fig. 1). One of the parents, FRA 364, also displayed a three-banded phenotype. PGI-2 was proposed to have a dimeric subunit structure in the strawberry (Arulsekar and Bringhurst, 1981). In heterozygotes, the polypeptide subunits encoded by alternate alleles associate at random to form dimers. As a result, besides the two possible homodimerbands, an intermediate, heterodimerband also appears on the gel. The three-banded phenotype in the FRA 364 parent and the transmission of different alleles to its F_1 plants GB and S (Fig. 1) indicated that FRA 364 was heterozygous for PGI-2.

Arulsekar and Bringhurst (198 1) assigned allele symbols *Pgi-2b*, *Pgi-2c*, and *Pgi-2d* for the three alleles found in California populations of *F. vesca*, and *Pgi-2a* for the slow allele unique to alpine varieties. By screening *Fragaria* germplasm, including representatives of California populations (collected in 1992 by Tom Davis), and comparing the band nobilities (unpublished data), we determined that the alleles in WC6 and WC40 and the fast allele in FRA 364 were *Pgi-2c*. The slow allele of FRA 364, which was slower than the alpine *Pgi-2a*, was also present in accessions FRA 341 and FRA 333 of *F. viridis* Duch., another diploid strawberry species. Arulsekar and Bringhurst (1981) earlier also noted the presence of alleles slower than *Pgi-2a* in *F. viridis*. The presence of this slow allele (denoted here as *Pgi-2s* for convenience) in FRA 364—found in *F. viridis* but never observed in *F. vesca*—indicated that FRA 364 might be an interspecific hybrid between these two species. Personal communication with R.S. Bringhurst, who originally donated FRA 364 to the germplasm repository, supported this proposition.

Linkage analysis was done for *r* and *Pgi-2*. The contingency chi-square test showed significant association of the two loci in all six populations (Table 1). The recombination frequency varied from 13. 1% to 24.8% and was 18.1%* 1.6% for the pooled data. The homogeneity chi-square among all populations was not sig-

Table 3. Alpine allele frequency for PGI-2 in diploid strawberry.

Population	Phenotypic frequencies[z]			N	χ^2 (1:2:1)	*P*	Alpine allele frequency[y]
	F	*H*	*S*				*F*
F_2 GB	35	48	7	90	17.82	<0.01	0.66
F_3 GB-2	44	64	15	123	13.87	<0.01	0.62
F_3 GB-12	31	49	9	91	11.57	<0.01	0.61
	S	*H*	*F*				*S*
F_2 S	94	115	28	237	36.96	<0.01	0.64
F_2 BS x WC6	39	45	13	97	14.44	<0.01	0.63
F_2 BS x WC40	22	38	6	66	9.27	<0.01	0.62
Mean							0.63

[z]S, H, and F denote the slow, heterozygous and fast type of the allozymes, respectively, and are population-specific.
[y]F and S denote fast and slow PGI-2 alleles, respectively.

Table 4. Alpine allele frequency for SDH in diploid strawberry.

Population	Phenotypic frequencies[z]			N	χ^2 (1:2:1)	*P*	Alpine allele frequency[y]
	F	*H*	*S*				*F*
F_2 GB	52	22	0	74	85.24	<0.01	0.85
F_3 GB-2	35	18	0	53	51.68	<0.01	0.83
	S	*H*	*F*				*S*
F_2 S	64	109	18	191	25.98	<0.01	0.62
F_2 BS x WC6	24	23	3	50	17.96	<0.01	0.71
F_2 BS x WC40	13	26	3	42	7.15	<0.05	0.62
Mean							0.70

[z]S, H, and F denote the slow, heterozygous and fast type of the allozymes, respectively, and are population-specific.
[y]F and S denote fast and slow SDH alleles, respectively.

nificant, supporting the 18.1% ± 1.6% recombination frequency and also indicating that the possible hybrid origin of parental FRA 364 was not a confounding factor in this study. The map distance calculated from the pooled data using the LINKAGE- 1 computer program was 18.9 ± 1.6 cM. This result established the second quantified linkage in the strawberry.

If conserved at the octoploid level, the linkage between runnering and PGI-2 may have practical implication in breeding programs. Brown and Wareing (1965) found that nonrunnering was associated with the bushy branching habit in the progeny of a cross involving alpine variety 'Bush White'; hence these two growth habit traits might be governed either by two closely linked genes or by one gene with pleiotropic effects. The bushy type has more fruit-bearing crowns; thus, the bushy branching habit could be a character related to strawberry yield. It would be of interest to know whether any correlation exits between PGI-2 genotypes and the bushy branching growth habit or runnering propensity in the cultivated strawberry *Fragaria ×ananassa.*

During the linkage analyses we found that monogenic segregation ratios were severely distorted for runnering and PGI-2. The segregation of phenotypes deviated significantly from the expected ratios in all populations based on a chi-square test (Tables 2 and 3). Further examination revealed that the distortion was systematic, with an excess of alpine-derived alleles compared with the expected proportion. The deviation was consistent across populations. The average alpine allele frequencies of the six populations were 0.67 and 0.63, respectively, for runnering and PGI-2, which was much higher than the expected 0.50.

Then, we examined another isozyme, SDH. Monogenic segregation for SDH also showed the same trend of deviation favoring the alpine allele (Table 4). The average frequency was 0.70 for the alpine allele. We found no linkages of SDH with either PGI-2 or runnering. Thus, the existence of the linkage between runnering and PGI-2 was not an artifact of the deviations from the expected segregation ratios at the monogenic level. We speculate that the distorted monogenic segregation ratios favoring alpine alleles may be a common phenomenon in the progeny of crosses involving both the perpetual (alpine) and seasonal flowering varieties. Such a distortion was not observed in previous alpine **x** alpine crosses used to detect the SDH–fruit color linkage (Williamson et al., 1995).

The observation of distorted monogenic segregation ratios is important and provides caution in future genetic studies of strawberry, particularly when using crosses involving alpine and nonalpine varieties as parents. For example, by looking at the segregation data for runnering (Table 2), which fit a 9:7 ratio, one might conclude that the runnering trait was controlled by two complementary, dominant genes. However, in the context of the previous inheritance study of nonrunnering (Brown and Wareing, 1965) and the distorted monogenic segregation pattern of the linked *Pgi-2* locus, a monogenic basis for runnering vs. nonrunnering is clearly indicated.

The distortion of monogenic segregation ratios was probably caused by the fundamental genetic differences between the two types of *F. vesca.* Using only alpine varieties as female parents in making crosses as we did might have provided cytoplasmic advantage for alpine alleles in our study. Further studies involving reciprocal crosses are needed to determine the cause of the observed distortion of monogenic segregation ratios.

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