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INHERITANCE AND DIVERSITY OF PGI IN CHESTNUT (*CASTANEA*)*

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Abstract Inheritance of PGI isozymes in chestnut species was analyzed using isoelectric focusing on thin-layer polyacrylamide slab gels and single-tree-progeny method. Three alleles at one *Pgi* locus (*Pgi*-1) were found to be codominantly inherited. Two additional alleles were detected in samples of natural populations. Considerable variations in allele frequency and heterozygosity were found in populations of the American (*Castanea dentata*) and Chinese (*C. mollissima*) chestnut species. Heterozygosity at the *Pgi* locus was generally higher in Chinese than in American chestnut. Significantly higher heterozygosity of the *Pgi* was detected in the southernmost location of the natural range of the American chestnut. The highest and lowest heterozygosity was observed in Chinese chestnut populations from the Changjiang River and southeast region of China, respectively.

Key words *Castanea*, PGI, Isozyme, Heterozygosity, Diversity

Considerable efforts are underway to develop a linkage map of chestnut genome integrating 5 morphological, 3 isozyme, 14 RFLP and 177 RAPD markers [1,2]. Because of the codominant nature and low sampling cost of allozymes, these markers have been used extensively in studies of the genetic diversity and gene structure in natural populations [3]. Polymorphic isozyme loci in American (*Castanea dentata* Borkh.) and Chinese chestnut (*C. mollissima* Bl.) species were evaluated using controlled crosses and the single-tree-progeny method of Gillet [4]. The single-tree-progeny method proved successful for isozyme genetic studies [5]. As part of our continuous efforts to evaluate the genetic diversity and population structures of chestnut species, the inheritance and variability of phosphoglucoisomerase (PGI) isozyme in American and Chinese chestnut was studied. PGI is known to be a dimeric protein and two loci are usually found in plants, one specific to the plastid and the other to cytoplasm [6,7]. Fineschi *et al.* [8] reported the presence of one PGI zone with 3 alleles in the European chestnut (*C. sativa* Mill.).

1 Materials and Methods

Single-tree-progeny families derived from five Chinese cultivars were used for genetic analysis (Table 1). Open-pollinated seeds were randomly harvested from each parent tree. Enzymes were ex-

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tracted from cotyledon tissue and were assayed for PGI using an isoelectric focusing polyacrylamide gel system with pH 4～9 [9] and the staining protocol of Wendel and Weeden [10] with modification of 1% agarose overlay. χ^2 tests were used to determine the goodness-of-fit of the segregation ratios to the expected relationships of progeny genotypes (Table 1).

Table 1 Genetic analysis at the PGI locus using single-tree-progeny in Chinese chestnut cultivars

Maternal tree		Progeny genotype							Expected progeny relationship*	χ^2
Name	Genotype	Total	*ac*	*cc*	*ce*	*ae*	*ee*	*cd*		
Cropper	*ce*	50		15	28		7		$N_{ce}=N_{cc}+N_{ee}$	0.50^{NS}
Homestead	*ce*	85	23	13	26	20	2	1	$N_{ae}=N_{ae}$	0.09^{NS}
									Expected progeny genotype	
A-2-2	*cc*	50		38	12				*cc*, *ce*, *ac*	
Black Beauty	*cc*	50		30	20				*cc*, *ce*, *ac*	
Leader	*cc*	50		36	14				*cc*, *ce*, *ac*	

* N = Total number of progeny genotype; NS. Nonsignificant.

American and Chinese chestnut populations were assayed for PGI polymorphism. Winter dormant or summer mature buds from 20～50 different trees, randomly collected in the natural range of the American chestnut, were used for enzyme extraction and assayed for PGI as described. The Northern population consisted of 11 trees collected in Connecticut and 14 trees in New York. The Central and Southern populations were collected in West Virginia and Alabama, respectively (Table 2). Three populations of the Chinese chestnut were obtained from the China National Chestnut Germplasm Plantation and Hubei Academy of Agricultural Science. These populations consisted of seedlings derived from open pollinated trees of each regional cultivar plantings. The US naturalized population of the Chinese chestnut consisted of the third generation seedlings derived from seeds introduced in 1933 by USDA from China to Alabama. Dormant buds were used for enzyme extraction. Allele frequencies and observed heterozygosity (h_{ob}) and unbiased expected heterozygosity (h_{ex}) values [11] were estimated for each population.

Table 2 Allelic frequencies and heterozygosity at *Pgi* in populations of the American and Chinese chestnut

Population	Population size	*Pgi-a*	*Pgi-b*	*Pgi-c*	*Pgi-d*	*Pgi-e*	h_{ob}	h_{ex}
Chinese chestnut								
Northern population	25	0.060	0.000	0.640	0.000	0.300	0.560	0.507
Changjiang river population	24	0.083	0.042	0.625	0.042	0.229	0.625	0.558
Southeast population	20	0.050	0.000	0.800	0.000	0.150	0.400	0.344
US naturalized population	20	0.075	0.000	0.700	0.000	0.225	0.600	0.465
American chestnut								
Northern population	25	0.000	0.000	0.900	0.000	0.100	0.200	0.184
Central population	22	0.000	0.018	0.911	0.018	0.054	0.179	0.170
Southern population	20	0.000	0.000	0.550	0.000	0.450	0.500	0.508

2 Results and Discussion

Results indicated that chestnut tissues showed one polymorphic PGI zone (Fig. 1). Single- or triple-

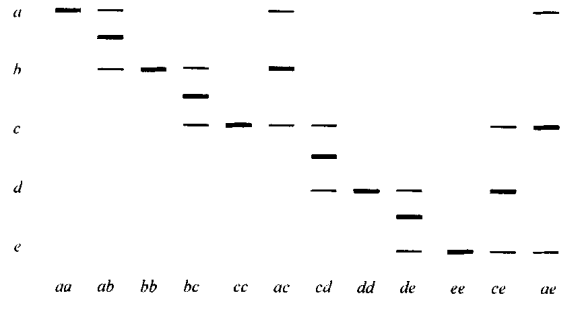

Fig. 1　Interpretative drawing of zymogram and allelic definition of PGI isozymes extracted from chestnut seeds

banded patterns were found in both species confirming the dimeric nature of this isozyme, agreeing with the results of Fineschi [8] in European chestnut (*C. sativa*). Of five Chinese cultivars used as parents for single-tree-progeny analysis, Cropper and Homestead are triple-banded heterozygotes. χ^2 test was used to determine the goodness-of-fit of segregation ratio to expected relationships of progeny genotypes (quantitative test for single-tree-progeny). The other Chinese chestnut cultivars, A-2-2, Black Beauty and Leader, are single-banded homozygote which can be used for qualitative single-tree-progeny test only. Results shown in Table 1 are consistent with the hypothesis that one codominant locus with three alleles controlling the variants of PGI phenotypes. The most anodal allozyme was designated as allele *a* and the other alleles as *c* and *e* (Fig. 1). The *ae* genotype exhibited a five-banded phenotype pattern. This could reflect the presence of a second locus (*Pgi-2*) overlapping with the *Pgi-1c*. The bands between *a* & *c* and *c* & *e* could reflect the following intergenic dimers *Pgi-1a* : *Pgi-2a* and *Pgi-1 e* : *Pgi-2a*. Heterodimeric five banded PGI patterns have been extensively studied in Clarkia species [12] and were examined using stem and pollen tissues. Additional studies are needed to clarify the heterodimeric banding patterns observed in the Chinese chestnuts. Two other alleles were found in population samples of the American and Chinese chestnuts and tentatively assigned as *b* and *d*. Fineschi *et al.* [8] reported that only one PGI zone could be revealed and three alleles were found in European chestnut. Considerable variation in allele frequencies and heterozygosity levels were detected in both the American and Chinese chestnut populations (Table 2). Allele *a* was not detected in any populations of the American chestnut, while allele frequencies of 5% ～8.3% were found in Chinese chestnut populations. Allele *c* was the most common allele in both species. Heterozygosity of *Pgi-1* was generally higher in the Chinese as compared to the American chestnut (Table 2). This is concordant with previous isozyme studies that showed Chinese chestnut, particularly populations in Changjiang River region, is the most genetically diverse species in genus *Castanea* [13]. A surprisingly high heterozygosity was noted in the population collected in Macon county, AL, the most southern location in the natural range of the American chestnut. The reason for this is unclear and needs additional study. Difference of *Pgi-1* heterozygosity levels was also detected among populations of the Chinese chestnut. A remarkably lower heterozygosity was found for the southeastern China population. This might have been the result of clonal propagation of only a few commercial cultivars in that area of China [14]. Chinese chestnut trees have been introduced into the United States since last century as a source of resistance to chestnut blight (*Cryphonectria parasitica*) [15]. Most introduction showed a high level of resistance to the fungus and are grown across the US. Although the breadth of the genetic base of the introduction continues to be debated [15], our results indicate that the naturalized populations of Chinese chestnut have a similar level of heterozygosity at the *Pgi-1* locus as other Chinese chestnut populations (Table 2). More markers are needed to accurately evaluate the overall genetic diversity of the American and Chinese species.

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栗的 PGI 遗传和多样性

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摘 要

采用超薄聚丙烯酰胺平板凝胶等电聚焦电泳和单株后代法,分析了栗属种的 PGI 同工酶的遗传。研究发现 *Pgi* 位点(*Pgi-1*)主要有 3 个等位基因并呈共显性遗传。在栗属的自然居群中还检测到了出现频率较少的另外 2 个等位基因。美洲栗(*Castanea dentata*)和中国板栗(*C. mollissima*)居群的 *Pgi* 基因频率和遗传杂合度存在较大的差异。中国板栗的 *Pgi* 位点的遗传杂合度通常高于美洲栗。在美洲栗自然分布区中,南端地区的居群存在显著高的 *Pgi* 遗传杂合度。在中国板栗居群中,长江流域居群 *Pgi* 杂合度最高,东南部居群最低。

关键词 栗属, 磷酸葡萄糖异构酶, 同工酶, 遗传杂合度, 多样性