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Genetic Studies in Peach: Inheritance of Sweet Kernel and Male Sterility

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Abstract. **Inheritance of the sweet kernel trait was studied in F_1 and F_2 families generated by crossing 'Summer Beaut' nectarine (sweet kernel) with 'Ellerbe' and 'Biscoe' peach. F_1 plants showed bitter kernel. Segregation in the F_2 fit a 3 bitter : 1 sweet phenotypic ratio, suggesting that sweet kernel is controlled by a single recessive gene, for which the symbol *sk* is proposed. Sweet kernel (*sk*) was linked to nectarine (*g*) at a map distance of 12 cM. Seed bitterness phenotype is controlled by the genotype of the maternal tree and not the genotype of the individual embryo. Inheritance of male sterility derived from plant introduction (PI) 240928 and allelism of male sterile genes found in 'Chinese Cling' and 'White Glory' were investigated. Analysis of F_1, F_1 open-pollinated, and BC_1 families derived from crossing PI 240928 with six different wild-type cultivars showed that male sterility in PI 240928 is controlled by cytoplasmic factors. Allelism studies showed that the male-sterile gene found in 'White Glory' is not allelic to *ps* found in 'Chinese Cling', and hence is designated *ps2*.**

Seeds of many species of *Prunus*, including peach [*Prunus persica* var. *persica* (L.) Batsch], almond (*P. dulcis* D.A. Webb), and apricot (*P. armeniaca* L.), are bitter to taste because of the accumulation of cyanogenic glucosides, primarily amygdalin (Mizutani et al., 1979). Wild-type peaches exhibit bitter seeds, although some accessions of peach and the closely related botanical variety nectarine [*Prunus persica* var. *nucipersica* (Suckow) C.K. Schneid.] (syn. var. *nectarina* (Ait. f.) Maxim.) have nonbitter seed, often referred to as sweet kernel. Seed bitterness in almond is controlled by a single recessive gene, designated *s* (Heppner, 1923, 1926). Kostina (1969) reported that seed bitterness in apricot is controlled by a single recessive gene, but recent studies by Bassi et al. (unpublished data) suggest a more complex mode of inheritance.

Male sterility in peach was reported to be controlled by a single recessive gene, designated *ps*, originating from 'Chinese Cling' (Blake and Conners, 1936; Conners, 1926). Chaparro et al. (1994) presented preliminary evidence suggesting that the nectarine 'White Glory' possesses a pollen sterility gene (designated *ps2*) that is nonallelic to *ps*. Recent hybridizations made by D.J. Werner using male-sterile peach plant introduction (PI) 240928 revealed segregation behavior inconsistent with the action of a single nuclear gene. We report on the frequency of the sweet kernel seed character in the peach PI collection and describe the inheritance of sweet kernel in peach and its linkage to the nectarine (*g*) gene. We also present data describing the inheritance of male sterility derived from PI 240928 and present further evidence confirming that 'White Glory' possesses a pollen-sterility gene that is nonallelic to *ps* derived from 'Chinese Cling'.

Materials and Methods

Sweet kernel. Forty-six peach and nectarine PIs were tested for seed bitterness by harvesting mature fruit, tasting the seed, and characterizing each as bitter or sweet. No difficulty was experienced in classifying any PI into these two categories. Inheritance of sweet kernel and its linkage to nectarine was investigated by generating F_1 and F_2 populations between 'Summer Beaut' x 'Biscoe' and 'Ellerbe' x 'Summer Beaut'. 'Summer Beaut' is a sweet kernel nectarine, and 'Biscoe' and 'Ellerbe' are bitter kernel peaches; hence, both F_2 populations were segregating for sweet kernel and nectarine. Each F_2 family was created from one F_1 individual. Fruit from progeny were harvested at maturity, and seeds were removed and tested for bitterness. A least two fruit per tree were sampled and scored. Segregation ratios were tested for goodness of fit to test ratios using chi-square tests. Data from the two different families were tested for departure from homogeneity before combining the data for linkage analysis. Linkage was tested using the product-ratio method (Fisher and Balmukand, 1928). To determine if the sweet kernel phenotype is controlled by the genotype of the individual seed or the genotype of the maternal tree that the seed is developing on, 'Summer Beaut' nectarine was crossed with pollen of 'Contender', a bitter kernel variety. Seeds from these fruit were harvested at fruit maturity and characterized for bitterness.

Male sterility. Inheritance of male sterility derived from PI 240928 was tested in six F_1 families derived by crossing PI 240928 with male-fertile testers 'Encore', 'Sunglo', 'Contender', 'Cresthaven', 'Redhaven', and 'Rutgers Red Leaf 2n' and in three families derived from open pollination of F_1 trees of PI 240928 x 'Contender', PI 240928 x 'Redhaven', and PI 240928 x 'Rutgers Red Leaf 2n'. Additionally, the backcross family (PI 240928 x 'Rutgers Red Leaf 2n') x 'Rutgers Red Leaf 2n' was generated. The backcross of the F_1 to PI 240928 could not be accomplished because both parents were male sterile. All progeny were examined for pollen fertility in the third growing season after establishment of trees in the field. Pollen fertility was determined by visually examining anthers on all progeny at full bloom. Prior microscopic examination of anthers of PI 240928 at full bloom showed no evidence of pollen production. Furthermore, anthers of PI 240928 are small and shriveled compared to wild type. The male-sterile phenotype of PI 240928 could be unambiguously discriminated from wild type by visual observation.

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Table 1. Segregation ratios and goodness of fit for sweet kernel in peach x nectarine crosses.

Cross	Family	Observed		Ratio tested	χ^2	P
		Bitter	Sweet			
Summer Beaut x Biscoe	F_1	4	0			
Summer Beaut x Biscoe	F_2	83	30	3:1	0.08	0.75–0.90
Ellerbe x Summer Beaut	F_1	3	0			
Ellerbe x Summer Beaut	F_2	47	14	3:1	0.12	0.50–0.75

To test for allelism between the male sterility genes in 'Chinese Cling' and 'White Glory', an F_1 family of 49 individuals was generated by crossing these two cultivars. 'White Glory' is heterozygous for its male-sterility gene and hence male fertile, and 'Chinese Cling' is homozygous and male sterile. Fifty percent male-sterile progeny would be expected in the F_1 if the male-sterility genes found in these two cultivars are allelic.

Results and Discussion

SWEET KERNEL. Two PIs—PI 129678 ('Stanwick' nectarine) and PI 34685 ('Quetta' nectarine)—out of a total of 46 examined, showed the sweet kernel character. 'Quetta' was widely used as a parent in early efforts to develop improved nectarines for California and is found in the pedigree of most nectarine cultivars developed in California, including 'Summer Beaut' used in this study. The prevalence of sweet kernel in many of the nectarines developed in California breeding programs probably traces back to the early use of 'Quetta'.

All F_1 plants obtained in the 'Summer Beaut' x Biscoe' and 'Ellerbe' x 'Summer Beaut' crosses (four and three F_1 plants, respectively) had bitter kernel. A chi-square test for heterogeneity between the two F_2 populations for segregation of the nectarine and sweet kernel traits was nonsignificant ($P = 0.90$–0.95), so data from the two populations were combined before linkage analysis. Segregation in each F_2 population closely fit the expected test ratio of 3 bitter : 1 sweet (Table 1). The F_1 and F_2 data indicate that bitter kernel is dominant to sweet kernel, and that sweet kernel is conditioned by a single recessive gene for which we propose the designation *sk*. Because of the lack of backcross data, this conclusion should be judged as tentative. Cosegregation analysis for combined F_2 data of *sk* and *g* revealed a significant linkage chi-square value ($P < 0.01$), suggesting linkage. Linkage analysis using the product-ratio method for markers in coupling revealed that *sk* and *g* are linked with a recombination frequency of 12% (SE = 7%).

Thirteen heterozygous *Sksk* embryos developing on a homozygous *sksk* maternal parent resulting from the pollination of 'Summer Beaut' nectarine (sweet kernel) with pollen from 'Contender' (bitter kernel) were phenotypically sweet. This demonstrates that the phenotype of the kernel is determined by the genotype of the maternal parent and not the genotype of the embryo. This finding agrees with results obtained in almond (Kester and Asay, 1975). It is not known if cyanogenic glucosides in species of *Prunus* are produced *de novo* in the seed or instead synthesized in other plant parts and transported into the developing seed (Frehner et al, 1990). The *sk* allele may inhibit the synthesis of cyanogenic glucosides in the seed or the transport of cyanogenic glucosides into the developing seed. Alternatively, sweet kernel individuals may have normal amounts of seed cyanogenic glucosides, but the *sk* allele may code for a defective cyanoglycoside catabolic enzyme, resulting in a seed that is acyanogenic. Studies by Patton et al. (unpublished data) have shown that PI 34685 produces normal amounts of cyanogenic glucosides in leaf tissue, showing that *sk* is organ specific in its action.

MALE STERILITY. Crosses of PI 240928 with six male-fertile testers yielded only male-sterile F_1 offspring (Table 2). All progeny examined in open-pollinated families derived from three different F_1 individuals and the backcross family with 'Rutgers Red Leaf 2n' were male sterile. The above evidence strongly suggests that male sterility in PI 240928 is controlled by cytoplasmic factors. We propose designating the cytoplasmic male sterile derived from PI 240928 as *cms-1*. The male-sterile phenotype in PI 240928 and its offspring differ from the male-sterile phenotype conferred by the nuclear gene *ps*. Male sterility conferred by *ps* results in shrunken anthers that appear very light yellow or white. In contrast, male sterility conferred by *cms-1* from PI 240928 results in anthers that are shrunken and light pink in color. Interestingly, Scott and Weinberger (1944) described a similar phenotype and male sterility in seedlings from crosses with peach PI 101676. The limited data presented in their manuscript suggests that PI 101676 may also be a cytoplasmic male sterile, although recent examination of PI 101676 at bloom revealed the presence of small amounts of pollen of unknown viability (T.G. Beckman, personal communication). Cytoplasmic male sterility in peach PI 240928 is interesting because of the rarity of documented cytoplasmic male sterility in woody plants. The authors have been unable to find another report of cytoplasmic male sterility in any other woody plant species. At this time, we believe that cytoplasmic male sterility has little applied importance in peach. Toyama (1974), Sanford (1983), and Scorza et al. (1993) have proposed the development of doubled-haploid derived true-breeding peach cultivars and of seed-propagated F_1 hybrid cultivars. The existence of male sterility would facilitate hybrid seed production. Male sterility conferred by *ps* would be preferred in this scheme because it is recessive and subsequent F_1 trees would be male fertile. Cytoplasmic male sterility conferred by *cms-1* would not be useful without the presence of a restorer gene.

All 49 progeny generated from the cross of 'Chinese Cling' x 'White Glory' were male fertile. Because 'Chinese Cling' is

Table 2. Incidence of male sterility in crosses of peach PI 240928 with six male-fertile testers[z].

Cross	Family	Male sterile	Male fertile
PI 240928 x Encore	F_1	14	0
PI 240928 x Sunglo[y]	F_1	8	0
PI 240928 x Contender	F_1	28	0
PI 240928 x Cresthaven	F_1	12	0
PI 240928 x Redhaven	F_1	6	0
PI 240928 x RRL-2n[x]	F_1	34	0
PI 240928 x Contender	OP[w]	33	0
PI 240928 x Redhaven	OP	51	0
PI 240928 x RRL-2n	OP	640	0
(PI 240928 x RRL-2n) x RRL-2n	BC	139	0

[z]PI 240928 is male sterile and was used as the female parent in all crosses.
[y]Nectarine cultivar.
[x]RRL-2n = Rutgers Red Leaf 2n.
[w]OP = family generated from open pollination of F_1.

homozygous *psps* and 'White Glory' is heterozygous for a male-sterility gene and male fertile, 50% male-sterile progeny would have been expected if the recessive male-sterile gene heterozygous in 'White Glory' was allelic to *ps*. This evidence confirms that 'White Glory' possesses a nuclear male-sterile gene, *ps2*, that is nonallelic to *ps* derived from 'Chinese Cling'.

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