A Reassessment of the Linkage of Red Leaf Trait and Incompatibility Loci in Hazelnut (*Corylus avellana* L.)

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Abstract

As in other species, it is possible that a homozygous red leafed (= redleaf) hazelnut (*Corylus avellana* L.) tree would have brighter red coloration than a heterozygote and thus be more desirable as an ornamental. However, 'Fusco Rubra' and 'Rode Zeller' both carry incompatibility allele S6 (thus cross-incompatible) and are heterozygous for the redleaf trait. Furthermore, the incompatibility trait is linked to the redleaf trait. To obtain a homozygous redleaf tree, it is necessary find recombination between S6 and the redleaf trait. Controlled crosses of green leafed parents and parents heterozygous for redleaf trait and incompatibility allele S6 were made to reassess the linkage of incompatibility and leaf anthocyanin. Leaf color was noted and presence of S6 was determined by fluorescence microscopy. The progenies had very close to a 1:1 redleaf to green leaf ratio and an averaged frequency of recombination of 19.7% between the redleaf (A locus) and incompatibility (S locus) traits. These results contradict an earlier report.

Introduction

Hazelnuts are an important crop in Oregon (37,700 mT in 1993), which produces 98% of the US crop (Hazelnut Marketing Order Annual Report, 1994). This amount is dwarfed by the hazelnut industry in Turkey, which grows approximately 70% of the world's crop (Thompson, *et al.,* 1996). Most of the European crop is imported

by Germany, Switzerland and France. Demand for processed hazelnuts is high in Europe, with German citizens eating a yearly average of six pounds of hazelnuts (Mehlenbacher, personal communication). In contrast, the average American consumption is only one ounce per person, equal to about fourteen kernels (Hazelnut Marketing Order Annual Report, 1994).

There are several ornamental forms of hazelnut: redleaf, yellow leaf, contorted, weeping, and cutleaf. The redleaf hazelnut has red foliage, husks and catkins. The color of each part is most prominent at different times. Leaves are most vibrantly colored in spring and the red color fades as the leaves mature. The showy husks (involucres surrounding the nuts) are most notable during summer, as they contrast with the now-faded leaves. The catkins are most visible in winter, when they are not covered by leaves. These traits differ slightly among the three sources of red leaf color: 'Fusco Rubra', 'Rode Zeller' and 'Purple Aveline'.

'Fusco Rubra' (= Rotblättrige) was first described by Goeschke (1887). Its leaves are a light red-brown in the spring and its color fades early in the season. The leaves of 'Rode Zeller' are a dull, dark red and its catkins are especially attractive in winter (Thompson, 1985). 'Purple Aveline' (S5 S10) has an intense coloration, equal to 'Rode Zeller', and the leaves appear glossy. It is a chimeral mutant of 'White Aveline' and was not used in this study, as it does not transmit its color to its progeny (Thompson, *et al.*, 1996). As the leaf, husk and catkin color may vary in a progeny, it is possible that modifier genes exist that affect expression of the major gene.

Thompson (1985) proposed that a homozygous redleaf hazelnut might have darker red coloration and thus be more desirable as an ornamental. Besides this potentially intensified leaf color, a homozygous redleaf cultivar could be useful in several other areas: tracking orchard pollen movement and use as a rootstock parent. Pollen movement could be tracked by planting a homozygous redleaf tree in an orchard, collecting nuts from trees in the orchard, germinating the seeds, recording the frequency of redleaf seedlings and relating this to the distance from the redleaf tree in the orchard. Since any hazelnut successfully pollinated by a homozygous redleaf tree would be a redleaf seedling, the movement of the homozygote's pollen through the orchard could be mapped in this manner. For the same reason, a homozygote could also be used as a rootstock parent since all of the seedlings would have red leaves. Mix-ups in commercial nurseries could be prevented, as no redleaf cultivars are grown for their nuts. Unfortunately, when breeding to obtain a homozygous redleaf tree, Thompson (1985) discovered that the redleaf trait (A locus, for anthocyanin) was linked to S6, a highly dominant incompatibility allele. This linkage blocked all crosses of trees bearing the A allele.

Linkage of genes to incompatibility is not uncommon. In particular, the tight linkage of incompatibility and anthocyanin has been well described in *Brassica* (Sampson, 1967; Ockendon, 1977; Ockendon, 1980).

In a cross of 'Ennis' and 'Fusco Rubra', Thompson (1985) found no crossover between the S- and A- loci in a population of 56 seedlings. She concluded that the crossover value must be lower than 1.8%. Thompson did find recombination in several crosses of *C. avellana* and interspecific *Corylus* hybrids, although the populations were small and non-random, as they experienced heavy rouging as part of the breeding program. She reported values of 17% and 18%, although again, these figures were based on small, non-random populations with interspecific hybrids in the parentage. Because of this seeming contradiction, Thompson proposed a second locus: the C-locus (C for complementary color gene). The dominant C allele would also have to be present in order to have red leaf color, so a green leaf tree could carry a dominant allele from either the A or C locus if it was also homozygous recessive at the other locus. As the A and S loci are tightly linked, the crossovers observed would actually have been between the A- and C- loci, not between A- and S- loci.

Hazelnuts exhibit sporophytic incompatibility which is controlled by a single, multi-allelic locus, called the S-locus (Thompson, 1979a). This system prevents self-pollination and crosses with most siblings. If the pollen grains and the recipient pistil express a common S-allele, pollen germination is delayed or inhibited, pollen tube growth is arrested and fertilization does not occur. These genes are thought to be either expressed by the transfer of tapetal proteins to the pollen grain wall during microsporogenesis or to form one component of the poral proteins, which are released at the beginning of pollen germination (Heslop-Harrison, *et al.*, 1986). This

means that the genotype of the pollen parent (sporophyte) determines what genes are expressed. Incompatibility alleles may be expressed dominantly or codominantly in pollen (that is, have one or both tapetal proteins), but both alleles are always expressed in the female flower. This system is further complicated by the large number of incompatibility alleles and the complex dominance hierarchy, as shown in Figure 1 (Mehlenbacher, unpublished). Since S6 is dominant to all alleles except S3 and S8, almost all trees carrying S6 will express it in their pollen. Due to the linkage between S6 and the A allele, the only way to obtain a redleaf tree without S6 is to find a recombinant type.

The stigmas of hazelnuts extrude from buds located on shoots or catkin peduncles. Each flower has two styles and each bud contains from 4 to 16 flowers (Hampson, *et al.*, 1993). Female flowers are receptive when they emerge (red dot stage) and the stigmatic surface is most receptive fifteen days after the beginning of anthesis (Germain, 1994). The stigmas remain receptive for up to three months if pollination is prevented (Germain, 1994), although as flower age increases, pollen tubes become increasingly difficult to discern under fluorescence microscopy. Stigmas of female flowers from some redleaf trees are significantly darker than flowers from green leaf trees, which also makes discerning pollen tubes more difficult. In addition, red leaf selections tend to bloom later in the season than most green leaf trees. This late flowering can affect quality of flowers (due to age) available from other trees used in S allele bioassay.

Materials and Methods

Controlled crosses were made by the OSU hazelnut breeding program to determine if crossover had occurred (Table 1). In each of these crosses one of the parents was heterozygous for both S6 and the redleaf trait. The redleaf parents were 'Fusco Rubra' (S6 S19) and 'Redleaf #3' (S2 S6). 'Redleaf #3' is a open-pollinated seedling of 'Barcelona'; we believe that 'Rode Zeller' is the pollen parent as 'Redleaf #3' resembles 'Rode Zeller' much more than it resembles 'Fusco Rubra'. A total of 210 trees were tested.

In progenies segregating for red leaf color, the first seeds to germinate give green leaf seedlings, while those germinating later give mostly redleaf seedlings. As the largest and most vigorous seedlings are usually planted by a breeding program, this can skew the redleaf to green leaf ratio of a progeny segregating for leaf anthocyanin (Mehlenbacher, unpublished).

Leaf color was noted from field observation in early May, when leaf color differences were most striking. Presence or absence of S6 was determined through stylar squashes with aniline blue dye and fluorescent light, as described by Thompson (1979a, 1979b). In a stylar squash of a compatible cross, many long, parallel pollen tubes are visible. In an incompatible cross, few pollen grains germinate and the pollen tubes rarely penetrate the stigmatic surface. Tubes from

incompatible pollen are short and curving. Those that do not penetrate the stigmatic surface sometimes end in a bulb-like shape.

Catkins were collected when fully elongated, then set out on paper overnight for the anthers to dehisce. The pollen deposited on the paper was then stored in cotton-stoppered vials and stored in a freezer at -20°. Pollen stored in this manner can remain viable for up to two years. To obtain usable flowers for the S-allele bioassay, entire trees or single branches were emasculated and isolated before the extended female bloom season (typically from late December to early March). Trees were enclosed in cages (2.4m x 2.4m x 2.4m) covered with white polyethylene. Individual branches were isolated with bags made from Tyvek (DuPont) housewrap fabric (Smith and Mehlenbacher, 1994). Female flowers were collected from bagged branches and refrigerated in Petri dishes lined with moistened filter paper.

In the lab, female flowers were dipped into pollen vials and incubated at room temperature overnight. After incubation, the stigmas were removed, placed on a microscope slide, stained with aniline blue dye and squashed under a plastic coverslip. The stigmas were then observed with fluorescent light under a microscope. Aniline blue dye stain the callose of the pollen tubes, making them easily visible under fluorescent light.

Seedlings carrying S6 were identified with stylar squashes either by using them as female parents or as pollen parents in crosses with genotypes whose

alleles were known. In each case, at least four flowers were pollinated: two flowers to test for S allele (= S6 tester) and two flowers (= pollen check) to test flower quality and pollen fertility. Trees used for pollen checks had no alleles in common with the progenies tested and the S6 testers had no S alleles in common besides S6.

If pollen from a seedling is placed on an S6 tester and stylar squashes show the cross is not compatible, this indicates that the seedling carries S6. If the cross is compatible, the seedling does not express S6 in the pollen. If the pollen is also incompatible with the pollen check , this indicates poor pollen fertility or poor flower quality (i.e., old flowers). When using flowers from seedlings, pollen from a tree expressing S6 is placed on individual flowers, as is pollen from a pollen check tree. Again, if the cross of the seedling's flower and the S6 pollen is incompatible, the seedling carries S6. If it is compatible, it does not carry S6. Incompatible crosses with both the tester and pollen check indicate either poor pollen fertility or poor flower quality. Tables 2 and 3 show examples of how these tests are carried out.

Recombinant trees were green leafed trees with S6 and redleaf trees without S6. These forms are distinct from the parental types (redleafs with S6 and green leafed trees without S6). The percentage of recombination was calculated as the number of recombinants over the total number of trees times one hundred.

Results and Discussion

The results for each progeny and the pooled data passed a homogeneity $\chi 2$ test, indicating the estimated recombination value was acceptable. Two progenies had to be eliminated from statistical analysis because of invalid incompatibility tests. These progenies (R101 x 'Fusco Rubra' and 49.083 x 'Fusco Rubra') were tested before it was established that S3 and S8 were dominant to S6. The tests used pollen from seedlings assuming that S6 would be expressed if it were present, so compatibility on an S6 tester may have been due to dominance of S3 or S8 over S6. The five remaining progenies (a total of 178 trees) were not affected.

The average percent of recombination was 19.7%. See Tables 4 and 5 for details. Several progenies had an unequal number of red to green leaf, due to selection by the breeding program and germination rate differences, but this did not appear to affect statistical analysis adversely. It is unclear why the progeny of 'Fusco Rubra' x 'Casina' had such a marked difference in percent recombination. Although unlikely (P = .10-.05), this could be due to random error. However, the data still passes a homogeneity $\chi 2$ test, allowing pooling of the data.

The discovery of a substantial rate of recombination in *C. avellana* shows that the proposed explanation involving the C locus is more complex than is necessary to explain the results seen here; a single dominant gene coding for leaf anthocyanin is sufficient. Furthermore, the progenies tested had 108 redleafs and 102 green, a

close fit to a 1:1 ratio (P = .70-.50), consistent with a single dominant gene for leaf anthocyanin. The C locus was proposed to explain the lack of recombination between the S and A loci in *C. avellana* in Thompson's original study. It is possible that random error was responsible for the lack of recombination (P = .05-.01) in the cross of *C. avellana*. However, her report of 17% and 18% recombination in two interspecific hybrid crosses is close to the 19.7% found here.

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Figures and Tables

Figure 1: Dominance Hierarchy of S-alleles in Hazelnut Pollen

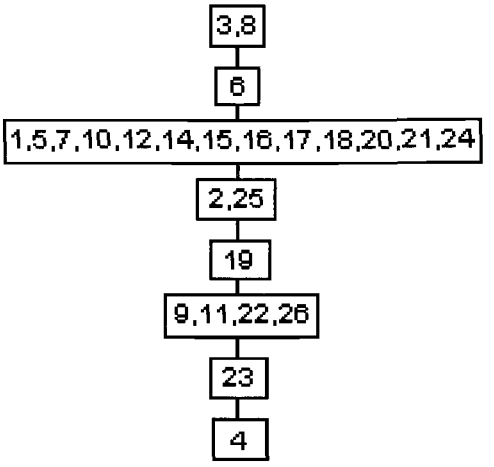

Table 1: Parentage and Incompatibility Alleles

Progeny	Progeny Size	Possible Allele Combinations				Alleles Expressed[y]
R101[x] x Fusco Rubra[u]	11	3,6	6,7	3,19	7,19	3, 6, 7
49.083[w,s] x Fusco Rubra[u]	21	1,6	6,8	1,19	8,19	1, 6, 8
23.017[v,s] x Fusco Rubra[u]	33	1,6	1,19	6,12	12,19	1, 6, 12
Fusco Rubra x Halls Giant[t]	26	5,6	5,19	6,15	15,19	5, 6, 15
Fusco Rubra x Romisondo G1[t]	27	6,18	6,20	18,19	19,20	6, 18, 20
Fusco Rubra x Casina[t]	29	6,10	6,21	10,19	19,21	6, 10, 21
Casina x Redleaf #3[t]	62	2,10	2,21	6,10	6,21	6, 10, 21

[z]Underlining denotes dominance in the pollen

[y]Alleles expressed in the pollen

[x]R101 is a seedling of 'Tonda Gentile delle Langhe' and 'Cosford'

[w]49.083 is a seedling of 'TombulGhiaghli' and a cross of 'Barcelona' x 'Daviana'

[v]23.017 is a seedling of 'Barcelona' and 'Extra Ghiaghli'

[u]Crosses made in 1987

[t]Crosses made in 1988

[s]Numbered selections from the Oregon State Univ. breeding program are listed by location of the original seedling tree (row number.tree number)

Table 2: Example of Bioassay Design (Seedling Pollen Parent)

	Progeny (pollen parent)[z]			
Female Flowers	10,19	6,10	19,21	6,21
179.061 2,6	++	--	++	--
Barcelona 1,2	++	++	++	++

Seedlings expressing S6 are incompatible on females of 179.061 (S2 S6) but compatible on 'Barcelona' (S1 S2)

++ Denotes a compatible cross

-- Denotes an incompatible cross

[z] From Table One

Table 3: Example of Bioassay Design (Seedling Female Flower Parent)

		Tester Pollen	
		179.061 2,6	Barcelona 1,2
Progeny Female Flowers	10,19	++	++
	6,10	--	++
	19,21	++	++
	6,21	--	++

Females of seedlings carrying S6 would be incompatible with 179.061 pollen but compatible with 'Barcelona' pollen.

++ Denotes a compatible cross

-- Denotes an incompatible cross

Table 4: Progeny and Pooled Recombination Results

Progeny	Red		Green		Percent	χ^2	
	w/ S6	w/out S6[z]	w/ S6[z]	w/out S6	Recombination	Value	*P*
R101 x Fusco Rubra	7	1	0	3	9.1%[y]	[y]	[y]
49.083 x Fusco Rubra	7	4	1	9	23.8%[y]	[y]	[y]
23.017 x Fusco Rubra	15	7	0	12	20.6	7.68[w]	.05-.01
Fusco Rubra x Halls Giant	11	3	2	10	19.2	0.28[w]	.90-.70
Fusco Rubra x Romisondo G1	5	4	4	14	29.6	5.02[w]	.10-.05
Fusco Rubra x Casina	10	1	1	17	6.9%	5.60[w]	.10-.05
Casina x Redleaf #3	28	5	8	21	21.0%	1.69[w]	.50-.30
Total[x]	69	20	15	74	19.7%	12.59[v]	.30-.20
Pooled						0.89[w]	.70-.50
Homogeneity						11.70[u]	.20-.10

[z]Recombinant
[y]Not used in statistical analysis
[x]Does not include R101 x 'Fusco Rubra' or 49.083 x 'Fusco Rubra'
[w]df = 2
[v]df = 10
[u]df = 8

Table 5: Progeny and Pooled Genotype Results

Progeny	Red	Green	χ^2 Value	χ^2 *P*	S6	not S6	χ^2 Value	χ^2 *P*
R101 x Fusco Rubra	8	3	2.27[y]	.10-.05	[z]	[z]	[z]	[z]
49.083 x Fusco Rubra	11	10	0.05[y]	.95-.90	[z]	[z]	[z]	[z]
23.017 x Fusco Rubra	22	12	2.94[y]	.10-.05	15	19	0.47[y]	.50-.30
Fusco Rubra x Halls Giant	14	12	0.15[y]	.70	13	13	0.00[y]	>.95
Fusco Rubra x Romisondo G1	9	18	3.00[y]	.10-.05	9	18	3.00[y]	.10-.05
Fusco Rubra x Casina	11	18	1.69[y]	.20-.10	11	18	1.69[y]	.30-.20
Casina x Redleaf #3	36	26	0.26[y]	.70-.50	36	26	1.61[y]	.30-.20
Total	108	102	10.36[x]	.20-.10	84	94	6.77[v]	..30-.20
Pooled			0.17[y]	.70-.50			0.56[y]	..50-.30
Homogeneity			10.19[w]	.20-.10			6.21[u]	..20-.10

[z]Alllele tests invalid
[y]df = 1
[x]df = 7
[w]df = 6
[v]df = 5
[u]df = 4