Distribution and frequency of a gene for resistance to white pine blister rust in natural populations of sugar pine

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The gametic frequency of a dominant allele (*R*) for resistance to white pine blister rust, a disease caused by an introduced pathogen (*Cronartium ribicola*), in natural populations of sugar pine was estimated by the kind of leaf symptom expressed after artificial inoculation of wind-pollinated seedlings from susceptible seed–parent genotypes (*rr*). Gene frequency increased clinally from near 0 in the southern Cascade Range to 0.08 in the southern Sierra Nevada, but it was not correlated with any major climatic gradient. Because *R* expresses a typical hypersensitivity response to pathogenesis, it has probably always functioned in disease resistance. A candidate pathogen for this function is *C. occidentale*, cause of pinyon blister rust, and a close relative of *C. ribicola*. Increase in allele frequency was positively associated with the proximity of sugar pine to single-leaf pinyon pine populations. Although sugar pine is not presently a natural host of pinyon rust, *R* may be a relict gene that protected sugar pine from this endemic pathogen in recent geologic epochs, when both pines are known to have been more intimately associated.

Key words: *Cronartium ribicola*, genetic resistance, *Pinus lambertiana*, population genetics.

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L'auteur a évalué la fréquence gamétique d'un allèle dominant de résistance (*R*) à la rouille du pin blanc, une maladie causée par un pathogène introduit, le *Cronartium ribicola*. L'étude a été effectuée dans des populations naturelles de *Pinus lambertiana* en examinant les types de symptômes foliaires après inoculation de plantules provenant de parents possédant des génotypes de susceptibilité (*rr*) et pollinisés par le vent. La fréquence des gènes augmente de façon graduelle à partir de 0 dans la partie sud des Cascade Range pour atteindre 0,08 dans le sud des Sierra Nevada, mais ne montre de corrélation avec aucune variable climatique importante. Parce que *R* permet l'expression d'une réaction d'hypersensibilité typique envers le pathogène, il a probablement toujours fonctionné dans la résistance à la maladie. Un candidat possiblement responsable de ceci serait le *C. occidentale*, responsable de la rouille sur le pin pignon, une rouille étroitement apparentée au *C. ribicola*. L'augmentation dans la fréquence de l'allèle montre une corrélation positive avec la proximité de population des ces deux espèces de pins. Bien que le *P. lambertiana* ne soit pas un hôte naturel pour la rouille du pin pignon, *R* pourrait être un gène vestigial qui aurait protégé le *P. lambertiana* contre ce pathogène endémique au cours d'époques géologiques récentes, puisqu'on sait que les deux pins ont été intimement associés.

Mots clés : *Cronartium ribicola*, résistance génétique, *Pinus lambertiana*, génétique ces populations.

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Mutations for disease resistance in plants occur spontaneously, but unless they have a positive selective value they are usually not expected to be maintained in large, outcrossing populations at frequencies substantially higher than the mutation rate. In natural pathosystems, genes for resistance–susceptibility in hosts are thought to co-evolve with complementary genes for avirulence–virulence in pathogens, as a result of mutually antagonistic selection pressures (Flor 1956; Person 1966). These interacting genes are expected to reach equilibrium at intermediate frequencies, because the fitness value of any one depends on the frequency of its complement (Mode 1958). The discovery of a major gene in sugar pine (*Pinus lambertiana* Dougl.) for resistance to white pine blister rust (Kinloch *et al.* 1970) was thus surprising, since the pathogen (*Cronartium ribicola* Fisch.) was not introduced to western North America until 1910 (Mielke 1943).

White pine blister rust ranks as one of the catastrophic plant disease epidemics in history (Klinkowski 1970). All North American white pines in section *Strobus* (Critchfield and Little 1966) are highly susceptible and the biological and economic impacts on them have been severe in their native habitats and wherever they have been planted as exotics.

The primary infection courts are leaves (needles), which are entered through stomata by germinating basidiospores. The pathogen establishes in mesophyll tissue, where it causes a bright yellow spot to form by destroying chlorophyll and unmasking carotene. Mycelium then penetrates the central vascular cylinder and grows down the needle in the phloem, invading bark tissues at the base of the needle fascicle and spreading into the stem or branch. Here, mycelial growth accelerates, successively produces two spore forms, and eventually kills the tissues directly, or indirectly by predisposing infected tissues to secondary invasion by insects and other fungi.

Sugar pines with a major, dominant allele (*R*) for resistance respond to infection with a hypersensitive reaction, wherein cells immediately surrounding invading hyphae collapse and become necrotic, usually arresting further development of the pathogen with negligible damage to the host. Very young seedlings sometimes develop bark symptoms when inoculated, but always of an incompatible type (Kinloch and Littlefield 1977).

Field tests of full-sib families from a small part of the range of sugar pine showed that *R* was uncommon but not rare (Kinloch *et al.* 1970). If the allele were pleiotropic for another adaptive trait, or closely linked in disequilibrium with a selected locus, its persistence in the population might be rationalized, especially if its frequency was correlated with a major environmental gradient, such as temperature or moisture. These gradients are common in the natural distribution

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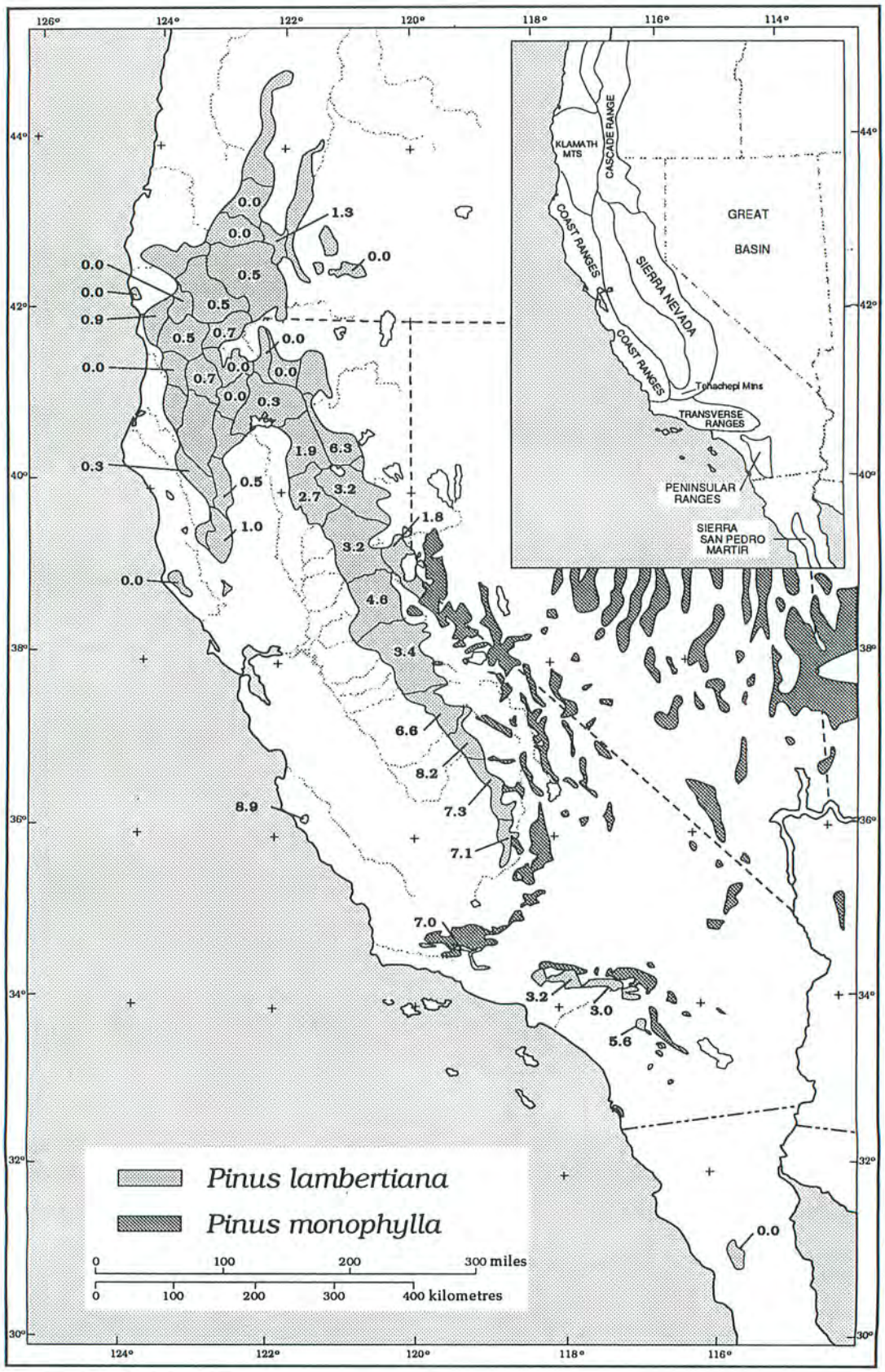

FIG. 1. Frequency (%) of an allele (*R*) for resistance to white pine blister rust in sugar pine averaged for all trees sampled over each seed zone. Seed zones were delineated after Buck *et al.* (1970). Tree distributions were redrawn from Critchfield and Little (1966), and Griffin and Critchfield (1972). Inset shows major physiographic regions discussed in the text.

of sugar pine, which ranges over 3000 m in elevation and 14° of latitude in the dissected topography of several major mountain ranges from Oregon to Baja California. The practical benefit to be realized by locating different sources of resistance for breeding improved sugar pine provided the opportunity to map the frequency of *R* in different areas and to evaluate the role of environmental factors in determining its distribution.

Materials and methods

Cone-bearing, rust-free trees were sampled extensively throughout most of the range of sugar pine and intensively in some areas. Most parent trees used in this study were individuals or small groups scattered over large areas called seed zones, units of land area designated for collection and internal transfer of tree seed by the U.S. Forest Service (Buck *et al.* 1970; see Fig. 1). Seed zones are the smallest unit in a hierarchy that includes physiographic regions and subregions. Major and often abrupt environmental changes occur between physiographic regions, such as the moisture gradient between the west and east slopes of the Sierra Nevada – Cascade mountain ranges, but gradients between subregions are apt to be more gradual. Although seed zones within subregions are usually demarcated by natural features such as rivers or ridges, their limits are somewhat arbitrarily set at roughly 80 km in latitude, and differences between adjacent zones may not be obvious or real. Nevertheless, the compact and relatively uniform size of seed zones made them expedient sampling units for assessing latitudinal trends in gene frequency. Numbers of parent trees sampled in each seed zone are given in Table 1. Seed zones were not sampled with equal intensity; those with denser populations of sugar pine were sampled more intensively, as were intermediate elevations within these zones. Large collections were made in some stands for different agencies involved in tree improvement programs. Gaps in sampling were conspicuous in the extreme northern part of the species' range and in parts of the Coast Ranges and Tehachapi Mountains, which extend southwest from the southern Sierra Nevada.

A major variable within all seed zones is the great range in elevation, with associated changes in temperature and precipitation. Accordingly, stands were sampled at low, middle, and high elevations along four elevational transects from north to south in the Sierra Nevada. These elevational bands were relative, reflecting the tendency of the lower and upper elevational limits of tree species to ascend with decreasing latitude. The limits for sugar pine ranged from 710 to 1600 m in the northernmost transect and from 1740 to 2320 m in the southernmost transect. In three of the transects, eight seed parents were sampled in each elevational band; in the fourth transect, sample sizes were increased to between 50 and 70 seed parents in each of the three stands. These stands, and an additional stand at Cone Peak in the south Coast Range with 93 seed parents (seed zone 120, Table 1), were used to determine whether or not genotype frequencies of seed parents were in Hardy–Weinberg equilibrium. Since blister rust was not present in any of these stands at time of sampling (unlike many of the other stands), parents were selected without bias with respect to phenotypic resistance.

Seed was extracted separately for each parent tree, soaked in aerated water at 20°C for 24 h, drained, then chilled at 2°C for 90 days before sowing two seeds in each small container (Ray Leach Supercells).[1] Some 28–98 seeds of each parent were sown and germination varied greatly among seed lots. Approximately 4 weeks after germination, seedlings were inoculated by spore casts from infected leaves of *Ribes nigrum* L., an alternate host of the pathogen, to determine their genotypes (Kinloch and Comstock 1980). Inoculum for *Ribes* plants was from bulked aeciospores collected from nearby sites in the Sierra Nevada. Sugar pines known to have *R* were included in the tests as controls to detect the presence of spores of a race of the pathogen genetically virulent to *R* (Kinloch and Comstock 1981).

TABLE 1. Distribution and frequency of a major gene (*R*) for resistance to white pine blister rust in sugar pine among different physiographic regions and seed zones

Seed zone[a]	Seed parents tested[b]	No. of seedlings evaluated			Gene frequency
		Total	Susceptible	Resistant	
Coast ranges (outer)					
081	2	15	15	0	0.0000
090	10	74	74	0	0.0000
091	36	1763	1748	15	0.0085
095	8	136	136	0	0.0000
120	93	2133	1944	189	0.0886
Coast ranges (inner)					
301	162	4752	4723	29	0.0061
302	5	137	137	0	0.0000
311	28	683	678	5	0.0073
321	141	5197	5159	38	0.0073
322	5	302	302	0	0.0000
331	3	44	44	0	0.0000
340	33	2030	2024	6	0.0030
371	4	190	189	1	0.0053
372	101	4065	4026	39	0.0096
Cascade – Sierra Nevada (west slope)					
491	24	430	430	0	0.0000
492	7	231	231	0	0.0000
501	8	237	234	3	0.0127
502	18	379	377	2	0.0053
511	12	204	203	1	0.0049
512	9	205	205	0	0.0000
516	8	265	265	0	0.0000
521	12	779	777	2	0.0026
522	264	15815	15512	303	0.0192
523	42	1869	1808	59	0.0316
524	85	3141	3055	86	0.0274
525	116	5099	4935	164	0.0322
526	222	7714	7359	355	0.0460
531	91	2976	2875	101	0.0339
532	16	941	879	62	0.0659
533	16	635	583	52	0.0819
534	124	2668	2474	194	0.0727
540	44	1077	1001	76	0.0706
Cascade – Sierra Nevada (east slope)					
703	8	157	157	0	0.0000
732	7	174	163	11	0.0632
741	9	538	538	0	0.0000
772	14	222	218	4	0.0180
Transverse and peninsula ranges					
992	8	644	599	45	0.0699
993	29	463	448	15	0.0324
994	31	337	327	10	0.0297
997	32	517	488	29	0.0561
—[c]	12	254	254	0	0.0000
Total	1901	69440	67542	1898	0.0220

[a]Buck *et al.* 1970.
[b]Excluding heterozygotes for major gene resistance.
[c]Sierra San Pedro Martir, Baja California, Mexico.

[1]Trade names and commercial enterprises or products are mentioned solely for information. No endorsement by the U.S. Department of Agriculture is implied.

TABLE 2. Frequency of a major gene (*R*) in sugar pine for resistance to white pine blister rust along four elevational transects in the Sierra Nevada

Seed zone[a]	Elevation: relative (absolute, m)		
	Low	Middle	High
524	0.006 (710)	0.010 (1151)	0.014 (1601)
526	0.020 (854)	0.066 (1707)	0.067 (2134)
531	0.024 (1439)	0.064 (1662)	0.046 (2119)
534	0.056 (1741)	0.073 (2002)	0.038 (2322)

[a]Buck *et al.* 1970.

Symptoms were evaluated a few weeks after inoculation. *R* is distinctively marked by the type of symptom expressed on leaves, including cotyledons. When challenged by *C. ribicola*, R^- genotypes develop small necrotic flecks, which contrast vividly with the bright yellow spots that typically form and enlarge on susceptible genotypes. These contrasting reactions are qualitative and not affected by inoculum density.

Because *R* is dominant, its frequency can be estimated directly in most wind-pollinated seedlots simply by counting the number of seedlings expressing the hypersensitive reaction. Since most seed parents are homozygous for susceptibility (*rr*), any resistant offspring among their progeny *must* derive their dominant allele from wind pollination by an unknown male parent and are heterozygotes (*Rr*). Infection data on seedlings from each susceptible (*rr*) seed parent thus represent genotype frequencies of a random sample of male gametes in the ambient pollen cloud. Heterozygous (*Rr*) seed parents, identified by families segregating in a 1:1 ratio of resistant to susceptible reactions, were ignored in the computation of gametic frequencies of *R* but were useful in computing zygotic frequencies of *R* in seed parents for determining Hardy–Weinberg equilibrium statistics.

Results

The frequency of *R* is low in the various subpopulations, with an overall mean of 2.2%, but ranging from undetectable to 8.9% (Table 1). Where large seed parent samples were taken in individual stands or small areas, *R* allele frequencies generally reflected overall averages of seed parents dispersed throughout the seed zone. For example, the frequency of *R* in a 164 parent tree sample (11 635 seedling offspring) from Latour Demonstration State Forest in the southern Cascades was 0.0193, nearly the same as the seed zone (522) average of 0.0187 based on 111 scattered parents (4180 seedlings). Similarly, Mountain Home Demonstration State Forest in the southern Sierra, with an *R* allele frequency of 0.076 (131 parents, 3126 seedlings), was very close to its seed zone (534) average of 0.073 (124 parents, 2668 seedlings). An exception was a stand of 46 parents with an *R* frequency of 0.0203, less than half of its seed zone (526) average of 0.0460.

Genotype frequencies were in equilibrium. In four intensively sampled stands, gametic *R* allele frequencies were estimated by averaging the frequencies of resistant seedlings from susceptible seed parent genotypes (*rr*). Expected genotype frequencies of seed parents were then calculated from gametic gene frequencies estimated from seedlings and compared with observed genotype frequencies of parents, with the result that no genotype frequencies deviated from expectation in any of the four stands (Table 3). In stands where blister rust was present, however, zygotic allele frequencies often exceeded gametic frequencies. The excess depended on the intensity of infection in the stand and thus the amount of phenotypic selection that could be exercised against infected trees (Kinloch and Dulitz 1991). In the entire study, 2 homozygous and 197 heterozygous resistant genotypes were detected of a total of 2100 seed parents tested, over double the expectation for each class at the average allele frequency of 0.022.

TABLE 3. Tests for Hardy–Weinberg equilibrium of sugar pine genotype frequencies in four stands

Genotype	Observed	Expected	χ^2
Crozier Loop: $R = 0.0254$			
RR	0	0.030	0.030
Rr	0	1.803	1.803
rr	46	46.693	0.122
Total			1.955
Pillikin Burn: $R = 0.0662$			
RR	0	0.285	0.285
Rr	5	8.034	1.146
rr	60	56.681	0.194
Total			1.625
Bunker Hill: $R = 0.0674$			
RR	0	0.263	0.263
Rr	10	7.288	1.009
rr	48	50.449	0.119
Total			1.392
Cone Peak: $R = 0.0886$			
RR	0	0.754	0.754
Rr	19	15.509	0.786
rr	77	79.737	0.094
Total			1.634

NOTE: Critical value of χ^2, 2 df, at $P = 0.05$ is 5.991.

There is no clear association of *R* allele frequency with elevation, even though stands at the lowest elevation have the lowest frequency in three of the four transects (Table 2). Although the simple correlation of *R* frequency with elevation is 0.700, the relationship is spurious, because in the Sierra Nevada the elevational limits of tree distribution increase with southerly latitude. When only *relative* elevation is considered, that is when the low, middle, and high elevational bands of the several transects are made equivalent to each other, the correlation of *R* frequency with elevation is only 0.252 and nonsignificant. There is a tendency for the highest frequency of *R* to shift from upper to middle elevation from north to south, but if real, its meaning is not clear.

The pattern of general increase in *R* allele frequency from north to south is also reflected in the more extensive data from the seed zones (Table 1). It is most evident on the west slopes of the Cascade – Sierra Nevada. The frequency of *R* is generally less than 0.01 in the northern part of sugar pine's range, increases from 0.02 to 0.04 in the northern and middle Sierra, and peaks at 0.06–0.08 in the southern Sierra. Frequencies are lower and more variable in the disjunct populations of the Transverse and Peninsular Ranges of southern California (0.03–0.06), and *R* was below levels of detection in the distant population in the Sierra San Pedro Martir in Baja California. The pattern in the Coast Ranges and east slope of the Cascade–Sierra is not as clear but seems to be similar. The population in the Coast Ranges (seed zone 120, Table 1), with a frequency of 0.09, is somewhat anomalous, as is the eastside Sierra population in seed zone 732 with a frequency of 0.06.

Discussion

The distribution of *R* in sugar pine subpopulations is not random, yet it does not conform to any obvious environmental gradients. The most likely gradients are temperature and moisture: from north to south in the range of sugar pine, and from higher elevations to lower, climate becomes warmer and drier. *R* does increase clinally from the northern to southern Sierra Nevada, the most conspicuous pattern in its distribution (Fig. 1), but then decreases abruptly in the even warmer and drier climates of southern California and Mexico. There is also no consistent increase in *R* on the eastern slopes of the Cascade – Sierra Nevada or in lower elevation stands of the western slope, both relatively dry environments. Although more subtle environmental gradients could be involved, it is difficult to invoke any specific contemporary agent of selection in the distribution of *R* when its frequency, while variable, is so generally low.

Nevertheless, the mean frequency of *R* (2.3×10^{-2}) is several orders of magnitude greater than that usually assumed for mutations ($10^{-6}-10^{-8}$) and its geographic distribution seems to follow a distinctly patterned cline. Such clines may represent the genetic legacy of historical events, or response to selection in past environments, when alleles that are now uncommon may have had higher selective values and frequencies.

An important component of fitness in plant populations is the ability to resist endemic pathogens. Because *R* is expressed as hypersensitivity, a typical defense reaction against pathogens in many plant taxa, its function has most likely always been in resisting disease. A candidate pathogen that could have selected for *R* in the past is *C. occidentale* Hedg., Bethel, & Hunt, cause of pinyon pine blister rust, which closely resembles *C. ribicola* in morphology and symptomatology and shares common alternate hosts in *Ribes* (Peterson 1967). The main pine hosts of *C. occidentale* are the single-leaf pinyon, *P. monophyla* Torr. & Frem., and *P. edulis* Engelm. These species, endemic to the arid mountain ranges and high plateaus of the American Southwest and Mexico, are in the same subgenus as sugar pine (*Strobus*; the white pines) but are in a distinctly different section (*Parrya*, subsect. *Cembroides*, the pinyon pines; Critchfield and Little 1966).

The distribution of single-leaf pinyon in the western Great Basin and eastern slope of the Sierra Nevada nearly parallels that of sugar pine on the western slope of the Sierra Nevada. This part of sugar pine's distribution is coincident with the steepest part of the cline in the frequency of *R* from north to south, which culminates near a few small areas of contact or close proximity (Fig. 1). Overlap is infrequent in the present distribution of these two pines, but evidence from rat midden fossils indicates that they grew in close association in cismontane California during the late Pleistocene (Cole 1983) and possibly also in earlier epochs.

Although *C. occidentale* is not known to be a natural parasite of sugar pine, no results of artificial inoculations have been reported. The hypothesis that *R* is a relict gene arising by selection in an earlier, more intimate association of this rust with both primary and alternate hosts would be strengthened if it could be shown that *C. occidentale* is able to establish a parasitic relationship with sugar pine and that *R* is able to protect sugar pine against this disease.

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