Allozyme variation of bishop pine associated with pygmy-forest soils in northern California

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Two races of bishop pine (*Pinus muricata* D. Don) meet in a narrow contact zone near sea level along the Sonoma County coast, northern California. The races previously were identified by foliar ("blue" in north, "green" in south), monoterpene, and allozyme differences. Disjunct stands of blue bishop pine were observed at higher elevations along a ridge south of the contact zone on patches of infertile pygmy-forest soils. Green bishop pine grew on fertile soils 0.5 km from the pygmy-forest patches. The frequency of trees with blue foliage in pygmy-forest stands was 0.73–0.86. One ridgetop stand south of the contact zone on fertile soils also had blue foliage. Allozyme differences at 7 of 46 loci distinguished blue and green races on different soils. Ridgetop blue and green stands were clearly related allozymically to lower elevation blue and green races in the same region. Selection for tolerance to soil conditions in the pygmy forest appears to maintain blue bishop pine in ridgetop pygmy forests.

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Deux races de *Pinus muricata* D. Don se rencontrent dans une zone de contact étroite près du niveau de la mer le long de la côte dans le comté de Sonoma, dans le nord de la Californie. Ces races ont déjà été identifiées par des différences foliaires (« bleu » au nord, « vert » au sud), de monoterpènes et d'allozyme. Des peuplements épars de *P. muricata* bleu ont été observés aux hautes altitudes le long d'un versant situé au sud de la zone de contact dans des pochettes de sols infertiles supportant une forêt de taille réduite. Des pins verts croissaient dans des sols fertiles dans des pochettes distantes de 0,5 km des précédentes. La fréquence des arbres pourvus d'un feuillage bleu dans les peuplements forestiers de taille réduite était 0,73 à 0,86. Un peuplement localisé sur un sommet à sol fertile au sud de la zone de contact avait aussi un feuillage bleu. Des différences d'allozyme à 7 parmi 46 loci ont permis de distinguer les races bleue et verte dans divers sols. Les peuplements bleus et verts localisés sur des sommets étaient facilement reliés d'une façon allozymique aux races bleue et verte de plus faible altitude dans la même région. La sélection vis-à-vis de la tolérance aux propriétés du sol dans la forêt de taille réduite semble maintenir le pin bleu dans les forêts de taille réduite localisées sur les sommets.

[Traduit par la revue]

Introduction

Bishop pine (*Pinus muricata* D. Don) is unusual among conifers because of its geographically abrupt pattern of genetic variation. A maritime and insular species, it ranges from Baja California to northern California in nine disjunct populations (Critchfield and Little 1966). The largest continuous population extends in a narrow coastal band 120 km long between Fort Ross and Fort Bragg in Sonoma and Mendocino Counties, California (Fig. 1). Thirty kilometres north of Fort Ross, near Sea Ranch, abrupt changes occur in frequencies of several morphological and biochemical traits. The most obvious is a change in frequency of foliage types, from all "green" to all "blue" (Duffield 1951). This change occurs over only 2 km distance. Distinct changes in monoterpene composition (Forde and Blight 1964; Mirov et al. 1966), flowering phenology, and allozyme frequencies (Millar 1983) coincide with the change in foliage types. A high frequency of allozyme heterozygotes and individuals intermediate in stomatal, monoterpene, and phenological traits occur in the narrow contact zone.

Initial studies (Millar 1983) led me to interpret these abrupt changes as the result of a recent meeting of two formerly isolated races whose ranges expanded and met at the present contact zone. There were few ecological or genetic clues, however, to the origin of the races: no obvious environmental differences were known north and south of the contact zone, blue and green races were interfertile (Millar and Critchfield 1988), and interracial gene flow was extensive (Millar 1983). Subsequently, blue bishop pine was found growing outside its main range on islands of infertile soils within the range of the green race. In this paper, I report an allozyme association of blue and green bishop pine in this region with distinct soil types.

Background

The topographic profile of the coast between Fort Bragg and Fort Ross is fairly uniform. For the first 3–8 km inland, the continent forms a ridge that comprises a series of disjunct and increasingly elevated terraces joined by moderate to steep slopes. The terraces are Pleistocene beaches that became sequentially exposed as tectonic events and sea-level changes created the continental ridge (Gardner and Bradshaw 1954; Gardner 1967; Jenny et al. 1969; Westman 1975). An unusual forest–edaphic association, known as the pygmy forest, occurs on some of the higher and older terraces. Extremely low soil pH, mineral toxicities and deficiencies, podsolized iron hardpans, and extreme water conditions on these sites contributed to the evolution of stunted forest communities, which are high in both endemic plants and stress-tolerant species (Gardner and Bradshaw 1954; Jenny et al. 1969).

Bishop pine occurs abundantly between Fort Bragg and Fort Ross on fertile soils of the lowest terrace (ca. 50 m). It grows scattered on the flat grasslands near the sea where

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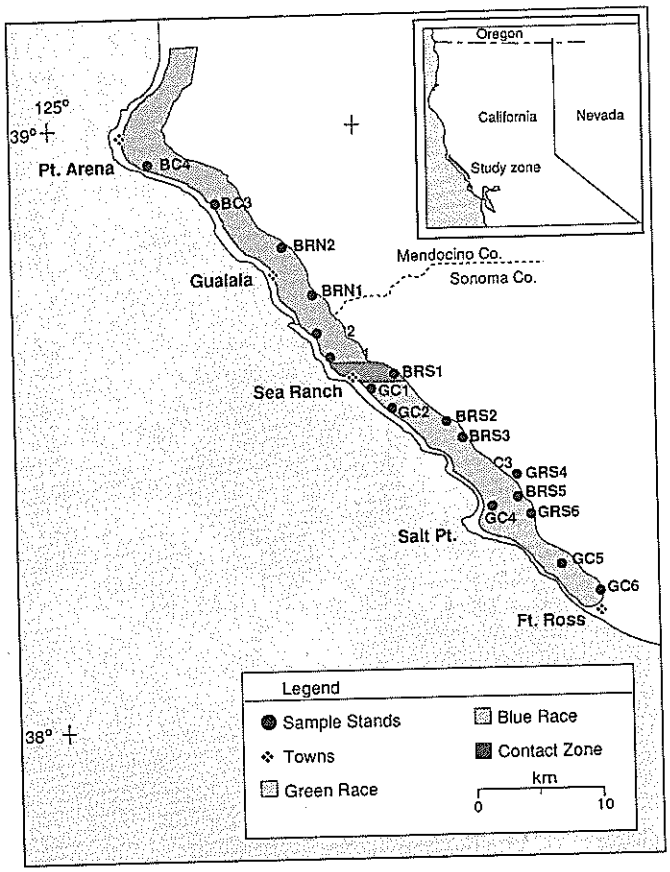

FIG. 1. Locations of sample stands of bishop pine in Sonoma and Mendocino Counties, California, and distribution of blue and green races in this region.

salt spray and high winds are common, and in dense pure stands at the inland edge of the terrace. In places it extends a short distance up the slope, but is soon replaced by forests of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and coast redwood (*Sequoia sempervirens* (D. Don) Endl.). On these fertile soils, bishop pine grows as a small timber tree, occasionally attaining heights of 25 m.

The floristics of the higher ridge elevations fall roughly into three zones between Fort Bragg and Fort Ross. In the northern zone, between Fort Bragg and Navarro (Fig. 1), the staircase development of successively higher and older terraces is most evident. On the highest terraces are the oldest, most impoverished soils of the Blacklock series. These support the most stress-tolerant plants. Bolander pine (*Pinus contorta* var. *bolanderi* (Paul.) Lemm.) and pygmy cypress (*Cupressus pygmaea* (Lemm.) Sarg.), which attain mature heights less than 2 m, are the primary trees. Bishop pine grows occasionally in the oldest pygmy forests, but with increasing abundance on successively younger, mid-elevation terraces on better soils.

In the middle zone, between Navarro and approximately Gualala (Fig. 1), the staircase development on the ridge is less prominent. Extremely impoverished soils (Blacklock series) are rare if not absent and are replaced by other terrace soils that support moderately stunted pygmy forests. Bolander pine is absent in this area, and pygmy cypress occurs on only two known sites near Gualala. Bishop pine grows abundantly on the terraces of this region, on soils of the Noyo series, where it attains mature heights of 5–10 m. More fertile soils on the slopes surrounding the terraces support Douglas-fir and coast redwood forests.

In the southern zone, between Gualala and Fort Ross (Fig. 1), the staircase succession is least developed. To my knowledge, there are no pygmy-forest patches between Gualala and the interracial contact zone at Sea Ranch. Single bishop pines occur occasionally on mid and upper elevations on the ridge, but there appear to be no patches of local abundance on stressful sites. Douglas-fir – coast redwood forests dominate the ridge. South of the contact zone, there are patches of Noyo soils on the mid and upper ridge that support locally abundant and variously stunted pine forests. In addition, there is one ridgetop site where Blacklock soils occur, and this site contains a severely stunted flora with bishop pine and pygmy cypress. At the middle and upper elevations throughout the area between Fort Bragg and Fort Ross, bishop pine also forms locally abundant stands on fertile soils around openings in the forest.

Previous work on bishop pine in this region focused on the continuous pine forests of the lowest coastal terrace between Fort Bragg and Fort Ross (Duffield 1951; Millar 1983). The abrupt contact zone between blue and green races near Sea Ranch was documented at this elevation near Sea Ranch; however, bishop pines growing on the mid and upper ridge, notably in pygmy-forest patches south of the contact zone, were not investigated.

In several pygmy forests south of the contact zone, high frequencies of blue trees were recently found in the region previously thought to be solely occupied by the green race. Adjacent to these patches, as close as 0.5 km, green bishop pine dominates on deep, fertile soils. These islands of blue bishop pine are the first suggestion of adaptive differences between the races.

Materials and methods

Stand descriptions

Samples for genetic analyses were obtained from 18 native bishop pine stands: 4 low-elevation coastal stands north of the contact zone, 6 low-elevation coastal stands south of the contact zone ("coast stands"), 2 ridge stands north of the contact zone, and 6 ridge stands south of the contact zone ("ridge stands") (Table 1, Fig. 1). The low-elevation coastal stands differed from those sampled previously (Millar 1983). New coast stands are included in the current study for comparisons with ridge stands. The sample size of the new coast collections is four times that of the previous study.

The coastal stands (BC1–BC4, GC1–GC6) were all located on the eastern edge of the lowest terrace adjacent to the broad grasslands that grow on the seaward side of the terrace. Bishop pine was the dominant conifer in each stand. Supporting these stands were deep, well-drained, moderately acidic soils that are typical of luxuriant bishop pine, Douglas-fir, and coast redwood forests at this elevation (Table 1).

The two ridge stands north of Sea Ranch (BRN1, BRN2) included disjunct patches of locally abundant bishop pine at middle elevations on the ridge (Table 1). Stand BRN1 was on flat Noyo soils, and was dominated by a suppressed but not stunted pine forest. Stand BRN2 was a stunted pygmy forest on impoverished sandy soils of the Anchor Bay series and contained pygmy cypress as well as pine. Douglas-fir – coast redwood forests surrounded both sites.

The six ridge stands south of Sea Ranch (BRS and GRS stands) also included small disjunct patches of locally abundant bishop pine (Table 1). Stand BRS1, at the southern end of the contact zone, was in a moderately stunted pygmy forest on the flat ridgetop.

TABLE 1. Forest characteristics, soil conditions, and frequency of foliage types for two races of bishop pine in northern California

Stand	Forest conditions	Elevation (m)	Soil			Foliage (%)[a]		
			Series	pH	Description	Green	Blue	Intermediate
BC1–BC4	Forest and meadow edge	50–70	Caspar, Hugo, and Empire	5.4–6.8	Deep, well drained[b]	0	100	0
BRN1	Pygmy	310	Anchor	4.8	Shallow, sandy[c]	0	100	0
BRN2	Mildly stunted	190	Noyo	4.8	Podsol, clay hardpan[d]	0	100	0
BRS1	Pygmy	240	Gualala	4.2	Shallow, sandy[d]	12	86	2
BRS2	Pygmy	300	Noyo	3.7–4.0	Podsol, clay hardpan[e]	22	73	5
BRS3	Prairie	295	Caspar	5.8	Deep, well drained[e]	13	86	1
GRS4	Intermediate	185	Noyo	4.8	Podsol, clay hardpan[e]	100	0	0
BRS5	Severe pygmy	285	Blacklock	2.8–3.9	Podsol, iron hardpan[e]	14	83	3
GRS6	Prairie	280	Caspar	5.9	Deep, well drained[e]	100	0	0
GC1–GC6	Forest and meadow edge	50–70	Caspar, Hugo, and Empire	5.4–6.8	Deep, well drained[b]	100	0	0

[a]Foliage frequency based on 120 trees per stand.
[b]Gardner (1964).
[c]McMillan (1956).
[d]Smith et al. (1978).
[e]DeLapp an Powell (1979).

Soils were shallow, undeveloped, sandy, and acidic. Bishop pine of normal stature grew adjacent to this site mixed with redwood–Douglas-fir forests.

Stand BRS2 was also on flat ground and was floristically similar to BRS1, but supported more highly developed, podsolized, and acidic soils of the Noyo series. Pines in stand BRS3, 0.6 km from stand BRS2, grew on deep, well-drained soils in a narrow band of pine forest surrounding a 25-ha grass-covered prairie. The pines were not stunted and the forests resembled the low-elevation coastal forests in stature and vigor of trees, floristic associates, and soils. Douglas-fir and coast redwood replaced the pines away from the prairie.

Stand GRS4, the lowest ridge stand, grew on Noyo soils but differed from other pygmy sites south of the contact zone in that trees were only slightly stunted, understory vegetation was dense, and the site sloped to the southwest. By the stature of the vegetation and the diversity of other species present, stand GRS4 appeared intermediate between a pygmy forest and a coastal pine forest. Adjacent to this stand were dense redwood–Douglas-fir forests.

Stand BRS5 was the most stunted of the pygmy-forest patches south of the contact zone. In many ways it resembled the severely stunted pygmy forest near Fort Bragg. Stand BRS5 was the only known location south of stand BRN2 where pygmy cypress grows, and the only site south of Navarro where the most severely podsolized soil series occur (Blacklock series with an iron hardpan).

Stand GRS6, 0.5 km from BRS5, resembled stand BRS3 in that the bishop pine forest fringed a large grass-covered prairie. Soils and associated vegetation were typical of the coastal forest, and trees were of normal height and stature. Surrounding stands BRS5 and GRS6 were redwood–Douglas-fir forests, although bishop pine grew more or less continuously between the stands.

Foliage analyses

Foliage was collected from 120 trees in each stand, and foliage type (blue, green, or intermediate) was determined with a dissecting microscope (drawings in Duffield, 1951). The most common intermediate type had partially closed epistomatal chambers filled with some wax.

Allozyme analyses

Two- to 4-year-old serotinous cones were collected from 40 trees in each coastal stand and in the blue ridge stands north of the contact zone. The trees were spaced at least 15 m apart and were over 25 years old. Cones from 20 trees were collected in each ridge stand south of the contact zone. Trees from which cones were collected were a subset of those used for foliage determinations.

Megagametophytes from each of the 600 trees were analyzed by starch gel electrophoresis using techniques of Conkle et al. (1982) and Millar (1985). The 45 loci from 22 enzyme systems used in this study were described previously (Millar 1985). Nineteen more loci were analyzed here than in the previous study (Millar 1983). Diploid genotypes of the female parent trees were estimated from five gametophytes per tree. The probability of misclassifying a heterozygote as a homozygote with this sample size is 0.06 (Morris and Spieth 1978). Allele frequencies for each stand were based on female-parent genotypes. Expected heterozygosities, Nei's unbiased genetic distance and diversity statistics (Nei 1973, 1978), and UPGMA cluster analyses (Sneath and Sokal 1973), based on Nei's unbiased distances, were calculated using BIOSYS-1 (Swofford and Selander 1981).

Results

Foliage analyses

All trees in the coastal stands (BC1–BC4) and the ridge stands north of Sea Ranch (BRN1, BRN2) had blue foliage; all trees in the coastal stands south of Sea Ranch (GC1–GC6) had green foliage. Trees in the ridge stands south of Sea Ranch had either all green foliage or primarily blue foliage (Table 1). Intermediate foliage types occurred in low frequencies in stands BRS1, BRS2, BRS3, and BRS5. Foliage type was mostly associated with soil and forest type in ridge

TABLE 2. Allele frequencies at 38 polymorphic loci for 18 stands of bishop pine in northern California

Locus and allele	Coastal stands										Ridge stands							
	North of contact zone (blue)				South of contact zone (green)						North of contact zone (blue)		South of contact zone					
													Blue				Green	
	BC4	BC3	BC2	BC1	GC1	GC2	GC3	GC4	GC5	GC6	BRN2	BRN1	BRS1	BRS2	BRS3	BRS5	GRS4	GRS6
Aco1																		
1	0.81	0.88	0.73	0.85	0.62	0.63	0.58	0.43	0.58	0.58	0.83	0.87	0.69	0.88	0.74	0.83	0.55	0.55
2	0.19	0.11	0.27	0.10	0.39	0.37	0.43	0.58	0.42	0.43	0.16	0.12	0.30	0.12	0.24	0.18	0.45	0.45
3		0.01		0.05							0.01	0.01			0.03			
Acp1																		
1	0.73	0.49	0.69	0.59	0.80	0.85	0.64	0.61	0.90	0.81	0.93	0.90	0.81	0.81	0.63	0.90	0.68	0.80
2	0.26	0.51	0.22	0.38	0.19	0.15	0.36	0.39	0.10	0.19	0.07	0.10	0.19	0.17	0.34	0.08	0.33	0.20
3	0.01				0.01									0.02		0.03		
4			0.01	0.03											0.03			
5			0.08															
Adh1																		
1	0.91	0.95	0.97	0.60	0.78	0.92	0.91	0.94	0.94	0.93	0.95	0.84	0.94	1.0	0.95	0.88	0.88	0.95
2	0.04	0.02	0.02	0.21	0.09	0.06	0.03	0.06	0.05	0.03	0.03		0.03			0.03	0.13	0.03
3	0.05	0.03	0.02	0.19	0.03	0.01	0.01		0.01	0.03	0.03	0.16	0.03		0.05	0.10		0.02
4							0.05			0.03								
Ala1																		
1	1.0	1.0	0.97	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2			0.03															
Ala2																		
1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2																		
Ald1																		
1	1.0	1.0	1.0	1.0	0.99	1.0	1.0	1.0	1.0	1.0	0.90	0.74	0.61	1.0	1.0	1.0	0.65	1.0
2					0.01						0.10	0.21	0.39				0.35	
Ald2												0.03						
1	0.78	0.95	0.83	0.63	0.80	0.77	0.75	0.53	0.80	0.56		0.02						
2	0.16	0.05	0.14	0.23	0.10	0.23	0.25	0.32	0.19	0.28								
3					0.03			0.03	0.01									
4	0.06		0.03	0.15	0.08			0.02		0.17	1.0	0.96	0.94	0.93	0.97	1.0	0.98	0.90
Cat1												0.04	0.06	0.05	0.03		0.02	0.10
1	0.99	1.0	0.97	1.0	1.0	0.96	0.99	1.0	1.0	0.98				0.02				
2	0.01		0.03	1.0		0.04	0.01			0.02								
3											1.0	1.0	0.67	0.91	0.79	1.0	0.90	1.0
Fdp1													0.33		0.21		0.10	
1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.97	1.0				0.09				
2									0.03									
3													0.50	0.62	0.63	0.55	0.98	0.93
Got1											0.90	0.30	0.50	0.38	0.37	0.45	0.03	0.08
1	0.14	0.51	0.39	0.33	0.99	0.99	0.98	0.95	0.92	0.90	0.10	0.69						
2	0.85	0.49	0.62	0.67	0.01	0.01	0.03	0.04	0.08	0.10		0.01						
3								0.01										
4																		

TABLE 2 (*continued*)

Locus and allele	Coastal stands										Ridge stands						
	North of contact zone (blue)				South of contact zone (green)						North of contact zone (blue)		South of contact zone				
													Blue			Green	
	BC4	BC3	BC2	BC1	GC1	GC2	GC3	GC4	GC5	GC6	BRN2	BRN1	BRS1	BRS2	BRS3	BRS5	GRS4	GRS6
Got2																		
1	0.89	0.95	0.97	0.94	0.95	0.94	0.95	0.99	0.99	0.90	0.90	0.95	0.89	0.95	0.97	0.95	1.0	0.98
2	0.11	0.05	0.03	0.06	0.05	0.06	0.05	0.01	0.01	0.01	0.10	0.05	0.11	0.05	0.03	0.05		0.02
Got3																		
1	1.0	1.0	1.0	1.0	1.0	0.98	0.94	0.95	0.95	0.96	0.99	1.0	1.0	1.0	1.0	1.0	0.93	0.98
2						0.01	0.05	0.04	0.05	0.04	0.01						0.07	0.02
3							0.01	0.01										
Gpd1																		
1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.93	1.0	0.90
2				0.99												0.07		0.10
Gpd2				0.01														
1	1.0	1.0	1.0	1.0	0.99	0.86	0.90	0.95	0.96	0.96	0.96	0.93	1.0	0.93	0.95	0.95	0.83	0.93
2		0.75	0.96		0.01	0.14	0.09	0.05	0.04	0.01	0.04	0.07		0.07	0.05	0.05	0.18	0.07
3		0.25	0.04				0.01											
Idh1																		
1	0.96	0.76	0.80	0.84	0.73	0.72	0.74	0.99	0.80	0.75	0.84	0.91	0.75	0.86	0.79	0.78	0.65	0.83
2	0.04	0.01											0.03			0.03		
3		0.23	0.21	0.16	0.27	0.28	0.26	0.01	0.21	0.20	0.16	0.09	0.22	0.14	0.21	0.20	0.35	0.18
Lap1																		
1	0.92	0.73	0.71	0.70	0.96	0.83	0.65	0.73	0.81	0.57	0.83	0.50	0.78	0.74	0.53	1.0	0.70	1.0
2	0.08	0.28	0.30	0.30	0.04	0.17	0.35	0.27	0.19	0.43	0.17	0.50	0.22	0.26	0.47		0.30	
Lap2																		
1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.97	0.95	1.0	1.0	0.98	1.0
2													0.03	0.05			0.02	
Mdh1																		
1	1.0	0.99	1.0	1.0	1.0	1.0	1.0	1.0	0.99	1.0	1.0	0.99	1.0	1.0	1.0	0.98	1.0	1.0
2		0.01							0.01			0.01				0.02		
Mdh2																		
1	1.0	1.0	0.99	1.0	0.86	0.97	0.96	0.94	1.0	0.99	0.99	1.0	0.89	0.98	0.97	0.93	0.98	0.98
2			0.01		0.13	0.03	0.04	0.06		0.01	0.01		0.11	0.02	0.03	0.05	0.03	0.02
3					0.01											0.03		
Mdh4																		
1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.99	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2								0.01										
Mmd1																		
1	0.99	1.0	1.0	1.0	0.95	0.96	0.93	1.0	0.92	0.90	0.98	1.0	0.75	0.95	0.92	0.85	0.85	0.80
2	0.01				0.05	0.04	0.07		0.08	0.10	0.02		0.25	0.05	0.08	0.15	0.15	0.20
Mnr1																		
1	1.0	0.98	0.99	1.0	1.0	0.96	0.99	0.94	1.0	0.96	1.0	0.99	1.0	1.0	1.0	0.98	1.0	0.98
2		0.02	0.01			0.04	0.01	0.06		0.04		0.01				0.02		0.02

TABLE 2 (*continued*)

Locus and allele	Coastal stands										Ridge stands							
	North of contact zone (blue)				South of contact zone (green)						North of contact zone (blue)		South of contact zone					
													Blue				Green	
	BC4	BC3	BC2	BC1	GC1	GC2	GC3	GC4	GC5	GC6	BRN2	BRN1	BRS1	BRS2	BRS3	BRS5	GRS4	GRS6
Mnr2																		
1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.99	1.0	0.98	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2								0.01		0.02								
Mnr3																		
1	0.99	0.98	0.95	0.99	1.0	1.0	0.98	0.95	0.96	0.93	0.91	0.97	1.0	0.81	0.53	0.98	1.0	1.0
2			0.01					0.05	0.03	0.03						0.02		
3	0.01	0.03	0.04	0.01			0.01			0.01	0.09	0.03		0.14	0.47			
4							0.01		0.01	0.04				0.05				
Mpi1																		
1	1.0	1.0	1.0	1.0	0.99	1.0	1.0	1.0	1.0	0.96	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2					0.01					0.01								
3										0.03								
Per1																		
1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.95	1.0	1.0	1.0	1.0
2														0.05				
Per2																		
1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.99	1.0	1.0	0.99	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2								0.01			0.01							
Per3																		
1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.99	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2											0.01							
Pgd1																		
1	1.0	0.98	0.90	0.97	1.0	1.0	1.0	1.0	1.0	1.0	0.98	0.93	1.0	1.0	1.0	0.98	1.0	1.0
2		0.02	0.10	0.03							0.02	0.07				0.02		
Pgd2																		
1	1.0	0.99	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2		0.01																
Pgi1																		
1	1.0	1.0	1.0	1.0	0.99	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2					0.01													
Pgi2																		
1	0.90	0.66	0.78	0.88	0.95	0.96	0.96	0.94	0.97	0.96	0.90	0.91	0.75	0.93	0.97	0.90	0.85	0.98
2	0.10	0.33	0.22	0.12	0.05	0.04	0.04	0.06	0.03	0.04	0.10	0.09	0.25	0.07	0.03	0.10	0.15	0.03
3		0.01																
Pgm1																		
1	1.0	1.0	1.0	1.0	1.0	0.99	0.99	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.98	1.0	1.0
2						0.01	0.01									0.02		
Pgm2																		
1	0.82	0.97	0.87	0.88	0.81	0.69	0.70	0.58	0.76	0.71	0.95	0.80	0.53	0.79	0.90	0.68	0.83	0.73
2	0.18	0.03	0.13	0.12	0.17	0.31	0.30	0.43	0.24	0.29	0.05	0.20	0.25	0.21	0.11	0.33	0.17	0.27
3					0.03								0.22					
Pgm3																		
1	0.98	1.0	1.0	0.96	0.95	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.95	0.90
2	0.02			0.04														0.10
3					0.05												0.05	

TABLE 2 (*concluded*)

Locus and allele	Coastal stands										Ridge stands							
	North of contact zone (blue)				South of contact zone (green)						North of contact zone (blue)		South of contact zone					
													Blue				Green	
	BC4	BC3	BC2	BC1	GC1	GC2	GC3	GC4	GC5	GC6	BRN2	BRN1	BRS1	BRS2	BRS3	BRS5	GRS4	GRS6
Skd1																		
1	1.0	1.0	0.96	1.0	0.94	0.96	0.94	0.93	0.92	0.98	0.98	0.99	1.0	0.88	0.92	0.98	0.98	0.98
2							0.01											
3			0.04		0.05	0.04	0.05	0.04	0.08	0.02	0.02	0.01		0.10	0.08	0.02	0.02	0.02
4					0.01			0.03						0.02				
Sod1																		
1	0.99	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2	0.01																	

NOTE: Coastal stands are arranged in order from north to south. Twenty trees were analyzed for the six ridge stands south of the contact zone; 40 trees were analyzed for all other stands.

TABLE 3. Nei's unbiased genetic distances between blue and green coast and ridge stands in northern populations of bishop pine (above diagonal) and average distances (below diagonal)

	Coast stands										Ridge stands							
	North of contact zone (blue)				South of contact zone (green)						North of contact zone (blue)		South of contact zone					
													Blue				Green	
	BC4	BC3	BC2	BC1	GC1	GC2	GC3	GC4	GC5	GC6	BRN2	BRN1	BRS1	BRS2	BRS3	BRS5	GRS4	GRS6
BC4	—	0.009	0.003	0.003	0.019	0.020	0.021	0.022	0.017	0.019	0.002	0.006	0.010	0.008	0.016	0.006	0.022	0.018
BC3		—	0.004	0.006	0.015	0.014	0.013	0.020	0.015	0.016	0.008	0.009	0.014	0.006	0.010	0.010	0.012	0.016
BC2	0.005		—	0.003	0.012	0.011	0.011	0.015	0.009	0.010	0.002	0.002	0.007	0.003	0.008	0.008	0.011	0.012
BC1				—	0.015	0.016	0.015	0.018	0.015	0.013	0.005	0.003	0.010	0.008	0.012	0.009	0.015	0.019
GC1					—	0.001	0.004	0.007	0.002	0.005	0.014	0.016	0.013	0.008	0.015	0.007	0.004	0.002
GC2						—	0.001	0.005	0.000	0.002	0.014	0.013	0.011	0.007	0.014	0.007	0.002	0.002
GC3							—	0.002	0.002	0.001	0.017	0.012	0.011	0.008	0.011	0.011	0.000	0.005
GC4		0.015				0.003		—	0.005	0.003	0.022	0.017	0.013	0.014	0.018	0.016	0.005	0.008
GC5									—	0.002	0.011	0.011	0.010	0.006	0.012	0.006	0.002	0.002
GC6										—	0.014	0.009	0.009	0.008	0.011	0.011	0.002	0.007
BRN2											—	0.003	0.010	0.003	0.010	0.004	0.017	0.014
BRN1		0.005					0.014				0.003	—	0.009	0.004	0.010	0.008	0.014	0.017
BRS1													—	0.009	0.016	0.008	0.009	0.013
BRS2														—	0.003	0.003	0.011	0.007
BRS3		0.009					0.011				0.007		0.009		—	0.013	0.013	0.015
BRS5																—	0.013	0.005
GRS4																	—	0.006
GRS6		0.016					0.003				0.016		0.011				0.006	—

TABLE 4. Genetic variability at 45 loci in northern bishop pine, including sample size, percent of polymorphic loci (where frequency of most common allele does not exceed 0.95), expected heterozygosities, and the ratio of observed to expected heterozygosities (H_o/H_e)

Population	Sample size (no. of trees)	Mean no. alleles/locus	Percent loci polymorphic	Expected heterozygosity	H_o/H_e
Coastal blue (BC1–BC4)	160	1.4 (0.05)	22.5 (2.2)	0.071 (0.01)	0.761
Coastal green (GC1–GC6)	240	1.5 (0.09)	25.0 (3.6)	0.076 (0.01)	0.789
Blue ridge north (BRN1, BRN2)	80	1.5 (0.07)	25.0 (1.6)	0.066 (0.01)	0.782
Blue ridge south (BRS1–BRS3, BRS5)	80	1.5 (0.06)	27.7 (2.1)	0.088 (0.02)	0.784
Green ridge south (GRS4, GRS6)	40	1.4 (0.01)	26.1 (3.1)	0.081 (0.02)	0.852
Total ensemble	600	1.5 (0.07)	24.9 (3.3)	0.077 (0.013)	0.792

NOTE: SE is given in parentheses.

stands. All pygmy forests had primarily blue foliage, and prairie stand GRS6 had all green foliage. The stand with intermediate floristics (GRS4) had all green foliage. One of the prairie stands (BRS3) had an unexpectedly high frequency of trees with blue foliage.

Allozyme analyses

Differences in allozyme frequencies between blue and green coastal populations were similar to those reported previously (Millar 1983) (Table 2). On average, the genetic distance (Table 3, Fig. 2) between the blue and green coastal populations (0.015) was three times larger than distances among stands within the blue population (0.005), and five times larger than distances among stands within the green population (0.003). In the coastal ensemble, 5.9% of the total variation was distributed between blue and green populations.

Allozyme frequencies differentiated blue and green ridge stands at the same loci that differentiated blue and green coastal populations (*Aco*, *Ald2*, *Got1*, *Got3*, *Pgd1*, *Pgi2*, and *Pgm2*; Table 2). The average genetic distances between the blue and green ridge stands (south of the contact zone, 0.011; north and south of the contact zone, 0.016; Table 3) were comparable to the distance between the blue and green coastal populations (0.015), but were greater than the following: the distance between the two green ridge stands (0.006), the distances among the four blue ridge stands south of the contact zone (0.009), and the distance between the two blue ridge stands north of the contact zone (0.003). In the ridge stands, 4.2% of the total variation was distributed between blue and green stands.

The blue ridge stands, including the five pygmy sites and one prairie-edge site (BRS3), had allozyme frequencies (Table 2) and genetic distances (Table 3) that clustered them with the blue coastal population (Fig. 2) . The two ridge stands north of the contact zone (BRN1, BRN2) had smaller genetic distances to the blue coastal population than did the blue ridge stands south of the contact zone. The distance between the blue ridge stands south of the contact zone and the blue coastal population (0.009) was slightly more than the distance among stands within the blue coastal population (0.005) and was the same as the distance among blue ridge stands south of the contact zone (0.009).

The two green ridge stands (GRS4 and GRS6) had

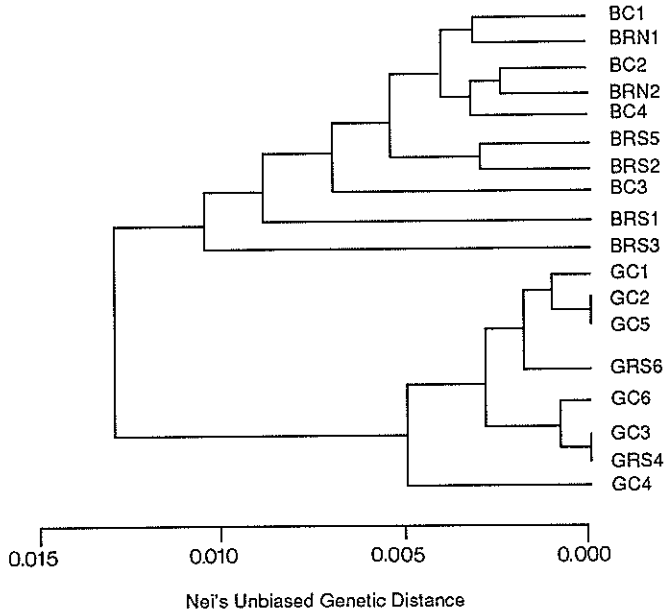

FIG. 2. Cluster phenogram (UPGMA) summarizing genetic distances among blue coastal, green coastal, and ridge stands of northern bishop pine. Ridge stands GRS4 and GRS6 are green; all other ridge stands are completely or mostly blue.

allozyme frequencies similar to the green coastal stands and clustered within the green coastal population (Fig. 2). Genetic distance (Table 3) between the green ridge stands and green coastal stands was low (average 0.003), the same as the distance among stands within the green coastal population (average 0.003) and less than the distance between the two green ridge stands (average 0.006).

In addition to the loci that clustered the blue and green ridge stands with blue and green coastal populations, another set of loci distinguished the blue ridge stands south of the contact zone from both coastal populations. Three loci (*Alap2*, *Gpd1*, and *Mdh1*) were monomorphic in the coastal populations, but polymorphic in at least one of the blue ridge stands (Table 2). Other loci with minor differences between the blue ridge stands south of the contact zone and the coastal populations were *Ald2*, *Cat1*, *Fdp1*, *Mnr3*, and *Mmd1*. These differences, although secondary to the loci that indicated affinity of the blue and green ridge stands to

respective blue and green coastal populations, weighted the genetic distances (Table 3) and clustering results (Fig. 2). Although these stands clustered with the blue coastal population, the distances between blue ridge stands south of the contact zone and the blue coastal stands were larger than distances among blue coastal stands, thus setting the blue ridge stands somewhat apart.

All populations in this study had low and similar levels of allelic variation (Table 4). The coastal blue and green populations had similar average numbers of alleles per locus, percent polymorphic loci, and expected heterozygosities, although in every measure the blue coastal population had the lowest values. The blue ridge stands north of the contact zone had values similar to the blue coastal population. The blue and green ridge stands south of the contact zone had similar levels of allelic variation to each other, but the values were consistently higher than both coastal populations. Observed heterozygosities were about 20% lower than expected heterozygosities in every population; this deficiency was greatest in the blue coastal population and least in the green ridge stands.

Discussion

Two races of northern bishop pine were known previously to meet only in a narrow zone (2 km wide) on fertile soils of the lower coastal terrace at Sea Ranch, California. The lack of any obvious environmental changes coincidental with this contact zone has obscured an understanding of the origin of the races. However, the association of races with ridgetop soil types described in this paper suggests edaphic evolution of the races. Pines that grow in patches of the most impoverished pygmy-forest soils on ridgetops north and south of the Sea Ranch contact zone clearly are the blue race in allozyme frequencies and stomatal anatomy. Similarly, with one exception, pines that grow on adjacent fertile ridgetop soils south of the Sea Ranch zone are clearly the green race.

The differences in bishop pine are unusual for conifers because they occur in parapatric stands and not in isolated populations. Soil factors of the pygmy forest are well known for their role in the evolution of distinct floras. Many characteristics of pygmy-forest soils exert strong selection on plant growth that has favored the evolution of stress-tolerant, endemic races and species. Of woody species, Bolander pine (McMillan 1956; Critchfield 1957; Wheeler and Guries 1982), pygmy cypress (McMillan 1956), and *Arctostaphylos nummularia* Gray grow naturally only in pygmy forests (Westman 1975).

Other soils have also been implicated in abrupt genetic differentiation. Serpentines and other ultramafic soils support tolerant endemics that do not compete well on fertile soils (Kruckeberg 1951, 1967, 1987). Abrupt genetic differentiation occurs in grasses such as *Agrostis tenuis* Sibth., where tolerant and nontolerant populations occur on and off toxic mine soils (Antonovics et al. 1971). It is not as common in tree species, however, to find genetic differentiation that is related to soil characteristics. The outbreeding habit, with wind-dispersed pollen and seeds, often counters the evolution of abrupt genetic differentiation even when large environmental differences occur (e.g., Ledig and Fryer 1972; Jenkinson 1977).

The general historic pattern of movement in bishop pine and related pines in California has been one of population expansion and fragmentation. This movement has been caused primarily by climatic changes of the glacial cycles during the last million years (Mason 1932; Axelrod 1980, 1981; Millar et al. 1988). During this time, the coastal ridge in northern California was being formed (Gardner 1967) and the pygmy-forest ecosystem was evolving (Jenny et al. 1969). Since bishop pine has been present in northern California for at least 5 million years (Dorf 1933), there were many times during the last million years when isolated bishop pine populations could have invaded pygmy-forest sites and racial differences evolved.

The blue race is most likely the derived taxon in bishop pine, since green is the only foliage morph in the southern, older populations of the species (discussed in Millar, 1983), and since severely impoverished pygmy-forest soils occur only in northern California. Although the species grows elsewhere on shallow, sandy soils (notably at Monterey and in Baja California), the suite of characteristics typical of the blue race does not occur on these soils.

The degree of differentiation between blue and green races is low in overall allozyme frequencies, but high in other traits. Allozyme distances between northern and southern California bishop pine are three times as great as distances between races in northern California. Similarly, allozyme differences among widespread populations in related knobcone pine (*Pinus attenuata* Lemm.) and Monterey pine (*P. radiata* D. Don) are almost twice as large (Millar et al. 1988). Differences in other traits, however, are as great as those that distinguish species in related pines (Millar 1986). The evolution of these large differences, despite only minor allozyme divergence, suggests that selection has been strong for certain traits. The hypothesis that some or all of these traits directly contribute to survival of blue bishop pine on pygmy-forest soils has yet to be tested. However, the repeated occurrence of the same genetic variation on several pygmy forests and the maintenance of genetic differentiation on these patches despite potential gene flow from nearby green bishop pine is strong circumstantial evidence for selection.

A clue to the migrational history of blue and green bishop pine in this area comes from the pattern of blue trees south of the contact zone. These blue bishop pine stands may either predate green bishop (relictual) or postdate them (colonizing). The presence of stand BRS3, where blue trees are common on fertile ridge soils, and the occurrence of occasional blue trees mixed in Douglas-fir – coast redwood forests on the ridge south of Sea Ranch, suggest that the blue stands are relictual and occupied the ridge before green bishop pine.

According to this hypothesis, northward migrating green bishop pine displaced blue bishop pine on most of the fertile sites, except in occasional areas like BRS3. If blue bishop pine stands postdated green bishop south of the contact zone, then it is unlikely that fertile sites would have been available for colonization by blue bishop. Green bishop pine would have occupied available sites, leaving areas like pygmy-forest sites, where it could not compete, unoccupied.

The near absence of the blue race on low elevations south of the contact zone suggests that it did not grow there in recent history. If it did, some major environmental shift or competitive factor restricted blue bishop pine to ridgetop sites. One way to resolve these alternative hypotheses would be to measure the physiological response of blue and green

races to edaphic factors associated with pygmy soils compared with fertile soils.

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