

HYBRIDIZATION OF FOXTAIL AND BRISTLECONE PINES

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The pines have been more successful than most of their coniferous relatives in occupying marginal habitats at the upper and lower edges of the forest zone in western North America. Among the groups restricted to such habitats is subsection *Balfourianae* of *Pinus*, comprising the foxtail and bristlecone pines. These pines characteristically grow on cold dry sites at high elevations, and in most places have few tree associates. Perhaps because of their inaccessibility and limited economic importance, not much was known about them until E. Schulman's discovery in the mid-1950's that some bristlecone pines reach greater ages than other higher organisms (Ferguson, 1968). Since then, much has been learned about the *Balfourianae*, and investigations of natural variation have generated two taxonomic proposals in the group. Bailey (1970) named the western populations of bristlecone pine *P. longaeva*, restricting the older name, *P. aristata* Engelm., to the eastern populations. Mastrogiuseppe (1972) proposed the subdivision of foxtail pine (*P. balfouriana* Grev. & Balf.) into two subspecies.

The first attempts to cross bristlecone and foxtail pines were made in 1940 by the U.S. Forest Service's Institute of Forest Genetics (IFG) at Placerville, California, but most of our exploratory crossing of the *Balfourianae* pines with each other and with other pines was carried out between 1963 and 1971. This paper summarizes the results of these crosses, examines variation in several characteristics of the *Balfourianae*, and relates this information to recent investigations of the systematics and evolution of the group.

THE TAXA OF *Balfourianae*

Subsection *Balfourianae* is a morphologically and geographically coherent group, with no close affinities to any other group of white pines in subgenus *Strobus* (*Haploxylon*). The needles mostly number five per fascicle, but this common feature of white pines is accompanied by the absence of marginal teeth on the needles, cone scales with dorsal umbos terminating in a mucro or spine, and seeds with long detachable wings—a combination of characters that readily differentiates the *Balfourianae* from other subsections of *Strobus*.

Among the named taxa in the *Balfourianae*, foxtail pine is the most limited in distribution. This Californian endemic grows only in the Klamath Mountains of northwestern California and a portion of the southern

Sierra Nevada centered on Sequoia National Park. The northern and southern stands are separated by more than 500 km. They differ in turpentine composition (Haagen-Smit, Wang and Mirov, 1950) and in morphology, particularly quantitative characteristics of cones and seeds (Mastrogiuseppe, 1972; Mastrogiuseppe and Mastrogiuseppe, 1975). Mastrogiuseppe (1972) summarized the evidence supporting their recognition as separate subspecies.

The bristlecone pines are much more widely distributed, ranging from California to the southern Rocky Mountains. The Colorado River separates eastern *P. aristata* from western *P. longaeva*. Eastern bristlecone pine grows in the mountains of Colorado and New Mexico, with a disjunct population more than 500 km west in the San Francisco Peaks of northern Arizona. Western bristlecone pine is widely distributed in the mountains of Utah, Nevada, and eastern California. At its western limits in the White and Inyo Mountains of California, it grows only about 25 km from the nearest foxtail pine stands on the east slope of the Sierra Nevada.

The eastern and western bristlecone pines differ in morphology (Bailey, 1970) and turpentine composition (Zavarin and Snajberk, 1973; Zavarin, Snajberk and Bailey, 1976). Bailey noted differences in cone morphology and color, resin odor, needle retention, and other features, but his separation of eastern and western bristlecone pines was documented primarily by characteristics of the needle resin canals. He found that most needles of western bristlecone and foxtail pines had two externally visible resin canals and ungrooved needle surfaces. In the needles of eastern bristlecone pine, the resin canals were located beneath shallow grooves in the needle surface, and Bailey estimated the number and distribution of resin canals by counting grooves. About half of the needles had only one groove. The rest had two or more, but in more than half of these needles all but one groove terminated below the apical quarter of the needle.

The resin canals of the eastern trees are also much smaller and closer to the needle surface. They often burst, exuding resin onto the surface. The dried resin forms a white fleck, and the eastern and western trees differ greatly in the incidence of these flecks. Only 5 percent of the needles Bailey sampled in western stands had flecks. In the east, flecks were present on 92–94 percent of the needles from Colorado and New Mexico and 47–63 percent of the needles from Arizona (Bailey, 1970; Zavarin et al., 1976).

Bailey also observed differences between eastern and western bristlecone pines in the shape of the cone base, stoutness and length of the cone bristles, and color of ripe pollen-cones and maturing seed-cones. Measurements of the length of bristles on cones in the IFG herbarium substantiate his statement that eastern bristlecone pines often have longer bristles. Cones from eastern trees averaged about 5 mm in bristle length,

and ranged from 3–8 mm. Cones from western trees had bristles averaging about 3 mm long (range 1.5–6 mm).

Polymorphism in the color of maturing reproductive structures is one of the most distinctive characteristics of western bristlecone pine, differentiating it from all other elements of the *Balfourianae*. The seed-cones of most western trees are dark purple during their second season of development, and the pollen-cones of the same trees are nearly always deep red at maturity. A minority of trees have green seed-cones and yellow (sometimes pink-tipped) pollen-cones. The green-cone trees lack an anthocyanin pigment that is present in the others (Mastrogiuseppe, 1976). This color variant is more common in some western stands than Bailey's (1970) estimate of less than 1%. We found that about 20% of the trees in the White Mountains had green cones (Johnson and Critchfield, 1974), and on Telescope Peak in the Panamint Mountains about 4% had green cones (Unpub. data, IFG). Eastern bristlecone pine and foxtail pine are not known to be polymorphic in cone color. Both have purple or greenish-purple seed-cones and yellow pollen-cones. Thus nearly all western bristlecone pines differ from the other *Balfourianae* pines in the color of either the ripening seed-cones (green) or the mature pollen-cones (deep red).

Bailey's classification of the bristlecone pines was supported by the results of the first detailed investigation of turpentine composition in the group (Zavarin and Snajberk, 1973). With a single exception in each region, turpentine of 37 eastern trees was mostly 3-carene (63–92%); that of 30 western trees was almost entirely α -pinene (95–97%).

A more complex picture has emerged from further work in which Zavarin et al. (1976) used larger samples and analyzed needle resin as well as the turpentine fraction of wood resin. Between 10 and 26% of the trees sampled in Arizona and the White and Inyo Mountains of California were chemically deviant in turpentine composition. The most remarkable finding of these authors was the turpentine composition of a California stand of *P. longaeva* recently discovered on Sentinel Peak in the Panamint Mountains (Johnson, 1976). All ten trees sampled were higher in 3-carene content than most eastern bristlecone pines. Needle resin composition showed three main groups: Arizona, Colorado–New Mexico, and west of the Colorado River. Arizona trees were closer to Colorado–New Mexico trees in a quantitative measure of chemical similarity, but in several constituents they were intermediate or resembled western trees. Foliage from the single Sentinel Peak tree sampled was most like Colorado–New Mexico trees in resin composition, but in some constituents it was apparently outside the range of other bristlecone pines. Thus there are four chemically identifiable groups of bristlecone pines: Colorado–New Mexico, Arizona, Utah–Nevada, and the highly variable California stands.

METHODS

Parent trees. Access is a problem in the controlled pollination of foxtail and western bristlecone pines, and all parent trees were in a few relatively accessible native stands. No arboretum trees of these taxa were used; none has survived to reproductive age in the warm foothill climate of Placerville.

The first crosses on western bristlecone pine were made in 1940 and 1948 at Telescope Peak in the Panamint Range (plot location: 36°10'N, 117°05'W, 3330 m). In the same years, crosses were made on foxtail pine at Onion Valley, on the east slope of the southern Sierra Nevada (36°46'N, 118°20'W, 2790 m). The fate of the seed harvested from these early crosses is not fully documented and they are not included in the summarized data. Two 1940 crosses of *P. monticola* females and Telescope Peak pollen are included in the data summary (Table 1).

In 1963 and 1971 crosses were made on six and five bristlecone pines about 1.5 km north of Schulman Grove in the White Mountains (plot location: 37°24'N, 118°11'W, 3095 m). Five of the trees were used as females in both years. Pollen was collected from other stands within 1.5 km of Schulman Grove at elevations of 2990–3200 m. All crosses with foxtail pine as female parent were made in 1965 on four trees at Onion Valley. Pollen was collected in several seasons within 0.5 km of Onion Valley at elevations of 2775–2955 m. Pollen from northern foxtail pine was collected in 1961 and 1964 on North Yolla Bolly Mountain at elevations of 2195–2285 m (40°12'N, 122°59'W). Pollen of eastern bristlecone pine was collected in 1970 by D. K. Bailey in three stands west of Denver, Colorado. The stands were 13–27 km apart, and ranged in elevation from 2990–3290 m (39°40'–52'N, 105°34'–44'W).

Crosses of western bristlecone and foxtail pines with other species utilized trees in natural stands and the IFG arboretum. Female parents of *P. bungeana* and *P. flexilis* were old arboretum trees, mostly of unknown origin. *Pinus monticola* and *P. monophylla* females grew in native stands in the central Sierra Nevada, *P. monticola* at 2130 m and *P. monophylla* on the east side at 1615 m. Arboretum trees supplied all pollen except *P. flexilis*, which was collected near Schulman Grove in the White Mountains.

Techniques and Terminology. Standard breeding and seed-processing techniques were used (Critchfield, 1963). Pollen was collected during the season before pollination and deep-frozen, unless otherwise noted. The data summaries (Tables 1–4) include all crosses for which we have complete information: numbers of strobili pollinated, cones harvested, and sound and hollow seeds.

An *attempt* is the pollination during a single season of a female parent with pollen from a single male parent (Tables 1–4) or with a mixture of pollen from more than one male parent (Table 1 only). *Crossability* is

TABLE 1. CROSSES OF *Balfourianae* WITH OTHER WHITE PINES.

Female parent	Male parent	
	monticola	longaeva
monticola	2 (—)/ 2	1/ 2 (2)/ 0
	79: 43	38:87
	76.8/ 94.4	0/ 92.2
flexilis	flexilis	balfouriana
	2 (2)/ 2	2 (2)/ 0 2/
	17: 59	18:0
balfouriana	3 (3)/ 2	3 (5)/ 3
	18: 78	44: 93
	0.2/ 42.8	46.4/ 55.4
bungeana	bungeana	balfouriana
	1 (1)/ 1 3/	1 (1)/ 0
	8: 100	3: 67
balfouriana	4.0/ 6.0	0/ 0.5
	3 (3)/ 0	3 (5)/ 3
	15: 73	47: 85
monophylla	0/ 13.0	53.6/ 62.3
	monophylla	balfouriana
	2 (4)/ 2	2 (6)/ 0
monophylla	25: 44	34: 29
	9.4/ 11.6	0/ 8.5

1/ wind pollination

2/ pollen frozen for 2 years

3/ self pollination

Number of attempts.....

Number of female strobili.....

Mean number of sound seeds per cone.....

0	(0)	0
0,	0	
0.0	/	0.0

Number of tree x tree combinations

Number of attempts producing sound seeds

Percent of strobili producing cones

Mean total number of seeds per cone

the mean yield of sound seeds per cone from crosses between two taxa, expressed as a percent of the yield from control crosses within the seed-parent taxon. All control crosses summarized in Tables 1–4 were made on the same seed parent in the same season as crosses between taxa.

The following abbreviations are used: northern foxtail pine—NFO, southern foxtail pine—SFO, western bristlecone pine (*P. longaeva*)—WBR, and eastern bristlecone pine (*P. aristata*)—EBR.

Seedling Observations. Seedlings of the *Balfourianae* are slow-growing, and do not regularly produce secondary leaves (needles in fascicles) until the third growing season. Needles were sampled at the end of that season. Comparisons of needle characteristics were based on five fascicles from each of five seedlings from each of these groups: WBR (White Mountains); EBR (Colorado); EBR (Arizona); WBR x EBR, and SFO (Onion Valley). The seedlings were as diverse as possible in origin and parentage.

Counts of grooves on the needle surface did not provide reliable data on the number and distribution of resin canals in the needles of EBR seedlings. All needles were hand-sectioned at one-fourth of the distance from the tip to the base of the needle, and about half were also sectioned at one-half and three-fourths of this distance. All resin canals were external, and with a few exceptions were adjacent to the abaxial face of the needle. Resin canal diameters were measured perpendicular to the abaxial surface. Measurements were made between the outer walls of the epithelial cells, since the cells in this layer were highly variable in degree of flattening.

Other observations. Several unnoticed or poorly documented characteristics of the *Balfourianae* pines were observed in California stands of fox-tail and western bristlecone pines or in herbarium collections and stored seeds at the IFG. The materials available limited comparisons to major groups within the *Balfourianae*. Cone specific gravity was determined from volume, measured by water displacement, and oven-dry weight. Measurements were made of single cones from ten trees in each of the following groups: NFO (trees scattered throughout range); SFO (all but two trees in Onion Valley area); WBR (three trees in Utah, one in Nevada, two in Panamint Range, and four in White and Inyo Mountains); EBR (Arizona); and EBR (Colorado/New Mexico: two in New Mexico, eight in four Colorado stands). Seed-wing length was measured on four seeds from each of ten trees or mass collections in these groups: SFO and NFO combined (two SFO, eight NFO); WBR (three Utah, two Nevada, one Panamint Range, four White and Inyo Mountains); and EBR (seven Arizona, two Colorado, one New Mexico). De-winged, air-dried seeds in cold storage were weighed to the nearest milligram. Five seeds were measured from each of eight trees or collections in these groups: SFO and NFO combined (five SFO, three NFO); WBR (two Utah, six White Mountains); and EBR (Arizona); and four trees or collections of EBR (Colorado/New Mexico: three Colorado, one New Mexico). Tree means were analyzed for seed weight and wing length. Stratification requirements were determined for seed samples of EBR (Kenosha Pass, Colorado); WBR (White Mountains); and SFO (Onion Valley). Samples of 100 seeds received one of three treatments (stratification for 45 or 90 days, or no stratification). All seeds were placed in

germinators at the same time, and germination was observed for four weeks.

RESULTS

Reproductive Phenology and Capacity. Bristlecone and foxtail pines flower later in the season than any other North American pines. Flowering (pollen shedding and/or maximum opening of ovulate strobili) was observed in natural stands of these pines during 12 seasons between 1940 and 1974. All observations fall within a narrow range of calendar dates: a three-week period beginning in mid-July and peaking during the last week in July. Western bristlecone pine flowers later than *P. flexilis* in the White Mountains, and foxtail pine is later than *P. flexilis* or *P. albicaulis* at Onion Valley. In Tilden Park, near Berkeley, California, planted western bristlecone pines were observed in two seasons to flower in mid-June, about six weeks earlier than in their native habitat.

The *Balfourianae* pines flower at almost the same time. In four years of observations on successive days in stands of foxtail pine at Onion Valley and bristlecone pine in the White Mountains, we found no differences in flowering time, nor are there any indications of differences between eastern and western bristlecone pines. Pollen was collected in three Colorado stands between July 25 and August 2, 1970. In an Arizona stand at 2925 m, pollen was shed on July 22–27, 1969 (Schubert and Rietveld, 1970), and on July 31, 1975 (Rietveld, pers. comm., 1975). More generalized dates for pollen shedding in Arizona at 3050 m, based on three seasons of observations, were July 20 to August 20 (Pearson, 1931).

Northern foxtail pine has been observed to flower later than southern stands. In 1963 and 1964, observations were made 3–4 days apart at Onion Valley and North Yolla Bolly Mountain. In 1963 the North Yolla Bolly stand was estimated to be at least a week behind the Onion Valley stand, but in 1964 flowering was nearly simultaneous. Western bristlecone pine stands at different elevations have also been observed to peak a few days apart. With these exceptions, there appear to be no substantial differences in flowering time in the *Balfourianae*.

These pines are also uniform in the timing of cone-opening. Cones of foxtail pine at Onion Valley and western bristlecone pine in the White Mountains open in a two-week period starting about September 20. Arizona cones mature at about the same time. Schubert and Rietveld (1970) found that seeds of Arizona trees matured (were germinable) between September 24 and October 2, 1969, and the cones opened between September 27 and October 10. They concluded that cone maturation is not complete until just before the cones open, and our experience with western bristlecone pines confirms this. Some control-pollinated cones of White Mountains female parents, still closed when they were collected at the end of September, failed to open and had to be discarded.

In crosses where genetic barriers were not encountered, controlled pol-

ination of western bristlecone pine produced more sound seeds per cone than wind pollination. Wind-pollinated cones from the bristlecone female parents yielded a mean of 38 sound seeds per cone (range 25–65)—close to the 36 sound seed per open-pollinated cone reported for Arizona trees (Schubert and Rietveld, 1970). Cones from controlled pollination of the White Mountains trees averaged about 30% more sound seed than wind-pollinated cones, and the most successful controlled crosses on individual parents averaged 70 sound seed per cone. A potential source of bias in these comparisons is the possibility that seed extraction was more complete for control-pollinated cones.

The reproductive capacity of bristlecone and foxtail cones can be conservatively estimated from these seed yields, together with available data on numbers of cone scales. Bailey (1970) estimated that western bristlecone pine averages 117 scales per cone. Since the most successful controlled crosses produced 70 sound seeds, a minimum of 30% of the scales can bear two sound seeds. Foxtail pine, averaging only 81 scales per cone (Bailey, 1970; Mastrogriuseppe, 1972), had a mean of 58 sound seed per cone in the most productive controlled crosses. Thus at least 35 or 36% of its cone scales can bear two sound seeds. Wind pollination utilizes only a fraction of this potential reproductive capacity—54% in our bristlecone pine female parents. Comparable data are not available for the foxtail female parents, but a few estimates of sound seed per cone in bulk collections suggest that about 40% of the cone's reproductive capacity is exploited by wind pollination.

Crossing the Balfourianae with other Pines. Like most other subsections of *Pinus* (Critchfield, 1975), the *Balfourianae* appear to be isolated from the rest of the genus by genetic barriers. With one doubtful exception, our limited attempts to cross them with members of other white pine groups failed (Table 1). Representatives of other groups include *P. monticola* and *P. flexilis* (subsection *Strobi*), *P. monophylla* (*Cembroides*), and *P. bungeana* (*Gerardianae*).

In two instances there were indications of strong barriers acting early in the reproductive process. *Pinus balfouriana* x *P. bungeana* and the species-reciprocal cross yielded very few hollow seeds in either direction (Table 1). Since seed coats form early in the second season of cone development, at about the time of fertilization, this drastic reduction in hollow seeds indicates a developmental breakdown before fertilization. Another suggestion of early barriers is the abortion of all *P. flexilis* strobili pollinated with *P. balfouriana* pollen, but here a genetic interpretation is confounded by pollen age (Table 1).

Of the crosses summarized in Table 1, only *P. balfouriana* x *P. flexilis* produced any sound seed. Two *P. balfouriana* parents crossed with the same *P. flexilis* pollen parent produced a total of three sound seeds with normal embryos (determined by X-ray radiographs). Only one of the

three seeds germinated. In its germination time and three-year height the seedling was similar to sibling *P. balfouriana* seedlings in adjacent nursery rows, but before its non-hybrid identity could be firmly established the seedling was accidentally destroyed.

Crossing Northern and Southern Foxtail Pines. Northern and southern stands of foxtail pine were almost fully crossable (Table 2). Onion Valley females pollinated with North Yolla Bolly pollen produced 84% as much sound seed as they did in combination with Onion Valley males. The reduction in seed yield was not statistically significant (four pairs, $0.20 > p > 0.10$) but it was consistent among female parents. Individual trees averaged 5–27% less sound seed in hybrid combinations than in control crosses.

The hybrid seedlings did not differ appreciably in size or other features from their non-hybrid siblings in the nursery, and it is doubtful whether they will be morphologically identifiable as hybrids until they reach reproductive maturity.

Crossing Foxtail and Western Bristlecone Pines. White Mountains bristlecone pine and Onion Valley foxtail pine were fully compatible in both directions (Table 3). Foxtail pine females produced nearly identical

TABLE 2. CROSSES BETWEEN SOUTHERN AND NORTHERN FOXTAIL PINES.
(See Table 1 for legend.)

Female parent	Male Parent	
	balfouriana (northern)	balfouriana (southern)
balfouriana (southern)	12 (12)/ 12 99: 84 43.4/ 55.4	7 (7)/ 7 63: 87 51.6/ 60.6

TABLE 3. CROSSES BETWEEN FOXTAIL AND WESTERN BRISTLECONE PINES.
(See Table 1 for legend.)

Female parent	Male parent		
	balfouriana (northern)	balfouriana (southern)	longaeva
longaeva	1 (1)/ 1 1/ 7: 43 5.3/ 21	23 (23)/ 23 191: 54 52.4/ 68.5	13 (13)/ 13 2/ 96: 65 36.9/ 47.3
balfouriana (southern)		7 (7)/ 7 63: 87 51.6/ 60.6	3 (3)/ 3 31: 90 52.9/ 65.4

1/ pollen frozen for 2 years

2/ fresh pollen used in 1971

amounts of seed in combination with bristlecone pines and with other foxtail pines (52.9 and 51.6 sound seeds per cone). And bristlecone females produced more seed when foxtail pine was the pollen parent than they did in control crosses with other bristlecone pines (52.4 and 36.9 sound seeds per cone). Single-tree combinations ranged from 40–66 sound seeds per cone in SFO x WBR crosses, and 4–116 in WBR x SFO crosses.

Although the reduced seed yield of crosses within bristlecone pine was not significant (eight comparisons, $0.10 > p > 0.05$), it was consistent in both breeding seasons. Within-bristlecone crosses averaged 40.6 and 30.9 sound seeds per cone in 1963 and 1971, compared to 49.1 and 57.8 seeds from crosses with foxtail pine. The 1971 reduction may have been due to the use of slightly abnormal bristlecone pine pollen, which formed enlarged pollen tubes when it was germinated in the laboratory, but the 1963 difference is unaccounted for. This anomalous reduction cannot be attributed to inbreeding between closely related neighbors: in both seasons the bristlecone pollen was collected in stands 1–2 km from the female parents.

A single cross between northern foxtail pine and western bristlecone pine yielded fewer sound seeds (5.3 per cone) than other crosses on the same bristlecone female (x foxtail pines: 34.8 seeds; x other bristlecone pines: 25.8). Although the foxtail pollen had been frozen for two years and its viability may have been slightly reduced, this cross provides the only suggestion in our data that the crossing behavior of northern and southern foxtail pines may differ in combination with western bristlecone pine.

By the end of three growing seasons most of the hybrids could be distinguished from their western bristlecone siblings. At this age most bristlecone pines (both western and eastern) resembled cushion plants, lacking an emergent leader. If a leader was present it usually lacked a definite terminal bud, and the stem was mostly concealed by branches, fascicles, and closely appressed primary leaves. The slightly taller hybrids and much taller foxtail pines of the same age nearly all had emergent leaders, and much of the stem was visible. This was due partly to fewer primary leaves on the third-season stems and partly to slightly longer internodes—1.2–1.5 mm compared to 1.0 mm for bristlecone seedlings. Many hybrids and nearly all foxtail pines had well-developed terminal buds at this stage.

Crossing Eastern and Western Bristlecone Pines. Unlike the combinations described above, crosses between California and Colorado bristlecone pines were relatively unsuccessful. Although 19 or 20 combinations produced filled seeds, the mean of all 20 crosses was only 6.1 filled seeds per cone, and the maximum for a single cross was 18.7 seeds. The filled seeds were routinely X-rayed before most of them were planted in the

nursery. The rest were later germinated in petri dishes. Germination was low in the nursery and only slightly higher in the laboratory. A total of 69 seeds from 11 crosses germinated. The mean number of germinated seeds per cone for all hybrid crosses was 1.6, compared to 19.8 for non-hybrid combinations (Table 4)—a crossability of 8%. This could be an overestimate because of the possibly abnormal pollen used in the non-hybrid crosses (see above); 4–6% may be a more realistic estimate of crossability.

X-ray radiographs of the seeds provided an explanation of the low germination. Most "filled" seeds contained fully developed or slightly shrunken female gametophytes, with well-defined embryo cavities extending almost the full length of the gametophyte. Nearly one-fifth of the filled seeds had empty cavities, another one-fifth contained embryo-like objects too small to identify with certainty, and the others contained identifiable embryos. Most of these embryos were smaller than the embryos of germinable seeds from control crosses, which ranged in size from about two-thirds to the full length of the embryo cavity. Although the hybrid seeds were not handled individually, a few that germinated must have had embryos as small as 35–40% of the length of the cavity. But the great majority of germinable seeds from hybrid combinations had embryos from 45–65% of the cavity length. Embryos of this size showed about 80% germination.

Nine of the 20 hybrid combinations failed to produce any germinable seeds. All but one produced filled seeds, but the embryos were mostly vestigial, with a few ranging in length up to a third of the embryo cavity. Only two of the nine failed crosses (both on the same female parent) produced ungerminable embryos in the 45–65% size range.

Third-year hybrid seedlings resembled unrelated Colorado and Arizona seedlings more closely than their western bristlecone siblings, although all of the seedlings were similar in size and appearance. The

TABLE 4. CROSSES BETWEEN WESTERN AND EASTERN BRISTLECONE PINES.

Female parent	Male parent	
	aristata	longaeva
longaeva	20 (20) / 11	5 (5) / 4
	113: 37	14: 79
	1.6 / 49.6	19.8 / 35.1

Number of attempts.....	0 (0) / 0	Number of tree x tree combinations
Number of female strobili.....	0, 0	Number of attempts producing germinable seed
Mean number of germinable seeds per cone.....	0.0 / 0.0	Percent of strobili producing cones
		Mean total number of seeds per cone

needles of the hybrids were more like those of eastern seedlings in having grooves overlying the resin canals and in the small size of the canals. The needles of western seedlings, like those of older western trees, lacked grooves. The mean diameter of resin canals in western, eastern, and hybrid seedlings was 0.138, 0.085, and 0.105 mm.

The expression of other needle characteristics was quite different in the seedlings than in older trees, as it often is in other pines. Two of the needle characteristics that Bailey (1970) found most useful in distinguishing *P. aristata* and *P. longaeva*—resin flecks and number of resin canals—were much more similar in seedlings than in the older trees he observed in natural stands.

Seedlings from (a) Colorado, (b) Arizona, and (c) the White Mountains had resin flecks on (a) 61, (b) 58, and (c) 27% of their needles. Corresponding percentages in the older trees that Bailey sampled were (a) 92–94, (b) 47–62, and (c) 3. Only 9% of the hybrid needles had flecks—fewer than either parental group of seedlings.

Eastern and western bristlecone pine seedlings were also more similar in resin canal number than the older trees that Bailey sampled in natural stands. Only 3% of the needles of eastern (and hybrid) seedlings had single canals extending the length of the needle, compared to 49% in older trees. Arizona and Colorado seedlings had single resin canals in the apical quarter of only 20 and 8% of their needles, compared to 84 and 87% in older trees (Bailey, 1970).

The presence of three or four resin canals per needle was fairly common in eastern bristlecone seedlings and occasional in hybrids, but none of the needles of western bristlecone or foxtail pine seedlings had more than two canals. Only 3 and 5% of Colorado and Arizona seedling needles had three canals near the tip, but near the base the frequency of three or more canals was 30 and 48%. The mean number of canals in Colorado and Arizona seedlings increased from 2.0 and 1.8 per needle near the tip to 2.3 and 2.6 near the base. Four resin canals—the highest number observed—were present in 15% of the needles of eastern seedlings. Where three or four canals were present, they were crowded together and often shared grooves. In seedlings, at least, the number of resin canals would be underestimated by counts of the grooves.

Variation in Morphological and Other Characteristics. Closer attention to the *Balfourianae* pines in recent years has turned up an array of differences that merit more detailed study:

Tree form—Most old foxtail pines have single, erect stems even under severe timberline conditions (Arno, 1966), although exceptions have been noted on dry sites (Bailey, 1970). Old bristlecone pines in the desert ranges of California have what Arno described as an “ungainly, weedy” form, usually twisted and multi-stemmed. To what extent this multi-stemmed form is characteristic of old bristlecone pines farther east is uncertain, although it is present in Colorado (Bailey, 1970; Fig. 1).

Bark—Young trees of eastern bristlecone pine differ from the other *Balfourianae* pines in having the smooth, blistered cortical bark and delayed onset of periderm formation that is characteristic of the firs and many white pines (Zavarin and Snajberk, 1973; Zavarin et al., 1976). Old foxtail pines in southern stands have thick, reddish-brown bark in squarish plates, a combination that has not been observed in other *Balfourianae* pines (Bailey, 1970; Mastrogioseppe, 1972).

Foliage—A vegetative difference noted by LeRoy C. Johnson (pers. comm., 1974) distinguishes living trees, including seedlings. The needles on a foxtail pine twig are painfully sharp to the touch; those of eastern and western bristlecone pines are not. This difference, difficult to quantify but useful in identification, appears to be due partly to sharper points but primarily to the greater stiffness of foxtail pine needles.

Cones—Eastern and western bristlecone pine cones project abruptly from the branch, and their straight axes form right or acute angles with the distal part of the branch. The cones of foxtail pine are pendent to varying degrees. This visually striking difference in orientation is due primarily to a difference in peduncle length. The peduncles of mature bristlecone pine cones in the IFG herbarium were usually flush with the basal cone scales, but occasionally extended beyond them a maximum of 4–5 mm. Intact peduncles of foxtail pine cones in the herbarium were 7–16 mm long, and several trees growing at Onion Valley had cone peduncles 14–16 mm long. An associated feature is the curved axis of 21–23% of foxtail pine cones (Mastrogioseppe, 1972).

The cones of foxtail pine have somewhat “fleshy” apophyses (Bailey, 1970; Mastrogioseppe, 1972), and are softer and more fragile than cones of the bristlecone pines. This difference can be expressed quantitatively in terms of cone specific gravity. Northern and southern foxtail pine cones did not differ significantly in specific gravity, nor did the western, Colorado–New Mexico, and Arizona samples of bristlecone pine. But the combined data showed a significant difference ($0.05 > p > 0.01$) between the less dense cones of foxtail pine (mean specific gravity 0.45) and the cones of the bristlecone pines (mean 0.49).

Seeds—Our data confirm Uyeki's (1927) observation that foxtail pine seeds have longer wings than seeds of bristlecone pine. His measurements of a few seeds of unspecified origins showed ranges of 18–20 mm and 8–11 mm for the two pines. In our samples, wing length was almost identical in eastern and western bristlecone pines: the means were 10.7 and 10.0 mm, and ranges were 7–15 mm in both samples. Seeds of foxtail pine have much longer wings (mean 17.2 mm), and the differences between it and the bristlecone pines were highly significant ($p = < 0.01$). Foxtail pine's range in wing length (13–24 mm) just overlapped that of the bristlecone pines. In disagreement with these data is Mastrogioseppe's (1972) observation that southern foxtail pine has rather short seed wings. His large samples of seeds from single localities of northern (Lake

Mountain) and southern (Timber Gap) foxtail pines had mean wing lengths of 17.4 and 11.5 mm, a highly significant difference. Timber Gap may be poorly representative of southern stands, however. The two southern foxtail pines in our sample, both collected by Mastrogioseppe (XIV-7, Onion Valley, and XI-5, Silliman Crest), had average (17.5 mm) or long (23.3 mm) seed wings—the latter the longest wings of any tree in the sample.

The *Balfourianae* pines were much more variable in seed weight. Foxtail and Arizona bristlecone pines had the heaviest seeds, with means and ranges of 25.4 mg (13–39) and 26.4 mg (18–38). The small sample of Colorado–New Mexico trees had somewhat lighter seeds (mean 20.8 mg, range 11–25), the difference between it and the Arizona sample approaching statistical significance ($0.10 > p > 0.05$). Western bristlecone pine seeds were by far the lightest—less than half the weight of the others (mean 8.8 mg, range 6–15). The differences between this and the other samples were highly significant ($p = < 0.01$).

A difference in seed color has been noted by Zavarin et al. (1976), eastern bristlecone pines having darker seed coats than western trees.

Stratification requirement—Bristlecone pine seeds germinate promptly without stratification, but the germination of foxtail pine seed—like that of most other white pines—is slow and incomplete without this pretreatment (Forest Service, 1974). Unstratified seed of Arizona bristlecone pine showed 75% germination within eight days (Schubert and Rietveld, 1970); untreated Colorado seed germinated 75–80% in 4–10 days (Reid, 1972); and untreated White Mountains seed showed 90% germination within six days (Wright, 1963). In foxtail pine, Mastrogioseppe (1972) obtained only 29–63 and 50–55% germination in three-month tests of unstratified seed from northern and southern stands. In germination tests at the IFG, 45 or 90 days of stratification reduced mean germination time of Colorado and White Mountains seed by 2–3 days (unstratified: 11.3 and 7.9 days; stratified: 8.3–8.4 and 5.5–5.8 days), but did not increase the amount of germination. The same pretreatments increased the germination of Onion Valley foxtail pine seed from 41 to 100% and reduced mean germination time by 8–9 days (unstratified: 11.9 days; stratified: 2.6–3.5 days).

DISCUSSION

The ability of pines to hybridize is generally restricted to taxa that are considered to be related on other grounds. Within groups linked by the ability to hybridize, however, the degree of crossability is sometimes highly discordant with other evidence of relationship. Among the pines, *P. muricata* (bishop pine) provides the closest parallel to the *Balfourianae* in this respect. The three races of this coastal Californian species—northern, central, and southern—exhibit little correspondence between crossability and other indicators of relationship (Critchfield, 1967). The

northern race differs morphologically from the other two, and all three are chemically distinct. These distinctions are nearly absolute except in the narrow zone where the northern and central races meet. Crossability is complete between northern and central races (the only two in contact), low between central and southern races, and close to zero between northern and southern races.

Among the *Balfourianae*, the crossing behavior of northern and southern foxtail pines is an exception. Their crossability of 84% is fairly typical of segments of a species that have diverged sufficiently to warrant taxonomic recognition. In *P. ponderosa*, the western (var. *ponderosa*) and eastern (var. *scopulorum*) races had an average crossability of 52% in small-scale reciprocal tests (Krugman, 1970). The coastal and Sierra Nevada races of *P. contorta*, usually given subspecific or varietal status but still occasionally considered two species (*P. contorta*, *P. murrayana*), has a crossability of 93% in an extensive series of reciprocal crosses (unpublished data, IFG).

No macrofossils of foxtail pine have been described from the Tertiary, although Axelrod (1976) noted that a fossil pine in the Thunder Mountain, Idaho, flora (Eocene age) resembles this species. Pollen identified as *P. balfouriana* was present in several pollen floras in and near the southern Sierra Nevada during the first recorded Pleistocene glaciation (Axelrod and Ting, 1961), but one problem with this identification is the assumption that the dimensions of the pollen have remained constant since then. Foxtail pine cones have recently been found near Clear Lake, California, in late Pleistocene (probably Illinoian) deposits (J. Wolfe, pers. comm., 1977). At this site, more than 100 km south of and more than 1000 m below the present northern distribution of foxtail pine, the abundance of cones suggests that this species was a major component of a high-elevation mixed-conifer forest.

Mirov (1967), Bailey (1970), and Mastrogriuseppe (1972) have speculated—apparently on the basis of geological history—that the northern and southern stands of foxtail pine have been separated since the end of the Tertiary or the early Pleistocene. If so, the accumulation of genes influencing crossing ability has been very slow in the two or three million years these groups have been isolated from each other. However, the recent Clear Lake find raises the possibility of contact between them as recently as one of the last major glacial episodes of the Pleistocene.

The most striking instance of discordance between genetic and other evidence of relationships in the *Balfourianae* is the complete crossability of western bristlecone and southern foxtail pines. This combination is also by far the best supported by crossing data; it is the only combination made in both directions and in more than one season. This level of crossability is remarkably high for pine species, although it is approached by the 69–85% crossability of the California closed-cone pines *P. attenuata*

and *P. radiata* (Critchfield, 1967) and a few other less fully investigated combinations.

Although genetic barriers to interbreeding are nonexistent in the White Mountains and Onion Valley stands, the morphological and other differences between foxtail and western bristlecone pines are of the same magnitude as those distinguishing most other closely related pine species. Two cone characteristics have traditionally been emphasized: foxtail cones have minute mucros but lack conspicuous bristles, and have fewer scales—about 80–81, compared to 117 for western bristlecone pine. But they also differ in peduncle length and the complex of associated characters (cone orientation, curvature), cone density, seed weight, seed-wing length, stratification requirement, foliage stiffness, and probably tree form.

The morphological distinctions between eastern and western bristlecone pines are less conspicuous, with the notable exception of the resin flecks on the needles. The principal differences between the two are in the complex of characters associated with the needle resin canals and in resin composition, but they also differ in cone color, bristle length, seed weight and color, bark, and other features noted by Bailey (1970). These taxa are also far less crossable than foxtail and western bristlecone pines, with a crossability of only a few percent, estimated from crosses made in one direction in a single season. A smaller number of crosses made by Bailey in the opposite direction in the same season were even less successful (Zavarin et al., 1976).

The reproductive barriers between the two bristlecone pines resemble those of many other white pine combinations in acting mainly or entirely after fertilization (Kriebel, 1975). The seed coats, which form at about the time of fertilization, showed no reduction in numbers in western x eastern combinations (Table 4). Post-fertilization barriers are also indicated by the many seeds with fully developed female gametophytes and embryo cavities, but lacking embryos or with very small embryos. Embryo cavities form after fertilization (Sarvas, 1962), and the female gametophyte usually degenerates rapidly after the death of the last developing embryo (Sarvas, 1962; Plym Forshell, 1974). Mature or nearly mature seeds with empty embryo cavities have been reported in only a few instances: in *P. sylvestris* by Ehrenberg et al. (1955), Sarvas (1962), and Plym Forshell (1974); and in the cross *P. jeffreyi* x *P. coulteri* by Krugman (1970). These authors interpreted such seeds as indicators of breakdown at a relatively late stage of embryo development. Another unusual feature of the cross between western and eastern bristlecone pines was the germination of seeds with embryos less than half the length of the embryo cavity. This has also been reported in *P. sylvestris* (Ehrenberg et al., 1955).

The *Balfourianae* group is an old lineage in western North America. Macrofossils resembling contemporary bristlecone pines have been found

in Eocene and Oligocene deposits in Nevada, New Mexico, Colorado, and Montana. Most of these fossils have been described under the name *P. crossii* Knowlton.

The oldest *P. crossii* fossils were part of the Eocene Copper Basin flora of northern Nevada (Axelrod, 1966), which has an estimated age of about 40 million years. Copper Basin is 120 km north of the present northern limits of western bristlecone pine. The five-needled fascicle illustrated by Axelrod (Plate 6, Fig. 6) has needles only 13 mm long—much shorter than most needles of contemporary *Balfourianae*. Needles this short were not observed on specimens of eastern bristlecone pine and were very rare on foxtail pine, but bristlecone pines in the White Mountains occasionally produce fascicles of this size on slow-growing branches. I have examined the Copper Basin fascicle (University of California Museum of Paleontology hypotype 8873), and one of the needles shows two linear impressions suggestive of resin canals extending the length of the needle.

According to Axelrod (1976), a mid-Oligocene flora at Hillsboro, New Mexico, was dominated by a pine that closely resembles contemporary *P. aristata* in its cones and fascicles. The nearby Hermosa flora, also mid-Oligocene, consists mostly of a “five-needled pine allied (distantly) to *P. aristata*.”

A pine resembling *P. aristata* is also abundantly represented in a fossil flora deposited near Creede, Colorado, near the end of the Oligocene, about 27 million years ago. This pine (*P. crossii*) is represented by a conelet, seed wing, and many needle impressions (Fig. 34–37, Bailey, 1970). Bailey and earlier authors consider it nearly identical with *P. aristata*, which grows nearby today.

A single fascicle that can probably be assigned to *P. crossii* was found in late Oligocene deposits in the Ruby Basin of southwestern Montana (Becker, 1961). It consists of three needles and the base of a fourth. These needles are also shorter (16–17 mm) than those of contemporary *Balfourianae* pines.

Impressed by the characteristics shared by foxtail and western bristlecone pines and by the similarity of the Creede fossils to eastern bristlecone pine, Bailey (1970) proposed a polyphyletic origin for the bristlecone pines. According to this scheme, two lineages arose in northwestern North America at the start of the Tertiary or earlier, and migrated south into the Rocky Mountain and Pacific regions. The eastern line—substantially unchanged since at least the late Oligocene (Creede flora)—is modern *P. aristata*, and the Arizona population is a Pleistocene offshoot. The western line resembled *P. balfouriana*. It gave rise to *P. longaeva* at the end of the Tertiary and beginning of the Pleistocene as the major uplift of the Sierra Nevada produced increasing aridity in the Great Basin.

A principal reason for rejecting Bailey's polyphyletic hypothesis is the

burden it places on convergent evolution, which must account for all of the diverse ways in which eastern and western bristlecone pines resemble each other and differ from foxtail pine. These include, in addition to cone-scale number and bristles, several characteristics noted above—peduncle; orientation and density of the cone; seed wings; absence of a stratification requirement; foliage stiffness; and perhaps tree form. Nor does recent chemical evidence (Zavarin et al., 1976)—particularly the chemical intermediacy of Arizona stands and the similarity of Sentinel Peak to Colorado–New Mexico trees—support the view that western bristlecone pine evolved from a foxtail-pine-like ancestor.

The tendency of western bristlecone pine to resemble foxtail pine in some respects (needle resin canals, chemistry) and eastern bristlecone pine in others can be explained more simply in other ways. Western bristlecone pine could be the product of ancient hybridization between eastern (*P. aristata*) and western (*P. balfouriana*) ancestral lines. One difficulty with this hypothesis is that western bristlecone pine is not intermediate in most respects (bristle length is an exception). Instead, it closely resembles either eastern bristlecone pine or foxtail pine. And in a few characteristics it resembles neither (cone-color polymorphism, seed size, great longevity). A more critical objection is the asymmetrical crossing behavior of western bristlecone pine in combination with its presumed parents. The hypothesis of a hybrid origin is difficult to reconcile with the complete absence of crossing barriers on the one hand (*P. balfouriana*) and the presence of strong barriers to interbreeding on the other (*P. aristata*).

An alternative—and more acceptable—hypothesis is that all of the elements of the *Balfourianae* are segregates of a single ancestral line most closely resembling western bristlecone pine (Eocene Copper Basin fossil). By the middle or late Oligocene a Rocky Mountain lineage split off and developed some of the distinctive attributes of contemporary eastern bristlecone pine (Hillsboro and Creede floras). Arizona bristlecone pine could have arisen as a very early offshoot of this lineage, or it could be (as Zavarin et al., 1976, suggested) the product of later contact between eastern and western bristlecone pines. It is uncertain when foxtail pine originated from the western bristlecone pine lineage, although separation must have been complete before the late Pleistocene (Clear Lake fossils). Foxtail pine has undergone pervasive changes in morphological and other characteristics, but divergence has apparently been too recent for the accumulation of genes influencing its ability to cross with the closest modern equivalent of its ancestor.

The crossing data summarized in this paper are incomplete in many respects. The exploratory hybridization of the *Balfourianae* should be extended to include Arizona *P. aristata*, *P. longaeva* of the Panamint Range, the untried combination of *P. balfouriana* and *P. aristata*, and more combinations of *P. balfouriana* and *P. longaeva*. Crosses between

P. aristata and *P. longaeva* also need to be repeated and extended, but our preliminary finding of very low crossability between these taxa provides tentative support for Bailey's (1970) proposal to recognize them as species.

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