

Natural Hybridization between Russian Stone Pine (*Pinus sibirica*) and Japanese Stone Pine (*Pinus pumila*)

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Abstract—A study was conducted on phenology and reproductive characteristics of Siberian stone pine (*Pinus sibirica*), Japanese stone pine (*Pinus pumila*), and their putative hybrids in high altitudes. The study demonstrated that the species were not reproductively isolated, and putative hybrids were identified. Seed development and production were very poor in the putative hybrids indicating that introgression is very slowly or not occurring beyond the first filial generation.

Key words: *Pinus sibirica*, *Pinus pumila*, stone pines, reproductive biology, natural hybridization

Introduction

In the Baikal region of Siberia, the natural ranges of Siberian stone pine (*Pinus sibirica* Du Tour) and Japanese stone pine (*P. pumila* Regel) overlap. Siberian stone pine grows to become a large tree and occurs in pure stands between 600 and 1,600 m in elevation but becomes increasingly scarce as altitude increases. Japanese stone pine generally grows as a shrub (1 to 4 m high) that can generate adventitious roots. A single individual can often form vast clones, up to 50 m in diameter. Japanese stone pine is an occasional understory species or occurs in pure populations on steep, stony slopes at altitudes between 1,000 and 1,600 m. The occurrence of this species in forest composition increases with altitude. At altitudes of 1,600 to 2,000 m, it forms a thick brushwood. At altitudes of 2,000 to 2,100 m (timberline), Japanese stone pine and dwarfed, sterile Siberian stone pine occur together.

The occurrence of natural hybridization between these species has been the subject of debate since putative hybrids were described 70 years ago (Pozdnjakov 1952; Galazij 1954; Molodjnikov 1975). Although natural hybridization has been refuted in many recent reviews (Bobrov 1978; Lanner 1990; Homentovski 1995), individual trees were recently found north of Khamar-Daban (15 km southwest of Baikal), which

were intermediate between the species in a number of traits including: form, growth, needle and shoot structure, and cone color (Goroshkevich 1999). The purpose of this research was to determine where this natural hybridization occurs and to study reproductive characteristics and seed production in these two pine species and their putative hybrids.

Material and Methods

Samples from trees were collected north of Khamar-Daban, 30 to 35 km southwest of Baikal, where Siberian stone and Japanese stone pines can be found growing together. Putative hybrids are occasionally found in intermixed populations. An area of approximately 5 by 5 km in the region of Cherskij peak, Serdze Lake, and the Podkomarnaja riverhead was inspected for putative hybrids. Putative hybrids were distinguished by a combination of characteristics from the parental species: multiple crooks in the stem (Japanese stone pine characteristic) and the violet color of 2-year-old cones (Siberian stone pine characteristic).

Studies on reproductive characteristics were conducted in populations at an altitude of approximately 1,500 m from June 30 to July 10, 1998, according to methodology of Titov (1982) and Nekrasova (1983). At this altitude, Siberian stone pine was common (approximately 200 cone-bearing trees per ha), but Japanese stone pine was less frequently encountered (approximately 10 clones per ha). In development of pollen cones and pollen dispersal, successive stages were distinguished as follows: (1) initiation of pollen shedding, (2) abundant pollen shedding, and (3) residual pollen shedding. In development of the female cone, successive stages of receptivity for pollination were distinguished by changes in the ovuliferous scales as follows: (1) onset of receptivity, (2) optimal receptivity, and (3) declining receptivity.

Cone samples were made from 10 Siberian stone pines, 10 Japanese stone pines, and 10 putative hybrids. Samples of cones (10 per individual) were collected at the late August 1998 from 106 individuals (27 Siberian stone pines, 59 Japanese stone pines, and 20 putative hybrids). Seed quality was studied by X-ray photography. Different characteristics were assessed including: cone length (cm), cone width (cm), average number of scales/cone, average number of initial ovules/cone, ovules/cone lost in the early stages of development (percentage), average number of seeds/cone, poorly developed seeds/cone (percentage), average number of filled seeds/cone, hollow seeds/cone (percentage), average number of seeds/cone with endosperm, seeds/cone with imperfect endosperm (percentage), average number of seeds/cone with

In: Snieszko, Richard A.; Samman, Safiya; Schlarbaum, Scott E.; Kriebel, Howard B., eds. 2004. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance; 2001 July 23–27; Medford, OR, USA. IUFRO Working Party 2.02.15. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

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perfect embryos, seeds/cone without embryos (percentage), average number of seeds/cone with embryo, seeds/cone with differentiated embryos (percentage), average number of seeds/cone with differentiated embryos, seeds/cones with undifferentiated embryos (percentage), and average weight of a single seed with complete endosperm (mg).

Significant differences ($p < 0.05$) among means were determined using an analysis of variance (ANOVA), and mean separation was by Duncan's multiple range test (Steel and Torrie 1980).

Results

The phenology studies indicated that putative hybrids were occasionally found in populations below 1,990 m in elevation (one clone per ha). In contrast, the number of hybrids increased with elevation with a density of two to three clones per ha found above 1,900 m in elevation. The observations on reproductive characteristics showed that timing of reproductive maturation of the two species and putative hybrids practically coincide. Reproductive maturation generally occurred during a 2 to 3 week period, which precludes reproductive isolation of either species, given that hybridization is possible.

The results of cones and seeds morphological analysis are presented in table 1. The seed analysis showed that Japanese stone pine was fertile up timberline, and Siberian stone pine became increasingly infertile above altitude of 1,750 m. Siberian stone pine was significantly different from Japanese stone pine in most of the cone and seed traits studied. Putative hybrids were found to differ from either Siberian pine or both of the parental species in all characteristics, except for the initial number of ovules. Putative hybrids had intermediate values between both parents for cone length, cone diameter, average number of scales per cone, and

average weight of seed. For characteristics related to seed quality (with the exception of seed weight), the putative hybrids had significantly higher poorly developed seeds/cone (percentage), hollow seeds/cone (percentage), and seeds/cone without embryos (percentage), and significantly lower seeds/cone with differentiated embryos (percentage), when compared to both parents. Differences of putative hybrids only from Siberian pine were apparent in a number of characteristics.

Discussion

It is known that taxonomically close pine species can be often crossed to form fertile hybrids (Critchfield and Little 1966) and that species with sympatric ranges can form hybrid swarms (Wright 1975). Siberian and Japanese stone pines belong to the same *Pinus* subsection (*Cembrae* Lond.), but their natural distribution overlaps only by 5 to 10 percent. This study shows that no reproductive isolation between the species occurs due to the timing of pollen flow and female cone receptivity, and occasionally morphologically intermediate individuals occur, that is, putative hybrids. Further introgression, however, does not appear to be rapidly occurring, if at all. Production of viable seed in the putative hybrids was proportionally lower than in the parental species. Additionally, the number of seed/cone in the putative hybrids was very low (on average one filled seed per cone), thereby indicating little introgression between these species beyond the first filial generation. Future studies using various combinations of controlled pollinations between Siberian and Japanese stone pines with corresponding genetic analyses could further corroborate the origin of the naturally occurring hybrids and better delineate limitations for introgression between these two species.

Table 1—Cones and seed characteristics in Siberian stone pine, Japanese stone pine, and putative hybrids.

Characteristic	Siberian stone pine	Putative hybrid	Japanese stone pine
Cone length (cm)	5.0a ¹	4.0b	3.0c
Cone diameter (cm)	4.3a	3.0b	2.1c
Average number of scales/cone	73.6a	55.9b	37.6c
Average number of initial ovules/cone	68.3a	57.0ab	35.9b
Ovules/cone lost in the early stages of development (%)	15.5a	32.2b	43.9b
Average seed number/cone	58.3a	38.6b	20.7b
Poorly developed seeds/cone (%)	14.6a	52.0b	27.9a
Average number of filled seeds/cone	49.5a	19.6b	14.1b
Hollow seeds/cone (%)	3.2a	24.6b	5.3a
Average number of seeds/cone with endosperm	48.0a	14.9b	13.5b
Seeds/cone with imperfect endosperm (%)	32.3a	79.2b	63.0b
Average number of seeds/cone with perfect endosperm	32.5a	3.6b	5.0b
Seeds/cone without embryos (%)	0.2a	48.5b	5.9a
Average number of seeds/cone with embryo	32.4a	1.6b	4.7b
Seeds/cone with undifferentiated embryo (%)	5.8a	35.3b	17.8b
Average number of seeds/cone with differentiated embryo	30.5a	1.0b	3.9b
Seeds/cone with differentiated embryo (%)	44.7a	1.8c	10.9b
Average weight of a single seed with complete endosperm (mg)	216.2a	142.4b	72.3c

¹Significant differences ($p < 0.05$) between means were determined using Duncan's multiple range test; values with different letters within a line differ significantly.

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