

Cone Sex Ratio and Pollen Traits in *Pinus densiflora* (Pinaceae)

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The pattern of variations in cone sex ratio and pollen traits such as pollen number and size was examined for two years in populations of red pines in Korea. We evaluated; (1) the relationship between tree size and cone sex ratio, (2) the relationship between year and cone sex ratio, and (3) the relationship between cone sex ratio and pollen traits. Larger trees in height and circumference at breast height produced more male as well as female cones. However, cone sex ratio was independent of these plant sizes. Across the two populations, both female cone numbers per tree and male cone numbers per new shoot increased 2.2 and 1.2 times, respectively, in 1999 compared to 1998, while the yearly pattern of male cone-bearing shoots per tree differed significantly between populations. Thus, cone sex ratio varied significantly between years in one of the two populations. Pollen traits such as the number of pollen grains and diameter did not vary significantly among populations. Pollen diameter and grain numbers per male cone were significantly negatively correlated with the cone sex ratio in two populations and one population, respectively. Trees which stopped female cone production in the first year and those which produced female cones in both years differed in the cone sex ratio and pollen size in the second year. The long duration of one reproductive episode and the potential of masting in red pines emphasize the need to conduct long-term studies on the dynamics of cone production, cone sex ratio, and simultaneous changes in pollen traits in red pines.

Higher allocation to male function is selected in anemophilous species than in entomophilous species because in the former male fitness increases linearly over a wide range of resource investments (Lloyd, 1984). In anemophilous outcrossed species, as the pollen dispersal distance increases with an increase in height, tall plants would invest more resources to male function than short ones (Burd and Allen, 1988). Resource investment to female function occurs during seed production after fertilization in most plants. If so, it is expected that the floral sex ratio of anemophilous, outcrossed species with unisexual flowers deviates from a 1:1 ratio (male to female flowers), and the floral sex ratio increases with plant height. Indeed, in several herbs, the proportion of male flowers increases with plant height (Ackerly and Jasienski, 1990; Lundholm and Aarssen, 1994; but see Dazos and Sandmeier, 1997). Four of the seven anemophilous, monoecious woody species such as birch, beech, and pine species also show a correlated increase in the ratio of male to female inflorescences with tree height (Fox, 1993).

On the other hand, if female fitness increases more rapidly than male fitness with an increase in resources (Charnov, 1982; Willson, 1983), sex ratios among individuals within populations or among populations may vary with environment or plant size reflecting the amount of resources available for reproduction. For example, among dioecious or sex-changing species, female plants are more abundant in habitats where light and water conditions are favorable than male plants (Willson, 1983; Freeman and Vitale, 1985; Dawson and Ehleringer, 1993). Even in hermaphroditic plants, larger plants function as females rather than as males (Devlin, 1988). Similarly, monoecious species such as oaks and junipers produce a higher number of female flowers in mesic habitats than in xeric habitats (Freeman et al., 1981).

Male or female cone production or cone sex ratio in pines appears to be more complex than in other species. In several pines male/female cone production varies greatly with climate, age, and growth form (Smith, 1981; Smith et al., 1988; Caron and Powell, 1989), resulting in yearly correlations in cone sex ratio changing among clones or populations (Schoen et al., 1986; Woodward et al., 1994). However, some other studies have shown that yearly cone sex ratios are positively correlated among clones or populations (Eis, 1967;

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Caron and Powell, 1989). Two aspects of the reproductive life history of pines may contribute to such complexity of cone production and cone sex ratio. In many pines, female function involving female cone bud formation (year^{-1}), pollination (year^0), and seed dispersal (year^{+1}) requires three years, and a large proportion of female allocation occurs after fertilization at year^{+1} . In contrast, male function lasts only until pollen shedding at year^0 . Reproductive periodicity is also well known in pines. *Pinus resinosa* and *P. banksiana* reproduce every two and 4-6 years, respectively, exhibiting good and bad years of seed production (Cremer, 1992; Houle and Filion, 1993). For pines with such a long temporal disparity of female vs. male allocation and reproductive periodicity, cone sex ratio predictions based on plant height and habitat quality may not be applicable. It is expected that many oaks and pines which demonstrate a strong pattern of reproductive periodicity exhibit a variable yearly relationship between cone or floral sex ratio and plant size or habitat quality. However, few studies have related the cone sex ratio to these two unique aspects of pine reproduction.

There is evidence that the male fitness of pines and related species is positively correlated with pollen production and pollen size. In *Picea mariana*, *P. glauca*, *Pinus contorta*, *Taxus canadensis*, and *Pseudotsuga menziesii*, seed production increases in a year with an increase in pollen production (Schoen and Stewart, 1986; Smith et al., 1988; Caron and Powell, 1989; Allison, 1990; Owens et al., 1991). Male fitness is also positively correlated with pollen size in one of two populations of *Pseudotsuga menziesii* (Nakamura and Wheeler, 1992). In pines, pollen production per tree is determined by a number of variables such as the number of pollen grains per male cone, the number of male cones per new shoot bearing male cones (male shoot), and the number of male shoots on the branches of the previous year. If a tradeoff relationship between pollen grain number and size exists as shown in a certain angiosperm (e.g., Stanton and Preston, 1986), a trade-off relationship is expected to occur between those male components. Furthermore, female and male cone production, or cone sex ratio may also affect male fitness by causing variations in pollen numbers and size. However, the relationship among cone sex ratio and male components such as male cone numbers, pollen grain numbers, and pollen size has rarely been simultaneously examined.

In this study, we examined the pattern of variation in male and female cone production for two years in three populations of *Pinus densiflora*; variations in pollen production and pollen size were also examined in the second year of the study. Specifically, we addressed the following questions: (1) is the cone sex ratio related to plant sizes such as height and circumference at breast height? (2) does the cone sex ratio vary between years and populations? and (3) is the cone sex ratio related to pollen production and pollen size?

Materials and Methods

Red pine (*Pinus densiflora* Sieb. et Zucc.) is a codominant tree species, together with oaks, in Korea. In Apr 1998, we tagged 33-35 red pines randomly selected from populations at Mt. Kwanak (KA) in Seoul ($37^{\circ} 26' \text{ N}$, $126^{\circ} 58' \text{ E}$), and at Yeosu (YJ) in Kyunggi-do ($37^{\circ} 17' \text{ N}$, $127^{\circ} 45' \text{ E}$). In May 1999, seven and eight individuals were added to KA and YJ, respectively, and 34 trees were randomly selected from an additional site, Hongneung (HN) in Seoul ($37^{\circ} 35' \text{ N}$, $127^{\circ} 03' \text{ E}$). These sites were mixed forests with *Pinus densiflora*, *P. rigida*, *Robinia pseudoacacia*, and various *Quercus* species. We measured both height and circumference at breast height of the tagged trees in 1998, while only circumference was measured in 1999. The number of male and female cones (strobili) was counted for the tagged trees at each site just before pollen shed in both years. All female cones on a tree were counted when they were relatively small in number. When female cones on individual trees were large in number, those cones on several branches of a tree were counted and multiplied by the total number of branches. A variable number of male cones were clustered near the base of a short shoot (male shoot), and one to several male shoots were clustered on the branches of the previous year. When male shoots were smaller in number, we directly counted the number of male shoots located throughout the tree; when the former was large in number, we counted the number of male shoots on a portion of the tree, e.g. one fourth of the canopy, and multiplied it by 4 to estimate the total number of male shoots per tree. We sampled at least five branches with male shoots. We tried to sample branches from various areas of the tree to reduce the potential effect of branch position on male shoots and male cones. In the lab, we counted the number of male cones per male shoot based on five to seven male shoots. Thus, we estimated the total number of male cones per tree by multiplying the number of male shoots per tree by the mean number of male cones per male shoot. We then estimated the cone sex ratio for each tree by dividing the number of male cones by that of female cones. The density of red pines in each population was also measured in 1999.

In 1999, ten cones were sampled from each tagged tree and were deposited into two scintillation vials (sets A and B). After a week of sampling, pollen grains in one set of vials (set A) were washed with 2% NaCl solution, and sonicated for 180 sec using an ultrasonicator (Branson 1210). The number and mean diameter of pollen grains in 1000 μL aliquot of each sample were measured three times with a Particle Counter (Elzone 180+). We measured the pollen mass per male cone using the other set of vials following the formula: the mass of male cones filled with pollen - mass of empty male cones $\times 1/10$. We also randomly selected four shoots bearing male cones, and estimated

the differentiation ratio of male cones on a shoot by dividing the number of male cones by the sum of needle fascicles and male cone numbers on a shoot.

The effects of year and site on male and female cone production and on cone sex ratio were examined with two-way ANOVAs using two-year data from the Kwanak and Yeosu populations. When the two-way interaction effects between main factors were significant, one-way ANOVAs with only one factor were subsequently conducted. A Nested ANOVA with year, site, and trees within a site as main factors was also conducted to identify the effect of tree identity within sites on the number of male cones. Almost all tagged trees at each site produced male cones consecutively for two years, whereas the pattern of female cone production was variable among trees. Thus, a third independent variable based on female cone production for two years, female cone production in one vs. both years, was incorporated into an ANOVA with year, site, repeated female cone production as main factors to further understand the variation in cone sex ratio. The effects of site and repeated female cone production on pollen production and size were analysed with a two-way ANOVA. Since the Hongneung site was added in 1999, the site difference in cone production and pollen traits measured in 1999 were analysed by one-way ANOVAs. Spearman rank correlation analysis was conducted to find the relationship between plant sizes such as height and circumference at breast height and reproductive characters such as cone production and pollen traits. All dependent variables except for plant height, circumference, the number of pollen grains per male cone, and pollen diameter were log-transformed to improve the normality of the data. All analyses were conducted with SAS procedures (1985).

Results

Differences in plant size and density among populations

In a simultaneous test of the effects of year and site

on the circumference at breast height, only site had a significant effect, showing that the red pines at YJ are larger in circumference than those at KA (Tables 1, 2). Red pines taller in height were larger in circumference at KA as well as at YJ when both tree height and circumference were measured in 1998 ($r=0.74$ and 0.79 , respectively, $P<0.0001$ for both): red pines at KA were also smaller in height than those at JY (Table 1, $F_{1,70}=20.37$, $P<0.0001$). Mean circumference also differed significantly among the three sites when the HN site was included in 1999 ($F_{2,112}=8.99$, $P=0.0002$): red pines at KA were smaller in circumference at breast height than those both at YJ and HN. The three sites did not differ greatly in density: 7.3, 7.8, and 10.8 trees/100 m² at KA, YJ, and HN, respectively.

Cone sex ratio

Not all tagged trees at all sites simultaneously produced male and female cones. In 1998, 81.1% and 74.3% of red pines at KA and YJ, respectively, produced female cones, whereas in 1999 red pines which produced female cones increased 1.2-fold (92.5, 90.5, 81.8% at KA, YJ, and HN, respectively). The increase in the proportion of red pines with female cones was marginally significant at YJ (contingency table analysis with year x female cone presence vs absence, KA $\chi^2=2.26$, $P=0.133$, $df=1$, total $N=77$; YJ $\chi^2=3.60$, $P=0.058$, $df=1$, total $N=77$). On the other hand, all trees observed for two years at both KA and YJ, and all trees at HN tagged in 1999 produced male cones.

The mean number of female cones per tree differed only between years, not between sites (Table 2). The female cone number per tree increased from 35.1 to 107.5 at KA, and from 63.2 to 106.1 at YJ (Table 1), i.e., an overall 2.2-fold increase in 1999. There was no significant difference in female cone numbers per tree among the three sites in 1999 ($F_{2,99}=0.22$, $P=0.8062$), though red pines at HN produced an average of only 89.2 female cones per tree.

The yearly pattern of male cone production per tree

Table 1. Means and 95% confidence intervals of vegetative and reproductive characters of *Pinus densiflora* trees measured at three sites in 1998-1999. Circumference was measured at breast height

Characters		Kwanak		Yeosu		Hongneung	
		1998	1999	1998	1999	1998	1999
Plant height (m)	\bar{x}	3.9	-	5.8	-	-	-
	CI	3.4-4.5	-	5.6-6.0	-	-	-
	N	37	-	35	-	-	-
Circumference (cm)	\bar{x}	42.2	42.6	47.8	52.7	-	58.3
	CI	36.7-47.8	37.6-47.6	38.6-66.6	46.9-58.5	-	57.7-62.9
	N	37	40	35	42	-	33
No female cones/plant	\bar{x}	35.1	107.5	63.2	106.1	-	89.2
	CI	24.1-53.9	169.9-168.6	47.4-87.0	103.4-111.0	-	4.9-133.7
	N	30	37	26	38	-	27
No male cones/plant	\bar{x}	11147	47811	105129	49556	-	23252
	CI	4295-25235	25789-88335	55347-199664	35555-69072	-	15011-36099
	N	35	40	35	42	-	33
Cone sex ratio	\bar{x}	310	427	1379	486	-	275
	CI	101-961	212-866	1149-1657	310-763	-	150-509
	N	28	37	26	38	-	27

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Table 2. Two-way ANOVA of the effects of year and site on vegetative and reproductive characters of *Pinus densiflora* trees. Data obtained from the two sites (Kwanak and Yeosu) for two years were used for analysis

Source of variation	Circumference			No female cones/plant			No male cones/plant			Cone sex ratio		
	df	F	P	df	F	P	df	F	P	df	F	P
Year	1	0.87	0.3526	1	12.38	0.0006	1	1.39	0.2403	1	0.91	0.3407
Site	1	7.91	0.0056	1	1.51	0.2212	1	14.58	0.0002	1	4.59	0.0342
Year × Site	1	0.64	0.4232	1	1.65	0.2008	1	13.67	0.0003	1	3.25	0.0737
Model	3,105	3.30	0.0221	3,127	5.27	0.0019	3,148	9.19	0.0001	3,125	2.51	0.0616

varied between sites with an overall mean of 37,400 male cones per tree (Tables 1 and 2). In separate analyses for each site, mean number of male cones per tree differed between years at both sites (KA $F_{1,73}=8.59$, $P=0.0045$; YJ $F_{1,75}=5.00$, $P=0.0283$). Male cone numbers rose 4.3-fold at KA, whereas those at YJ declined by 53%. Since the variation in male and female cone numbers per tree did not coincide between years and sites, a year × site interaction effect on cone sex ratio was marginally significant (Table 2). At KA, cone sex ratio tended to increase from 310:1 to 427:1 over two years, though statistically not being significant ($F_{1,63}=0.29$, $P=0.5932$). On the other hand, at YJ, cone sex ratio declined significantly from 1379:1 to 486:1 for the same period ($F_{1,62}=5.13$, $P=0.0270$). Cone sex ratio of 275:1 was relatively low at HN in 1999 compared to other sites, but there was no significant difference among the sites ($F_{2,99}=0.91$, $P=0.4078$).

The two components which determined the number of male cones per tree, the number of male cones per shoot and the number of shoots with male cones per tree, exhibited a complex pattern of variation between years and sites. The year × site interaction effect on the number of male shoots was highly significant (Table 3). The number of male shoots per tree increased 3.2-fold, from 413 to 1318, at KA over two years, while it decreased by 62%, from 3391 to 1288, at YJ (Fig. 1). This yearly difference was significant at both sites (KA $F_{1,73}=6.42$, $P=0.0134$; YJ $F_{1,75}=9.79$, $P=0.0025$). The mean number of male cones per shoot varied signifi-

cantly between years, not between sites (Table 3): mean number of male cones per shoot incremented from 31.6 to 38.9, i.e., a 1.2-fold increase in 1999. In 1999, red pines at HN had only one half the number of male shoots per tree, an average of 657 male shoots per tree, compared to those at other sites, showing a marginally significant difference among the sites (Fig. 1; $F_{2,112}=3.03$, $P=0.0524$). However, an average of 36.6 male cones was borne on each male shoot at HN, demonstrating no significant difference among the three sites ($F_{2,112}=0.73$, $P=0.4856$). Thus, the total number of male cones per tree at HN was smaller than those at other sites, and the site effect was marginally significant ($F_{2,112}=3.02$, $P=0.0526$).

In an analysis of the effects of year, site, and tree identity within sites on male cone numbers per male shoot, both year and tree identity exerted strongly significant effects, suggesting that male cone numbers per male shoot differed not only between years but also among trees within sites (Table 3). For example, in 1998, mean male cone numbers per male shoot showed a 5-6 fold difference among trees within sites, ranging from 10.3-49.3 at KA and 9.4-56.4 at YJ.

Trees which produced a larger number of female cones in 1998 also produced more female cones in 1999 at both KA and YJ (KA $r=0.57$, $P=0.0055$; YJ $r=0.76$, $P=0.0001$, $N=27$ and 34 , respectively). Since the 1998 and 1999 production of both male shoots per tree and male cones per male shoot were also positively correlated among red pines at KA, the ranking of trees regarding the total male cone numbers per tree was consistent between years ($r=0.55$, $P=0.0030$). At YJ, the two-year production of male shoots was not significantly correlated among red pines ($r=0.19$, $P=0.2889$) unlike that of male cone numbers per male shoot ($r=0.38$, $P=0.0283$). Despite the yearly difference of male cone numbers per tree between KA and YJ, 1998 and 1999 cone sex ratios were strongly positively correlated among red pines at both sites ($r=0.74$, $P=0.0001$, $N=21$ and $r=0.62$, $P=0.0013$, $N=24$, for KA and YJ, respectively).

The differentiation ratio of male cones on a shoot was $56.7 \pm 0.09\%$, 55.2 ± 10.2 , and $54.6 \pm 9.4\%$ for KA, YJ, and HN, respectively, showing no significant difference among the sites ($F_{2,27}=0.13$, $P=0.8766$). Thus, across the three sites, 55.5% of buds on a new shoot were differentiated into male cones.

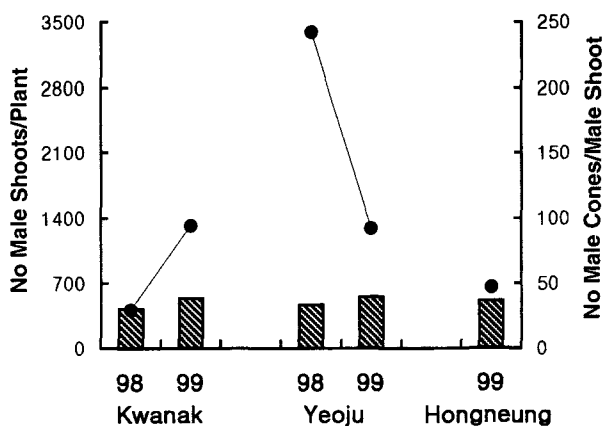


Fig. 1. Mean number of male shoots per plant (●) and mean number of male cones per male shoot (▨) in *Pinus densiflora* examined in 1998 and 1999, according to site.

Table 3. Two-way ANOVA of the effects of year and site on the number of male shoots per plant and mean number of male cones per male shoot (A); Nested ANOVA of the effects of the year, site, and plants within sites on the number of male cones per male shoot (B). Male shoots indicate new shoots bearing male cones at base

Source of variation	No male shoots/plant			Mean no male cones/male shoot			No male cones/male shoot		
	df	F	P	df	F	P	df	F	P
Year	A.			B.			C.		
Site	1	0.12	0.7278	1	14.24	0.0002	1	60.79	0.0001
Year × Site	1	14.32	0.0002	1	1.21	0.2722	1	0.59	0.4454
Plant (Site)	1	14.96	0.0002	1	0.24	0.6265	1	2.21	0.1371
Model	3,148	9.09	0.0001	3,148	5.51	0.0013	92,826	3.77	0.0001

Pollen traits

Pollen diameter, including sacchi, ranged from 36.5-52.4 µm with an overall mean of 45.6 µm (Table 4). Pollen grain numbers per male cone varied from 45,400-370,400 with a mean of 166,900 across the sites. Both pollen size and grain numbers per male cone did not vary significantly among the sites (Table 4). Mean male cone mass ranged from 6.60 to 8.81 mg across the three sites, but mean pollen mass per male cone did not vary among sites (Table 4). As a result, pollen mass accounted for 44.8% of male cone mass across the sites.

Correlations among tree size, cone sex ratio, and pollen traits

Tall and large trees tended to bear a larger number of male and female cones than short and small trees across the sites (Table 5). This relationship appeared to vary between years and sites. For example, the correlations at YJ, unlike those at KA, were consistently significant for two years. In 1999, the positive correlation between plant size and cone production was significant at KA and YJ, but not at HN. Male cone numbers per male shoot were independent of tree height and circumference across all sites ($P > 0.05$ for all pairwise correlations), indicating that tall trees

had more male cones by an increment in male shoot numbers than short trees. At all sites, female cone numbers per tree were not significantly correlated with any male cone components such as the total male cone numbers per tree, male shoot numbers per tree, or mean male cone numbers per male shoot ($P > 0.05$ for all pairwise correlations). Cone sex ratio was independent of tree height and circumference at all sites for two years.

Except for a negative correlation between circumference and pollen size at YJ in 1999, pollen size and pollen grain numbers per male cone were not correlated with circumference at other sites (Table 5). Pollen size decreased significantly (KA) or tended to decrease (YJ) with an increase in male cone numbers per tree. Thus, pollen size decreased as the cone sex ratio became male-biased at KA and YJ. Both male shoot numbers per tree and male cone numbers per male shoot were negatively, though not significantly, correlated with pollen size at KA ($r = -0.29$ and -0.31 , $P = 0.071$ and 0.052 , respectively), while only male shoot numbers tended to be negatively correlated with pollen size at YJ ($r = -0.16$, $P > 0.05$). The number of pollen grains per male cone was correlated with neither male shoot numbers per tree nor male cone numbers per male shoot (KA $r = 0.24$ and 0.02 , respectively, $P > 0.05$ for both; YJ $r = 0.18$ and 0.28 , respectively, $P > 0.05$ for

Table 4. Means and 95% confidence intervals for male cone and pollen traits of *Pinus densiflora* trees at three sites in 1999. One-way ANOVAs of the effect of sites were conducted for each class variable

Characters		Sites			Note
		Kwanak	Yeoju	Hongneung	
Pollen diameter (µm)	\bar{x}	45.9	45.8	44.0	
	CI	45.1-46.8	44.8-46.8	42.3-45.7	
	N	40	38	16	
$F_{2,91} = 2.51, P = 0.0866$					
No pollen grains/male cone	\bar{x}	174300	155700	174900	
	CI	155000-193700	132800-178600	133000-216800	
	N	40	38	16	
$F_{2,91} = 0.88, P = 0.4189$					
Male cone mass (mg)	\bar{x}	8.06	6.60	8.81	
	CI	7.32-8.81	5.76-7.45	7.99-9.63	
	N	39	32	10	
$F_{2,78} = 5.57, P = 0.0055$					
Pollen mass (mg)/male cone	\bar{x}	3.63	3.31	3.74	
	CI	3.09-4.18	2.66-3.97	2.49-4.99	
	N	35	23	10	
$F_{2,65} = 0.37, P = 0.6899$					
Kwanak, Hongneung > Yeoju					

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Table 5. Spearman rank correlation coefficients of vegetative and reproductive characters of *Pinus densiflora* trees sampled in 1998 (upper half) and 1999 (lower half). The first, second and third rows of values at each column and year represent the three sites (Kwanak, Yeosu, and Hongneung, respectively). Sample size is 30-40 (Kwanak), 26-42 (Yeosu), and 16-33 (Hongneung)

Characters	Circumference	No female cones /plant	No male cones /plant	Cone sex ratio	Pollen diameter
Plant height	0.74**** 0.78****	0.15 0.62***	0.20 0.50**	0.15 -0.08	--
Circumference	--	0.22 0.53**	0.31 0.52**	0.26 -0.00	--
No female cones/plant	0.56*** 0.45** 0.21	--	-0.17 0.06	-0.47° -0.68****	--
No male cones /plant	0.47** 0.51*** 0.24	0.13 0.30 0.14	--	0.90**** 0.57**	--
Cone sex ratio	-0.05 -0.03 -0.01	-0.50** -0.68**** -0.57**	0.74**** 0.41* 0.65***	--	--
Pollen diameter	0.12 -0.41** 0.01	0.19 0.22 0.23	-0.37* -0.11 0.38	-0.46** -0.37° 0.12	--
No pollen grains /male cone	0.12 0.19 0.45	0.08 0.24 0.35	0.18 0.21 -0.29	0.13 0.04 -0.71**	0.13 -0.16 -0.04

* P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001.

both). The relationship between both pollen size and grain numbers and cone sex ratio at HN, where sample size for pollen traits was relatively limited, was opposite to that at KA and YJ. At HN, pollen size was largely independent of cone sex ratio while pollen grain numbers per male cone were strongly negatively correlated with the latter (Table 5).

Female cone production in one vs. both years

Female cone production in one or both years was associated not only with male cone numbers in 1998, but also with male and female cone numbers and pollen size in 1999. Upon simultaneously considering the effects of year, site, and repeated female cone production on female cone numbers per tree, the interaction of year x repeated female cone production was significant (Table 6). Fig. 2A showed that trees

Table 6. Three-way ANOVA of the effects of year, site, and female cone production in one vs. both years on the number of female and male cones per plant of *Pinus densiflora*. Data obtained from the two sites (Kwanak and Yeosu) for two years were used for analysis

Source of variation	No female cones/plant			No male cones/plant		
	df	F	P	df	F	P
Year	1	57.46	0.0001	1	0.46	0.4990
Site	1	0.32	0.5713	1	6.27	0.0138
Year x Site	1	0.45	0.5022	1	10.92	0.0013
One vs. both years	1	68.60	0.0001	1	1.43	0.2353
Year x One vs. both years	1	20.90	0.0001	1	0.71	0.4020
Site x One vs. both years	1	0.19	0.6604	1	0.61	0.4377
Year x Site x One vs. both years	1	0.64	0.4252	1	1.14	0.2885
Model	7,106	19.32	0.0001	7,104	3.47	0.0022

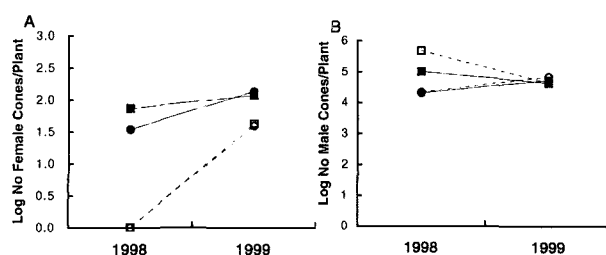


Fig. 2. Log mean number of female (A) and male cones (B) per plant of *Pinus densiflora* in 1998 and 1999, according to site and female cone production in one vs. both years. ●=Both years/KA; ○=One year/KA; ■=Both years/YJ; □=One year/YJ. F-test on A: in 1999, overall $F_{3,53}=2.33$, $P=0.0845$, site $F_{1,53}=0.01$, $P=0.9416$, repeated female cone production $F_{1,53}=6.72$, $P=0.0123$, two-way interaction $F_{1,53}=0.06$, $P=0.8026$; F-test on B: at KA, overall $F_{3,48}=1.33$, $P=0.2758$, year $F_{1,48}=2.31$, $P=0.1347$, repeated female cone production $F_{1,48}=0.06$, $P=0.8112$, two-way interaction $F_{1,48}=0.02$, $P=0.8967$ and on B: at YJ, in 1998 $F_{1,26}=4.51$, $P=0.0428$, and in 1999 $F_{1,26}=0$, $P=0.9613$.

which produced female cones for two years bore a higher number of female cones in 1998 as well as in 1999 compared to those which did not produce female cones in 1998, though the latter recovered the production of female cones rapidly in 1999. The year x site interaction effect on male cone production was significant (Table 6). In a separate analysis for KA, the overall effect of the two-way ANOVA with year and repeated female cone production as main factors was not significant (Fig. 2B). At YJ, red pines which stopped female cone production in 1998 produced a 4.3-fold higher number of male cones in 1998 than those which produced female cones, but such a difference in male cone production disappeared in 1999. The overall effect of a two-way ANOVA with site and repeated female cone production as main factors on cone sex ratio in 1999 was not significant, but repeated female cone production had a significant effect (overall $F_{3,53}=1.78$, $P=0.1627$; site $F_{1,53}=0.25$, $P=0.6174$; repeated female cone production $F_{1,53}=5.30$, $P=0.0253$; two-way interaction $F_{1,53}=0.14$, $P=0.7079$). Thus, red pines which stopped female cone production in 1998 demonstrated a 3.5 times higher cone sex ratio in 1999 than those which produced female cones for both years. In addition, trees produced female cones in one or both years differed in mean pollen size, but not in pollen grain

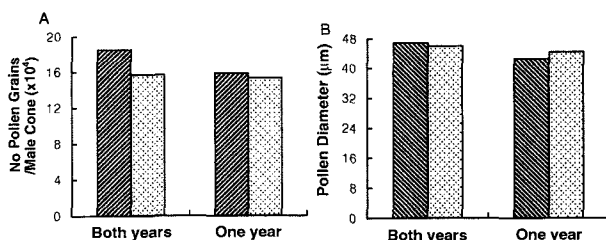


Fig. 3. Mean number of pollen grains per male cone (A) and pollen diameter (B) in *Pinus densiflora* trees produced female cones in one vs. both years, according to site. ▨=KA; ▩=YJ. F-test on A: overall $F_{3,50}=0.76$, $P=0.5205$, site $F_{1,50}=0.46$, $P=0.5018$, repeated female cone production $F_{1,50}=0.38$, $P=0.5412$, two-way interaction $F_{1,50}=0.22$, $P=0.6429$; F-test on B: overall $F_{3,50}=3.35$, $P=0.0262$, site $F_{1,50}=0.28$, $P=0.5982$, repeated female cone production $F_{1,50}=8.57$, $P=0.0051$, two-way interaction $F_{1,50}=1.97$, $P=0.1665$.

numbers. The former produced smaller grains than the latter (Figs. 3A, B).

Discussion

Sex ratio in general is controlled by either size advantage or quality of the environment (Lundholm and Aarssen, 1994; Bertin and Kerwin, 1998; Mendez, 1998). From an evolutionary point of view, height of anemophilous species is an important trait contributing to male success. Burd and Allen (1988) argued that tall plants invest more resources to male function than to female function, since pollen dispersal distance increases with plant height. The concordant increase in the ratio of male to female flowers with plant height in four of the seven anemophilous, monoecious species (Fox, 1993) partially supports their argument. The cone sex ratio of red pines was greatly deviated toward maleness, ranging from 275:1 to 1379:1 across the three sites for two years. For anemophilous pines which are largely outcrossing, cone sex ratio should exceed 1:1 when seed production which typically represents female function is not considered. However, cone sex ratio of red pines was independent of both tree height and circumference at breast height. Thus, unlike other anemophilous species, the height of red pines is not a good predictor for cone sex ratio.

On the other hand, certain red pine trees, regardless of tree size, produced more female than male cones and vice versa. Furthermore, this tendency was consistent for two years. A similar pattern of gender biased cone production has been observed in other pines and related species (e.g. Eis, 1967; Caron and Powell, 1989; but see Schoen et al., 1986). This pattern may be related to a functional role specialization among trees. In studies using marker genes (Muller-Starck and Ziehe, 1984; Schoen et al., 1986; Apsit et al., 1989), certain trees or clones contribute as males, and others contribute as females. If gender-biased cone production leads to a functional role specialization, information on maleness or femaleness of individual trees is critical to maintain or improve genetic diversity in red pine populations.

Female allocation increases with an increment in resources available for reproduction since male fitness increases rather slowly compared to female fitness with an increase in resources (Charnov, 1982; Willson, 1983). This statement has been confirmed in species with varying sexual systems. Female plants are more abundant in moist and lighted condition than male plants (Freeman and Vitale, 1985; Dawson and Ehleringer, 1993; Mendez, 1998); large hermaphroditic plants are more likely to function as females than small ones (Devlin, 1988); monoecious species such as oaks and junipers in moist habitats produce relatively more female flowers than those in xeric habitats (Freeman et al., 1981). In this study, the three sites were somewhat different in soil depth and competition for light. The soil

was shallower in depth and relatively infertile at KA than at YJ and HN. On the other hand, among these three sites of similar density, light competition appeared to be less severe at KA because it is composed of smaller trees compared to YJ and HN. Although we did not quantitatively measure environmental conditions at these sites, our data revealing variation in the cone sex ratio of red pines between years within sites and among the three adjacent sites suggest that site-specific environmental conditions may not be an important factor affecting the variation in the cone sex ratio of red pines.

In pines, the relationships of plant size with both male and female cone productions, rather than with cone sex ratio, were more frequently observed perhaps because cone sex ratio is a derivative, ratio variable, confounding the dynamic pattern of male and female cone production. In *P. ponderosa*, *P. contorta*, *P. radiata*, and *Picea glauca*, large trees in height or dbh produced higher numbers of male and female cones than small ones (Smith, 1981; Nienstaedt, 1985; Cremer, 1992). It is not possible to deny that selection for male success through longer pollen dispersal might have shaped the relationship between tree size and male cone production as stated by Burd and Allen (1988). In this study, there also was a tendency of both male and female cone production to increase with tree size, though a statistical significance was consistently detected for two years only at YJ. However, our data suggest that male and female cone productions may also change by proximate mechanisms such as architecture and weather.

Female and male cones are spatially separated within a tree: female cones are on the tip of the leading shoots and male cones are on the base of the new shoots. Tall trees are usually large in dbh in various species of pines (Rim and Shidei, 1974; Hong et al., 1991; Kang, 1999). Thus, larger trees with more leading and secondary shoots tend to produce more male and female cones, and more seeds than smaller trees (Daniel et al., 1979). However, female cones are distributed over the top of the crown not evenly but quite locally: they usually occur on the shoots exposed to light. Male cone distribution on the tree was also local. Stoll and Schmid (1998) found that the cone sex ratio on sun vs. shade branch of *Pinus sylvestris* was 3:79 and 30:23, respectively, confirming that male and female cone production varies greatly depending upon the level of light received within a tree. The local distribution of male and female cones within trees appears to be responsible for the lack of correlation between male and female cone productions per tree despite the positive correlation between tree size and both male and female cone productions.

In general, temperature and rainfall at the time of bud initiation at year⁻¹ and for several months before pollen shedding at year⁰ are closely related to cone production. For example, temperature affects cone pro-

duction in *Tsuga mertensiana*, and temperature and rainfall affect positively and negatively, respectively, the bud initiation, especially female buds of *P. banksiana* (Houle and Fillion, 1993; Woodward et al., 1994; Despland and Houle, 1997). In 1999, compared to 1998, not only trees producing female cones increased in number but also both female cone numbers per tree and male cone numbers per male shoot increased at both KA and YJ. Thus, weather conditions in summer at year⁻¹ and/or those in spring at year⁰ might have been favorable for cone production in red pines.

However, male shoot numbers per tree may be controlled by different factors from those controlling male cone numbers per male shoot. For example, male shoots per tree increased 3.2 times in number at KA during 1998-1999, while the former declined by 62% at YJ. In pines, growth of new shoots is based on reserved resources in nearby needles (Kozlowski and Winget, 1964), and one male cone is produced at the expense of one fascicle of needles (Cremer, 1992). About 56% of buds on male shoots were differentiated into fascicles of needles in red pines, in contrast to 13% in *P. radiata* (Cremer, 1992). Then it is likely that the excessive production of male shoots in 1998 at YJ weakened the shoots, suppressing the growth of new shoots in 1999. Caron and Powell (1989) stated that poor cone production in *Picea mariana* with a two-year periodicity occurred due to a decline in shoot growth, number of buds initiated, and number of cones matured, though the pattern changed depending upon weather. Considering the large contribution of the number of male shoots to the total number of male cones per tree, it is necessary to further examine the pattern of male shoot production per tree among years at each site.

Pine species require three years from cone bud formation to seed dispersal, and seed maturation occurs largely at a masting year. For example, *Pinus resinosa* and *P. banksiana* are known to have distinctive cycles of seed production (Cremer, 1992; Houle and Fillion, 1993). Although information on a masting cycle for red pine does not exist, the two-way interaction effect of year x female cone production in one vs. both years suggests the potential effects of reproductive periodicity on female cone production, and cone sex ratio in pines. If repeated female cone production for two years reflect, to some extent, the level of resources available for reproduction, red pines which stopped reproduction in one year are more likely to be the ones subjected to resource limitation for reproduction than trees which reproduced repeatedly. Indeed, trees which stopped female cone production in 1998 still produced male cones, and male cone production did not differ between trees producing female cones for one vs. two years. These results are expected if male flower or cone production is cheap in cost (Smith, 1981; Charnov, 1982; Thomas and LaFrankie, 1993). Although the sample size for trees which stopped female cone

production in one year is limited, these results suggest that female cone production in one year might not be independent of female cone production and cone sex ratio in immediate past years.

Lloyd (1984) predicted a linear relationship between male success and male investment in anemophilous species. Indeed, male success increases with more male cone production in *Picea mariana*, *P. glauca*, *Pinus contorta*, and *Taxus canadensis* (Schoen and Stewart, 1986; Smith et al., 1988; Caron and Powell, 1989; Allison, 1990), providing empirical support for Lloyd (1984). Quantitative information on pollen grain numbers per male cone and the total number of male cones per tree is quite lacking for pine species (e.g. Richards, 1997). In red pines, each male cone produces an average of 166,900 pollen grains, which is higher than a corresponding structure, i.e., flower of most entomophilous species (Richards, 1997). Red pines also produce a great number of male cones per tree with an extremely male-biased cone sex ratio in comparison to other gymnosperms such as *Picea mariana* with a cone sex ratio of 6.6:1 by the age of 18 (Caron and Powell, 1989). Despite the great production of pollen in most pines, many of them suffer a low seed set or fruit set. Owens et al. (1991) found that the major loss of seeds was resulted from insufficient pollen in the ovules of *Pseudotsuga menziesii*. Similarly, if pollen limitation is responsible for about 90% of early abortion of ovules in red pines (Kang, 1999), both the great number of pollen grains per male cone and the extremely male-biased cone sex ratio might be a strategy to improve pollination efficiency, and consequently male success.

Like the production and size variation of pollen in several angiosperms which are subjected to environmental conditions such as light and nutrients (e.g. Devlin, 1988; Young and Stanton, 1990; Lau and Stephenson, 1993), pollen traits in pines may also be influenced by environmental, especially weather, conditions. For example, in *P. banksiana*, through a positive correlation between male cone production and the summer temperature at year⁻¹, pollen production at year⁰ increased (Lee et al., 1996). Pollen production per male cone and pollen size were independent of each other in red pines. However, relatively more male-biased trees tended to produce smaller grains (two populations) or less number of grains per male cone (one population) of the three red pine populations examined. Thus, male investment of red pines appears to be controlled by a trade-off relationship between cone sex ratio and pollen size or pollen number. When large pollen grains are more likely to deposit onto female cones (Whitehead, 1983), and seeds fertilized by large pollen are more likely to mature (Nakamura and Wheeler, 1992), the effects of variation in pollen production and size on male success of pines should be examined in relation to the cone sex ratio.

Mast flowering usually precedes mast seeding. For

most flowering to be effective in anemophilous species, male and female allocations should increase concordantly (Smith et al., 1990). However, Smith et al. (1990) predicted that among pines in temperate areas the effect of pollen masting was not great due to relatively high species diversity, therefore male allocation was quite constant over years. Our two-year study shows that male and female cone productions of red pines may be partly influenced by both plant size and weather conditions as shown in many other pines, though those cone numbers do not vary in a concordant pattern. Thus, our results do not provide evidence on the simultaneous increase/decrease of male and female cone productions nor constant male cone production. To understand the dynamics of cone production, cone sex ratio, and concordant changes in pollen traits, it is necessary to conduct long-term studies because of the long duration of one reproductive episode and of the potential of masting in red pines.

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