Journal of Plant Biology, April 2007, 50(2) : 181-189

Changes in Gender Expression in Korean Populations of *Pinus densiflora* over a Five-Year Period

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Male and female cone crop patterns and gender expression in *Pinus densiflora* were examined in three adjacent Korean populations over four to five years. Three parameters -- cone ratio, sexual system, and standardized phenotypic gender -- were assessed according to cone production. Both male and female cone crops and cone ratios varied by year and by site. After controlling for plant size, population differences in cone crops and cone ratios became stronger. At all three sites, the proportion of trees with differing sexual systems tended to fluctuate among years. Overall, 37% of all tagged trees changed their sexual system at least once, mostly ranging between monoecy and male. These particular trees were relatively small and exhibited slow radial growth compared with the non-sex-changing trees, suggesting a trade-off between vegetative and reproductive growth. Despite annual variations in their standardized phenotypic genders, the rankings of trees remained concordant between successive years at all sites. These results indicate that, in *P. densiflora*, male and female cone crops and, consequently, gender expression, may be affected by diverse factors that include plant size, trade-offs between vegetative and reproductive growth, and genetic components.

Keywords: dbh, gender expression, male and female cones, *Pinus densiflora*, trade-off

Wind-pollinated monoecious plants, including pines, may selectively invest more energy toward male function in order to increase pollination efficiency (Allison, 1990; Klinkhamer et al., 1997; Shibata et al., 1998). Indeed, several conifers show increased maleness with plant size (Caron and Powell, 1989; Arista and Talavera, 1997). However, contrasting reports of increased female allocations in larger trees are available in other conifers (Rapp and Cabanettes, 1981; Linhart and Mitton, 1985). Thus, even within conifer species, no general conclusion may be made about the effect of plant size on sexual investment.

Sexual systems of many conifer species may not be strictly constrained even though those trees generally are monoecious. For example, several populations of conifer species comprise monoecious, male, female, or non-flowering trees (Allison, 1991; Arista and Talavera, 1997; Tapias, 1998). Sequential monoecy, i.e., the state of bearing only male or female cones when young and then producing both types several years later, appears to be one of the mechanisms that contributes to mixed sexual systems, as shown in *Cupressus sempervirens* (Lev-Yadun and Liphschitz, 1987) and *Pinus edulis* (Floyd, 1983). Alternatively, conifers may change sex under environmental stresses such as resource limitation (Shapcott et al., 1995) or arid habitats (Ortiz et al., 2002). Their variable sexual systems likely cause further complexities in annual male and female cone production, which then defines their gender expression. Information regarding sex changes in conifer species, however, is relatively scarce when compared with the habits of angiosperms (Freeman et al., 1984; Costich, 1995; Yamashita and Abe, 2002).

The relative investment to male and female functions in pines and, thus, gender expression of individuals within sites, can be estimated by male to female cone ratios. Such cone ratios may make it difficult to reflect precise functional gender, however, because male cone crops generally are far larger than female cone crops (Allison, 1991). Moreover, for a species with flexible sexual systems, sex change patterns also should be considered. A standardized phenotypic gender (Lloyd, 1980) is employed to compare gender expression among trees within populations, regardless of the sexual system and the absolute numbers of male and female cones (Allison, 1991).

Pinus densiflora is a dominant tree species in Korea that undergoes drastic seed crop fluctuations at two-year intervals (Kang, 2005). In wind-pollinated species, mast seeding is often preceded by mast flowering (Koenig et al., 1994), and flowering intensity varies greatly among years (Shibata et al., 1998; Ortiz et al., 2002). Nevertheless, annual male and female cone crop patterns and *P. densiflora* cone ratios rarely have been examined over a sufficient number of years, particularly in relation to plant size or sexual system (Kang and Yoon, 2000). In the current study, the following questions were addressed: (1) Does the size of *P. densiflora* affect cone production patterns and cone ratios? (2) What are the patterns of sexual systems for *P. densiflora* across sites and years? and (3) To what extent is the standardized phenotypic gender of individual trees variable among years?

MATERIALS AND METHODS

Species and Study Sites

P. densiflora (Korean red pine) is a major component of temperate forests that extend throughout most of the Korean peninsula (33° 07′N - 43° 0′N, 124° 11′E - 131° 53′E). Although most of its populations usually occur below 500 m in elevation, this species may range from 100 to 900 m, depending on latitude. Trees can be 35 m tall, and are

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characterized by red bark and two rather soft needles per fascicle. Male and female cone primordia are initiated from early July to September in Year 1, while needle primordia are initiated either before the Winter of Year 1 or at the beginning of Year 2 (Hanawa, 1966). Male and female cones then expand and become receptive in May of Year 2. Following fertilization during the Summer of Year 3, seed cones mature in the Fall of the same year. This study was carried out at three sites in the middle of the Korean peninsula: (1) Mt. Kwanak (KA; 37° 6′N, 126° 58′E) in Seoul; (2) Hongneung (HN; 37° 35′N, 127° 03′E) in Seoul; and (3) Yeoju (YJ; 37° 17′N, 127° 45′E) in Kyunggido. *P. densiflora* trees at these sites were mostly 20 to 40 years old, perhaps being naturally regenerated after the Korean War (1950 to 1953). Red pines at both YJ and HN were located at around 100 m in elevation, while those at KA were sampled below 300 m. Although all three sites comprised mixed forests of *P. densiflora, P. rigida, Robinia pseudoacacia*, and various *Quercus* species (Kang and Yoon, 2000), their canopies consisted largely of *P. densiflora*. Data for mean annual precipitation and temperatures over the study period (1998 to 2002) were obtained from two weather stations at Seoul and Icheon, near Yeoju (KMA, 2006). Both parameters show strong seasonality, i.e., hot and humid summers followed by cold and dry winters. Mean annual precipitation was slightly a bit higher in Seoul (1608.7 mm) than at Icheon (1404.0 mm) mostly because of frequent August thunderstorms at the former location. The mean temperature was highest in July at Seoul and Icheon (25.7°C vs. 24.4°C), and lowest in January (-1.6°C vs. -2.8°C).

Data Sets

In Spring 1998, 35, 41, and 31 red pines were randomly tagged at KA, YJ, and HN, respectively. Due to unexpected events, including tag loss and some mortality, sample sizes varied somewhat over the years. The numbers of male and receptive female cones (strobili) on each tagged tree were counted just before pollen shed (late April to early May), with these data being obtained for five years (1998 through 2002) at KA and for four years (1998 through 2001) at YJ and HN. When their numbers were small, the female cones were counted through binoculars (x 7), but when those tallies became too large on individual trees, the cones from several branches on a particular tree were counted and multiplied by the total number of branches. A variable number of male cones was grouped near the base of a lateral shoot (male shoot), and one to several male shoots could be found clustered on branches from a previous year. When male shoots were not too numerous, all were counted. For a larger tree, numbers were estimated by counting the male shoots only on a portion, e.g., one-fourth, of the canopy and then multiplying that number by four to estimate the total. Up to 10 male shoots were sampled from each tree in order to determine the number of male cones after they were taken to the lab. Whenever possible, male and female cones were counted or sampled from various areas within a tree to reduce the potential effect of branch positioning. Finally, the total number of male cones per tree was estimated by multiplying the number of male shoots on that tree by the mean number of male cones per male shoot. The cone ratio for each tree was then obtained by dividing [male cone + 1] with [female cone + 1]. Diameter at breast height (dbh) for each tree was measured at 120 cm aboveground in the Spring when observations were recorded for male and female cones. Based on the presence or absence of male and female cones, *P. densiflora* was categorized into four sexual groups: monoecious, bearing both male and female cones; female, bearing only female cones; male, bearing only male cones; or non-flowering, bearing no cones at all. Slightly more than half of the tagged trees did not change sex over the study period, while others changed sex at least once, mostly from monoecious to male or vice versa. Thus, two types of monoecy were established: "consistent monoecy", i.e., no alterations in the sexual system, and "facultative monoecy", where sex was changed at least once in four or five years. Standardized phenotypic gender (G, phenotypic gender hereafter) was calculated according to the formula of Lloyd (1980):

$$G = (m_i/M) / (f_i/F + m_i/M)$$

where m_i was the number of male cones on plant i; f_i, the number of female cones on plant i; and M and F equaled the total corresponding numbers of male and female cones in the entire population. G ranged from 0 (female) to 1 (male). In addition to any sex changes, trees exhibiting shifts in $G > |0.4|$ between two consecutive years were defined as being in a phenotypic gender shift, as described by Allison (1991).

Data Analyses

One-way ANOVAs were used to examine differences in male and female cone production and cone ratios among sites for each year. To control for the effect of plant size on cone crops and cone ratios, ANCOVAs with dbh as a covariate were employed. Following these ANOVAs and ANCOVAs, means were compared pair-wise by Tukey tests. These analyses employed data pooled over all trees within the sites. When considering any correlation among years within trees, annual cone crops and cone ratio patterns were analyzed with ANOVAs for two different sets of data, including trees with complete information for either four (KA, YJ, and HN) or five consecutive years (KA). The significance of each contrast in ANOVAs was tested after a Bonferroni correction to maintain an overall alpha of 0.05 (von Ende, 2001). Associations between sexual systems and sites were examined for each year using two-way contingency table analyses. When considering repeated observations of tagged trees, three-way log-linear analyses were conducted, using site, year, and sexual system as the three factors. Because most trees were either monoecious or male, only trees in those systems were included in the log-linear analyses. Due to a significant site x year interaction regarding sexual systems, the association of year and sexual system was re-examined separately for each site. The yearly pattern of sexual systems was determined only at YJ and HN because not all 10 cases of monoecious and male trees across five years were available at KA. Using data pooled over trees, differences in dbh among sexual systems were examined by one-way ANOVAs for each site and year. Subsequently, differences in dbh increments from the same trees between 1998 and 2001 were tested with a two-way repeated-measure ANOVA, with site and monoecy type as the main factors. At KA, the dbh increment between 1998 and 2002 also was tested with a one-way repeated-measure ANOVA, using monoecy type as a factor. Relationships between dbh and phenotypic gender were analyzed for each site and year via Spearman's rank correlations. Between-year gender concordances among trees within sites also were examined by the same correlation analyses. All dependent variables were log-transformed to improve data normality. Means were provided with 1 SD in the study. All analyses were conducted with SAS 9.1 software (2004).

RESULTS

Site Effects on Cone Production

Among the three study sites, mean dbh of *P. densiflora* differed significantly when first measured in 1998 ($F_{2,102}$ = 7.52, P <0.001; X (± 1 SD) = 12.4 ± 1.4 cm, 15.4 ± 1.4 cm, and 17.2 ± 1.3 cm at KA, YJ, and HN, respectively). Nevertheless, trees at both YJ and HN were larger than those at KA after a couple of trees were later added at each site (P <0.001 for all years from 1999 to 2001). Before considering dbh, site differences in cone crops and cone ratios were significant in only 5 out of 12 ANOVAs with site as a main factor (Table 1). For example, trees at YJ bore significantly more male cones than those at other sites only in 1998, while trees at HN carried about four-fold more female cones than at the other sites in 2001 (Table 1). Consequently, trees at YJ had higher cone ratios than at HN in 1998 and 2001.

When controlling for dbh, site differences were significant in all but two tests, i.e., female cones in 1998 and cone ratio in 1999 (Table 1). Additionally, site difference patterns also changed after controlling for dbh. Trees produced more male cones at both KA and YJ than at HN over the study period, although the difference between HN and YJ in 2000 was not significant. Female cone crops were larger at KA than at HN (1999) and YJ (2000), and those crops also were larger at HN than at YJ (2001). Thus, cone ratios were lower at HN than at YJ (1998, 2000) as well as being smaller than at both KA and YJ (2001).

Year Effects on Cone Production

Site x year interactions in repeated-measures ANOVAs indicated that annual patterns for both male and female cone crops and cone ratios differed among sites (Table 2A). For example, that ratio varied greatly among years, by 11.8-, 28.7-, and 68.2-fold at KA, YJ, and HN, respectively, over the study period (Table 1). In subsequent analyses for each contrast, significant changes in male cone crops between years due to site occurred only between 1998 and 1999 (Table 2B). After that year, male cone crops increased until 2000 before decreasing in the following years across sites. Changes in female cone crops between years due to site were significant for contrasts 2000-1999 and 2001-2000 (Table 2B). These interactions resulted largely because of a decline in female cones at YJ in 2000 and at KA in 2001, compared with the immediately preceding year at each site (Table 1). Cone ratio changes between years due to site were significant for all contrasts examined, although the contrast 2000-1999 became insignificant after a Bonferroni correction (Table 2B).

ANOVAs on five-year data at KA revealed significant year effects for all variables examined (Table 3A). Both male and female cone crops changed significantly at every interval except for contrast 2002-2001 (Table 3B). However, their patterns were not concordant. Therefore, cone ratios were at their highest levels in 2000, but decreased afterward (Table 1).

Site Effects on Sexual System

After pooling data over sites and years, the majority of *P.*

Table 1. Male and female cone crops and cone ratios (means ± 1 SD) of *P. densiflora* trees observed at KA (1998 - 2002) and at YJ and HN (1998 - 2001) are shown in columns labeled "Before"; for each year, differences among sites were tested by one-way ANOVAs followed by Tukey tests (P <0.05). Adjusted means (± 1 SD) of cone crops and cone ratios after controlling for plant size are shown in columns labeled "After"; for each year, differences among sites were tested by ANCOVAs using dbh as a covariate, followed by Tukey test (P <0.05). KA, YJ, and HN represent the three sites examined.

		N	Male cones		Female cones		Cone ratio	
			Before	After	Before	After	Before	After
1998	KA	33	9143 ± 15 b	14365 ± 8 b	20 ± 5 a	29 ± 8 a	493 ± 23 b	527 ± 9 b
	YJ	40	113963 ± 6 a	113836 ± 9 a	24 ± 9 a	23 ± 8 a	4684 ± 21 a	4682 ± 10 a
	HN	29	3569 ± 25 b	2562 ± 8 c	18 ± 6 a	15 ± 8 a	239 ± 16 b	213 ± 9 b
1999	KA	37	49302 ± 7 a	87329 ± 9 a	84 ± 5 a	110 ± 8 a	593 ± 13 a	764 ± 9 a
	YJ	43	44414 ± 4 a	40218 ± 9 a	70 ± 6 a	66 ± 9 ab	634 ± 8 a	613 ± 10 a
	HN	33	18315 ± 8 a	11815 ± 8 b	44 ± 7 a	34 ± 8 b	466 ± 12 a	399 ± 9 a
2000	KA	36	196723 ± 13 a	298781 ± 8 a	38 ± 5 a	51 ± 8 a	5007 ± 13 a	5521 ± 9 ab
	YJ	43	174904 ± 11 a	158800 ± 9 ab	10 ± 9 b	10 ± 9 b	15653 ± 20 a	15129 ± 10 a
	HN	33	116809 ± 16 a	71389 ± 8 b	31 ± 8 ab	23 ± 8 ab	3774 ± 18 a	3173 ± 9 b
2001	KA	37	7568 ± 24 a	10470 ± 9 a	16 ± 5 b	19 ± 8 ab	561 ± 18 a	639 ± 9 a
	YJ	43	8801 ± 6 a	7420 ± 9 a	15 ± 7 b	14 ± 9 b	545 ± 10 a	514 ± 10 a
	HN	33	2647 ± 11 a	1510 ± 8 b	53 ± 5 a	38 ± 8 a	55 ± 7 b	45 ± 9 b
2002	KA	36	4294 ± 38	---	13 ± 7	---	424 ± 24	---

Table 2. Repeated-measures ANOVA (A) and ANOVA of contrast variables (B) on male cones, female cones, and cone ratios of *P. densiflora* trees. Analyses were conducted using four years of data from all three sites.

	Male cones			Female cones			Cone ratio		
	df	F	P	df	F	P	df	F	P
A. Repeated-measures ANOVA									
Between subjects									
Site	2	5.43	0.0058	2	0.81	0.4477	2	5.28	0.0067
Error	98			98			98		
Within subjects									
Year	3	90.07	<0.0001	3	19.26	<0.0001	3	58.86	<0.0001
Year x Site	6	11.29	<0.0001	6	8.03	<0.0001	6	7.12	<0.0001
Error (Year)	294			294			294		
B. ANOVA of contrast variables									
1999 - 1998									
Mean	1,98	15.60	0.0001	1,98	37.61	<0.0001	1,98	1.55	0.2158
Site	2,98	20.46	<0.0001	2,98	1.33	0.2695	2,98	9.59	0.0002
2000 - 1999									
Mean	1,98	85.37	<0.0001	1,98	36.76	<0.0001	1,98	129.93	<0.0001
Site	2,98	1.06	0.3514	2,98	9.15	0.0002	2,98	3.42	0.0366
2001 - 2000									
Mean	1,98	240.37	<0.0001	1,98	0.10	0.7525	1,98	192.30	<0.0001
Site	2,98	1.52	0.2248	2,98	12.15	<0.0001	2,98	8.17	0.0005

Table 3. Repeated-measures ANOVA and ANOVA of contrast variables on male cones, female cones, and cone ratios of *P. densiflora* trees observed for five years at KA.

	Male cones			Female cones			Cone ratio		
	df	F	P	df	F	P	df	F	P
A. Repeated-measures ANOVA									
Year	4	26.48	<0.0001	4	16.30	<0.0001	3	58.86	<0.0001
Error (Year)	124			294			294		
B. ANOVA of contrast variables									
1999 - 1998									
Mean	1,31	24.46	<0.0001	1,31	32.28	<0.0001	1,31	0.29	0.5952
2000 - 1999									
Mean	1,31	32.23	<0.0001	1,31	8.23	0.0073	1,31	31.34	<0.0001
2001 - 2000									
Mean	1,31	63.31	<0.0001	1,31	23.16	<0.0001	1,31	22.65	<0.0001
2002 - 2001									
Mean	1,31	1.76	0.1937	1,31	0.55	0.4633	1,31	0.24	0.6297

densiflora trees were monoecious across sites, with an overall mean of 81.6% (Fig. 1). This was followed by the system of male trees, which comprised an average of 15.2%. Female trees or trees without any cones occurred at an overall frequency of less than 2% for any site and year. Testing for the homogeneity of sexual system distribution across sites showed that the proportion of trees with different sexual systems did not change among sites in 1998 and 1999, but was altered in 2000 and 2001 (1998 X^2 = 6.97, P = 0.3234, N = 99; 1999 X^2 = 5.52, P = 0.2380, N = 110; 2000 X^2 = 18.48, P = 0.0010, N = 109; 2001 X^2 = 14.20, P = 0.0275, N = 110). In 2000, 37.2% of the trees at YJ were male, while no males were present at KA. Female trees were conspicuous only at KA in 2001 and 2002.

Year Effects on Sexual System

When only trees for which repeated observations were available were considered, 62.2% of all *P. densiflora* were consistently monoecious for the entire period (69.9, 57.5, and 62.1% at KA, YJ, and HN, respectively). In contrast, only two trees at YJ remained male throughout the study. Furthermore, none remained female or non-flowering during this multi-year examination. Thus, 30.1 to 42.5% of the trees, depending upon site, changed sexual systems at least once

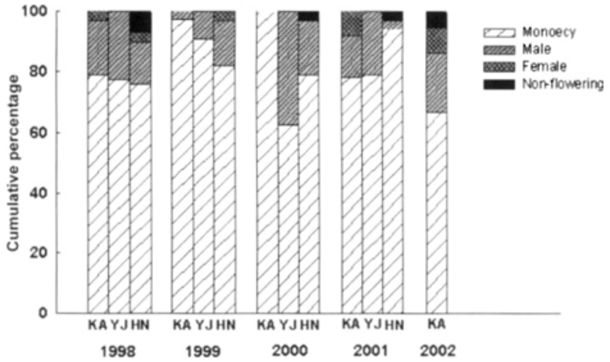

Figure 1. Cumulative percentages of *P. densiflora* trees with different sexual systems at each site over four years at YJ and HN (1998 through 2001) and five years at KA (1998 through 2002). All trees for which sexual systems were observed at each site and year are included in data. N ranges from 30-34 at KA, 40-43 at YJ, and 29-33 at HN.

in the study period. The most common fluctuation was between monoecy and male, although these patterns differed somewhat among sites. For example, across five years at KA, sex changes between male and monoecy occurred 14 times in 12 trees. Likewise, 15 and 8 trees, respectively, at YJ and HN changed sex over four years, largely from male to monoecy rather than the opposite trend (15 vs. 8 at YJ, and 7 vs. 3 at HN). Conversions from monoecy to female or vice versa were rare, being observed in only two (HN) or three (KA) trees but never at YJ. No changes between male and female occurred at any of these sites.

In repeated-measurement analyses for the association of site x year x sexual system, the relationship of year with sexual system differed upon site (Table 4A). Separate, two-way log-linear analyses of years and sexual systems for YJ and HN showed that the proportion of monoecious and male trees differed among years at YJ, but not at HN (Table 4B). At YJ, the proportion of male trees did not vary between 1998 and 1999 but did increase four-fold in 2000 relative to 1999, before decreasing by 40.8% the following year (Fig. 1). Although male trees were reduced from six to one between 2000 and 2001 at HN, this was not statistically significant, perhaps due to small cell counts.

Table 4. The contribution of site and year to heterogeneity in the distribution of sexual systems (monoecy vs. male). Repeated-measures log-linear analyses were conducted for data pooled over two sites (YJ and HN) (A) and for data from each site (B).

Source	df	X^2	P
A. Overall model			
Intercept	1	580.03	<0.0001
Site	1	1.76	0.1845
Year	3	19.71	0.0002
Site x Year	3	10.84	0.0126
B. YJ			
Intercept	1	234.39	<0.0001
Year	3	15.86	0.0012
HN			
Intercept	1	360.62	<0.0001
Year	3	5.97	0.1129

Table 5. One-way ANOVAs of the effects of sexual system on dbh at each site and year. All trees for which sexual systems were observed every year are included in the analyses. Analysis was not conducted for KA in 2000 because all trees exhibited the same sexuality, i.e., all were monoecious. For KA in 2002, none of the pair-wise mean comparisons were significant because the experiment-wise alpha level was 0.05.

Source	F	P	N	Note
KA 1998	4.15	0.0268	30	Monoecy > Male
1999	2.87	0.0998	34	---
2000	---	---	---	---
2001	3.05	0.0615	34	---
2002	3.07	0.0435	33	---
YJ 1998	0.09	0.7630	40	---
1999	3.45	0.0705	43	---
2000	0.75	0.3918	43	---
2001	0.01	0.9386	43	---
HN 1998	6.27	0.0025	29	Monoecy, Male > Non-flowering
1999	8.90	0.0009	33	Monoecy, Male > Female
2000	7.88	0.0018	33	Monoecy, Male > Non-flowering
2001	8.52	0.0012	33	Monoecy, Male > Non-flowering

When the correlation among years within trees was disregarded, those with different sexual systems significantly differed in their dbh in 6 out of 12 one-way ANOVAs for each site and year (Table 5). Monoecious trees had larger dbh values than did male trees at KA in 1998, while both monoecious and male trees were larger than either female or non-flowering trees at HN. Close examination of these data, however, showed that the significance detected at HN occurred largely because of a single tree or two small non-flowering trees. When disregarding that the same trees were repeatedly observed, consistently monoecious trees tended to be larger than facultative monoecious ones at all sites during the study period (Fig. 2). A subsequent repeated-measures ANOVA examined the effects of site and monoecy type on dbh increments of the same trees between 1998 and 2001. There, both year and monoecy type exerted significant effects without significant 2-way and 3-way interactions, except for the year x monoecy interaction (Table 6A). That particular interaction indicated that dbh change between years depended on monoecy type. For example, consistently monoecious trees grew about two-fold more than did facultative monoecious trees across the three sites for four years (1.17 ± 1.58 cm and 0.63 ± 1.52 cm, respectively). When only the KA trees that had been observed for five years were considered, both year and monoecy type significantly affected dbh without the year x monoecy interaction (Table 6B). At KA, consistently monoecious trees grew slightly more, though not significantly, than did facultative ones over the five years (1.66 ± 1.57 cm vs. 1.08 ± 1.49 cm). Thus, the trend was that consistently monoecious trees were larger and grew more than facultative monoecious trees at all sites.

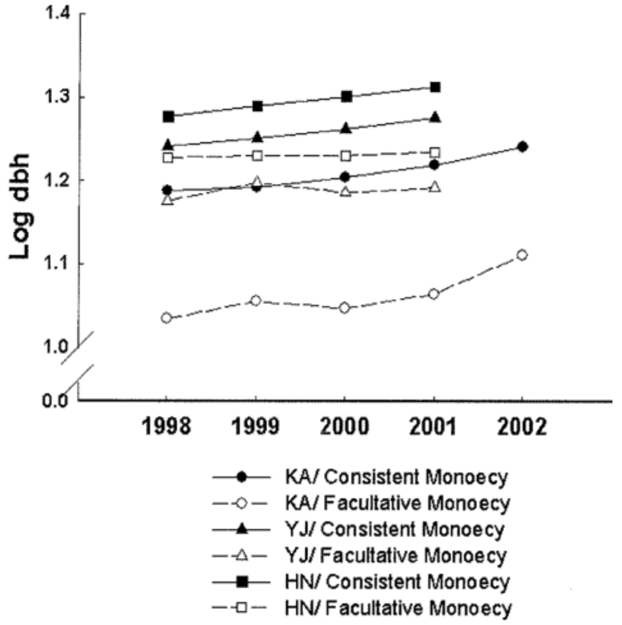

Figure 2. Log dbh of *P. densiflora* trees for five years (1998 through 2002), according to site and monoecy type. Line is drawn to illustrate trend in dbh changes over study period. Only trees with complete data on sexual systems are included. N for consistent vs. facultative monoecy is 20 vs. 9, 23 vs. 17, and 18 vs. 11 at KA, YJ, and HN, respectively.

Phenotypic Gender Variations within Populations

Phenotypic gender-ranking correlations proved significant between successive years, with only one exception -- between 1998 and 1999 at HN (Table 7). Phenotypic gender was not correlated with dbh for any combination of site and year (P >0.05 for all correlations).

Between years in this study period, however, 21.3% of all trees across sites shifted their phenotypic gender. This gender shift frequency differed little among sites (30 out of 135 cases at KA, 26 of 120 cases at YJ, and 15 of 78 cases at HN). Phenotypic gender shifts between two consecutive years occurred either in monoecious trees (42 cases at all three sites) or in trees that experienced changes in their sexual system, e.g., from monoecious to dimorphic (male and female) or vice versa (22 cases). Phenotypic gender shifts coupled with sexual system changes were more frequent at YJ and HN than at KA (42.3, 40.0, and 16.7%, respectively).

Table 7. Spearman's correlation coefficients of phenotypic gender between successive years at three sites. Only trees with complete data are included in the analyses for each site.

	1998*1999	1999*2000	2000*2001	2001*2002
KA (N = 27)	0.44*	0.52**	0.57**	0.34*
YJ (N = 40)	0.49****	0.55****	0.46****	
HN (N = 27)	0.16 ns	0.69****	0.52***	

* = P <0.05; ** = P <0.01; *** = P <0.001; **** = P <0.0001; ns = not significant.

DISCUSSION

If plant size is a major determinant of sexual investment toward male and female cones, one would expect the three populations of *P. densiflora* in this study to show that their differences in mean male and female cone production were consistent throughout this test period. Conversely, with everything else the same, all sites would have been similar to each other after controlling for plant size. However, except for 1998, the small trees at KA and the large trees at YJ were similarly fertile in terms of their male cone numbers. In contrast, large trees at HN were relatively infertile in both male and female cones for most years, although female cone crops can be very responsive to resource levels (Lloyd and Bawa, 1984; McDowell et al., 2000). Here, site differences became stronger after controlling for plant size. Furthermore, under the same constraints, cone ratios varied by 11-fold (KA) to 71-fold (HN) across years. Thus, despite plant size exerting an influence over annual male and female cone production, no consistent pattern revealed that large plants invested more toward female or male function.

Table 6. Repeated-measures ANOVA on dbh of *P. densiflora* trees. Analyses were conducted twice, using two-year data for the three sites (**A**) (1998 and 2001) and for KA (**B**) (1998 and 2002).

Source	A. dbh (1998 – 2001)			Source	B. dbh (1998 – 2002)		
	df	F	P		df	F	P
Between subjects				Between subjects			
Site	2	7.44	0.0010	Monoecy type (Monoecy)	1	7.11	0.0128
Monoecy type (Monoecy)	1	12.21	0.0007	Error	27		
Site x Monoecy	2	0.94	0.3924				
Error	92			Within subjects			
				Year	1	67.43	<0.0001
Within-subjects				Year x Monoecy	1	0.40	0.5342
Year	1	64.72	<0.0001	Error	27		
Year x Site	2	0.55	0.5765				
Year x Monoecy	1	6.41	0.0131				
Year x Site x Monoecy	2	1.39	0.2552				
Error (Year)	92						

In various conifers and oaks, the initiation of cone or flower buds is positively and negatively affected by temperature and rainfall, respectively (Caron and Powell, 1989; Sork et al., 1993; Dickson et al., 2000). However, climatic conditions at the three sites described for this study were not responsible for the discordance of male and female cone crops and cone ratios, because those sites, especially KA and HN, were geographically quite close. Although tree-ring growth can show increased sensitivity to climate as plants age (Carrer and Urbinati, 2004), differences in size among trees at all three sites did not appear large enough to influence their physiology. Thus, there was little possibility for differential effects of climate on size and, subsequently, on cone crops and cone ratios among sites.

Density, architecture, and habitat quality also can affect sexual investment in conifers and several angiosperms (Smith et al., 1988; Allison, 1990; Stromberg and Patten, 1990; Bickel and Freeman, 1993; Arista and Talavera, 1997). Despite the similarity in tree densities in this current study (Kang and Yoon, 2000), their mean plant size and, to some extent, their architecture did vary. For example, unlike for YJ trees, which were relatively tall and narrow, both KA and HN trees had broad canopies. Conifer species with wide canopies have more lateral branches, which allow for increased production of male and female cones. However, even after controlling for plant size, small KA trees had more abundant male and female cones in most years than did the HN trees, thereby limiting any potential impact of architecture on sexual investment. Pines demand high light levels, and crops of male and female cones, in particular, increase with the amount of available light (Shapcott et al., 1995; Stoll and Schmid, 1998). Compared with the KA site, the canopies of the HN trees tended to interfere with those of nearby trees due to size. Thus, HN trees may have been constrained by the reception of light, and in their production of male and female cones, whereas the open trees at KA may have been able to produce more male and female cones than trees at the other two sites. Six ecotypes of Korean *P. densiflora* have been identified that are distinct in their canopy architecture (Uyeki, 1928). Therefore, if the YJ trees had a different ecotype than the one represented by both KA and HN trees, the sexual investment in *P. densiflora* may have been influenced in part by both genetic components and environmental factors, such as light.

This study revealed that *P. densiflora* is primarily monoecious, although it also retains a sexual system flexibility similar to that found with other conifers, including *Abies pinsapo* (Arista and Talavera, 1997), *Cupressus sempervirens* (Lev-Yadun and Liphschitz, 1987), *P. edulis* (Floyd, 1983), *P. halepensis* (Tapias, 1998), and *Taxus canadensis* (Allison, 1991). In *P. densiflora*, about two-thirds of the trees (62.2%) examined here showed consistent monoecy over the period. Except for two trees that were always male, all other trees changed sexual systems at least once during the experiment. The changes occurred largely between monoecy and male, rarely between monoecy and female, and never between male and female. This lack of change between male and female may have been a consequence of architectural constraints caused by the inherent positioning of male and female cones on a tree. That is, *P. densiflora* bears female cones on the top leading branches and male cones on lower branches (Kang and Yoon, 2000). Therefore, transitions between male and female in successive years may prove difficult to accomplish, requiring rather drastic changes in branch growth and tree architecture within a single year.

In comparing consistent vs. facultative monoecy, however, consistently monoecious trees were larger and their dbh values increased more than did those of facultative monoecious trees (Fig. 2; Table 6). This trend was concordant among sites exhibiting different annual patterns of gender expression. Small plants with fewer reserves (Trabaud and Valina, 1998) must allocate more energy to maximum vegetative growth than to reproduction (Shapcott et al., 1995). In general, that preference toward secondary growth is more important than that of other vegetative organs, such as branches, new foliage, and roots (Waring and Schlesinger, 1985). If sex changes in facultative monoecious trees occur primarily between monoecy and male, then small *P. densiflora* trees must invest in radial expansion, perhaps at the expense of female cone production in certain years. The apparent absence of consistently female trees, in contrast to consistently male trees, further demonstrates the high reproductive cost in the former. By procuring resources after being male one year, small trees might be able to turn monoecious in the following growing season. Such a trade-off has indeed been reported among very young trees of *Cupressus sempervirens* (Lev-Yadun and Liphschitz, 1987). The fact that dbh increments over five years did not differ significantly between monoecy types at KA also reflects the fact that, unlike for the other sites, almost half of the trees at KA were male in both 2001 (45.5%) and 2002 (54.5%). Nonetheless, large trees with greater reserves might afford the production of both male and female cones while increasing their girth as well. Sex changes confer survival advantages in *Atriplex canescens* (Freeman et al., 1984). Similar alterations in small *P. densiflora* trees might also be a survival strategy as well as a means to reproduce at least as a male parent.

The standardized phenotypic gender of individual trees, which controls for sexual systems, was not constant over years at all sites. An average of 21% of all trees shifted their phenotypic gender (G changes $> |0.4|$) across the three sites. This average was higher than the 7 to 18% reported in *Taxus canadensis* (Allison, 1991), but lower than the 43% for *Abies pinsapo* (Arista and Talavera, 1997). Data showed that even trees that were monoecious for two consecutive years experienced such shifts in this study. Furthermore, a substantial proportion of both groups of consistent and facultative monoecious trees that differed in size shifted phenotypic gender. In other words, *P. densiflora* appears to be quite flexible not only in its sexual systems, but also in phenotypic gender expression, a result of variations in relative investment toward male and female cones. Such flexibility within trees seems to be responsible for the lack of any correlation between plant size and phenotypic gender. Weak connections also have been expected between those two parameters (Lloyd and Bawa, 1984).

Despite fluctuations in annual cone crops and phenotypic gender changes in a substantial portion of the study trees, their phenotypic gender rankings within sites were positively correlated between all pairs of years except for one case at HN. Rankings have also been found to be concordant in *Pinus ponderosa* (Linhart and Mitton, 1985) and *T. canadensis* (Allison, 1991). This agreement in gender ranking across years suggests that, whereas gender may be labile, it also is at least partially determined genetically, as suggested for other conifers (Schoen et al., 1986; Apsit et al., 1989).

This study measured gender expression as a function of cone ratios, sexual systems, and standardized phenotypic gender. Although *P. densiflora* is generally monoecious, sex changes were prevalent at all sites. Alterations to their dimorphic systems, whether male or female, may have reflected a trade-off relationship between vegetative and reproductive growth. Such changes also can cause variation in subsequent reproductive success, e.g., seed production. For example, if a large proportion of the trees becomes male, such as did those at YJ in 2000, the result would be a rapid decline in seed crops the following year. The relationship between parameters of phenotypic gender and functional gender remains to be tested in order to confirm the evolutionary meanings of variations in gender expression in *P. densiflora*.

ACKNOWLEDGEMENTS

The author wishes to thank reviewers for comments on an earlier version of the manuscript, and for financial support from Sungshin Women's University in 2003 (Grant No. 03-10-11).

Received December 12, 2006; accepted February 27, 2007.

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