

## Paternal gene flow in *Cryptomeria japonica* seed orchards as revealed by analysis of microsatellite markers

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Seed orchards, which are stocked with superior tree clones, are important sources of materials for afforestation. Ideally, such materials should have high genetic value and diversity. In order to maintain high genetic value of their seeds, rates of contamination by external pollen and self-fertilization within the orchards should be minimal, while the paternal contribution of the constituent clones should be roughly equal to ensure the seeds have high diversity.

Sugi (*Cryptomeria japonica* D. Don) is an allogamous, wind-pollinated conifer species that is frequently used for commercial afforestation in Japan. To ensure a supply of high-quality reforestation materials, wind-pollinated clonal seed orchards of *C. japonica* have been established in various parts of Japan by propagating superior clones. In the present study, we estimated the levels of pollen contamination, self-fertilization and paternal contribution, using microsatellite markers, in three common and two miniature clonal seed orchards of *C. japonica*. To obtain basic data that could be used to further improve seed orchards, we attempted the comparison of gene flow data among seed orchards. We analyzed gene flow in each of the seed orchards and investigated reasons for observed differences in pollen contamination, self-fertilization and paternal contributions to elucidate the scale of these potential problems and to help identify possible ways to counter them.

Two types of seed orchards have been established in Japan: common clonal orchards and miniature clonal orchards (Longman and Dick 1981, Itoo and Katsuta 1986). The latter type has been used since 1982. Miniature clonal seed orchards are more efficient, owing to their smaller labor requirements and shorter establishment times. Five clonal seed orchards of *C. japonica* (three common types and two miniature types) were investigated (Moriguchi et al. 2005b). The common seed orchards had average tree heights for each clone of about 5 m; the spacing was 5 × 5 m. The miniature clonal seed orchards had average tree heights for each clone of about 2 m; spacing was 1 × 1 m. In each seed orchard, crude DNAs were extracted from needle tissues of all the constituent parental clones and from germinated seedlings collected from 12 randomly selected ramets. DNA extraction was carried out using the CTAB

method (Murray and Thompson 1980) and the modified CTAB method (Tsumura et al. 1995). Thirty seeds per mother tree were analyzed using microsatellite markers in five seed orchards. We selected microsatellite markers that show high stability and polymorphism (Moriguchi et al. 2003, Tani et al. 2004). The multi-paternity exclusion probability (Weir 1996) for the selected markers was high (more than 0.999) in all seed orchards. PCR amplifications were carried out using the method of Moriguchi et al. (2005b). Paternity was determined by a simple exclusion method (Moriguchi et al. 2004).

The average proportions of the seeds originating from contaminating pollen in the seed orchards varied between 35.0 ( $\pm 3.47$ )% and 65.8 ( $\pm 4.11$ )%. The high contamination rate detected in this study was similar to rates reported for other conifer seed orchards of dominant species (El-Kassaby et al. 1989, Yazdani and Lindgren 1991, Wang et al. 1991, Adams et al. 1997, Burczyk and Part 1997, Stoehr et al. 1998, Pakkanen et al. 2000). The area of *C. japonica* forests in the vicinity of the seed orchard (ha) was correlated with the pollen contamination rate (Table 1). The contamination rates seem to be affected by the rate of pollens from outside the seed orchard in the cloud of pollen. Because even the seed orchard located in an excellent environment had pollen contamination levels of about 30%, it may be impossible to avoid pollen contamination levels below 30% in outdoor seed orchards.

**Table 1** Average pollen contamination rates and the area (in ha, and as a percentage of the total area) of artificial *C. japonica* forests within 3, 5, 8, 10 and 15 km of the five seed orchards. (Moriguchi et al. 2005b)

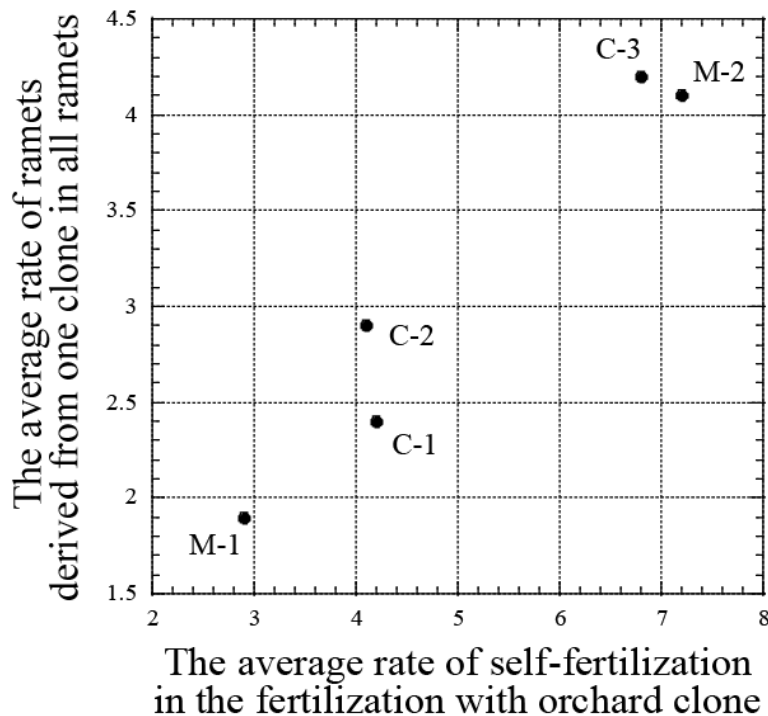
	radius of 3km (ha (%))	radius of 5km (ha (%))	radius of 8km (ha (%))	radius of 10km (ha (%))	radius of 15km (ha (%))	Contamination (%)
C - 1	612.8 (21.7)	1796.4 (22.9)	3373.5 (16.8)	4151.6 (13.2)	6430.7 (9.1)	47.8
C - 2	782.8 (27.7)	1582.1 (20.2)	5000.4 (24.9)	7970.0 (25.4)	19770.3 (28.0)	65.8
C - 3	8.2 (0.3)	197.5 (2.5)	326.9 (1.6)	476.6 (1.5)	2702.4 (3.8)	35.0
M - 1	0.0 (-)	0.0 (-)	851.7 (4.2)	1521.0 (4.8)	5815.4 (8.2)	40.8
M - 2	585.6 (20.7)	1755.8 (22.4)	3918.8 (19.5)	5616.9 (17.9)	15169.2 (21.5)	50.0
Spearman's $\rho$ ( <i>p</i> value)	0.472 (<0.001)	0.217 (0.096)	0.562 (<0.001)	0.562 (<0.001)	0.562 (<0.001)	

The average self-fertilization rates in the seed orchards varied between 1.4 ( $\pm 0.64$ )% and 4.4 ( $\pm 2.02$ )%. The number of ramets per clone was correlated with the self-fertilization rates (Table 2, Fig. 1, Moriguchi et al. 2005b). The self-fertilization rates seem to be affected by the rate of self pollen in the cloud of orchard pollen. The self-fertilization rate in conifer seed orchards is thought to be generally less than 5 % (Ritland and El-Kassaby 1985; Rudin et al. 1986; Goto et al. 2002). However, a much lower self-fertilization rate (0.426%) was observed in the progeny test (Moriguchi et al. submitted). Therefore, self-fertilization does not

appear to present a major threat in conifer seed orchards, despite its potentially adverse effects, as reported by Goto et al. (2005).

**Table 2** Average self-fertilization rate, the average rate of ramets derived from one clone in all ramets and the average rate of self-fertilization in the fertilization with orchard clone.

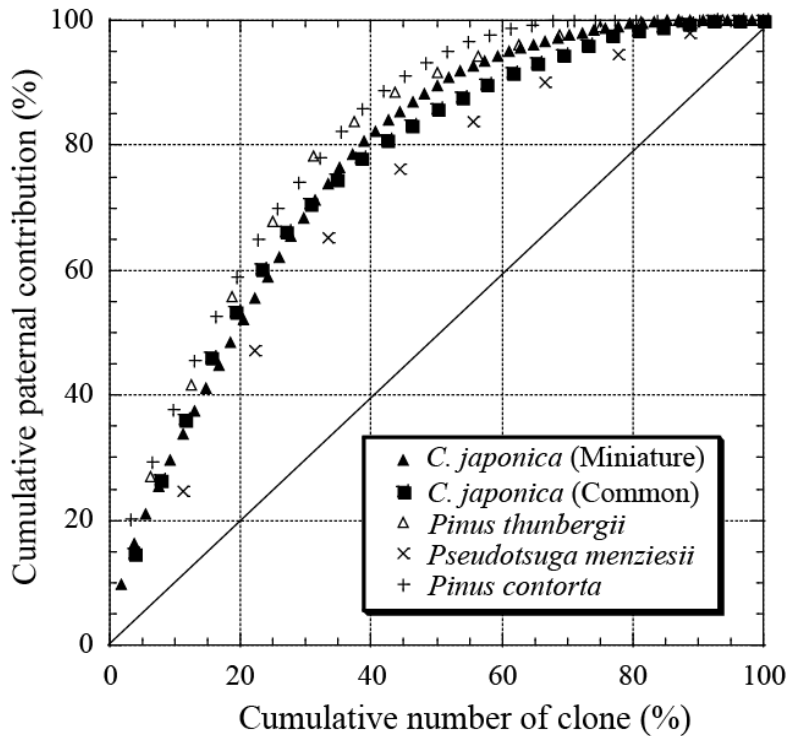
	Self-fertilization rate (%)	The average rate of self-fertilization in the fertilization with orchard clone	The average rate of ramets derived from one clone in all ramets
C - 1	2.2	4.2	2.4
C - 2	1.4	4.1	2.9
C - 3	4.4	6.8	4.2
M - 1	1.7	2.9	1.9
M - 2	3.6	7.2	4.1



**Fig.1** Relationship between the average rate of ramets derived from one clone in all ramets and the average rate of self-fertilization in the fertilization with orchard clone.

In the result of  $\chi^2$  test, paternal contributions to seed production by the constituent clones differed significantly in all seed orchards ( $p < 0.001$ ). In spite of the differences in the types of seed orchard and their locations, the same tendency was revealed for all of the seed orchards, i.e. about 20% of the clones accounted for about 60% of the total gene flow and about 30% of total clone made no contribution (Moriguchi et al. 2005b). Similar results have been found in seed orchards of other conifer species, such as *Pinus contorta* Dougl., *Pinus thunbergii* Parl. and *Pseudotsuga menzeisii* Franco (Fig. 2, Moriguchi et al. 2005a). Paternal

contribution is affected by the male flower production, floral synchrony, distance between parents, wind direction and pollen competition (Shen et al. 1981, Schoen and Stewart 1986, Erickson and Adams 1989, Burczyk and Prat 1997, Stoehr et al. 1999, Nikkanen et al. 2000, Aronen et al. 2002, Goto et al. 2002). In *C. japonica*, total male flower production strongly affects male reproductive success and the inter-tree distance also has some effect (Moriguchi et al. 2007).



**Fig.2** Relationship between cumulative number of clones (%) and cumulative paternal contribution (%) (Moriguchi et al. 2005a, in Japanese). The numbers of clones were cumulated sequentially from the clones with the highest contributions. The data of *Pinus thunbergii*, *Pseudotsuga menziesii* and *Pinus contorta* obtained from Goto et al. (2002), Stoehr et al. (1998) and Stoehr and Newton (2002), respectively.

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