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# Inheritance of male flower production in *Cryptomeria japonica* (sugi) estimated from analysis of a diallel mating test

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## Abstract

• **Context** Clonal variation of flower production has been investigated thoroughly in coniferous tree species because of the importance of flowering in seed production from seed orchards. However, the inheritance of flower production by progeny test has not been previously studied well, so the heritability of male flower production of *Cryptomeria japonica*, the most important species for forestry and the main cause of pollinosis in Japan, was estimated to provide fundamental data for pollen control in seed orchards and plantation forests.  
• **Methods** Male flower production in *C. japonica* was investigated for three years using 20 full-sib families created by a diallel mating design with six parents using gibberellin treatment to promote flowering.

• **Results** The narrow-sense individual tree heritability of male flower production in each year was estimated as ranging from  $0.777 \pm 0.339$  to  $1.050 \pm 0.346$  by sib analyses. The contribution of general combining ability to the total variance varied from 24.1 to 35.6 %. The highest contribution of specific combining ability was 1.42 %. By selecting the top 50 % of parental clones for reduced male flower production, the genetic gain in lowering male flower productivity was predicted as 46.1–53.0 %.

• **Conclusion** Our results suggest that male flower production in *C. japonica* is under strong genetic control and that control of male flowering by breeding is possible with a well-designed program.

**Keywords** *Cryptomeria japonica* · Male flower · Pollinosis · Heritability · Genetic gain

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**Contribution of the co-authors** Miyoko Tsubomura: writing the paper and running experiments. Eitaro Fukatsu: writing the paper and analyzing the data. Ryogo Nakada: writing the paper and supervising the work. Yoko Fukuda: writing the paper and designing the experiments.

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## 1 Introduction

In tree breeding, clarifying the mode of inheritance of flowering is important because flowering characteristics determine the productivity of genetically improved seeds and seedlings, and the extent of flowering of each individual affects its genetic contribution to progeny, especially in production from seed orchards. A lot of studies have examined the production of male strobili and female cones at the level of clonal variation in seed orchards and field trials (Bilir et al. 2006; Choi et al. 2004; Kang 2000; Matziris 1993; Nikkanen and Ruotsalainen 2000). However, few reports have analyzed the inheritance of flowering characteristics by progeny analysis following artificial mating (Hannerz et al. 2001).

The long time from seed germination to the onset of flowering and the fluctuation of flowering from year to year

make evaluation of the inheritance of flowering characteristics difficult. Reproduction in many conifers begins 5–10 years after planting (Williams 2009) and the number of flowers is not stable at such an early stage. The year-to-year fluctuation in flowering means that studies need to measure the flower production repeatedly over several years in various conditions. Promotion of flowering offers a solution to these problems. Flower induction by stem girdling, root pruning, heat treatment, and phytohormone treatment has been developed for several coniferous species (Bonnet-Masimbert and Weber 1995). Exogenous application of gibberellins was thought to be effective for flowering promotion in the Cupressaceae and Pinaceae (Pharis 1975). Many reports describe the induction of flowering by the application of gibberellins, especially gibberellin A4/7, in pine, larch, and Douglas fir (Almqvist 2003; Cherry et al. 2006; Philipson 1995). Gibberellins are effective on mature trees or grafted trees with another treatment, such as heating or girdling, but do not have strong effects on seedlings in pine or other coniferous species.

Gibberellin A3 (GA<sub>3</sub>) has a strong effect on flowering and can induce flowering even in 1-year-old seedlings in *Cryptomeria japonica* (Hashizume 1959). To investigate flowering characteristics, *C. japonica* would be a model coniferous species because of its ease of the flowering induction by GA<sub>3</sub> treatment. The study of flowering of *C. japonica* is important mainly from two standpoints: its importance for the forestry and wood industry in Japan, and the serious problem of allergies caused by its pollen. *C. japonica* is the most important species in Japanese forestry and accounted for approximately 22 % of total forestation in 2009 (Forestry Agency 2011). Its rotation age is generally 40–50 years. Many clonal seed orchards have been established over large areas for this species and many breeding programs with various objectives have been executed. On the other hand, Cupressaceae trees produce the most allergenic conifer pollen and the number of affected patients is increasing rapidly in Europe, North America, and Asia (D'Amato et al. 1998; Charpin et al. 2005). In Japan, approximately 20 % of adults suffer from *C. japonica* and *Chamaecyparis obtusa* pollinosis (Okuda 2003), which has become a serious health problem. Many studies have been performed on the flowering of *C. japonica*, such as histological analysis (Hashizume 1962), estimation of clonal variation of flower production (Saito and Kawasaki 1984), and recently, genetic analysis including establishment of cDNA or expressed sequence tag libraries (Futamura et al. 2008; Kurita et al. 2011) from male reproductive organs.

GA<sub>3</sub> treatment has often been used for investigation of flowering in *C. japonica* (Nagao 1985; Tsurumi and Ohba 1988; Miyazaki et al. 2009) because it enables obtaining consistent data regardless of meteorological conditions and clonal variation is maintained as in natural flowering. In *C. japonica*, reproductive onset under natural conditions without

any promoting treatments may occur at 3–4 years old, but it can require 20 years in some clones (Hashizume 1959). In *C. japonica*, male flowers are easily induced by GA<sub>3</sub> application in July and female flowers from the end of July to the end of August (Hashizume 1959). The flowers are initiated 1 month after GA<sub>3</sub> treatment and later development is similar to natural flowering. We postulate that GA<sub>3</sub> treatment for flowering promotion is effective for the investigation of the inheritance of male flower production.

There are still only a few detailed reports on the mode of inheritance of male flower production. Since male flower production, which is closely associated with pollen productivity, have large variation among clones (Saito and Kawasaki 1984), the Japanese tree-breeding program has selected “less-pollen clones” bearing only small numbers of male flowers (Kondo 1997). The male flower production of 1,700 plus tree clones, which are superior trees selected in forest stands based on their growth characteristics and stem forms, was evaluated in clonal trials and at clonal test sites for several years using a flowering index. The flowering index for male cone clusters was measured by observing and categorizing the number of male cone clusters into five or six classes from zero (no flower setting) to four or five (abundant; Forestry Agency 1991). To date, 135 less-pollen clones have been selected based on these data. The less-pollen clones are introduced into seed orchards and the seedlings are used for forestation mainly in the Kanto region, where pollinosis is the most serious. However, the male flower production of the seedlings is difficult to predict because of a lack of detailed inheritance studies (Tsurumi and Ohba 1988; Kondo 1998). Not only Cupressaceae species but also Betulaceae or Oleaceae species can cause pollinosis (D'Amato et al. 1998). The case of *Cryptomeria* could be a model for the inheritance of pollen production in tree species, which are perennials with a long maturation age.

In this study, we investigated the male flower production of progeny from a full diallel cross with six parents induced by GA<sub>3</sub> application and estimated several genetic parameters (heritability, general combining ability (GCA) and specific combining ability (SCA)). We also estimated the genetic gain of male flower productivity by mass selection.

## 2 Materials and methods

### 2.1 Plant materials

The progeny test site used in this study included the progeny from a full diallel cross with six parental plus tree clones of *C. japonica*. The test site was established in 2001 on the campus of the Forest Tree Breeding Center, Forestry and Forest Products Research Institute, located in Hitachi, Ibaraki, Japan (36° 69'N, 140° 69'E; elevation, 52 m). The experimental design consisted of randomized blocks with six replications and 2.5×1.5 m

spacing between trees. In each replication, various numbers of individuals for each full-sib family were planted randomly. The tree age was 7 years old in 2004. Three of the six blocks, containing 225 individuals from 20 families, were investigated in this study. The number of individuals in each family averaged 11.3 and ranged from 1 to 26 (Table 1). The number of individuals within one family per block averaged 3.75 and ranged from 0 to 26. The individual trees were allowed to grow naturally except for the promotion of flowering during this study.

The parental clones used for the progeny test were also investigated. One to three ramets from the parental clones were investigated at a crossing garden on the same campus. The crossing garden was established in 1995 and the plus tree clones were planted in lines with 2.5 × 1.5 m spacing between trees. They had been topped and hedged as seed orchard trees.

## 2.2 GA<sub>3</sub> induction and measurement of male flower production

In 2006, the mean tree height ( $\pm$ standard deviation) for all measured individuals in the progeny test was 4.11 $\pm$ 0.67 m and the diameter at breast height (DBH) was 5.15 $\pm$ 1.48 cm. The trees were treated with GA<sub>3</sub> to promote stable, early flowering in order to investigate male flower production. We chose three branches on each tree at a height of approximately 1.5–1.8 m for treatment. We soaked a length of approximately 30 cm from the distal tip of each selected branch in a 100 ppm aqueous solution of GA<sub>3</sub> (Kyowa-Hakko, Tokyo, Japan) using 5 L beakers. Branches were treated twice a year in the beginning and middle of July of 2004, 2006, and 2007 (the two treatments were separated by a 2-week interval). Parental clones were treated at the same time in 2006 and 2007. The primordia of male strobili are initiated from late June to late September and the formation of pollen grains is observed from mid-September to early November at typical sites in Japan (Hashizume 1962). Flowering begins in February and lasts to April, although it differs among clones and sites. Female

**Table 1** Diagram of the mating design

Female	Male					
	1,178	1,136	957	890	900	920
1,178	–	20	3	11	5	15
1,136	8	–	5	–	13	–
957	–	4	–	–	10	–
890	12	2	7	–	1	16
900	13	21	18	15	–	26

The parents are identified by plus tree numbers. The numerals in the table are numbers of individuals within families. The parental clones are presented in the order of mean NMC in 2006 and 2007 (Fig. 4)

strobili set on the top of the shoots and small male cones cluster on the top of lower twigs (Fig. 1). The number of male flowers setting on each GA<sub>3</sub>-treated branch from each tree was investigated in November or December in the same year, when the cones had almost fully developed (Hashizume 1973).

We obtained the number of male cone clusters (NMC) on each GA<sub>3</sub>-treated branch of each tree by counting male cone clusters and performed statistical analysis. The NMC was defined as the number of groups of male cones clustering on the tip of a small shoot (Fig. 1). They were counted on the three GA<sub>3</sub>-treated branches, using tally counters. The investigated flowers located 1.5–2.5 m or more above the ground. The mean of the NMC on the three branches was defined as the estimate for each tree. To avoid counting a particular cluster twice, the shoots already counted were held away from the uncounted ones by hand each individual tree. In further analysis, we referred to NMC as a trait of an individual tree.

To confirm the validity of using NMC as an estimation of the number of cones of each individual, the number of male cones (NM) setting on each individual GA<sub>3</sub>-treated branch was also investigated in 2004. The NMC of the three untreated branches for each individual was also investigated in 2007, the only year with abundant natural flowering, to evaluate the correlation between male flower production in the presence and absence of GA<sub>3</sub> treatment. The three untreated branches were chosen from sunny places of each investigated tree ( $n=225$ ). Their conditions were similar to that of GA<sub>3</sub> treated branches.

## 2.3 Statistical analysis

The distribution of NMC and NM were skewed toward zero and included some zero values (individuals that set no male flowers). Thus, we transformed the values for NMC and



**Fig. 1** The male flowers of *C. japonica*. A cluster (circled) sitting on the tip of a shoot consists of approximately 10 male cones on average

NM to logarithmic-scale values using the following formula (Yamamura 1999):

$$\log \text{NMC} = \log_e(0.5 + \text{NMC})$$

$$\log \text{NM} = \log_e(0.5 + \text{NM})$$

where  $\log \text{NMC}$  and  $\log \text{NM}$  are logarithmically transformed NMC and NM, respectively. The following analyses were executed based on the values for  $\log \text{NMC}$  and  $\log \text{NM}$  in each year. We analyzed the phenotypic correlations between  $\log \text{NMC}$  and  $\log \text{NM}$ , and those between  $\log \text{NMC}$  with and without  $\text{GA}_3$  treatment, for individuals in progeny test to evaluate the validity of our method. Two-way analysis of variance (ANOVA) was performed for  $\log \text{NMC}$  in year 2007 with family, flowering-promotion treatment (no treatment/ $\text{GA}_3$ ) and their interaction as explanatory variables. Two-way ANOVAs for  $\log \text{NMC}$  with  $\text{GA}_3$  treatment with family or clone, year and their interaction as explanatory variables were performed in progeny test or clonal archives. Data were analyzed using the statistical package R (R Development Core Team 2008).

To estimate genetic parameters, we fit the data to the following linear mixed model:

$$Y_{ijkl} = \mu + B_i + G_j + G_k + S_{jk} + P_{ijk} + e_{ijkl}$$

where  $Y_{ijkl}$  is an observation of each trait of a tree  $l$  in a cross with parent  $j$  and parent  $k$  in block  $i$ ;  $\mu$  is the overall mean,  $B_i$  is the fixed effect of block  $i$ ;  $G_j$  and  $G_k$  are the random effects of GCA of the parents  $j$  and  $k$ , respectively;  $S_{jk}$  is the random effect of SCA of the combination of the parents  $j$  and  $k$ ;  $P_{ijk}$  is the random effect of replication by a specific cross-interaction; and  $e_{ijkl}$  is the random residual. Random factors were assumed to be distributed normally with an expectation of zero. Previous reports (Saito and Kawasaki 1984; Tsurumi and Ohba 1988) indicated that the differences between female and male parental effects are not significant; thus, the reciprocal effect was not included in the model. We treated families in corresponding reciprocal crosses as a single family. The number of individuals in each family averaged 17.3 and ranged from 2 to 34. The number of individuals within one combination per block averaged 5.77 and ranged from 0 to 26. The variance components of each random factor were estimated using the restricted maximum likelihood method, and the best linear unbiased prediction (BLUP) of each random factor was calculated (Xiang and Li 2001). Genetic parameters for growth traits (height and DBH) were also calculated in the same way. The calculation was performed using the SAS MIXED and IML Procedure (SAS Institute Inc. 1996).

The individual tree heritability of each trait was calculated using the following formula:

$$h_i^2 = 4\sigma_G^2 / (2\sigma_G^2 + \sigma_S^2 + \sigma_p^2 + \sigma_e^2)$$

where  $h_i^2$  is the narrow-sense heritability of an individual tree,  $\sigma_G^2$  is the variance of GCA,  $\sigma_S^2$  is the variance of SCA,  $\sigma_p^2$  is the variance of replication by a specific cross-interaction, and  $\sigma_e^2$  is the within-replication variance. GCA covariance between trait  $x$  and trait  $y$  were estimated using the following formula (Xiang et al. 2003):

$$\text{Cov}_{G(x) \cdot G(y)} = \left( \sigma_{G(x)+G(y)}^2 - \sigma_{G(x)}^2 - \sigma_{G(y)}^2 \right) / 2$$

where  $\text{Cov}_{G(x) \cdot G(y)}$  is the GCA covariance between trait  $x$  and  $y$ ;  $\sigma_{G(x)+G(y)}^2$  is the GCA variance of the sum value of trait  $x$  and  $y$ ;  $\sigma_{G(x)}^2$  is the GCA variance of the trait  $x$ ; and  $\sigma_{G(y)}^2$  is the GCA variance of trait  $y$ . The genetic correlation between two traits ( $x, y$ ) was calculated as follows:

$$r_{G(x,y)} = \text{Cov}_{G(x) \cdot G(y)} / \left( \sigma_{G(x)}^2 \cdot \sigma_{G(y)}^2 \right)^{1/2}$$

where  $r_{G(x,y)}$  is the genetic correlation between two traits. The genetic correlations between  $\log \text{NMC}$  with and without  $\text{GA}_3$  treatment in 2007, and between  $\log \text{NMC}$  and growth traits (DBH and height) were estimated.

The BLUP of the breeding value of each parent was estimated by doubling the BLUP value for the general combining ability of male flower production. The additive genetic variance ( $\sigma_A^2$ ) was calculated as  $4\sigma_G^2$ .

The predicted genetic gain ( $\Delta G_{\log \text{NMC}}$ ) of mass selection was estimated as follows (Falconer and Mackay 1996):

$$\Delta G_{\log \text{NMC}} = ih_i \sigma_A$$

where  $i$  is the selection intensity.

Relative genetic gain was calculated as the genetic gain divided by the family mean of the original population. To calculate the genetic gain of the NMC ( $\Delta G_{\text{NMC}}$ ), we transformed the genetic gain of  $\log \text{NMC}$  to  $\Delta G_{\text{NMC}}$  as follows:

$$\Delta G_{\text{NMC}} = \exp(\Delta G_{\log \text{NMC}} + \mu) - \exp(\mu)$$

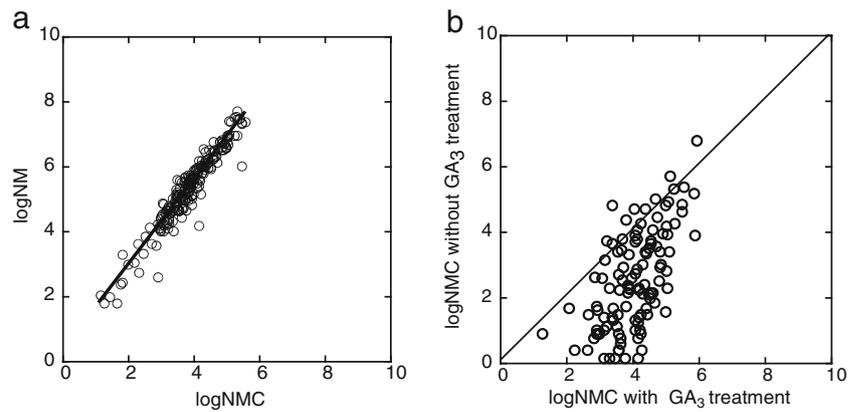
where  $\mu$  is the total mean of  $\log \text{NMC}$  calculated from each family  $\log \text{NMC}$  value before selection. Relative genetic gain was calculated as the genetic gain divided by the mean value of the original population.

### 3 Results

#### 3.1 Correlation between $\log \text{NMC}$ and $\log \text{NM}$ , and male flower production with and without $\text{GA}_3$ treatment

In 2004,  $\log \text{NMC}$  and  $\log \text{NM}$  of progeny from the diallel mating were compared for individuals (Fig. 2). NMC ranged from 2.67 to 261 (median, 46.0), and NM ranged from 5.67 to 2,268 (median, 228). The Pearson's product-moment

**Fig. 2** Correlation between logNMC and logNM, and male flower production with and without GA<sub>3</sub> treatment. **a** Correlation between NMC and NM in transformed logarithmic scale (see text). Each symbol shows an individual tree. Data from 2004 ( $n=225$ ,  $r=0.960$ ). **b** Correlation between logNMC with and without GA<sub>3</sub> treatment. Data from 2007 ( $n=225$ ,  $r=0.596$ )



correlation coefficient between logNMC and logNM was 0.959 ( $n=225$ ,  $p<0.001$ ). This means that NMC may be used as a reliable estimate for the NM. It was easier to count NMC than NM. Thus, in this study, NMC was used to evaluate male flower production.

The phenotypic correlation between logNMC with GA<sub>3</sub> treatment and under natural flowering without GA<sub>3</sub> treatment was analyzed (Fig. 2). The Pearson's product-moment correlation coefficient between logNMC with and without GA<sub>3</sub> treatment was statistically significant ( $n=225$ ,  $r=0.596$ ,  $p<0.001$ ). Based on two-way ANOVA on the effect of family and GA<sub>3</sub> treatment, the variations between family and between treatments were statistically significant ( $p<0.001$ , both), but the interaction between family and treatment was not significant (Table 2). The genetic correlation between logNMC with and without GA<sub>3</sub> treatment was estimated 0.955 in 2007. In this study, the logNMC value obtained for the GA<sub>3</sub> treatment was used as an estimation of the male flower production of each individual for further analyses.

### 3.2 Parental clonal variation and year-to-year correlation of male flower production

The clonal mean for NMC varied from 30.2 to 173 in 2006 and from 11.9 to 252 in 2007 (Fig. 3). Based on the results

**Table 2** Two-way ANOVA for logNMC in year 2007 with family, flowering-promotion treatment

Factor	Degree of freedom	Sum of square	Mean square	F value
Family	19	470	24.7	11.6*
Treatment	1	998	998	479*
Family × treatment	19	51.8	2.73	1.28 ns
Residuals	635	1349	2.12	

ns not significant

\*  $p<0.001$ , level of significance

of two-way ANOVA on the effects of clones and years, the variation between clones and between years, and the interaction between clone and year in the logNMC values were statistically significant. Clone 920 produced the largest NMC on average in two of the years and this value was approximately 9.82 times the value for clone 1178, which showed the lowest NMC. The ranking of clones was nearly stable for 2 years and Spearman's rank correlation coefficient was 0.943 ( $n=6$ ,  $p<0.05$ ).

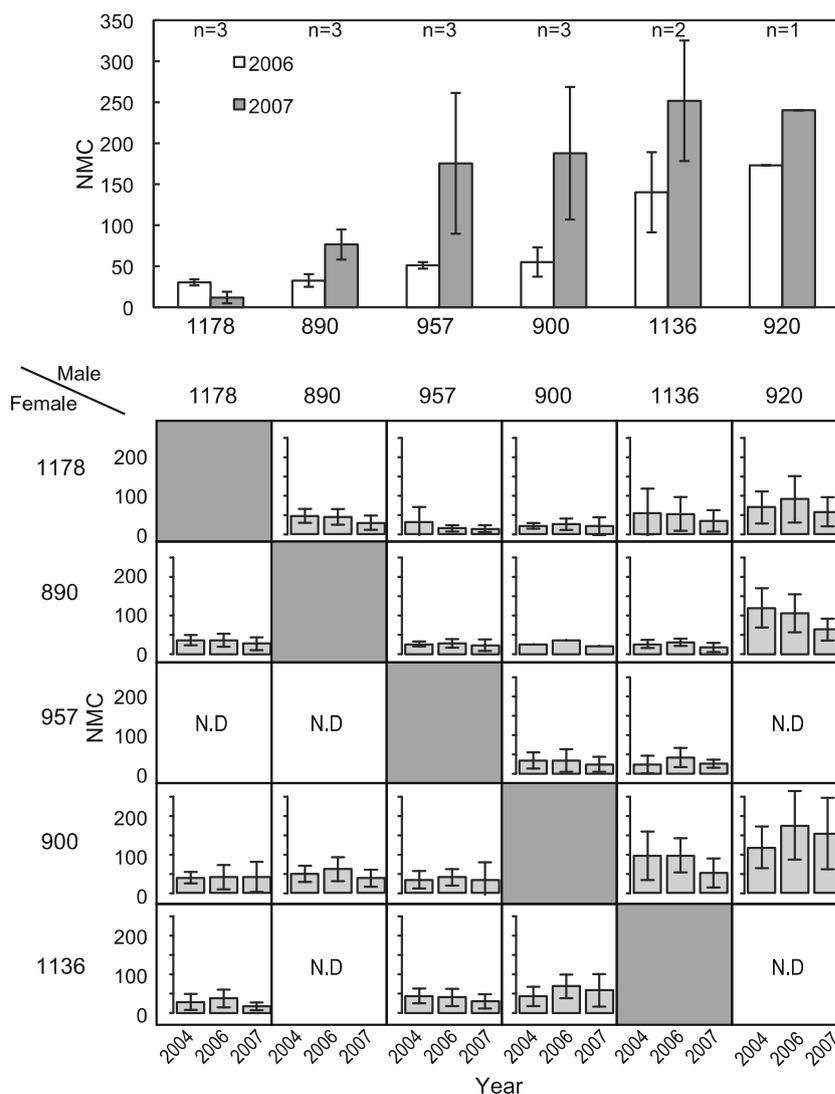
### 3.3 Family variation in progeny and year-to-year correlation of family male flower production and GCA

The mean of the NMC for progeny from each specific combination of parents (the family NMC) is shown in Fig. 3 for each year. The results of two-way ANOVA of logNMC showed that the differences between families and between years were significant ( $p<0.001$  for both), but the interaction between family and year was not significant. Family NMC varied from 22.3 to 120 in 2004, from 15.8 to 176 in 2006, and from 15.2 to 155 in 2007. Pearson's product-moment correlation coefficients among years for the family logNMC and the GCA of the logNMC of the six parents were statistically significant (Table 3).

### 3.4 Heritability and genetic gain

Figure 4 shows the variance components of the logNMC and growth traits, and the narrow-sense individual tree heritabilities with standard errors estimated from the results of ANOVA. The contribution of the GCA of logNMC to the total variance varied from 24.1 % in 2007 to 35.6 % in 2006. The contribution of SCA was only 1.42 % in 2004, which was its highest value. The highest estimated narrow-sense individual tree heritability ( $1.050\pm 0.346$ ) was observed in 2006. The heritability of logNMC under natural flowering in 2007 was  $0.471\pm 0.271$ . In contrast to logNMC, the heritabilities of growth traits (DBH and height) were very low in 2006. The family-mean phenotypic correlation was not significant

**Fig. 3** The mean and standard deviation (*error bars*) of NMC of the six parental clones and the family NMC of progeny obtained with GA<sub>3</sub> treatment. The parental clones are presented in the order of mean NMC in 2006 and 2007. The family NMC of progeny in 2004, 2006, and 2007 with GA<sub>3</sub> treatment are listed in the same order as their parents



( $r=0.331, p=0.154$ ), and genetic correlation was not estimable because of no GCA variance in DBH (Fig. 4), between DBH and logNMC. The family-mean phenotypic correlation between height and the logNMC was estimated as  $r=0.478$  ( $p<0.05$ ), and genetic correlation was estimated 0.672 in 2006. The genetic gain based on the NMC with selection of the top 50 % of parental clones bearing few male flowers was

**Table 3** Year-to-year correlation coefficients of logNMC for 3 years

Year	2004	2006	2007
2004	–	0.880*	0.809*
2006	0.975*	–	0.917*
2007	0.982*	0.978*	–

The correlation coefficients of family logNMC ( $n=20$ ) are shown in the upper right, and the correlation coefficients of GCA of logNMC of the six parents are shown in the lower left

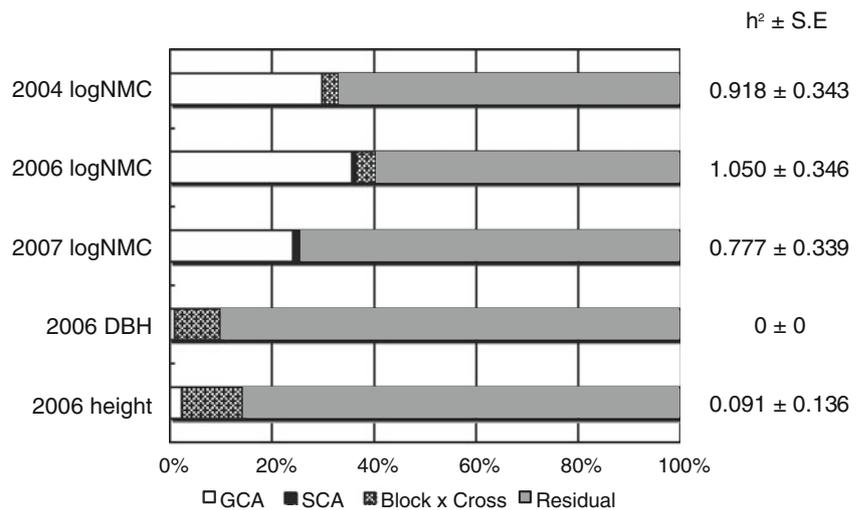
\*  $p<0.001$ , level of significance

estimated to range from  $-15.7$  to  $-27.6$  and varied between years (Table 4). The relative genetic gain by selecting the top 50 % of parental clones would be  $-46.1$  to  $-53.0$  %.

**4 Discussion**

The logNMC value of progeny was closely correlated with logNM (Fig. 2), which should be closely correlated with actual pollen production, and NMC counts were shown to be well suited for quantitative evaluation of male flower production. The logNMC value of progeny obtained with GA<sub>3</sub> treatment was significantly correlated with the logNMC of progeny undergoing natural flowering, and was generally larger (Fig. 2). Since the interaction between family and treatment was not significant (Table 2), the responses to GA<sub>3</sub> were not significantly different between families. In addition, the genetic correlation between logNMC with and without GA<sub>3</sub> treatment was high (0.957). We could obtain stable data for 3 years by GA<sub>3</sub>

**Fig. 4** Variance components of family logNMC for 3 years and that of height and DBH in 2006. The contributions to the total variance of GCA, SCA, interaction between block and cross, and residual are indicated as a percentage. The narrow-sense individual tree heritability ( $h^2$ ), with the standard error (SE) estimated from the results of ANOVA, is shown on the right



treatment even though the extent of natural flowering fluctuates every year. We concluded that GA<sub>3</sub>-treated pollen production is a good estimator for the natural pollen production, due to its stable expression, their high heritability and the high genetic correlation between GA<sub>3</sub>-treated and natural pollen production.

Both parental clones and their progeny showed a large variation in male flower production in spite of the relatively small number of individuals and families used in this study (Fig. 3). The high contribution of GCA to the total variance indicated that male flower production of *C. japonica* is under strong additive genetic control, and that the presence of low-pollen individuals was due to the high GCA values inherited from the parents and not due to the specific cross. Tsurumi and Ohba (1988) investigated the inheritance of flowering characteristics of *C. japonica* using a complete full diallel cross with three parental plus-tree clones. They could not estimate GCA of male flower production, and they suggested that this limitation occurred because they used only three parents for the analysis and the 2-year-old seedlings examined were too young to estimate genetic parameters. Kondo (1998) estimated significant GCA of male flower production using a half diallel cross with five parental plus-tree clones, but did not estimate heritability. In our study, significant and quite high GCA values were obtained using six parental clones for mating.

**Table 4** The genetic gain based on NMC estimated from several selection intensities

Selection percentage	Selection intensity	Genetic gain based on NMC		
		2004 (%)	2006 (%)	2007 (%)
50 %	0.798	-22.1 (-48.6)	-27.6 (-53.0)	-15.7 (-46.1)
40 %	0.966	-25.2 (-55.4)	-31.2 (-59.9)	-18.0 (-52.8)
30 %	1.159	-28.2 (-62.1)	-34.8 (-66.7)	-20.2 (-59.4)
20 %	1.400	-31.4 (-69.1)	-38.3 (-73.6)	-22.6 (-66.5)

Numerals in parentheses represent relative genetic gain

The estimated narrow-sense individual tree heritability of logNMC were high for all 3 years (Fig. 4) and that of logNMC under natural flowering was lower than that of logNMC with GA<sub>3</sub> treatment (0.471±0.271). Saito and Akashi (1998) evaluated natural male flower production of open-pollinated progeny using a flowering index and estimated the individual tree heritability to be 0.027–0.076. In lodgepole pine, the narrow-sense heritability for male flower production of open-pollinated seedlings was estimated as 0.32–0.64 (Hannerz et al. 2001), and the broad-sense heritability of female cone set for clones was estimated as 0.37–0.55 in Norway spruce (Almqvist et al. 2001). In Scots pine, individual broad-sense heritability of male strobili production by clones was very low because of high environmental effects (Bilir et al. 2006). In our study, since the male flowering potential of individuals was thought to be maximized by GA<sub>3</sub> treatment, the clonal and family variation became clear and the estimated heritabilities were very high compared to previous reports for *C. japonica* and other conifers. However, the heritability in 2006 was greater than 1.0, raising the possibility that it was overestimated. The six parental clones used in our study were selected from the plus tree population in the Kanto breeding region based on the male flower production. It is probable that six clones cannot represent the variation in male flower production of the whole population due to the small number of clones. However, the heritability and the contribution of GCA of male flower production were considerably higher than those of growth traits (Fig. 4) and this fact indicates a relatively high inheritance of male flower production.

The reduction in male flower production of seedlings from improved seed orchards is one of the current breeding objectives in Japan to address the allergenicity of *C. japonica* pollen. An estimated -50 % genetic gain in male flower production of progeny with such a weak selection percentage (50 %) of parental clones is an enormous achievement (Table 4). For the practical production of seedlings in Japan, an open-pollinated seed orchard is a reasonable approach.

Contamination by pollen originating outside of seed orchards is problematic (Moriguchi et al. 2005) because it reduces the genetic gain of male flower production. Since low-pollen individuals produce only small amount of male flowers, a number of individuals pollinate with the pollen originating outside of seed orchards, which capability is unknown. Nonetheless, some extent of genetic gain is expected in seedlings from open-pollinated seed orchards consisting of less-pollen clones; based on our results that male flower production is a highly inheritable and additive genetic trait, the additive effect of the maternal parents will be inherited by seedlings. To estimate more precisely the effect of improvement in male flower production of low-pollen trees, further studies are necessary, including an assessment of genotype by environment interactions detected in other conifers (Choi et al. 2004).

For commercial use of forest trees, it is necessary to investigate the correlations between reproductive traits and other traits, especially growth traits. Bilir et al. (2006) reported that DBHs and the numbers of strobili are weakly correlated in *Pinus sylvestris*. Almqvist et al. (2001) reported that genotypic correlations between cone-set and height are weak and not significant in *Picea abies*. In our study, the logNMC value was not correlated with DBH but was moderately correlated with the mean of the height in 2006. However, we found extremely low heritabilities in growth traits (Fig. 4) and could not obtain clear results from this study. It will be necessary to clarify the correlation between reproductive traits and growth traits by additional investigation.

This study is only one case, but our results provide fundamental data for the prediction of male flower production of progeny. Such studies address Japanese pollen allergies caused by *C. japonica* and can be used in breeding of other coniferous species to deal with pollen and seed productivity, especially for management of seed orchards.

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