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# Differences in Flowering Characteristic among Clones of *Cunninghamia lanceolata* (LAMB.) Hook

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**Abstract:**

Using data from a seed orchard of Chinese fir (*Cunninghamia lanceolata* (LAMB.) Hook) located at Chongyang, Hubei, China, we studied the differences in flowering characteristics among clones. The results show that for each flowering characteristics there is no difference among trees within the same clone. The differences in morphological characteristics of male and female cones among clones are clear, but the distributions of these morphological characteristics are not normally distributions. Male cones can be described by diameter, length and the number of microstrobilus, and the female cones by diameter, length and the number of macrosporophyll. Using principal components analysis, their cumulating contribution percentages are 95.8 % and 92.6 % respectively and the contributions of other morphological characteristics are very small and can be ignored. There are obvious differences in male and female cone numbers among both clones and their distribution in the tree crown levels. Using the ratio of female to male cones, the clones can be classed into mainly female clones, female and male clones and mainly male clones. Using flowering percentages of female and male cones among different clones at different dates, clones can be divided into early, mid and late flowering clones. Based on the timing of male and female flowering coincidence, clones can be grouped into early female flowering late male flowering clones, coincident male and female flowering clones and late female flowering early male flowering clones. These flowering characteristics are important in improving or designing a new seed orchard. Only in this way can we be able

to manage self -fertilization and produce higher genetic quality seeds.

*Key words: Cunninghamia lanceolata* (LAMB.) Hook/Chinese fir; seed orchard; clones; flowering habit

A seed orchard is established with clones of superior trees in order to produce high genetic quality seeds. Selection of superior trees and establishment in a seed orchard are very important steps during normal tree breeding and seed production. According to the information from Chinese fir Superior Trees & Seed Orchard Research Group, the wood volumes of 3 to 12 year-old trees from 337 clones are 37.8 % to 81.6 % greater than those of unimproved forests (SHEN XIHAN, 1990).

*Cunninghamia lanceolata* (LAMB.) Hook is a very important timber tree because of its characteristics of fast growth, high quality timber and wide use in southern part of China. The clone component and their ratio are very important for seed genetic quality and seed production. R. C. SCHMIDTLING (1983) reported that only 20 % of the clones produced 80 % seeds in a *Pinus taeda* seed orchard, and Beer reported 20 % of the clones produce 56 % seeds in a *Pinus elliottii* seed orchard in 1974 (R. C. SCHMIDTLING, 1983). CHI JIAN (1987) reported only 20 % of the 31 clones are high yielding, and the seed quality differs among clones. Based on the data from mother trees in a seed orchards located at Chongyang, we studied the male and female cone numbers and their distribution within the crown, the ratio of female to male cones, and the female cone and male cone flowering characteristics, such as flowering date, percentage, and coincidence of female to male cones.

## Materials and Methods

### *1. A Description of Seed Orchard*

Research was carried out in a seed orchard located at Chongyang, Hubei, China. The geographic location of this seed orchard is latitude 29° 38' 27", longitude 114° 8' 19" and at an elevation of 148 to 226 m. The seed orchard is on a sunny slope. The area is about 80.000 $m^2$, and is divided into 5 sections. Rootstock were local *Cunninghamia lanceolata* seedlings, planted at 5 × 5 m spacing, and they were grafted between 1991 and 1995. The scions came from selected superior trees in Fujian, Yunnan, Hunan, Hubei and Guizhou provinces. There are a total of 32 clones in the seed orchard. The status of mother trees in this seed orchard is shown in *Table 1*.

*Table 1.* – The Status of Mother Tree in Seed Orchard.

| Index of Statistics | Tree Height (m) | BHD (cm) | Number of Branches on Trunk |
|---|---|---|---|
| Number of Samples | 99 | 99 | 99 |
| Mean | 6.39 | 10.42 | 42.99 |
| SD | 1.69 | 3.05 | 13.86 |
| CD% | 26.45 | 29.27 | 32.24 |

### *2. Research Methods*

We chose 20 clones, and 5 trees of each clone at random in the seed orchard to measure. We divided the crown into upper, middle and lower levels, selecting 4 average size branches in the east, west, north and south directions. After counting the female and male cone numbers on these branches, we then estimated the female and male cone numbers of whole mother tree. With these data we analyzed the cone number differences among both clones and crown levels by means of variance analysis (FU WUYU and CHENG HUHANG, 1980).

On 20 to 50 female and male cones of each clone were selected at random respectively for each clone, we measured each morphological characteristics 20 times, analyzed the differences among clones by means of variance analysis and found the principal components of female and male cones by means of principal components analysis (LU WENDAI, 2000; ZHONG HUNGNAN, 1999).

Using Euclidean distance cluster analysis (LU WENDAI 2000), clones were classed 1) gender into groups with the ratio of female cones to male cones (R), 2) clones into flowering date types with flowering percentages of female and male cones in each tested tree among different clones at different dates (we counted the percentages every other day during the flowering season from March to April), and 3) group clones into kinds of the male and female flowering coincidence with the ratio of the female cone flowering percentage (fp) to the male cone flowering percentage (mp) for each clone.

## Results

### *1. Female and Male Cone Morphological Differences among Clones*

#### 1.1. Morphological Characteristics of Female and Male Cone

The average of diameter, length, macrosporophyll number, macrosporophyll width, macrosporophyll length and macrosporophyll thickness of female cones were 28.95 mm, 30.81 mm, 34.43, 12.97 mm, 15.47 mm and 0.51 mm respectively, and average diameter, length, microstrobilus number, microstrobilus diameter, microstrobilus length and microsporophyll number of male cones was 16.37 mm, 21.98 mm, 20.41, 2.79 mm, 13.97 mm and 43.61 respectively. Using the morphological characteristics of female and male cones, the normal distributions of these morphological characteristic of female and male cones have been tested by the method of A.H.KOЛ′′ MOГOPOB (FU WUYU; CHENG HUHANG, 1980). The results show that these distributions are not normal distributions because of different clones (*Tables 2* and *3*). For each morphological characteristics of female and male cones there are no differences among trees in a same clone, but there are differences among clones. Apart from the differences of morphological characteristics of female and male cones, there are differences among clones in the shape of crown, the colour of leaves, the branching habit (number, size and angle) and the growth (diameter, height and formal factor), and the data is too complex to be presented here.

#### 1.2 Morphological Characteristics of Female and Male Cone Differences

There are differences in each of the morphological characteristics of female cone (diameter, length, macrosporophyll number, macrosporophyll width, macrosporophyll length and macrosporophyll thickness) and male cone (diameter, length, microstrobilus number, microstrobilus diameter, microstrobilus length and microsporophyll number) among clones. The variance analysis Table is omitted because of too large.

#### 1.3 Principal Components Analysis of Female and Male Cones

The result shows that male cones can be described by diameter, length and microstrobilus number, and female cones by diameter, length and macrosporophyll number. Their cumulative contribution percentages are 95.8 % and 92.6 % respectively. The contributions of other morphological characteristics both of female and male cones are very small and can be ignored. We can class female and male cones using morphological size with above principal component indexes.

*Table 2.* – Morphological Characters and Normal Distribution Test of Male Cone of Chinese fir.

| Diameter (mm) | | Length (mm) | | Microstrobilus Number | | Microstrobilus Diameter (mm) | | Microstrobilus Length (mm) | | Microsporophyll Number | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | Frequency | Group | Frequency | Group | Frequency | Group | Frequency | Group | Frequency | Group | Frequency |
| < 8 | 0.00196 | < 10 | 0.00196 | < 6 | 0.00588 | < 1.6 | 0.00195 | < 6 | 0.00520 | < 27 | 0.00325 |
| 8-10 | 0.02745 | 10-12 | 0.00784 | 6-8 | 0.01176 | 1.6-1.8 | 0.01429 | 6-7 | 0.01365 | 27-30 | 0.01754 |
| 10-12 | 0.06275 | 12-14 | 0.03333 | 8-10 | 0.01765 | 1.8-2.0 | 0.02534 | 7-8 | 0.03509 | 30-33 | 0.02274 |
| 12-14 | 0.20392 | 14-16 | 0.06471 | 10-12 | 0.0333 | 2.0-2.2 | 0.08317 | 8-9 | 0.04483 | 33-36 | 0.06173 |
| 14-16 | 0.22549 | 16-18 | 0.13353 | 12-14 | 0.06078 | 2.2-2.4 | 0.15140 | 9-10 | 0.09487 | 36-39 | 0.12865 |
| 16-18 | 0.19412 | 18-20 | 0.12157 | 14-16 | 0.12745 | 2.4-2.6 | 0.14815 | 10-11 | 0.10201 | 39-42 | 0.16959 |
| 18-20 | 0.12549 | 20-22 | 0.15490 | 16-18 | 0.10196 | 2.6-2.8 | 0.13190 | 11-12 | 0.11371 | 42-45 | 0.19623 |
| 20-22 | 0.06863 | 22-24 | 0.17147 | 18-20 | 0.15490 | 2.8-3.0 | 0.10136 | 12-13 | 0.10851 | 45-48 | 0.17024 |
| 22-24 | 0.03922 | 24-26 | 0.11176 | 20-22 | 0.14510 | 3.0-3.2 | 0.10201 | 13-14 | 0.08317 | 48-51 | 0.11371 |
| 24-26 | 0.02549 | 26-28 | 0.08039 | 22-24 | 0.08235 | 3.2-3.4 | 0.08902 | 14-15 | 0.09617 | 51-54 | 0.06173 |
| 26-28 | 0.01765 | 28-30 | 0.05490 | 24-26 | 0.07843 | 3.4-3.6 | 0.06693 | 15-16 | 0.07432 | 54-57 | 0.02664 |
| > 28 | 0.07851 | 30-32 | 0.04706 | 26-28 | 0.07451 | 3.6-3.8 | 0.04159 | 16-17 | 0.05718 | 57-60 | 0.01689 |
| | | 32-34 | 0.01569 | 28-30 | 0.04118 | 3.8-4.0 | 0.02079 | 17-18 | 0.05523 | > 60 | 0.01104 |
| | | > 34 | | 30-32 | 0.02941 | 4.0-4.2 | 0.01235 | 18-19 | 0.04873 | | |
| | | | | 32-34 | 0.02353 | > 4.2 | 0.00975 | 19-20 | 0.02794 | | |
| | | | | > 34 | 0.01766 | | | 20-21 | 0.01689 | | |
| | | | | | | | | 21-22 | 0.01494 | | |
| | | | | | | | | > 22 | 0.00234 | | |
| N | 510 | 510 | | 510 | | 1539 | | 1539 | | 1539 | |
| X | 16.37 | 21.98 | | 20.41 | | 2.79 | | 13.27 | | 43.61 | |
| S | 4.01 | 4.93 | | 6.14 | | 0.55 | | 3.61 | | 6.49 | |
| $(n)^{1/2}Dn$ | 1.2979 | 0.5693 | | 1.1366 | | 2.3970 | | 1.9525 | | 0.7167 | |
| 1-Q( $\lambda$ ) | 0.068092 | 0.901344 | | 0.148606 | | 0.00002 | | 0.00096 | | 0.677735 | |

*Table 3.* – Morphological Characters and Normal Distribution Test of Female Cone of Chinsese fir.

| Diameter ( mm ) | | Length ( mm ) | | Macrosporophyll Number | | Macrosporophyll Width (mm) | | Macrosporophyll Length (mm) | | Macrosporophyll Thickness (mm ) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| group | frequency | group | frequency | group | frequency | group | frequency | group | frequency | group | frequency |
| < 20 | 0.00370 | < 20 | 0.00185 | < 21 | 0.00741 | < 9.0 | 0.00371 | < 12.0 | 0.01544 | < 0.38 | 0.02901 |
| 20-22 | 0.03704 | 22-22 | 0.01111 | 21-24 | 0.02778 | 9.0-9.5 | 0.00864 | 12.0-12.5 | 0.02037 | .38-.40 | 0.05370 |
| 22-24 | 0.07222 | 22-24 | 0.05185 | 24-27 | 0.09815 | 9.5-10.0 | 0.01852 | 12.5-13.0 | 0.0444 | .40-.42 | 0.07346 |
| 24-26 | 0.14815 | 24-26 | 0.08333 | 27-30 | 0.12222 | 10.0-10.5 | 0.03704 | 13.0-13.5 | 0.05802 | .42-..44 | 0.05802 |
| 26-28 | 0.17037 | 26-28 | 0.13148 | 30-33 | 0.17037 | 10.5-11.0 | 0.05309 | 13.5-14.0 | 0.10062 | .44-.46 | 0.08765 |
| 28-30 | 0.16852 | 28-30 | 0.18148 | 33-36 | 0.20370 | 11.0-11.5 | 0.09012 | 14.0-14.5 | 0.09012 | .46-.48 | 0.7654 |
| 30-32 | 0.17037 | 30-32 | 0.5556 | 36-39 | 0.12593 | 11.5-12.0 | 0.07593 | 14.5-15.0 | 0.11667 | .48-.50 | 0.10864 |
| 32-34 | 0.08704 | 32-34 | 0.14815 | 39-42 | 0.11111 | 12.0-12.5 | 0.09877 | 15.0-15.5 | 0.10432 | .50-.52 | 0.08086 |
| 34-36 | 0.09630 | 34-36 | 0.12037 | 42-45 | 0.07778 | 12.5-13.0 | 0.08765 | 15.5-16.0 | 0.09872 | .52-.54 | 0.10123 |
| 36-38 | 0.04704 | 36-38 | 0.05741 | 45-48 | 0.03148 | 13.0-13.5 | 0.13148 | 16.0-16.5 | 0.10309 | .54-.56 | 0.06358 |
| > 38 | 0.00555 | 38-40 | 0.02963 | 48-51 | 0.01852 | 13.5-14.0 | 0.10185 | 16.5-17.0 | 0.06481 | .56-.58 | 0.10247 |
| | | 40-42 | 0.00926 | > 51 | 0.00555 | 14.0-14.5 | 0.09691 | 17.0-17.5 | 0.05494 | .58-.60 | 0.05679 |
| | | 42-44 | 0.00370 | | | 14.5-15.0 | 0.10556 | 17.5-18.0 | 0.03210 | .60-.62 | 0.02099 |
| | | 44-46 | 0.00185 | | | 15.0-15.5 | 0.05247 | 18.0-18.5 | 0.02901 | .62-.64 | 0.00802 |
| | | 46-48 | 0.00556 | | | 15.5-16.0 | 0.02222 | 18.5-19.0 | 0.01728 | .64-.66 | 0.01481 |
| | | < 48 | 0.00741 | | | 16.0-16.5 | 0.00926 | 19.0-19.5 | 0.01481 | .66-.68 | 0.00556 |
| | | | | | | > 16.5 | 0.00680 | 19.5-20.0 | 0.01667 | > 0.68 | 0.01546 |
| | | | | | | | | > 20.0 | 0.01857 | | |
| N | 540 | 540 | | 540 | | 1620 | | 1620 | | 1620 | |
| X | 28.95 | 30.81 | | 34.43 | | 12.97 | | 15.47 | | 0.51 | |
| S | 4.114 | 4.775 | | 6.463 | | 1.906 | | 1.882 | | 0.08 | |
| $(n)^{1/2}Dn$ | 0.6853 | 0.6646 | | 0.8094 | | 1.3898 | | 1.4763 | | 1.5053 | |
| 1-Q( $\lambda$ ) | 0.7278 | 0.7764 | | 0.528 | | 0.04196 | | 0.0250 | | 0.02092 | |

*Table 4.* – Variance Analysis of Male and Female Cone Number among Clones in Chinese fir Seed Orchard (number / per branch).

| Variance | | Male cone | | | Female cone | | |
|---|---|---|---|---|---|---|---|
| Origin | df | Sum of Squares | Mean Square | F | Sum of Squares | Mean Square | F |
| Clone | 19 | 11837.125 | 623.0066 | 7.91** | 25869.8238 | 1361.5697 | 20.26** |
| Within group | 1152 | 90707.056 | 78.7388 | | 77406.0823 | 67.1928 | |
| Total | 1171 | 102544.181 | | | 103275.9061 | | |

F α 0.01=1.93

*Table 5.* – Variance Analysis of Female and Male Cones Distribution in Crowns in Chinese fir Seed Orchard (number / per branch).

| Variance | | Male cone | | | Female cone | | |
|---|---|---|---|---|---|---|---|
| Origin | df | Sum of Squares | Mean Square | F | Sum of Squares | Mean Square | F |
| Clone | 2 | 23325.969 | 11662.9845 | 172.11** | 2457.897 | 1228.9935 | 14.25** |
| Within group | 1169 | 79218.212 | 67.7658 | | 100817.919 | 86.24 | |
| Total | 1171 | 102544.181 | | | 103275.9061 | | |

F α 0.01=4.61

*2. Numbers of Female and Male Cones in a Clone*

There are 6.26 male cones and 10.28 female cones per branch on average in the seed orchard, and there are differences among clones (*Table 4*).

*3. Female and Male Cone Distribution in Crowns*

The number of female cone are 8.38, 11.91 and 10.51, and the number of male cone 0.94, 5.95 and 11.87 in upper, middle and lower levels of tree crown respectively. There are also differences in the numbers of female and male cones among crown levels (*Table 5*). The female cones are located in the middle to upper crown levels and male cones in middle to lower levels as age increase (ZHANG ZHUOWEN et al., 1990 and CHEN XIAOYANG et al., 1991). Cone distribution is an important way not only to allow young cones to develop with good light and aeration, but it also serves to prevent self fertilization by pollen falling from upper levels onto female flowers in lower levels or the crown of the same tree.

*4. Ratio of Female Cone Number to Male Cone Number and Clone Classification*

The R value (average R of 8.91, 2.00 and 0.89 in different levels of crown), decreases from upper to lower crown layer of trees in seed orchard. Using the R value, we can class clones into 3 groups by means of Euclidean distance cluster analysis and we also find both female trees and male trees which produce only either female or male cones.

**1. mainly female type: R>=2.4.** the following clones belong to this group: 7430, 7431, 7415 and 7416

**2. Both female and male clone type: R=1.5 to 2.3.** the following clones belong to this group: 7305, 431, 7444, 7405, 16 and 7407.

**3. mainly male type: R<=1.4.** the following clones belong to this group: 7420, 7316, 7408, 04, 13, 07, 33, 7434, 25 and 43.

In general, seed production depends on the density of trees in the seed orchard, the number of female cones produced by each mother tree, the number of macrosporophyll of a female cone (there are 3 ovules or seeds in a productive macrosporophyll), pollen dispersal patterns and the pollination efficiency. The larger the ratio of the mainly female type trees to mainly male a trees in seed orchard, the more seeds it will produce, so we would like to have a good ratio of mainly female clones to mainly male clones when establishing or rebuilding a seed orchard in order to maximize seeds yields and produce high genetic quality seeds.

*5. Flowering Date of Female and Male Cones and Their Types*

The male cones of mother trees in general release pollen during March 15 to April 5, but there is some variation in different years. There are difference of up to 5 days at the beginning of flowering and 7 to 8 day differences at the ending of flowering over the last 4 years. The female flowering is about 5 to 6 days earlier than those of males at the beginning of flowering stage, and about 7 to 8 days later than those of males at the end of flowering stage. The female and male flowering among different mother trees of a clone in the same year are almost the same, with only a 1 or 2 day difference. There are differences in female and male flowering among clones. Using flowering percentages of females and males in each tested tree among different clones at different dates, the clones can be divided into early, mid and late flowering types by means of Euclidean distance cluster analysis (*Table 6*).

*Table 6.* – Clone Flowering Date Type in Seed Orchard of Chinese fir

| Cone | Type | Clone | Percentage in Seed Orchard |
|---|---|---|---|
| Females | Early | 431 | 5 % |
| | Mid | 7431, 7405, 7444, 25 | 20 % |
| | Late | 16, 13, 7430, 7416, 33, 07, 7434, 04,7420, 7316, 7305, 43, 7407, 7408, 7415 | 75 % |
| Males | Early | 7405 | 5 % |
| | Mid | 7434, 7407, 25 | 15 % |
| | Late | 33, 07, 43, 13, 431, 16, 7305, 7430, 7416, 7316, 7420, 04, 7415, 7431, 7444, 7408 | 80 % |

*6. The Male and Female Cones Flowering Coincidence among Clones*

Using the ratio of the female flowering percentage (fp) to the male flowering percentage (mp) for each clone, clones can also be classed into 3 types by means of Euclidean distance cluster analysis. In this way we can classify the male and female flowering coincidence among clones.

**1. male flowering early and female cone late type: fp/mp =< 1.5.** the following clones belong to this group: 7434, 7430, 7416, 16, 7407, 33, 07 and 13.

**2. female and male flowering coincidence: 1.5 < fp/mp < 5.0.** the following clones belong to this group: 7408, 7405 and 25.

**3. female flowering early and male late type: fp/mp >= 5.0** the following clones belong to this group: 7420, 43, 04, 7316, 7431, 7444, 431 and 7415.

## Discussion

When the seed orchard of Chinese fir at Chongyang was established, there was little knowledge about the flowering characteristics, so the seed production was low and the self-fertilization could not be avoided. Knowledge of flowering characteristics is very useful in the improvement or establishment a new seed orchard (e.g. the clone selection and the number of clones, the ratio of mainly female clones to mainly male clones, the ratio of mainly female trees to pollen trees, the number of clones). We need to have as many mainly female clones as possible together with enough mainly male clones to produce enough pollen for dispersal and good fertilization depending on the seed orchard size, the main wind in pollen dispersal season, and the topography of this seed orchard. The reasonable ratio of pollen trees to female can be estimated (ZHANG ZHUOWEN et al., 1990, 2001; CHEN XIAOYANG et al., 1991 and 1996). This can be improved by using clones which female cones and male cones do not flower coincidence at the same time to avoid self-fertilization and produce high genetic quality seeds. Only in this way can we be able to control or avoid self-fertilization in seed orchard.


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# Improving Precision of Breeding Values by Removing Spatially Autocorrelated Variation in Forestry Field Experiments


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## Summary

Adjustment for micro-environmental heterogeneity in inadequately blocked field experiments is desirable to improve estimates of genetic parameters and to maximize genetic gains from selection. In three forestry field trials of red alder (*Alnus rubra* Bong.) we removed spatially autocorrelated variation with kriging and evaluated the effect on estimates of treatment means and heritabilitiy compared to standard analysis. Kriging removed block effects and reduced the family × block interaction in all traits. The variation due to interactions was recovered in simple family or provenance variance components, which increased by up to 40 % in some traits. Heritability estimates and expected gains from selection increased accordingly, while the standard error of the estimate for family and provenance means decreased. The improvement was largest in experiments where blocking was clearly inadequate to capture site variation, when block size was large, and for traits that could be influenced by variation in soil properties. Bud break and leaf abscission, which are presumed to be independent from variation in soil, were spatially independent. Heritabilities estimated from an experiment with incomplete block design with nine trees per block could only be minimally improved. We recommend that variograms should routinely be constructed in the analysis of forestry field trials to test if residuals from standard models are spatially autocorrelated. If they are, kriging is proposed as a useful supplement to ANOVA in tree breeding experiments and other forestry field trials.



*Key words:* Kriging, heritability, tree breeding, red alder.



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