The Variation Characteristics and Blooming Phenophase of Monoecious *Pistacia chinensis* Bunge

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**Abstract.** *Pistacia chinensis* Bunge is a pioneer tree for afforestation, and of high value as an ornamental and for timber and medicine. It has also become the preferred biofuel tree in northern China in recent years, with a broader development prospect. However, this development is seriously limited due to its dioecious character (separate sexes), because the male (nonfruit) trees are indispensable for pollination, and this leads to a waste of field and low yields. Fortunately, this bottleneck can be broken with the emergence of rare monoecious (having both female and male flowers, and even bisexual flowers) resources in Hebei Province, China. To determine their cultivation characteristics, the variation characteristics and blooming phenophase of local *Pistacia* were recorded with text, data, and images, by germplasm resources survey, telephone interviews, and field visits. Results showed that 1) 23 monoecious *Pistacia* were found, with very complex morphological features. 2) The branches of different gender types, ordered by inflorescence length were female > monoecious trees > bisexual flowers > inflorescence with male and female > male on monoecious trees > male. Ordered by inflorescence width: female > bisexual flower > female on monoecious trees > inflorescence with male and female > male on monoecious trees > male. Among these, the inflorescence length, inflorescence width, floret diameter, and floret spacing of bisexual flowers were significantly greater than that of male flowers, providing a basis to judge gender type without destructive sampling. 3) Gender types were unstable in successive years—female, male, mixed, or bisexual flowers could convert to another gender within 1 year, except that no female became male, and the overall trend was from male to mixed or bisexual gender in recent years. 4) The blooming phenophase changed a lot among different strains and sex types, which could enlarge the blooming period.

*Pistacia chinensis* Bunge belongs to *Pistacia* genus (Anacardiaceae) which consists of at least 11 species or variant (Al-Saghir, 2010a; Wang, 2005). The following botanical characteristics are common to *Pistacia* species: most are trees, but some are shrubs; leaves are alternate, pinnate, and leathery; female and male flowers are on separate trees (i.e., they are dioecious); pistillate flowers are borne in loose axillary panicles and staminate flowers are axillary and more compact; and the fruit is a monocarpic drupe (Avanzato and Quarta, 2004).

It is generally believed that all *Pistacia* trees are dioecious (Zohary, 1952), so a reasonable proportion of male and female trees must be present to increase production, which greatly reduces fruit yield and increases labor compared with some monoecious plants in China (Duan et al., 2012). Several cases of monoecious *Pistacia* species have appeared in China and elsewhere (Avanzato and Quarta, 2004; Crane, 1974; Hou, 2009; Isfendiyaroglu, 2007; Kafkas et al., 2000; Ozek and Ayfer, 1958; Zhao, 2011). Because *Pistacia terebinthus* and *Pistacia atlantica* are used as rootstocks for pistachio, a few subsequent researches about grafting, hybridization, and molecular markers (Gercheva et al., 2008; Isfendiyaroglu and Ozeker, 2009; Kafkas et al., 2003; Marra et al., 2007) with *Pistacia vera* were carried on, but the mechanism of sex determination and variation in monoecious *Pistacia* is still unclear. However, the previous papers about monoecious *P. chinensis* have only involved preliminary observation of the inflorescences, our research is the first systematic study of monoecious *P. chinensis*, and we focus on the traits among different sex types and the situation of the sex change, aiming to provide new clues and insights on sex expression in *Pistacia*. A preliminary germplasm resource investigation and a study of the fertility of male and female gametes by pollination experiment were completed in 2013. The results showed that pollen and ovaries from monoecious genotypes were fertile, and that crosses with male trees as male parents and monoecious trees as female parents resulted in larger fruit than other crosses (Wang et al., 2015). These results demonstrated that monoecious genotypes could be grown without pollinizers trees. Hence, the field of investigation was extended; detailed observations and investigations were made for specific variation characteristics and blooming data in 2014. Branches of different genders were marked with different colors, and their phenotypic stability of gender observed in 2015.

**Materials and Methods**

*Plant materials.* The test field was located in Tang County of Hebei Province, and materials were all natural *P. chinensis* in the area where monoecious individuals were discovered. Ages of female plants were about 60 years and several strains were over 100 years, while the male trees were about 20 years. Ages of monoecious trees were closer to that of females. We surveyed 162 strains, including 139 normal trees and 23 monoecious trees.

*Investigation on normal trees.* All the local *Pistacia* trees were numbered from N1 to N139 (N: normal), the gender and flowering phenophase of each tree were recorded, and some typical trees were selected and observed regularly. Investigated factors involved inflorescence length and transverse and longitudinal diameters and number of...
The blooming process was recorded from beginning to end.

Observation and measurement of monoecious trees. Monoecious trees were numbered from M1 to M23 (M: monoecious). The types and distribution, inflorescence length, transverse and longitudinal diameter, and number of florets were investigated, and the blooming phenophase was recorded. The different types were also tagged for subsequent sampling and observation. Flowering characteristics were recorded during the survey, including text and images. Three monoecious trees (M2, M7, and M23) containing male, female, and mixed gender were selected during the blooming period in 2014. The different gender types were marked with different colors (female was red, male was white, and mixed was yellow) and flowering was observed in 2015 again, with any changes in gender recorded. Also, many local people were interviewed face-to-face or by telephone for more information about sex change in Pistacia.

Statistical analysis methods. SPSS18.0 software (SPSS Inc., Chicago, IL) was used for variance analysis, multiple comparisons, and bivariate correlations.

Results and Discussion

Distribution traits and morphological characteristics. As can be seen in Fig. 1, mutant plants accounted for 15%, 17 strains of which bore bisexual flowers, showing that bisexual flower occupied a large proportion in the mutant plants, but the specific direction of evolution remains to be further explored.

In the preliminary germplasm resources survey, we removed the inflorescence for anatomical observation. Detailed comparison showed some differences in phenotypic traits between normal plants (Fig. 2A and B) and monoecious inflorescence (Fig. 2C and D). Female flower inflorescence was loose, while that of male flowers was compact. The bisexual flower inflorescence seemed more like the male but slightly more spread out.

The observations showed many variations in characteristics of florets on monoecious trees, such as bisexual florets being fertile, some anther deformities and degradation, and anther number in the range of 1–6 (Fig. 3A); however, there were normally four anthers in male flowers (Li, 2009). The emergence of more anthers (Fig. 3B) may increase the amount of pollen.

All recorded cases of monoecious Pistacia are listed in Table 1. For the 23 monoecious trees, including all variation types and characteristics above, there were significant differences among different individuals. This will be important for germplasm resources and breeding of improved varieties.

Biological characteristics. A series of investigations were carried out to confirm the existence of differences and obtain their.
detailed characteristics. Investigated branches were divided into six types according to gender: normal male, normal female, male on monoecious (monoecious male), female on monoecious (monoecious female), male and female in the same inflorescence (mixed inflorescences), and bisexual flower. The branch length, basal diameter, top diameter, inflorescence number, inflorescence length, inflorescence width, and floret number (in a single inflorescence) were measured for all six types.

Table 2 indicated that the floret number and inflorescence width were highly correlated, and there was also a significant correlation between gender types and inflorescence length, showing that floret number of individual inflorescences varied with branch types. Inflorescence number was highly correlated with branch length and with basal diameter, and significantly correlated with top diameter. This showed that the overall amount of flowers was high on trees with long and strong branches.

Type was only significantly correlated with floret number, which is difficult to measure, thus the gender difference on monoecious branches could be estimated by inflorescence length and width because of its relationship with floret number. The length...
and width of bisexual inflorescences were significantly greater than that of male flowers, while their difference in floret number was not obvious (Table 3), because the floret spacing of bisexual inflorescence was greater. Using these characteristics, flower types were predicted by appearance and then checked through anatomical observation. The predicted bisexual flower inflorescences were actually bisexual inflorescences or bisexual flowers mixed with a small amount of male inflorescences, and this could be used to determine the type according to appearance, without requiring anatomical examination and destructive sampling.

Normal male and female trees had significant differences among all examined factors, except for floret number (Table 3). There were significantly fewer florets on a single inflorescence of monoecious compared with normal trees, with the exception of M7 (monoecious). There were no significant differences between the floret number of male flowers on M7 and normal trees, but it was significantly greater than on other monoecious trees, and floret diameter and longitudinal diameter were greater (Table 3), so the flower quantity was considerable.

The floret number, inflorescence length, and width were similar for different genders on the same tree, illustrating that the differences among different germplasm were greater than that between different genders in the same germplasm. In general, the branches of gender types were ordered by inflorescence length as follows: female > monoecious female > bisexual flower > mixed inflorescence > monoecious male > male; and for inflorescence width: female > bisexual flower > monoecious female > mixed inflorescence > monoecious male > male. Judging from the inflorescence, male and female monoecious trees were similar to corresponding normal male and female trees; while the inflorescence of bisexual flowers was closer to that of the normal female, and bisexual floret diameter was significantly greater than for normal male and female inflorescences.

### Stability of gender variant on monoecious Pistacia

Changed gender status was found by the presence of fruit in 2013 and bisexual

![Characteristics of fruits and inflorescences on monoecious Pistacia chinensis: (A) fruits and male inflorescences and (B) fruits and bisexual inflorescences (bar = 1 cm).](image)

Fig. 4. Characteristics of fruits and inflorescences on monoecious Pistacia chinensis: (A) fruits and male inflorescences and (B) fruits and bisexual inflorescences (bar = 1 cm).

![The changes of various gender types on monoecious Pistacia chinensis. The female, male, and mixed or bisexual branches were marked and counted in 2014, and their gender was again determined in 2015.](image)

Fig. 5. The changes of various gender types on monoecious Pistacia chinensis. The female, male, and mixed or bisexual branches were marked and counted in 2014, and their gender was again determined in 2015.

![The gender changes of various monoecious Pistacia chinensis strains. All the branches of different gender in the same area of three monoecious P. chinensis were counted, respectively, in 2014 and 2015. M1, M7, and M22 are monoecious strains.](image)

Fig. 6. The gender changes of various monoecious Pistacia chinensis strains. All the branches of different gender in the same area of three monoecious P. chinensis were counted, respectively, in 2014 and 2015. M1, M7, and M22 are monoecious strains.
or pure male inflorescences in 2014 that occurred together (Fig. 4), showing that branch gender type could change within only 1 year. This sex change phenomenon was further confirmed by use of paint marks to distinguish sex in 2014 and 2015.

The branches of three gender types marked in 2014 changed in 2015 (Fig. 5). Half of the female branches from 2014 bore no flowers in 2015, the rest were mostly still bearing female flowers and a small amount produced hermaphrodite flowers. Of the male branches, nearly three-quarters did not change gender in 2015, 11.36% of them bore mixed or bisexual flowers and only a few became female. Of the mixed or bisexual flowers, 61.27% kept their original gender in 2015, but some also became female and male.
Although it is too early to draw a conclusion about the cause, it was evident that gender changes of single branches on monoecious trees could happen within only 1 year, suggesting that gender was influenced by environment or physiological status of the branch.

Furthermore, most nonflowering branches came from (or became) female or bisexual with few males. This resulted in a phenomenon of alternate bearing (i.e., alternating years of high and low yields) as in pistachio (Kallisen et al., 2007), which suggests that female organs may be more related to nutrition.

All branches within the same region (broken branches excepted) of M1, M7, and M22 were counted according to gender. From 2014 to 2015, the bisexual flowers on three trees significantly increased, while the number of males had different rates of decline and the number of females on M1 and M7 also declined, but there was a significant increase in the number of females on M22 (Fig. 6). This showed that overall plants tended to turn male into bisexual flowers, and this may be the dominant direction of gender evolution in recent years.

Although the sex determination mechanism of *P. chinensis* is not clear, Huang et al. (1986, 1989) have reported that the chromosome number of *P. chinensis* is 24 (Al-Saghir, 2010b), while Wang (2013), Wu and Yang (2014), and Yang et al. (2013) have shown that both their female and male chromosome number are 2n = 30, in which a pair of the 15 homologous chromosomes have not obvious morphological differences in females, but are visibly different in males, inferring that chromosome 15 could be the sex chromosome. Nevertheless, many local people pointed out more than one tree which was fully female before becoming monoecious, meaning that monoecious trees likely originated from female trees. Why do they possess labile systems instead of strictly genetic systems that determine a fixed sex expression? A widely accepted explanation for sex change is the sex allocation theory; reproducing individuals increase their fitness by facultatively adjusting their relative investment toward the rarer sex in response to population shifts in operational sex ratio, accounting for the adaptiveness of environmental sex determination, life histories in particular (Charnov and Bull, 1977; Fisher, 1930; Freeman et al., 1980; López and Domínguez, 2003; Sinclair et al., 2012; Zhao et al., 2015). This theory has reflected the plasticity of genders and the important influence of environment. In addition, many studies involving epigenetics have demonstrated new ways of sex determination, such as transposon-induced epigenetic change (Martin et al., 2009) and Y-chromosome-encoded small RNA (Akagi et al., 2014). According to the local people, the nonfruiting male trees which they thought were useless were almost cut out for wood furniture and some female trees gradually transformed into monoecious trees after that. Therefore, it is presumed that environmental pressures might influence the sexual differentiation through activating one or several “agent” genes (ancient silent genes, may also be restructuring or mutations of existing genes) which were hidden in the female trees, thus resulting in male organs. This strategy might be one of the explanations for the emergence of monoecious *P. chinensis*.

To obtain the blooming phases of each type of local *Pistacia* trees, the blooming phase of every monoecious and normal typical tree was recorded. Among these, the flowering data of normal female and male trees were of most value, and all gender types of monoecious trees were separately recorded.

Flowering of normal female trees lasted 7–8 d and was commonly during 7–14 Apr., typically preceding the male flowers by 1 d. Female flowers that were pollinated browned more rapidly than that of unpollinated flowers (Fig. 7). The pollen dispersal period of normal male trees was generally during 9–11 Apr., and lasted for 3–4 d; the pollen dispersal period of the whole tree was relatively tidy, and the inflorescences dropped after dispersing pollen. Female flowering of monoecious trees was during 9–16 Apr., about 2 d later than normal female trees, while its duration was similar to that of normal female flowers. Male flowers of monoecious trees dispersed pollen during 9–11 Apr., slightly later than normal male trees. Male organs of mixed inflorescence and bisexual flowers emerged partly pollen dispersing (i.e., was incomplete), and dispersing pollen was slightly later than for other male flowers; the female organ development was later than for normal female flowers.

Accordingly, the blooming period of normal male and female trees generally overlapped during 9–11 Apr., and so could meet the demand of pollination; hermaphrodite flowering was variable, but the female flowers generally bloomed ahead of the male flowers on the same monoecious tree by 1–3 d, and could achieve pollination by themselves. In addition, female flowers of monoecious trees can accept pollen from normal male flowers, and the male flowers can also be used for pollination of normal females, which extends the whole flowering period and improves overall efficiency of pollination, and also provides a basis for pollination tree selection and determining the configuration of different monoecious trees.

**Conclusion**

In this study, 23 monoecious *P. chinensis* were found and observed, and showed very complex morphological features. Our findings showed that dioecism was not the only form of *P. chinensis* Bunge.

Many regular characteristics among various types were demonstrated. Gender types, ordered by inflorescence length were female > monoecious female > bisexual flower > mixed inflorescence > monoecious male > male; and for inflorescence width: female > bisexual flower > monoecious female > mixed inflorescence > monoecious male > male. Careful observation, measurement, and data analysis showed that bisexual inflorescence, flower diameter, and spacing were significantly greater than for normal males, suggesting a method to determine gender type by these morphological characteristics without destructive sampling.

Gender types were unstable in successive years: female, male, mixed, or bisexual flowers could convert to another gender within 1 year, except that no female became male. Additionally, the overall trend in recent years was for male branches to change to mixed or bisexual gender. However, the sex-determining and change mechanisms remain unclear, and we will expand related work to the genetic level in the future.

**References**


