



# Sex effect on growth performance and marker-aided sex discrimination of seedlings of *Populus deltoides*

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**Abstract** Sex has a significant effect on various traits among dioecious plants. In this analysis of the sex effect on the radial growth and wood density of numerous 20-year-old trees of *Populus deltoides* growing in a common garden, male trees performed better than the females in radial growth, but sex did not significantly affect wood density. Growth rate and wood density were weakly negatively correlated. Sex selection is also critical for controlling seed-hair pollution from *P. deltoides* plantations. However, because the juvenile period of *P. deltoides* lasts for years, a reliable technique to determine the sex of juveniles has been needed.

Here we developed a marker-aided technique to discriminate the sexes of *P. deltoides* seedlings. This study provides essential information on target traits and a highly desirable genetic toolkit for accelerate breeding programs for this important tree species.

**Keywords** Sex effect · Wood quality · Growth performance · Marker-aided selection · Sex discrimination

## Introduction

Dioecy (the presence of distinct male and female plants) has evolved repeatedly from cosexual ancestors in nearly half of the angiosperm families (Geber et al. 1999). Dioecious plants exhibit sexual dimorphism in both reproductive organs and vegetative traits (Liu et al. 2021). Selection for outcrossing and compensatory resource reallocation following the loss of one sexual function are thought to be the principal mechanisms favoring sex dimorphism (Miller and Venable 2000). Resource allocation commonly differs between males and females, and these differences are assumed to be associated with trade-offs between reproductive cost and other functions (Seethapathy et al. 2021). For example, many studies have shown that growth performance differs between males and females (reviewed by Lloyd and Webb 1977).

*Populus deltoides* (cottonwood poplar) is native to North America and an important fast growing tree that can grow up to 40 m (Kartesz and Meacham 1999). Due to its extraordinary growth rate, *P. deltoides* and its hybrids have been widely used as a commercial source of fiber and fuel in many regions of the world, including North America, Europe, China, India, New Zealand and Australia (Zsuffa et al. 1996; Jain and Singh 2000; Broeck 2005; Wan et al. 2015). *P. deltoides* is fully dioecious; the androecia are

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produced on different individuals than the gynoecia. Early empirical observations revealed male superiority in vigor and form in natural populations of poplars (Farmer 1964; Grant and Mitton 1979); thus, the sex of a poplar tree may play an important role in its growth performance. In addition, female poplars produce abundant seed hairs when they reach sexual maturity. A mature female tree bears a huge number of female catkins and produces more than a kilogram of seed hairs. Seed hairs disperse in May of each year through mid-August, causing severe airborne pollution. For the above reasons, it is more desirable to breed elite male cultivars. However, it takes 6–7 years for *P. deltoides* to reach sexual maturity, so a method to discriminate the sex of *P. deltoides* at the seedling stage is in demand.

Sex in *P. deltoides* is governed by the XY determination system. The proximal end of the Y-chromosome harbors a male-specific sequence containing two genes, *FERR-R* and *MSL*. *FERR-R* is the female repressor and *MSL* is found to function as the male promotor. Expression of *FERR-R* and *MSL* triggers the differentiation of the opposite sexes of floral primordia (Xue et al. 2020). This research advance enables us to develop a marker-aided technique to discriminate between sexes in juvenile *P. deltoides*.

Using the trees in our large germplasm collection of *P. deltoides* that have been grown in the field for 20 years, we analyzed the effect of sex on growth performance of *P. deltoides* and developed a marker-aided technique to discriminate the sex of seedlings of *P. deltoides* to provide essential information and accelerate improvement programs.

## Materials and methods

### Field trial

A total of 270 *P. deltoides* clones were collected from natural populations in the United States: 29 from Texas, 95 from Louisiana, and 146 from Mississippi. These *P. deltoides* clones were maintained at Sihong Forest Farmer, Jiangsu

Province, China. The location lies in a transition zone of north subtropical to warm temperate zone, with yellow fluvo-aquic soil and mean annual temperature of 14.6 °C and precipitation of 893.9 mm. Four ramets for each clone were planted in the field using a randomized block design. After 20 years, some of the stands had died, and 914 stands remained in this field trial.

### Phenotypic data collection

We recorded sexes of these *P. deltoides* trees based their floral characteristics; male flower buds expand faster than the female ones, yielding a larger bud. When the flower buds are fully flushed, the most distinctive feature is that the male inflorescences bear florets only with stamens, and females bear florets only with pistils (Fig. 1). At this stage, the sex of the tree can be clearly discerned. We also measured the diameter at breast height (DBH) of each tree. To obtain the basic wood density (BWD), we took a tree core at a stem height of 1.3 m from each genotype. The cores were cut into pieces < 6 cm long, and three pieces per core were used to measure the BWD using the method and formula described by Olesen (1971).

### Marker-aided sex discrimination

In an early study (Xue et al. 2020), we discovered the Y-specific hemizygous sequence (~ 34.8 kb) in the sex determination region on the telomeric end of chromosome 19 in the genome of *P. deltoides*. In our 2020 study, we re-sequenced the genome of 115 *P. deltoides* trees (68 females and 47 males) and determined a conserved Y-specific sequence that harbored only two genes: *FERR-R* (~ 16 kb) and *MSL* (~ 3 kb). Based on the Y-specific hemizygous sequence, primers were designed and synthesized to amplify DNA from three male and three female trees in a PCR as described by Yin et al. (2008). Primers that generated a distinct amplicon in the males but no amplicon in the females were further verified using natural stands of *P. deltoides*.

**Fig. 1** Male and female inflorescence of *P. deltoides*



## Statistical analyses

We used Microsoft (Redmond, WA, USA) Excel to calculate the basic statistics for the phenotypic data. The sex ratio was analyzed using a  $\chi^2$  goodness of fit test. Fisher's least significant difference (LSD) test was used to compare the DBH or BWD significant differences among different provenances. Phenotypes were tested for significant differences between sexes using independent-sample *t*-test in SPSS 19.0 (Coakes and Steed 2003).

## Results

### Sex ratio of *P. deltoides* germplasm based on morphology

Among the 270 clones, 141 were discriminated as females, 129 as males; thus, females were more abundant in this *P. deltoides* collection. However, the  $\chi^2$  test revealed that the sex ratio did not significantly departure from 1:1 ( $\chi^2 = 0.5333 < \chi^2_{0.05} = 3.841$ ). Over the 20 years of growth, 66 trees had died; 32 were females, and 34 were males, indicating that mortality was not associated with sex.

### Variation in DBH and sex effect on DBH

The DBH frequency distribution of each group had a bell-shaped form (Fig. S1), and statistics for radial growth for these *P. deltoides* are listed in Table 1. The mean DBH was  $31.45 \pm 4.14$  cm and varied from 20.8 to 45.9 cm, with a CV of 13.17%. The maximum was > twofold larger than the minimum, indicating great breeding potential for radial growth. Clones from Texas had higher absolute radial growth than those from Louisiana or Mississippi. However, an analysis of variance revealed no significant difference in radial growth among the three provenances ( $P > 0.05$ ).

For the males, the mean DBH was  $32.28 \pm 3.74$  cm, varied from 22.2 to 43.3 cm with CV of 11.58%; mean DBH of females was  $30.69 \pm 4.36$  cm (20.8–44.9 cm), with CV of 14.19%. An independent-sample *t*-test indicated a

significant difference in radial growth between the two sexes ( $P = 0.002$ ). Therefore, our results clearly indicated that gender had a significant effect on radial growth of *P. deltoides*.

### Variation in BWD and sex effect on BWD

The shape of the BWD frequency distribution of each group was represented by a unimodal curve (Fig. S2). Statistics for BWD for this *P. deltoides* collection are listed in Table 2. Our measurements showed that the BWD values were more stable than that of DBH, with a CV of 5.61% vs. 13.17%. The mean BWD for these *P. deltoides* was  $0.38 \pm 0.02$  g cm<sup>-3</sup>, varied from 0.34 to 0.47 g cm<sup>-3</sup>. The highest BWD was 138% greater than the lowest. Thus, there is a considerable variance for BWD in the natural *P. deltoides* population. Correlation analysis revealed a weakly negative correlation between BWD and DBH (correlation coefficient = -0.143, significant at  $P < 0.05$ ). For the three provenances, there was little difference in mean BWD values, and LSD analysis showed no significant differences for BWD among the three provenances ( $P > 0.05$ ).

For the males, the mean BWD was  $0.39 \pm 0.02$  g cm<sup>-3</sup> (0.33–0.46 g cm<sup>-3</sup>) with CV of 5.75%; females had the same mean BWD as the males (0.34–0.47 g cm<sup>-3</sup>) with CV of 5.49%. The independent-sample *t*-test revealed that sex had no significant effect ( $P = 0.569$ ) on BWD.

### Marker-aided discrimination of the alternate sexes

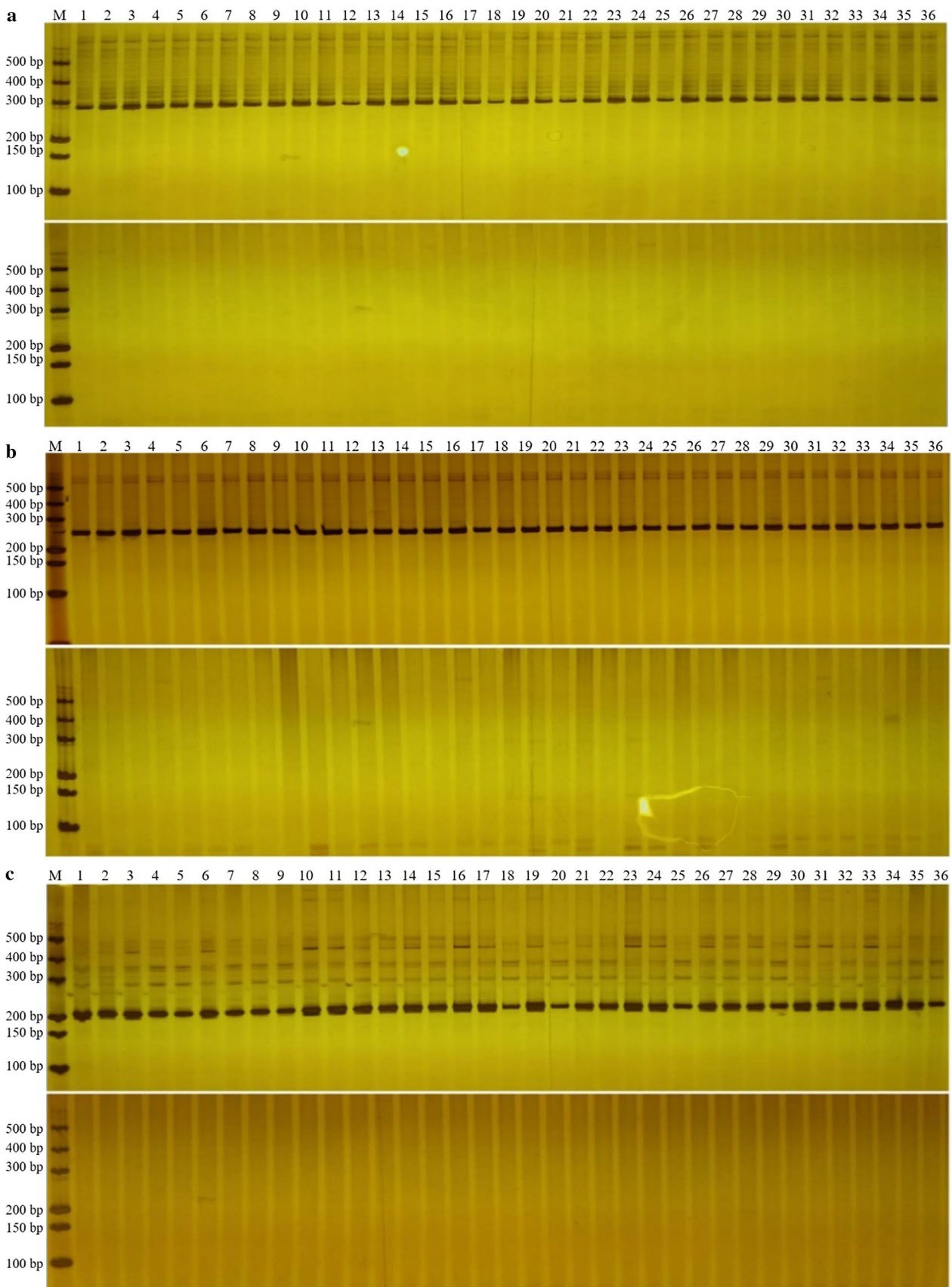
The PCR using the primer pairs based on the conserved Y-specific hemizygous sequence of *P. deltoides* (Xue et al. 2020) yielded the expected sizes for the PCR products (200–300 bp). Using DNA from three male and three female trees, we further screened primer pair N1869 (F: GGACAA CACACAAGGCTCTGC; R: TCGTGGGTTGATGTGTAG AGG), N1871 (F: TGCAAGTACAACAGGAGTTCAA; R: TCCACCGACAAGAGACAAAGA), and N1875 (F: CAA TATTTGTATTGCCCGTAG; R: CCACTGTTCCATGTT TTAAGG), which generated distinct bands of the expected sizes. Based on their positional information, N1871 and N1875 are located within the genic region of *MSL*, and

**Table 1** Statistics of radical growth of the *P. deltoides* collections

Germplasms	Number of clones	DBH (cm)	Range (cm)	CV (%)
Total	270	$31.45 \pm 4.14$	20.8–45.9	13.17
Texas	29	$32.89 \pm 4.37$	21.8–45.9	13.28
Louisiana	95	$31.17 \pm 4.44$	21.1–42.3	14.23
Mississippi	146	$31.34 \pm 3.86$	20.8–43.3	12.31
Females	129	$30.69 \pm 4.36$	20.8–44.9	14.19
Males	141	$32.28 \pm 3.74$	22.2–43.3	11.58

**Table 2** Statistics of density of wood for the *P. deltoides* collections

Germplasms	Number of clones	BWD (g cm <sup>-3</sup> )	Range (g cm <sup>-3</sup> )	CV (%)
Total	270	$0.38 \pm 0.02$	0.34–0.47	5.61
Texas	29	$0.39 \pm 0.03$	0.34–0.44	6.46
Louisiana	95	$0.39 \pm 0.02$	0.35–0.47	5.69
Mississippi	146	$0.39 \pm 0.02$	0.34–0.47	5.38
Females	141	$0.39 \pm 0.02$	0.34–0.47	5.49
Males	129	$0.39 \pm 0.02$	0.33–0.46	5.75





**Fig. 2** Polyacrylamide gel electrophoresis of PCR products from the alternate sexes of *P. deltoides* trees. Note **a** corresponds with N1869; **b** corresponds with N1870; and **c** corresponds with N1875. The upper panel (lanes 1–36) shows the males and the lower panel shows the females in each chart. M represents the molecular weight standard

N1869 is in the intergenic region between *MSL* and *FERR-R*. Since the hemizygous sequence is Y-specific, the expected PCR products were only generated in the males. Thus, the sex of *P. deltoides* was determined by the PCR products generated, and the three primer pairs provide a triple confirmation of the result.

We further verified the reliability of this marker-aided sex discrimination technique using 36 male and 36 female *P. deltoides* trees for which the genome had been re-sequenced. Results showed that all males had the expected bands and the females had none (Fig. 2). The technique thus had 100% accuracy for discriminating the sex of trees of *P. deltoides* and will be a reliable marker-aided tool to determine the sex of very young seedlings and accelerate breeding programs for *P. deltoides*.

## Discussion

Numerous studies have shown sex-based differences in secondary traits, including morphological, physiological and ecological characteristics (e.g., Dawson and Bliss 1989; Laporte and Delph 1996; Espirito-Santo et al. 2003), and the deployment of sexual function into different morphs may be modified by environmental stressors (Liu et al. 2021). Many studies have pointed to the possibility that males perform better than the females in terms of growth, vigor and form, but many others have reported that females performed similarly or even better than the males (e.g., Grant and Mitton 1979; Popp and Reinartz 1988; Hou et al. 2017). Therefore, there is no regular pattern of sex-based differences in performance among dioecious plants. This inconsistency might be related to biological and genetic differences or to experimental error. For tree species, the age and environmental habitat may vary greatly for the natural stands. To investigate a sex effect, it is essential to carry out a common garden experiment, but many studies have used only a limited number of trees, and conclusions were based on responses of juvenile trees due to the large size of mature trees. Here, many replicate trees of *P. deltoides* had been planted in a common garden spaced at 6 m × 6 m. These 20-year-old trees provided desirable plant materials in a large-scale field trial to investigate sex effects on growth performance of *P. deltoides*.

Conflicting sex effects have also been documented for species in Salicaceae. The expected sex ratio is 1:1. However, this ratio can be altered by stressors such as water supply and nutrient availability (Aber Krischik and

Denno 1990; Costich 1995). In our collections of *P. deltoides*, the sex ratio did not significantly depart from 1:1, in accordance with early empirical observations of natural populations of *P. deltoides* (Farmer 1964) and *P. tremuloides* (Einspahr 1960). It was noteworthy that our study revealed male superiority in radial growth of *P. deltoides* as reported by Farmer (1964), but no sexual difference in growth performance was observed in *P. tremuloides* (Einspahr 1960). For *Salix rigida*, males were reported to grow faster than the females (Elmqvist et al. 1991), but females of some other willow species grew as fast as or faster than males (e.g., Crawford and Balfour 1990; Åhman 1997; Hou et al. 2017). Thus, sex-based effects may vary among species and among the traits investigated. As noted already, *P. deltoides* has the XY sex determination system (Xue et al. 2020). *S. suchowensis* (Hou et al. 2015), *S. viminalis* (Pucholt et al. 2015) and *S. triandra* (Li et al. 2020) all have the ZW sex determination system. Males of *P. deltoides* were shown to have superior growth in our study and by Farmer (1964), whereas females were superior for a variety of traits in the three ZW willow species mentioned above (Åhman 1997; Hou et al. 2017; Yang et al. 2020). Since the sex determination system of plant species has been unclear in most previous studies, whether sex-based differences are associated with the sex determination systems among dioecious plants remains unknown.

For breeding commercial trees, growth rate and wood quality are among the most important traits. The present study showed that great variations in DBH and BWD in the natural stands of *P. deltoides*, indicating a great potential for improvement. In a study of numerous poplar hybrids, faster growth was generally related to lower wood density (Zhu et al. 1993), but no significant correlation between growth rate and wood density was found for *S. suchowensis* (Hou et al. 2017). Here, we found a weak negative correlation between DBH and BWD, indicating it is possible to breed elite *P. deltoides* cultivars with fast growth rate and good wood quality. Wood density is a fundamental wood quality trait. To date, studies of sex effects on wood quality have been scarce. However, our study showed no sex-based difference in wood density of *P. deltoides*, consistent with the findings for *S. suchowensis* (Hou et al. 2017).

Of the three sex-discriminating markers based on the Y-specific hemizygous sequence of *P. deltoides*, two were located within the genic region of *MSL*. Both the ZZ/ZW and XX/XY sex-determining systems have been found in *Populus* species. Although a Y-specific hemizygous region is also present in other *Populus* species that have an XX/XY system, such as *P. davidiana* (Xue et al. 2020), *P. tremula* (Pakull et al. 2015) and *P. trichocarpa* (Müller et al. 2020), the Y-specific hemizygous sequence varies greatly in different poplar species. Therefore, a specific sex discrimination toolkit needs to be developed for different *Populus* species;

the sex-discriminating toolkit developed in this study can be only used for *P. deltoides*.

In conclusion, male trees of *P. deltoides* were superior to females in radial growth; thus, considering with seed-hair pollution from the females, male sex should be a selection focus in breeding programs of this dioecious plant. We developed a reliable technique to discriminate the two sexes in seedlings of *P. deltoides*, which will accelerate breeding programs to improve this important commercial tree.

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**Author contributions** TY conceived and designed the research; HW, XD, WL, and YQ performed the experiments; YC analyzed the data and drafted the manuscript; TY revised the paper.

**Data availability** Sequence data have been deposited in NCBI WGS: JABCQW000000000 (*P. deltoides* female tree), JABEKP000000000 (*P. deltoides* male tree).

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## References

- Aber Krischik V, Denno RF (1990) Differences in environmental response between the sexes of the dioecious shrub *Baccharis halimifolia* (Compositae). *Oecologia* 83:176–181. <https://doi.org/10.1007/BF00317749>
- Åhman I (1997) Growth, herbivory and disease in relation to gender in *Salix viminalis* L. *Oecologia* 111:61–68. <https://doi.org/10.1007/s004420050208>
- Broeck AV, Villar M, Van Bockstaele E, VanSlycken J (2005) Natural hybridization between cultivated poplars and their wild relatives: evidence and consequences for native poplar populations. *Ann For Sci* 62:601–613. <https://doi.org/10.1051/forest:2005072>
- Coakes S, Steed L (2003) SPSS without anguish. John Wiley and Sons, Australia
- Costich DE (1995) Gender specialization across a climatic gradient: experimental comparison of monoecious and dioecious *Ecbalium*. *Ecology* 76:1036–1050. <https://doi.org/10.2307/1940914>
- Crawford RMM, Balfour J (1990) Female-biased sex ratios and differential growth in Arctic willows. *Flora* 184:291–302. [https://doi.org/10.1016/S0367-2530\(17\)31625-0](https://doi.org/10.1016/S0367-2530(17)31625-0)
- Dawson TE, Bliss LC (1989) Patterns of water use and the tissue water relations in the dioecious shrub, *Salix arctica*: the physiological basis for habitat partitioning between the sexes. *Oecologia* 79:332–343. <https://doi.org/10.1007/BF00384312>
- Einspahr DW (1960) Sex ratio in quaking aspen and possible sex-related characteristics. In: proceedings of the fifth world forestry congress held at the university of Washington, Seattle
- Elmqvist T, Cates RG, Harper JK, Gardfjell H (1991) Flowering in males and females of a Utah willow, *Salix rigida* and effects on growth, tannins, phenolic glycosides and sugars. *Oikos* 61:65–72. <https://doi.org/10.2307/3545407>
- Espírito-Santo MM, Madeira BG, Neves FS, Faria ML, Fagundes M, Fernandes GW (2003) Sexual differences in reproductive phenology and their consequences for the demography of *Baccharis dracunculifolia* (Asteraceae), a dioecious tropical shrub. *Ann Bot* 91:13–19. <https://doi.org/10.1093/aob/mcg001>
- Farmer RE Jr (1964) Sex ratio and sex-related characteristics in eastern cottonwood. *Silvae Genet* 13:116–118
- Geber MA, Dawson TE, Delph LF (eds) (1999) Gender and sexual dimorphism in flowering plants. Springer-Verlag, Berlin
- Grant MC, Mitton JB (1979) Elevational gradients in adult sex ratios and sexual differentiation in vegetative growth rates of *Populus tremuloides* Michx. *Evolution* 33:914–918. <https://doi.org/10.2307/2407654>
- Hou J, Ye N, Zhang DF, Chen YN, Fang LC, Dai XG, Yin TM (2015) Different autosomes evolved into sex chromosomes in the sister genera of *Salix* and *Populus*. *Sci Rep* 5:1–6. <https://doi.org/10.1038/srep09076>
- Hou J, Guo Z, Liu H, Yin T (2017) Gender effects on *Salix suchowensis* Cheng ex Zhu. growth and wood properties as revealed by a full-sib pedigree. *Can J Plant Sci* 97:594–600. <https://doi.org/10.1139/cjps-2016-0153>
- Jain SK, Singh P (2000) Economic analysis of industrial agroforestry: poplar (*Populus deltoides*) in Uttar Pradesh (India). *Agroforest Syst* 49:255–273. <https://doi.org/10.1023/A:1006388128069>
- Kartesz JT, Meacham CA (1999) Synthesis of the North American flora. Chapel Hill, NC
- Laporte MM, Delph LF (1996) Sex-specific physiology and source-sink relations in the dioecious plant *Silene latifolia*. *Oecologia* 106:63–72. <https://doi.org/10.1007/BF00334408>
- Li W, Wu H, Li X, Chen Y, Yin T (2020) Fine mapping of the sex locus in *Salix triandra* confirms a consistent sex determination mechanism in genus *Salix*. *Hortic Res* 7:64. <https://doi.org/10.1038/s41438-020-0289-1>
- Liu M, Korpelainen H, Li C (2021) Sexual differences and sex ratios of dioecious plants under stressful environments. *J Plant Ecol* 14:920–933. <https://doi.org/10.1093/jpe/rtab038>
- Lloyd DG, Webb CJ (1977) Secondary sex characters in plants. *Bot Rev* 43:177–216. <https://doi.org/10.1007/bf02860717>
- Miller JS, Venable DL (2000) Polyploidy and the evolution of gender dimorphism in plants. *Science* 289:2335–2338. <https://doi.org/10.1126/science.289.5488.2335>
- Müller NA, Kersten B, Leite Montalvão AP, Mähler N, Bernhardsson C, Bräutigam K, Lorenzo ZC, Hoenicka H, Kumar V, Mader M, Pakull B, Robinson KM, Sabatti M, Vettori C, Ingvarsson PK, Cronk Q, Street NR, Fladung M (2020) A single gene underlies the dynamic evolution of poplar sex determination. *Nat Plants* 6:630–637. <https://doi.org/10.1038/s41477-020-0672-9>
- Olesen PO (1971) Water displacement method; a fast and accurate method of determining the green volume of wood samples. *For Tree Improv* 3:3–23
- Pakull B, Kersten B, Lüneburg J, Fladung M (2015) A simple PCR-based marker to determine sex in aspen. *Plant Biol* 17:256–261. <https://doi.org/10.1111/plb.12217>
- Popp JW, Reinartz JA (1988) Sexual dimorphism in biomass allocation and clonal growth of *Xanthoxylum americanum*. *Am J Bot* 75:1732–1741. <https://doi.org/10.1002/j.1537-2197.1988.tb11249.x>

- Pucholt P, Rönnerberg-Wästljung AC, Berlin S (2015) Single locus sex determination and female heterogamety in the basket willow (*Salix viminalis* L.). *Heredity* 114:575–583. <https://doi.org/10.1038/hdy.2014.125>
- Seethapathy GS, Wold CW, Ravikumar K, de Boer HJ, Wangenstein H (2021) Ethnopharmacology, biological activities and chemical compounds of *Canarium strictum*: an important resin-yielding medicinal tree in India. *Fitoterapia* 152:104920. <https://doi.org/10.1016/j.fitote.2021.104920>
- Wan ZB, Li YR, Liu M, Chen YN, Yin TM (2015) Natural infectious behavior of the urediniospores of *Melampsora larici-populina* on poplar leaves. *J For Res* 26:225–231. <https://doi.org/10.1007/s11676-015-0021-4>
- Xue LJ, Wu HT, Chen YN, Li XP, Hou J, Lu J, Wui SY, Dai XG, Olson MS, Liu JQ, Wang MX, Charlesworth D, Yin TM (2020) Evidences for a role of two Y-specific genes in sex determination in *Populus deltoides*. *Nat Commun* 11:1–12. <https://doi.org/10.1038/s41467-020-19559-2>
- Yang G, Xu Q, Li W, Ling JH, Li XP, Yin TM (2020) Sex-related differences in growth, herbivory, and defense of two *Salix* species. *Forests* 11:450. <https://doi.org/10.3390/f11040450>
- Yin TM, DiFazio SP, Gunter LE, Zhang XY, Sewell MM, Woolbright SA, Allan GJ, Kelleher CT, Douglas CJ, Wang MX, Tuskan GA (2008) Genome structure and emerging evidence of an incipient sex chromosome in *Populus*. *Genome Res* 18:422–430. <https://doi.org/10.1101/gr.7076308>
- Zhu XY, Wang RL, Tong YC, Huang DS (1993) Study on the genetic variance of wood basic density and fiber properties of poplar hybrids. *For Res* 6:131–135. <https://doi.org/10.13275/j.cnki.lykxyj.1993.02.003>
- Zsuffa L, Giordano E, Pryor LD, Stettler RF (1996) Trends in poplar culture: some global and regional perspectives. In: Stettler RF, Bradshaw HD Jr, Heilman PE, Hinckley TM (eds) *Biology of Populus and its implications for management and conservation*. NRC-CNRC, Ottawa, ON, Canada, pp 515–539

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