Morphological and physiological differences in heteromorphic leaves of male and female Populus euphratica Oliv. Postprint

Authors: LI Xiu, ZHAI Juntuan, LI Zhijun, LI Zhijun

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Abstract

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Full Text

Preamble

Morphological and Physiological Differences in Heteromorphic Leaves of Male and Female *Populus euphratica* Oliv.

LI Xiu[1,2], ZHAI Juntuan[1,2], LI Zhijun[1,2*]

[1] College of Life Sciences and Technology, Tarim University, Alar 843300, China
[2] Key Laboratory of Protection and Utilization of Biological Resources in Tarim Basin of Xinjiang Production & Construction Corps, Alar 843300, China

Abstract: Leaf traits directly reflect plant adaptation strategies to environmental conditions. However, limited knowledge exists regarding the adaptive strategies of heteromorphic leaves in male and female *Populus euphratica* Oliv. in response to individual developmental stages (i.e., diameter class) and canopy height changes. This study investigated morphological and physiological properties of heteromorphic leaves in male and female *P. euphratica*. Results showed that both sexes exhibited increased leaf area (LA), leaf dry weight (LDW), leaf thickness (LT), net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (gs), proline (Pro), and malondialdehyde (MDA) concentration, along with decreased leaf shape index (LI) and specific leaf area (SLA), as diameter and canopy height increased. Leaf water potential (LWP) increased with diameter but decreased significantly with canopy height in both sexes, while carbon isotope fractionation (δ^{13}C) increased significantly with canopy height, all showing distinct resistance characteristics.

Males exhibited greater LA, LT, Pn, Tr, and Pro than females at the same canopy height. At the same canopy height, males also showed significantly higher LA, SLA, LT, Pn, Tr, gs, and MDA, but lower LWP and δ^{13}C than females, suggesting that male *P. euphratica* possess stronger photosynthetic and osmoregulatory abilities while being more sensitive to water deficiency. Moreover, differences between male and female *P. euphratica* are closely related to increases in individual diameter class and canopy height. In summary, male plants demonstrated higher stress tolerance than female plants, and differences in Pn, gs, Tr, Pro, MDA, δ^{13}C, and LWP between sexes were related to changes in leaf morphology, diameter class, and canopy height. These results provide a theoretical framework for understanding differential growth adaptation strategies during individual development in *P. euphratica*.

Keywords: arid area; canopy height; dioecious plants; morphology; physiological characteristics

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1 Introduction

Leaf traits exhibit high ecological plasticity and effectively reflect plant adaptation under different environmental conditions (Funk et al., 2013). Plants that display different leaf morphologies at various growth periods or under different environmental conditions are known as heteromorphic-leaf species (Bai, 2003). Heteromorphic leaves play a crucial role in adaptation to environmental changes; for instance, morphological changes in aquatic plant leaves facilitate gas exchange under submerged conditions (Kuwabara and Nagata, 2002; Mommer and Visser, 2005).

Previous studies have shown that changes in leaf morphological characteristics relate to tree height, as light utilization and evapotranspiration requirements change significantly with vertical position in the canopy (Russo and Kitajima, 2016). Kenzo et al. (2015) found significant linear relationships among leaf morphology, biochemical properties, and tree height. Additionally, Zhai et al. (2020) reported that morphological and physiological characteristics of poplar leaves exhibit significant differences as individual developmental stages and tree height increase, representing an adaptation to water stress. In higher plants, water stress causes accumulation of osmoregulatory substances such as proline (Pro) and malondialdehyde (MDA) (Kishor et al., 2005). Koch et al. (2004) found that leaf water potential (LWP) decreases with increasing tree height, and this reduction limits leaf expansion and photosynthesis. The latter change was directly caused by increasing δ^{13}C and indirectly by altered leaf structure under water stress conditions.

P. euphratica belongs to the genus *Populus* in the Salicaceae family and represents an important dioecious tree species with heteromorphic leaves (Wei, 1999), widely distributed in arid and semi-arid desert regions. This species plays a key role in ecosystem function, sand fixation, and climate regulation in oases (Keyimu et al., 2018). To improve adaptability to arid environments, *P. euphratica* modifies leaf morphological and functional traits, such as gradually increasing leaf area and thickness while decreasing specific leaf area, and increasing dry matter content and leaf dry weight (Huang et al., 2010; Wang et al., 2011). Previous studies found that heteromorphic leaves of *P. euphratica* exhibit enhanced photosynthetic capacity, higher osmoregulatory substance content, and stronger osmoregulatory capacity with increasing tree height under water stress (Zhai et al., 2020). While the morphological, physiological, and biochemical characteristics of *P. euphratica* heteromorphic leaves have been extensively studied (Liu et al., 2015; Li et al., 2017; Zhai et al., 2020), most investigations have not considered plant sex differences. However, previous research has documented sex differences in morphological characteristics, resource use efficiency, and other aspects (Espírito-Santo et al., 2003; Lei et al., 2017), and differences in morphological and physiological characteristics between male and female plants across different environments represent important factors influencing plant population composition, structure, and distribution (Li et al., 2019). The adaptive characteristics and sex differences in heteromorphic leaves of male and female *P. euphratica* have not been previously reported. Therefore, we investigated differences in morphological characteristics, photosynthesis, water use efficiency, and osmoregulatory substances of heteromorphic leaves in male and female *P. euphratica* across different developmental stages and canopy heights to elucidate their adaptation strategies. We focused on three questions: (1) What patterns of variation exist in the morphological and physiological characteristics of heteromorphic leaves in male and female *P. euphratica* during individual developmental stages? (2) Are there sex differences in the morphological and physiological characteristics of heteromorphic leaves of *P. euphratica* at different diameter classes and canopy heights? (3) Are the morphological and physiological differences in heteromorphic leaves between sexes related to individual diameter and canopy height? The results of this study may provide a theoretical basis for exploring adaptation strategies of heteromorphic leaves in both male and female *P. euphratica* during individual development.

2.1 Study area

The study area is located in an artificial *P. euphratica* forest (40°32$'36'$N, 81°17'56"$E; 980 m a.s.l.) at the northwestern edge of the Tarim Basin, Xinjiang, containing male and female *P. euphratica* individuals across different diameter classes. In late March 2020, when *P. euphratica* began flowering, we identified and tagged male and female individuals based on their distinct inflorescences (Fig. S1). The regional climate is hot and dry, with average annual precipitation of approximately 50 mm, mean annual temperature of 10.80°C, potential evaporation exceeding 1900 mm, and average annual sunshine duration of 2900 h, representing a typical temperate continental hyperarid desert climate zone. Habitat conditions include 27.53% soil water content, 1.50 m groundwater depth, 31.09°C daily average air temperature, and 36.78% daily average air humidity.

2.2 Experimental design and sampling

To minimize environmental factor influences, we selected male and female *P. euphratica* individuals of different diameter classes under identical habitat conditions. Sample plants were categorized into diameter classes using diameter at breast height (DBH) at 4-cm intervals. Three females and three males from 8, 12, 16, and 20 cm diameter classes were selected from the study site, with DBH and height measured using a tape measure and laser height measuring device (Haglöf, Sweden; Table S1). DBH and mean age (A) for each diameter class of female and male *P. euphratica* followed the relationship: A = 13.679/(1 + 3.3476 × exp(–0.2099 × DBH)) (Gu et al., 2013). Starting from the base of the main trunk of each plant (near ground level), we established sampling points at 2-m intervals along the vertical canopy profile (2, 4, 6, 8, 10, and 12 m, representing canopy height of sampling points). Considering the influence of light and other factors on leaf growth in different directions, we collected three annual branches at each sampling point from eastern, southern, western, and northern orientations, selecting leaves at the 4th node of each branch from base to tip as sample leaves for determination of morphological and physiological parameters of heteromorphic leaves.

2.3 Measurement of leaf morphological parameters

Leaf length (LL), leaf width (LW), and leaf area (LA) were measured using a portable leaf area meter LI-3000C (Li-Cor Inc., Lincoln, USA). Leaf thickness (LT) was measured using a vernier caliper (three measurements at the top, middle, and bottom of each leaf, averaged to obtain final thickness). Sample leaves were heated at 105°C for 10 minutes, then at 80°C to constant weight, and weighed to obtain leaf dry weight (LDW). Leaf shape index (LI) was calculated as the leaf length/width ratio (Tsukaya, 2002), and specific leaf area (SLA) was calculated as leaf area/leaf dry weight (Roderick et al., 1999).

2.4 Measurement of leaf stomatal exchange parameters

Using the LI-6400 photosynthesis measurement system (Li-Cor Inc., Lincoln, USA), we measured net photosynthetic rate (Pn), stomatal conductance (gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr) of leaves between 09:30–11:30 (local solar time). Measurements were conducted with a photosynthetic photon flux density of 1200 mol/($m^2 \cdot s$), leaf temperature of 25°C, and ambient CO_2 concentration of 400 mol/mol. Instantaneous water use efficiency (WUE) was calculated as the ratio of net photosynthetic rate to transpiration rate (WUE = Pn/Tr) (Zhai et al., 2020). Twelve leaves were measured at each sampling site, with measurements repeated three times.

2.5 LWP and carbon isotopic composition ($\delta^{13}C$) measurements

Leaves were selected from the 4th node of each branch from base to tip, and LWP was measured using a portable plant water pressure chamber (600-EXP). Measurements were conducted before dawn and completed within 2 hours. Sample leaves on selected branches were cut and measured immediately, with 12 leaves measured at each sampling point and averaged. After measuring morphological parameters of leaves from each sample tree, we rinsed samples with distilled water, subjected them to 105°C for 10 minutes, then oven-dried at 60°C for 48 hours to constant weight. A pulverizer was used to grind the dried material, which was passed through a 100-mesh sieve, and a glass vacuum system was used to prepare plant samples for carbon isotope analysis. The combustion furnace was connected to a power supply and maintained at 1000°C. After evacuation, oxygen was supplied to the system. A porcelain spoon containing the sample was placed in a combustion tube and burned at high temperature for 2 minutes. The CO_2 gas was then collected, purified by freezing, and analyzed for carbon isotope composition using an isotope mass spectrometer (Finnigan MAT, San Jose, CA, USA).

2.6 Determination of leaf Pro and MDA concentrations

A mixture of leaves from the 4th node of annual branches within the same canopy was selected as test samples, and microvolume measurements were performed using a kit (Suzhou Kemin Biotechnology Co., Ltd., China) for determination of leaf Pro and MDA concentrations.

2.7 Data analysis

Normality tests were conducted prior to statistical comparisons, and data were log-transformed to correct deviations. SPSS v.23.0 (SPSS, Chicago, IL, USA) was used to test for individual differences between means at a significance level of $P < 0.05$. Multi-factor ANOVA was employed to test effects of sex, canopy height, diameter class, and their interactions. Principal component analysis (PCA) was conducted using Canoco 5 (Microcomputer Power, USA) to further reveal effects of individual developmental stage and canopy height on leaf traits between sexes.

3.1 Sex differences in morphological characteristics of heteromorphic leaves

LA and LDW of male and female *P. euphratica* increased significantly with diameter class, while SLA decreased significantly. Additionally, LA and LT were significantly influenced by sex (Table 1). LA and LT differed significantly between sexes within the same diameter class, with both parameters being higher in males than females, though LI, SLA, and LDW showed no significant sex differences. As canopy height increased, LI, LA, and LT increased significantly in both sexes, while SLA decreased significantly. Sex, canopy height, and their interactions had significant effects on LA, SLA, and LT (Fig. 1 [Figure 1: see original paper]). At the same canopy height, LA, SLA, and LT of heteromorphic leaves were all significantly greater in males than females. These results indicate that with increasing individual developmental stage and canopy height, male plants produce larger and thicker leaves than female plants.

3.2 Sex differences in photosynthetic characteristics of heteromorphic leaves

Pn, Tr, Ci, and gs of heteromorphic leaves across different diameter classes were influenced by sex (Fig. 2 [Figure 2: see original paper]), with significant differences in Pn and Tr between male and female *P. euphratica* in the 12-cm diameter class. As canopy height increased, Pn, gs, and Tr increased significantly in both sexes (Fig. 3a–c [Figure 3: see original paper]), while Ci and WUE decreased significantly (Fig. 3d and e [Figure 3: see original paper]). The interaction among sex, canopy height, and diameter class had significant effects on Pn, Tr, and gs (Fig. 3 [Figure 3: see original paper]). Differences in Pn, gs, and Tr between sexes at the same canopy height were significant, with males showing substantially higher values than females, indicating that male *P. euphratica* exhibit greater photosynthetic capacity than female plants.

3.3 Sex differences in water physiological characteristics of heteromorphic leaves

Figure 4 [Figure 4: see original paper] presents water characteristics of heteromorphic leaves in male and female *P. euphratica*. δ^{13}C of heteromorphic leaves across different diameter classes was significantly influenced by sex (Fig. 4c [Figure 4: see original paper]). In the 8–16 cm diameter classes, δ^{13}C differences between sexes were significant, with female plants showing higher δ^{13}C than males, indicating that long-term WUE of females exceeded that of males at the same developmental stage. LWP of both sexes was significantly affected by diameter class, with LWP at the 20-cm diameter class being significantly higher than other classes (Fig. 4a [Figure 4: see original paper]), suggesting that LWP tended to increase with individual developmental stage. As canopy height increased, LWP decreased significantly while δ^{13}C increased significantly in both sexes. Sex, diameter class, canopy height, and their interactions had significant effects on LWP and δ^{13}C (Fig. 4b and d [Figure 4: see original paper]). Differences in LWP and δ^{13}C between sexes at the same canopy height were significant, with both parameters being higher in females than males, suggesting that males had greater water uptake capacity while females exhibited higher long-term water use efficiency.

3.4 Sex differences in Pro and MDA of heteromorphic leaves

Pro and MDA concentrations in heteromorphic leaves of male and female *P. euphratica* tended to increase with diameter class (Fig. 5 [Figure 5: see original paper]), though differences between sexes across diameter classes were not significant (Fig. 5a and c [Figure 5: see original paper]). As canopy height increased, Pro and MDA concentrations increased in both sexes, with significant differences between top and bottom canopy positions, showing higher values at the top. At different canopy heights, Pro and MDA concentrations were significantly influenced by sex (Fig. 5b and d [Figure 5: see original paper]), with significant differences in Pro concentrations between sexes at the same canopy height in the 12-cm diameter class, and higher Pro concentrations in males than females. Sex, diameter class, canopy height, and their interaction had significant effects on MDA (Fig. 5d [Figure 5: see original paper]). MDA concentration differences between sexes at the same canopy height were significant, with all male *P. euphratica* showing higher MDA concentrations than females, indicating that male plants were more sensitive to stress.

3.5 Relationship among functional traits of heteromorphic leaves, developmental stage, and canopy height

Correlations among functional traits of heteromorphic leaves, developmental stage, and canopy height are shown in Table 2 . LA, LDW, and gs of heteromorphic leaves in both sexes were positively correlated with diameter class (DC) and canopy height (CH). SLA was negatively correlated with DC and CH. LT, Pn, Tr, Pro, MDA, and δ^{13}C were positively correlated with CH, while LI and Ci were negatively correlated with CH in both sexes. LWP of female plants was negatively correlated with DC, whereas LWP of male plants was positively correlated with DC. Additionally, Pn and WUE of female plants were positively correlated with DC, while MDA of male plants was positively correlated with DC.

LI and SLA in heteromorphic leaves of both sexes were negatively correlated with Pn, Tr, Pro, and δ^{13}C, and positively correlated with Ci. LA, LDW, and LT all showed positive correlations with Pn, gs, Tr, Pro, and MDA, and significant negative correlations with Ci (Table 3). Furthermore, LA of female plants was positively correlated with WUE and negatively correlated with LWP. WUE was positively correlated with LDW and negatively correlated with SLA. LI of male plants was negatively correlated with gs and MDA.

3.6 PCA of functional traits of heteromorphic leaves

To identify the main factors influencing variation in functional traits of heteromorphic leaves between sexes, we performed PCA using indicators of each functional trait. Results showed that female and male plants were well separated at different developmental stages and canopy heights (Fig. 6 [Figure 6: see original paper]). Two principal components explained 75.73% (Fig. 6a [Figure 6: see original paper]) and 98.95% (Fig. 6b [Figure 6: see original paper]) of total variation, respectively. At different developmental stages, female and male plants separated along the first principal component (PC1) axis; at different canopy heights, they separated along the second principal component (PC2) axis. PC1 was primarily influenced by LA, LT, LDW, SLA, δ^{13}C, LWP, gs, Pn, Tr, and MDA, while PC2 was more influenced by LI, Ci, WUE, and Pro. These results indicated that LA, LT, Pn, Tr, gs, MDA, and Pro of male plants showed more pronounced changes across developmental stages and canopy heights than those of female plants.

4 Discussion

This study revealed significant differences in LA, SLA, LT, Pn, Tr, gs, LWP, δ^{13}C, Pro, and MDA of heteromorphic leaves between male and female *P. euphratica* as individual developmental stage and canopy height changed. These sex differences were closely related to increases in individual developmental stage and canopy height, particularly for different canopy heights. At the same canopy height, male plants showed higher LA, SLA, LT, Pn, Tr, gs, Pro, and MDA, and lower LWP and δ^{13}C compared to female plants, indicating that males made more morphological and physiological adjustments at different canopy heights to enhance resource acquisition, use, and allocation, thereby ensuring growth and survival under adverse conditions.

Sex differences in plant leaves reflect sex-specific strategies arising from differential reproductive investments and may produce variations in stress tolerance under unfavorable conditions (Espírito-Santo et al., 2003). Many studies have documented differences in growth strategies between male and female plants across various environmental conditions (Li et al., 2007; Juvany and Munné-Bosch, 2015; Lei et al., 2017; Liu et al., 2020). Generally, the proportional distribution of reproductive structures and aboveground vegetative growth in dioecious plants varies by sex and developmental stage (Teitel et al., 2016).

This study confirmed that morphological variation in heteromorphic leaves is closely related to individual developmental stage and canopy height increase. At different developmental stages and canopy heights, LA, LT, and SLA were significantly higher in male than female plants. Under drought conditions, males maintained higher LA and lower SLA than females (Xu et al., 2008a). As tree height increases, water stress is induced by increased gravitational and path-length resistance (Koch et al., 2004). Large, thick leaves help increase the distance and resistance of water diffusion from leaf interior to leaf surface, reducing water loss and enhancing carbon capture to adapt to water stress caused by tree height (Zhai et al., 2020), while reduced SLA enhances drought resistance (Ne'eman et al., 2011) and facilitates survival. Thus, male plants appear more tolerant to water stress caused by tree height, while female plants are relatively less tolerant.

Considering the distribution of effective light at different canopy heights (Peavey et al., 2020), LA, LT, and LDW were substantially higher at the top canopy than at the bottom for both sexes, indicating that increasing canopy height significantly affected leaf morphology. Plant leaves can adapt morphologically to different light conditions, and male plants are more susceptible to photoinhibition than females (Sánchez-Vilas and Retuerto, 2009). Wu et al. (2021) found that plants with high resource acquisition capacity can compensate for the inhibitory effects of environmental stress on growth. Furthermore, photosynthetic properties of leaf area relate to tree height and drought stress degree (Kenzo et al., 2015). This study showed that Pn, Tr, and gs of heteromorphic leaves in both sexes were significantly and positively correlated with LA, LDW, LT, and CH, indicating that changes in stomatal exchange parameters were related to leaf morphology and canopy height. At different canopy heights, sex differences in Pn, Tr, and gs were evident, with male plants showing higher values at the same canopy height. Previous studies on *Pistacia lentiscus* L. found that males exhibited higher photosynthesis and stomatal conductance than females (Correia and Díaz, 2004), consistent with our findings. Additionally, water stress caused by increased tree height may ultimately limit leaf expansion and photosynthesis (Koch et al., 2004; He et al., 2008; Zhang et al., 2009; Zhai et al., 2020). Therefore, male plants possess high photosynthetic capacity, which may facilitate acquisition and use of carbon resources under adverse conditions.

In some cases, plants exhibit more conservative water use strategies under unfavorable conditions (Retuerto et al., 2000; Alvarez-Cansino et al., 2010; Grossiord et al., 2017). Koch et al. (2004) suggested that water potential decreases with increasing tree height and that reduced LWP leads to decreased growth, assimilation, and hydraulic conductivity (Ratzmann et al., 2019). Increased plant leaf $\delta^{13}C$ favors photosynthetic productivity in bright, dry upper canopy conditions (Kenzo et al., 2015). This study found that $\delta^{13}C$ of heteromorphic leaves was significantly and positively correlated with CH, while LWP was significantly and negatively correlated with CH. As canopy height increased, LWP decreased significantly and $\delta^{13}C$ increased significantly in both sexes, supporting these previous results. Additionally, at the same developmental stage and canopy height, sex differences in δ^{13}C and LWP were evident, with females showing higher values than males. Under relatively well-watered conditions, efficient water transport through the xylem allows for higher carbon acquisition rates and conversion to more effective carbohydrates, while female leaves appear to have high metabolic costs that require greater water transport capacity (Espírito-Santo, 2003). However, this acquisition strategy may entail long-term costs. Females maintain a more sensitive water balance and more liberal water use efficiency than males, which may help compensate for female reproductive costs (Obeso, 2002; Barrett and Hough, 2013; Lei et al., 2017). Dawson and Bliss (1989) found that potential water use efficiency of female *Salix* is higher in arid habitats. Furthermore, heteromorphic leaf LA and SLA of female plants were significantly and negatively correlated with LWP and δ^{13}C, suggesting that sex differences in δ^{13}C and LWP relate to leaf morphological adjustments. This indicates that female plants can modify leaf morphology to enhance long-term water use efficiency to meet greater growth demands.

In higher plants, water stress causes accumulation of osmoregulatory substances such as Pro and MDA in response to water scarcity (Kishor et al., 2005). Generally, MDA serves as an indicator of lipid peroxidation degree induced by oxidative stress and is important for plant adaptation to environmental stress (Apel and Hirt, 2004; Xu et al., 2008b; Sies and Jones, 2020). Previous studies have shown that drought stress significantly increases leaf MDA concentrations in both sexes (Xu et al., 2008b). This study demonstrated that Pro and MDA concentrations in heteromorphic leaves were significantly and positively correlated with CH, LA, LDW, and LT. As canopy height increased, Pro and MDA concentrations in both sexes tended to increase, being significantly higher at the top than at the bottom of the canopy. This indicates that as canopy height increases, water deprivation intensifies, resulting in greater water stress that significantly affects morphological construction of heteromorphic leaves and accumulation of osmoregulatory substances. Additionally, gender differences in MDA concentrations of heteromorphic leaves existed at the same canopy height, with males showing higher values than females, indicating that male plants are more sensitive to water deprivation.

Early evidence suggests that male plants tend to be more abundant than females under unfavorable environmental conditions (Hutline et al., 2016; Melnikova et al., 2017). Sex differences may reflect trade-offs between resource-exploration strategies across resource gradients (Lei et al., 2017), wherein female plants improve long-term water use and meet greater reproductive costs through leaf morphological adjustments, while male plants engage in efficient nutrient storage and enhance organic carbon acquisition and water uptake through synergistic changes in heteromorphic leaf morphology and physiology that improve stress tolerance and survival chances in resource-limited habitats (Hultine et al., 2016). Differences in resource uptake, allocation, and utilization strategies between sexes may represent evolved responses to meet specific resource needs associated with reproduction, thereby maintaining population structure and ecosystem stability.

5 Conclusions

This study revealed significant differences in morphological and physiological characteristics of heteromorphic leaves of *P. euphratica* to accommodate different developmental stages and canopy heights. With increasing developmental stage and canopy height, male plants demonstrated greater photosynthetic and water-absorbing capacity compared to female plants, thereby increasing access to and utilization of organic carbon resources under adverse conditions and improving stress tolerance and survival chances in unfavorable habitats. Conversely, female plants showed relatively less leaf morphological adjustment but greater long-term water utilization as a means of meeting or compensating for greater reproductive costs. Additionally, differences in resource uptake, allocation, and utilization strategies between sexes were associated with individual developmental stages and increased canopy height. Therefore, male and female *P. euphratica* evolved responses to meet specific reproduction-related resource requirements through feedback regulation between heteromorphic leaf morphological adjustments and physiological responses to adapt to changes in individual developmental stage and canopy height. These results may provide a basis for management of dioecious plant populations.

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Appendix

Fig. S1 Female and male inflorescences of *P. euphratica*. (a) male inflorescence; (b) female inflorescence.

Table S1 Basic information of male and female *P. euphratica*

Diameter class (cm)	Average diameter at breast height (cm)	Average tree height (m)	Average tree age (a)
Female 8	8.33e	7.53e	8.10e
Female 12	14.30d	9.47d	9.30d
Female 16	17.67c	11.27b	10.37b
Female 20	23.23ab	12.87a	11.17a
Male 8	9.33e	7.97e	8.37e
Male 12	14.37d	10.00cd	9.70cd
Male 16	17.33c	10.93be	10.13bc
Male 20	24.83a	12.70a	11.10a

Note: Different lowercase letters within the same column indicate significant differences among females or males at $P < 0.05$ level.

Note: Figure translations are in progress. See original paper for figures.