Lee et al. Journal of Ecology and Environment (2018) 42:7
https://doi.org/10.1186/s41610-018-0067-2

Journal of Ecology and Environment

RESEARCH Open Access

A rare duodichogamous flowering system in monoecious *Toona sinensis* (Meliaceae)

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Abstract

Background: Duodichogamy is the rarest form of dichogamy in angiosperms, which is characterized by flowering in the sequence of male→female→male. Disentangling factors promoting duodichogamy require the discovery of more duodichogamous species in angiosperms. However, extremely limited information on duodichogamous species makes it difficult to make general conclusions. Given the inflorescence morphology and flowering characteristics, the Meliaceae family is highly likely to contain duodichogamous species.

Methods: We selected 48 individuals from 20 populations in Korea and investigated their flower morphology, arrangement of flowers by sexual condition within inflorescences, and flowering phases and duration of male and female flowers of *Toona sinensis* (Meliaceae) for 5 years (2011–2015) to determine if the species shows duodichogamous flowering.

Results: *Toona sinensis* belonging to Meliaceae possessed functionally unisexual flowers with rudimentary parts of the opposite sex. The floral organs in male were larger than those in female, except for ovary length and width. In dichasium, male flowers were observed on primary or lateral branches, whereas female flowers were borne only on lateral branches. Overall, individuals from six different populations flowered in the male→female→male sequence, thereby male is blooming far longer than female flowers at the level of individual trees (male vs. female = 17–20 days vs. 2–4 days).

Conclusions: This is the first study to report a duodichogamously flowering species, *T. sinensis*, within Meliaceae. Several flowering characteristics observed from *T. sinensis* may be important clues used to discover additional duodichogamous Meliaceae species. Short flowering period and relatively small number of female flowers, which is analogous to reduced ovule numbers observed in other duodichogamous species, may intensify male–male competition in *T. sinensis*. This study contributed to narrowing down potential candidates of duodichogamy based on their geographic distributions and flowering time.

Keywords: Duodichogamy, Monoecy, Reproductive biology, Synchronous dichogamy, *Toona sinensis*

Background

Dichogamy is a temporal flowering system in which male and female reproductive organs mature at different times within a single flower or across multiple flowers. When the female reproductive organ (or female flower) matures first, it is called protogyny. Conversely, protandry refers to instances when the maturation of the male reproductive organ precedes that of the female. Potential selective factors that influence the evolution of dichogamy include the avoidance of self-fertilization (Bawa and Beach 1981; Wyatt 1983), inbreeding depression (Routley and Husband 2003; Sargent et al. 2006), pollen–stigma interference (Wyatt 1983; Lloyd and Webb 1986; Harder et al. 2000; Barrett 2002; Sargent et al. 2006), and sex-ratio selection (Sargent et al. 2006). However, it is still difficult to determine the major selective factors associated with dichogamy despite the common occurrence of dichogamy in angiosperms. This may reflect that these selective forces are not mutually exclusive (Lloyd and Webb 1986) and there is not sufficient information for diverse dichogamous systems.

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Lloyd and Webb (1986) identified diverse types of dichogamy based on several criteria. For example, depending on the degree of synchrony of male and female anthesis within a plant, they distinguished asynchronous, hemisynchronous, and synchronous dichogamy. Synchronous dichogamy is further subdivided into one-cycle dichogamy, duodichogamy, multi-cycle dichogamy, and heterodichogamy, depending on the sequence and cyclicity of maturation of male and female flowers (unisexual) or each reproductive organ (bisexual) (Lloyd and Webb 1986). One-cycle dichogamy is flowering in the sequence male→female or female→male, duodichogamy is flowering in one-and-a-half cycles, usually in the sequence (male→female→male), and multi-cycle dichogamy has repeated flowering cycles alternating between male and female. Heterodichogamy has two morphs (protandrous and protogynous morphs) that flower synchronously within unisexual or bisexual populations.

Duodichogamy is the rarest form of dichogamy that has so far only been reported in a few species from seven genera of four families (i.e., *Bridelia* and *Cleistanthus* in Phyllanthaceae, *Castanea* in Fagaceae, *Cladium* in Cyperaceae, and *Acer*, *Dipteronia*, and *Paullinia* in Sapindaceae) (Luo et al. 2007; Li et al. 2014; Renner 2014; de Lima et al. 2016). Since these genera are largely composed of species with unisexual or functionally unisexual flowers, the sequence of flowering male→female→male is highly likely to represent greater numbers of male flowers (unisexual flowers) or greater investment to male function (bisexual flowers). There is no universal conclusion about the evolution of duodichogamy associated with more male flowers than female flowers. The most supported hypothesis suggests that it resulted from competition between male gametes for a small number of ovules (Lloyd and Webb 1986; Luo et al. 2007). Indeed the reported duodichogamous species in the aforementioned seven genera all have a small number of ovules (1–4) per female flower. The discovery of more duodichogamous species in angiosperms, in particular, studies on detailed aspects of duodichogamous flowering including flower sexual identity and flowering sequence along the inflorescence, will aid our ability to make general conclusions about the conditions that promote duodichogamy.

The Meliaceae family (ca. 600 species; Christenhusz and Byng 2016), also known as the mahogany family, is an excellent candidate for the identification of additional duodichogamous species. The family is distributed in tropical, in subtropical, and rarely in warm-temperate regions (eFloras 2017), and species produce highly valued timber. The monoecious tribe Cedreleae (e.g., *Cedrela* and *Toona*; Gouvêa et al. 2008; Muellner et al. 2009), within the subfamily Cedreloideae, has a high likelihood of containing duodichogamous species based on their inflorescence morphology and flowering characteristics. Firstly, the species in this tribe typically have complex inflorescence such as thyrse (a racemose scaffold holding several determinate compound dichasia; Weberling 1992) that contains functionally unisexual male/female flowers with rudimentary reproductive organs of the opposite sex (Pennington and Styles 1975). Sapindaceae, which is closely related to Meliaceae within Sapindales, has such inflorescence and includes several duodichogamous species (eFloras 2017). Cedreleae species also generally form large inflorescences with a high number of male flowers (Bahadur 1987; Edmonds and Staniforth 1998). This is very similar to the flowering pattern of duodichogamy, which is characterized by far more male than female flowers. Secondly, Cedreleae species generally form dichasia with a female flower at the primary branch (Pennington and Styles 1975; Bahadur 1987; Gouvêa et al. 2008), which is indicative of protogyny, but some species are known to be protandrous (e.g., *Toona sinensis* (Juss.) M. Roem.; Edmonds and Staniforth 1998), possibly indicating duodichogamous flowering. Thirdly, Styles (1972) reported that Meliaceae species exhibit sex-reversal flowering in which all the flowers blooming within an individual are the same sex at a certain time, and this occurs before all flowers change to those of the opposite sex. Close examination of such flowering may reveal a typical pattern of duodichogamy (i.e., sequential batches of male–female–male flowering within the same individuals).

Between the two Meliaceae species in Korea, *Melia azedarach* and *Toona sinensis*, only *T. sinensis* belongs to the tribe Cedreleae (Muellner et al. 2009). *T. sinensis* is native to Southeast Asia (Edmonds and Staniforth 1998) and was introduced to Korea from China before the fifth or sixth century. The species has several uses, including timber, medicinal, horticultural, and vegetable usages. In villages along the coast of South Korea, one or two *T. sinensis* trees are typically planted by each household, and households rarely have more than three trees. *T. sinensis* produces large thyrses (up to 1 m in length) that contain a mixture of functional male or female flowers (hereafter referred to as male flowers and female flowers) (Bahadur 1987; Edmonds and Staniforth 1998). Edmonds (1993) considered *T. sinensis* in Southeast Asia to be protandrous, with several cycles of male flowering followed by a brief period of female flowering. However, Edmonds was unable to closely inspect the course of flowering because of the height of the examined *T. sinensis* trees. Thus, information about the flowering sequence and arrangement of flowers based on the sexual condition within inflorescences, which is essential for defining duodichogamy in *T. sinensis*, is not available in Korea or China.

In the present study, we investigated the type of dichogamy associated with *T. sinensis* to answer the following questions: (1) Does morphology differ between male and female *T. sinensis* flowers? (2) How are male and female flowers arranged within inflorescences? (3) Is the flowering pattern consistent with duodichogamy? To answer these questions, we observed flower morphology, the arrangement of flowers based on sexual condition within inflorescences, and flowering phases and duration of male and female flowers.

Methods

Study species and sites

In order to inspect the rarity and phylogeny of duodichogamous systems, we extracted information about duodichogamous species from previous studies (Table 1). Renner (2014) reported that the five genera exhibiting duodichogamous flowering systems (*Acer*, *Bridelia*, *Castanea*, *Cladium*, and *Dipteronia*) belonged to mutually independent families (i.e., five families). However, according to the APG IV system (The Angiosperm Phylogeny Group 2016), both *Acer* and *Dipteronia* belong to the Sapindaceae family. Therefore, in the present study, we describe a total of five angiosperm families, including the Meliaceae, as those that contain duodichogamous species.

Toona sinensis (Juss.) M. Roem. grows up to 40 m in height (eFloras 2017), and individuals produce unisexual flowers of both sexes (Bahadur 1987). *T. sinensis* fruits are capsules with five locules, and each locule contains six ovules (eFloras 2017). *T. sinensis* trees in West Java produced a low number of fruits (mean ± SD = 232.7 ± 133.7) compared to the high number of flowers per inflorescence (mean ± SD = 8104.3 ± 5853.8) (fruit-set 2.9%) (Pramono et al. 2016). According to these authors, less than half the ovules within an ovary produced seeds (seed-set 39.7%), and the seed germination rate was very low (Bahadur 1987).

Korea has a monsoon climate with heavy rain concentrated in the summer (June–August). *T. sinensis* flowering begins before the start of the summer rainy season (in early June in lower latitudes and in mid-June in higher latitudes); flowering ends at the start or peak of the rainy spell (in late June in lower latitudes and in early-to-mid July in higher latitudes), and the fruit matures in September or October. During the flowering seasons (June–July) of 2011, 2013, 2014, and 2015, we collected and observed 48 individuals out of 106 individuals in 20 populations in South Korea, including those in southern, inland, and coastal regions. These populations were distributed in relatively warm, low-lying mountain regions, along the coast, and near farmlands

Table 1 List of duodichogamous species in angiosperms reported in previous studies and in this study. The numbers within the parentheses in the first column indicate approximate numbers of species and genus of each family (Christenhusz and Byng 2016)

Family	Genus	Species	Reference
Cyperaceae (5500, 90)	*Cladium*	*Cladium jamaicense*	Snyder and Richards (2005)
Fagaceae (927, 8)	Castanea	*Castanea mollissima*	Stout (1928), Godley (1955)
		Castanea crenata	Hagman (1975), Jaynes (1975)
Meliaceae (600, 53)	*Toona*	*Toona sinensis*	This study
Phyllanthaceae (2050, 57)	*Bridelia*	*Bridelia retusa*	Borges et al. (1997)
		Bridelia tomentosa	Luo et al. (2007)
		Bridelia stipularis	Li et al. (2014)
	Cleistanthus	*Cleistanthus sumatranus*	Li et al. (2014)
Sapindaceae (1860, 142)	*Acer*	*Acer* spp.	de Jong (1976)
	Cupania	*Cupania guatemalensis*	Bawa (1977)
	Deinbollia	*Deinbollia* spp.	de Jong (1976)
	Dipteronia	*Dipteronia* spp.	de Jong (1976)
	Hippocastanum	*Hippocastanum* spp.	de Jong (1976)
	Koelreuteria	*Koelreuteria* spp.	de Jong (1976)
	Litchi	*Litchi chinensis*	Khan (1929)
	Paullinia	*Paullinia weinmanniifolia*	de Lima et al. (2016)
	Sapindus	*Sapindus emarginatus*	Subba Reddi et al. (1983)
	Serjania	*Serjania* spp.	Acevedo-Rodriguez (1993)
	Talisia	*Talisia* spp.	Acevedo-Rodriguez (2003)
	Urvillea	*Urvillea ulmacea*	Zapata and Arroyo (1978)

(range 10–322 m asl) (Table 2). The mean annual temperature in these regions was 11.1 to 13.8 °C, and the mean annual precipitation was 1031.7 to 1505.6 mm (Korea Meteorological Administration, KMA) (Table 2).

Male and female flower characteristics

We selected one to five individuals (48 individuals in total) from each region between June and July of 2011. One inflorescence was picked at random from each of the 48 selected individuals (mean inflorescence length, mean ± SE = 52.5 ± 1.5 cm, $N = 48$). The inflorescences were divided into three to four sections, and one mature or almost-mature male and female flower was collected from each section ($N = 320$ each for male and female flowers). Then, the morphology of those flowers was observed. Measurements of each part (petal length and width, filament length, anther length and width, and ovary length and width) were taken from mature male and female flowers, and measurements were statistically compared ($N = 184$ each for male and female flowers). A stereoscopic microscope (× 10 to × 30, Nikon SMZ-1000) and measurement program (iWorks Lite, Image Measuring & Analysis Software) were used to observe and measure each flower part under 5 mm in size.

Arrangement of male and female flowers within inflorescences

Thyrses (Fig. 1a) were collected (three thyrses per individual, total $N = 144$) from the 48 individuals from 20 populations described above, and ten single dichasium containing three flowers were cut from each thyrse ($N = 1440$) (Fig. 1b). The cut inflorescences were stored in FAA solution (formaldehyde/acetic acid/70% ethanol = 5:5:90) and were later removed from the solution at the laboratory for observation with the naked eye or with a stereoscopic microscope (× 10 to × 30, Nikon SMZ-1000). Finally, after determining the sex of the flower by inspecting its rudimentary parts, we analyzed the arrangement of male and female flowers within inflorescences.

Flowering phases and duration of male and female flowers

A large number of inflorescences (mean 36 per individual, range 2–165) and flowers (mean 8000 per individual, range 951–27,315) and tall trees (above 40 m) of *T. sinensis* (Pramono et al. 2016) make it difficult to determine the course of flowering for male and female flowers. Therefore, in 2013, in the Gangneung region on the east coast of central Korea, we selected and tagged two individuals, of which height of inflorescences were

Table 2 Population sizes and physical conditions of *Toona sinensis* of 20 populations examined in South Korea during flowering season (June to July) for 5 years (2011–2015). Inflorescences of a total of 48 individuals were observed or collected among 106 individuals in these populations. Data on annual mean temperature and precipitation (1980–2010) were obtained from Korea Meteorological Administration (KMA)

Population	Population size	Alt. (m)	Weather observatory location	Temp. (°C)	Precip. (mm)	Examined year
Chuncheon	6	126	Chuncheon	11.1	1347.3	2011
Yeongok	5	10	Gangneung	13.1	1464.5	2011
Gangneung	2	10				2011, 2013
Seongsan	5	58				2011
Suwon	1	39	Suwon	12.0	1312.3	2011
Daebudo	10	20	Incheon	12.1	1234.4	2011, 2014, 2015
Yeongheungdo	27	10				2011, 2014, 2015
Taean	4	40	Seosan	11.9	1285.7	2011
Uljin	2	10	Uljin	12.6	1119.0	2011
Cheongsong	1	309	Euiseong	11.2	1031.7	2011
Mirayng	2	10	Miryang	13.3	1229.4	2011, 2014, 2015
Yangsan	1	30				2011
Wanju	5	30	Jeonju	13.3	1313.1	2011
Jeongeup	5	22	Jeongeup	13.1	1317.3	2011
Namwon	2	322	Namwon	12.3	1380.4	2011, 2014, 2015
Beopseong	5	10	Gwangju	13.8	1391.0	2011
Yeonggwang	14	20				2011, 2014, 2015
Nampyeong	3	14				2011
Donggang	3	22				2011
Boseong	3	50	Jangheung	13.0	1505.6	2011, 2014, 2015

Alt. altitude, *Temp.* mean annual temperature, *Precip.* mean annual precipitation

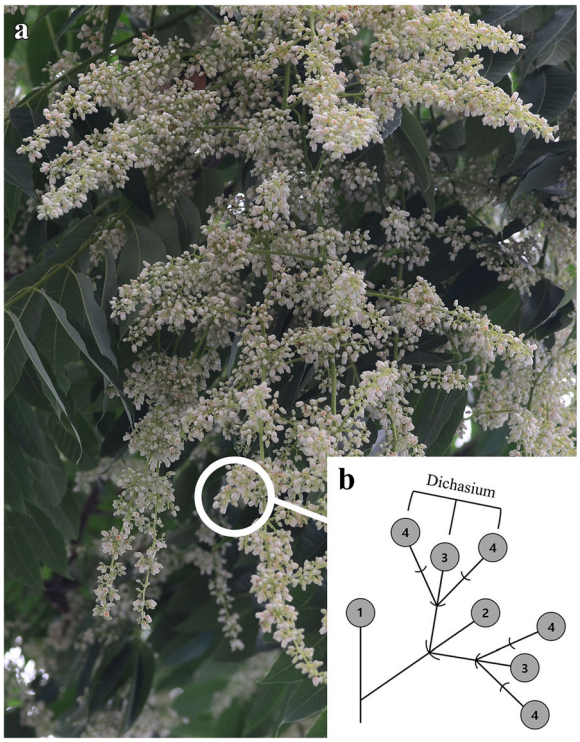

Fig. 1 Inflorescence structure of *Toona sinensis*, including **a** the number of compound dichasium and **b** the flowering phases (1 = primary, 2 = secondary, 3 = tertiary, and 4 = quaternary). Male flowers occur through the whole phases, but female flowers only occur in the quaternary phase

low enough to reach by a ladder (3–4 m above the ground) (T1 and T2; height 14 vs. 5.4 m, respectively; DBH 44 vs. 28.6 cm, respectively). Due to a limited number of thyrses being reached, we marked two thyrses in each individual (T1-1 and T1-2 and T2-1 and T2-2, respectively), although the individuals had many more thyrses. Each thyrse was divided into five even parts along the axis, and one compound dichasium, which is the basic inflorescence unit of thyrse (Fig. 1b), was tagged in each part. During a preliminary investigation in 2012, when flowering had finished for five tagged compound dichasia, almost all the flowers on the thyrse had finished flowering. Therefore, the tagged compound dichasia were used as units of measure in the flowering survey. The compound dichasia, consisting of only eight flowers, were categorized into four phases (primary, secondary, tertiary, and quaternary phases) based on their position on the branch (Fig. 1b). From June 11 to July 7 of 2013, the course of flowering was observed at a 1- or 3-day interval until abscission occurred in all the flowers. We additionally observed the flowering phases for a total of 48 individuals (Table 2) from six populations (Miryang, Namwon, Boseong, Yeonggwang, Daebudo, and Yeongheungdo) in early June to mid-July of 2014 and 2015. To confirm conversion between male and female flowering and synchrony in sexuality within the individual, we collected five to seven inflorescences per individual or photographed inflorescences and flowers with telephoto lens at once in a week.

In compound dichasia from the T1 and T2 individuals in Gangneung, flower longevity was calculated for male flowers ($N = 97$), which flowered during all four phases (Fig. 1b), and female flowers ($N = 15$), which flowered only in the quaternary phase. Flower longevity was defined for a given flower as the duration from the start of flowering to abscission. For combined T1 and T2 measurements, there was very little variance between the flower longevity of male and female flowers [male vs. female = 0 vs. 0.80 (SD)], so the mean and range of flower longevity of male and female flowers were obtained across both individuals. Flowering duration per thyrse was defined as the number of days between the first flower and last flower over the five compound dichasia. We then calculated the duration for each sex and phase. The flowering duration per individual was calculated using a method that was similar to that used to determine the duration per thyrse. However, ten compound dichasia from two inflorescences were combined, because the start and abscission of flowering within the two inflorescences was regarded as a basic unit of an individual's flowering, despite some degree of deviation in flowering among the thyrses of an individual.

Statistical analysis

Measurement data are presented as mean ± SE. Statistical differences between male and female flower measurements were analyzed using an unpaired Student's t test (SPSS 19, 2010). Statistical significance was tested at the level of $\alpha = 0.05$.

Results

Male and female flower characteristics

We found several common characteristics between male and female *T. sinensis* flowers. The flowers had a weakly developed androgynophore above the calyx, and five separate, creamy-white, carinate petals were attached to the androgynophore (Fig. 2). The intrastaminal nectary disk, which had a swollen discoid shape surrounding the androgynophore, was pale green in the bud stage, and it changed, in order, to yellow, orange, and then red as the flower matured. The nectaries were divided into five rows, and each was fused with one of the five filaments. Latrorse anthers were attached to the top of the filaments, and there were five thread-like, shrunken staminodes alternating with the filaments. The stigma at the end of the style had a large discoid shape (Fig. 2), and papillae were located along the edge and underside of

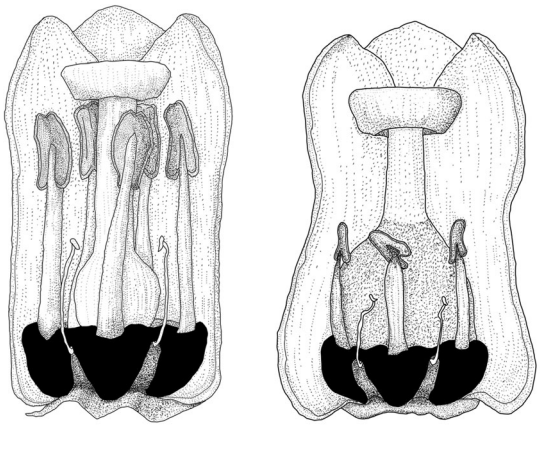

Fig. 2 Functional male (left) and female (right) flowers of *Toona sinensis*. Both types of flowers have five thread-like staminodes alternating with stamens (male) or antherodes (female). The ovary color of the female flower is shiny green, whereas that of the male is pale white. The nectary around the androgynophore is green initially but turning bright red when opened (scale bar = 1 mm)

the stigma. The carpel has five locules, each with four to six ovules.

Male and female flowers appeared to be bisexual flowers externally, but parts of opposite sex flowers were rudimentary. In male flowers, the anthers were pale yellow, adjacent to each other, and they dehisced during flowering. Conversely, in female flowers, the anthers were dark yellow, relatively small, and far apart, and they did not dehisce even after flowering (thus being called antherodes). The ovary was white in male flowers at the time of flowering, and it was green in female flowers. The ovules in male flowers had a shriveled appearance inside the ovaries (thus forming a pistillode), whereas the former in female flowers was round. All floral parts of male flowers except for ovary length and width were significantly larger than those of female flowers (Table 3). The anther length and the filament length in male flowers were 2-fold and 1.6-fold larger, respectively, than those in female flowers. On the other hand, the ovary length and the ovary width were both 2-fold larger in female flowers than those in male flowers.

Arrangement of male and female flowers within inflorescences

Male flowers were observed on both the primary and lateral branches of dichasia or compound dichasia (N = 1440), whereas female flowers were only borne on the lateral branches of dichasia with three flowers (Fig. 3). As a result, there were three possible combinations for flowers on the two lateral branches of dichasia (male + male, female + male, and female + female) (Fig. 3a, b, c). Based on our observations of flowering on collected inflorescences, when the flowers on the lateral branches were of the same sex, both flowers bloomed (Fig. 3a, c). However, when the flowers were of different sexes, they flowered at different times (Fig. 3b).

Flowering phases and duration of male and female flowers

On the tagged compound dichasia from the two individuals in Gangneung (T1 and T2), the males flowered in sequence from the primary to the tertiary phase. Furthermore, in the quaternary phase, the flowering sequence was either male→female→male or female→male→female (Fig. 4). For all inflorescences, with the exception of T1-2, the males flowered first in the quaternary phase and were then immediately followed (or after an interval of a few days) by females and then by males. In T1-2, there was no flowering from day 177 to day 182 immediately after the end of the tertiary phase. Females then flowered on day 183 and were followed by males and then females.

When two pairs of flowers in the quaternary phase were of the same sex (male + male or female + female) (Figs. 1 and 3a, b, c), they flowered at the same time. When the flowers were of different sexes (male + female), the males usually flowered later than the females. In some inflorescences (T1-1 and T1-2), the overlap of male and female flowers was observed in the quaternary phase, whereas such overlap was not observed in the remaining two inflorescences from T2 (Fig. 4). When we observed individual trees in six different populations (Miryang, Namwon, Boseong, Yeonggwang, Daebudo, and Yeongheungdo) in 2014 and 2015, most flowers within individuals showed sexual synchrony with a negligible overlap, and the flowering sequence in the quaternary phase was mostly male→female→male.

Table 3 Mean sizes of male and female floral structures in *Toona sinensis*. Mean ± SE is provided. N = 184 each for male and female flowers

	Petal (mm)		Stamen (mm)		Ovary (mm)	
	Length	Width	Filament length	Anther length	Length	Width
Male	4.74 ± 0.46	2.85 ± 0.36	2.66 ± 0.27	1.39 ± 0.20	0.82 ± 0.15	0.81 ± 0.15
Female	4.42 ± 0.42	2.61 ± 0.30	1.67 ± 0.26	0.71 ± 0.19	1.55 ± 0.29	1.62 ± 0.19
t	6.959	6.989	36.394	33.918	30.132	45.863
df	366	366	366	366	366	366
P	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

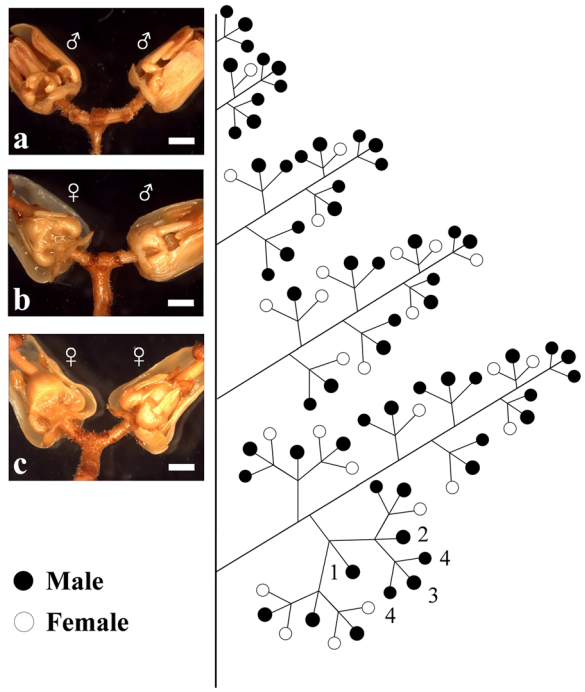

Fig. 3 The arrangement of male and female flowers in two lateral branches of a dichasium (left) and thyrse (right) (**a** male (left) + male (right), **b** female + male, **c** female + female; scale bar = 1 mm). Numbers on the compound dichasium correspond to the flowering phases described in Fig. 1

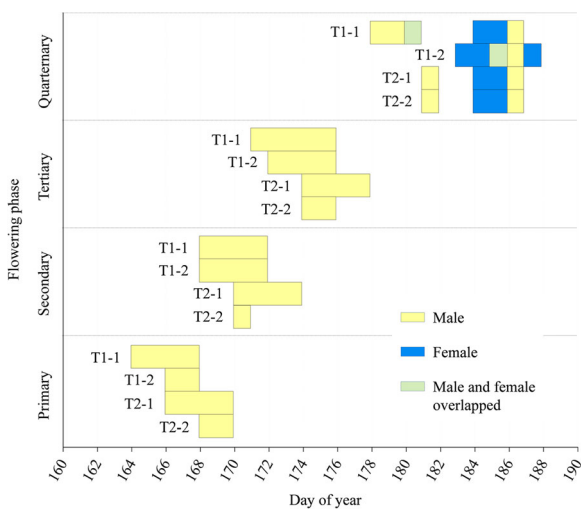

Fig. 4 The flowering phases and duration of male and female flowers on two thyrses of two *Toona sinensis* individuals (T1 and T2) in Gangneung on the east coast of South Korea. Two thyrses of T1 and T2 were again tagged as T1-1 and T1-2, and T2-1 and T2-2

In T1 and T2, the flower longevity of all male flowers was 1 day ($N = 97$) and it was 2 days for female flowers (1.93 ± 0.21, $N = 15$). From the primary phase to the tertiary phase, males flowered for an average of 3.33 ± 0.36 day per phase per thyrse (range 1–5 days, $N = 12$) (Fig. 4). In the quaternary phase, T1-2 females flowered the longest (4 days) in which females flowered twice. Furthermore, the T1-1 females flowered for 3 days, and other inflorescences (T2-1 and T2-2) flowered for 2 days. Conversely, in the same phase, T1-1 males flowered the longest (4 days) and other inflorescences (T1-2, T2-1, and T2-2) flowered for 2 days. Thus, the flowering per individual persisted for almost 1 month [T1 24 days (June 13–July 7); T2 21 days (June 15–July 6)] (Fig. 4). At the individual level, the males flowered for 17 to 20 days and the females flowered for only 2 to 4 days.

Discussion

Duodichogamous flowering pattern of *T. sinensis*

This is the first study to demonstrate that *T. sinensis* of the Meliaceae is a duodichogamous species. Monoecious taxa within the Meliaceae have been known to contain only protandrous or protogynous species (Styles 1972; Bahadur 1987; Pennington and Muellner 2010). Duodichogamous flowering with the order male→female→male was observed in both individuals at Gangneung and in all individuals ($N = 58$) that were monitored for 2 years in six other populations. Therefore, we concluded that *T. sinensis* exhibits duodichogamy, which is "the rarest (form of dichogamy) in angiosperms" (Luo et al. 2007). Meliaceae contains approximately 600 species from 53 genera (Christenhusz and Byng 2016), and most of them are monoecious species other than two tribes (Agalaieae and Guareeae) and seven genera (*Aphanamixis*, *Ekebergia*, *Guarea*, *Pterorhachis*, *Ruagea*, *Trichilia*, and *Walsura*) that contain dioecious species (Pennington and Styles 1975; Renner 2014). Given the fact that the Meliaceae belongs to the same order (Sapindales) as the Sapindaceae, which tends to include a relatively large number of duodichogamous species, more duodichogamous species are likely to be discovered within the Meliaceae. Currently, in the global sexual system database compiled by The Tree of Sex Consortium (2014), there is a lack of information about Meliaceae, including *T. sinensis*. If *T. sinensis* is included, duodichogamous flowering has now been detected in 17 genera from five families (Table 1), and this information should be reflected in the database.

Although dichogamy can increase the outcrossing rate and reduce pollen–stigma interference, it does not function perfectly in every inflorescence within individuals of *T. sinensis*. In this species, male and female flowerings are not overlapped in compound dichasia. While as seen in the two inflorescences of T1, there was a partial

overlap of male and female flowerings at the level of inflorescence (Fig. 4); flowering overlap occurred only in two flowers. However, at the individual level, at least some degree of geitonogamous selfing will be unavoidable in *T. sinensis* as in some duodichogamous species (*Bridelia tomentosa*, Luo et al. 2007; *Paullinia weinmanniifolia*, de Lima et al. 2016).

Because duodichogamous flowering of *T. sinensis* is dominated by male flowers, the flowering time and duration of the female flowers and the flowering sequence of individuals within a population are very important factors of successful pollination and seed production. In *T. sinensis*, the overall flowering duration of female flowers is much shorter (male vs. female 17–20 days vs. 2–4 days) because female flowers bloom only once for on average 2 days during a season. If there are few active pollinators visiting female flowers when their stigmas are receptive, or if they are not supplied with pollen from other individuals during the male phase, outcrossing would be difficult and possibly result in a low seed-set rate. In dichogamous species, cross-fertilization is only possible when the individuals within populations flower asynchronously. Verification of this characteristic will require further research that compares the pollinator activity and fitness levels in several natural *T. sinensis* populations.

Sexual arrangement within thyrse

The arrangement of male and female flowers in dichasia appears to be related to the dichogamous characteristics in the Meliaceae. *Cedrela* species. (Meliaceae) have female and male flowers at the primary and lateral branches of their dichasium, respectively, and they are known to be protogynous, with female flowers at the primary branches flowering first (Cruden 1988; Pennington and Muellner 2010). Conversely, in *T. sinensis*, the flower on the primary branch of the dichasium was always male, and there were three possible combinations of sex for the lateral branches (male + male, male + female, and female + female). When one examines dichogamy of other species belonging to Meliaceae, duodichogamous flowering can be inferred from such complex sex combinations on the lateral branches within dichasia. Species in Meliaceae typically produce unisexual flowers and exhibit a pattern of synchronous dichogamy (Styles 1972). Then, the arrangement of male and female flowers within dichasia could be a useful clue used to distinguish one-cycle dichogamy, duodichogamy, and multi-cycle dichogamy in Meliaceae species.

Floral structure of *T. sinensis* mistaken as bisexual flower

The flowers of Meliaceae species are often mistaken as bisexual flowers (e.g., Troup 1921; Lamb 1960). In *T. sinensis*, the identification of the precise sexual system has been hampered by its long flowering duration at the individual level and apparently bisexual flowers. However, the ovaries with rudimentary ovules in male flowers and non-dehiscing anthers in female flowers indicate that they are pistillodes and staminodes, respectively, that have lost their function. Hence, *T. sinensis* is a monoecious species producing distinct male and female flowers. Bahadur (1987) and Edmonds and Staniforth (1998) recognized the morphological differences between male and female flowers of *T. sinensis*, but they could not verify whether the anthers in the female flowers were still actually functioning. In the present study, we found that both rudimentary ovary and anthers in each male and female flower are unable to set seeds or disperse pollen, respectively. Therefore, *T. sinensis* in South Korea is a monoecious species producing male and female flowers within individuals. This result is concordant with previous studies that have described this species as having unisexual flowers (Pennington and Styles 1975; Bahadur 1987; Edmonds and Staniforth 1998; eFloras 2017).

Evolution of duodichogamy and potential candidates in Meliaceae

Regarding the conditions that promote evolution to duodichogamy, the most supported hypothesis is the male–male competition (Lloyd and Webb 1986; Luo et al. 2007). However, there are still ongoing debates about the conditions that promote a male–male competition. Luo et al. (2007) claimed that the conditions promoting the competition were not pollinator limitation but resource limitation, and a small number of ovules (1–4 ovules per flower) may contribute to male–male competition in duodichogamous species. While a low number of ovules does not appear to apply to *T. sinensis* (30 ovules per flower), short flowering period and relatively small number of the female flowers, analogous to reduced ovule numbers, may intensify male–male competition. We can further draw a potential correlation between duodichogamy and an additional potential factor promoting male–male competition based on geographic distributions of *T. sinensis* and other duodichogamous species (Table 1). Firstly, duodichogamous species (Table 1) mostly inhabit monsoon climates, which are characterized by large volumes of rain that fall over brief or long periods. Secondly, in the duodichogamous species comprehensively examined so far, the female flowers opened during the summer monsoon (rainy season) [e.g., *Cladium jamaicense* (Snyder and Richards 2005), *Bridelia tomentosa* (Luo et al. 2007), and *Paullinia weinmanniifolia* (de Lima et al. 2016)]. Regarding *T. sinensis*, the female flowers opened at the start of the summer monsoon (late June or early July) in South Korea. Heavy rain hampers the receptivity of the stigma, pollen germination, pollen dispersal (particularly

in wind-mediated flowers), and the activity levels of pollinators (Kjøhl et al. 2011). Ultimately, severe weather, such as heavy rain, may reduce the number of fertile female flowers, thus intensifying male–male competition. Therefore, we hypothesize that among protandrous (male–female) individuals subjected to severe monsoon conditions, those individuals with an extra half cycle of male flowering would have been selected for. However, due to the limited studies on duodichogamous species among angiosperms, it is still difficult in this study to draw any firm conclusions on their underlying evolutionary mechanism. Alternatively, this study suggested that monoecious species under monsoon climate, with the female flowering in the start of wet monsoon season, may be potential candidates of duodichogamy.

Conclusions

This study reveals that *T. sinensis* of Meliaceae exhibit duodichogamous flowering which is the rarest form of dichogamy in angiosperms. That is, we add 5th family and 17th genera that contain duodichogamous species among angiosperms. In this study, we did not investigate natural *T. sinensis* populations. In order to prove male–male competition to promote duodichogamy, it is necessary to determine flowering sequences, pollinator activities, and finally their selective outcome in natural populations with sufficient sample size. This is particularly important because species with duodichogamous flowering systems tend to change their sexual expression among individuals or between years (Luo et al. 2007; de Lima et al. 2016) and fitness is realized through pollinator activities. This study will contribute to finding more duodichogamous species in Meliaceae based on sex combinations within dichasium. If dichogamous systems are comprehensively examined further in the remaining *Toona* species and other taxonomic groups within Meliaceae, such findings will provide critical information needed to disentangle the associated promoting factors. Phylogenetic relationship among the known duodichogamous species and environmental or ecological factors affecting formation of additional male flowers in duodichogamous species are needed to understand the evolution of duodichogamy.

Abbreviations

DBH: Diameter at breast height

Acknowledgements

We especially thank Hyeseon Lee and Jangheum Yun for the assistance with field collections and observations. We also thank the anonymous reviewers for providing helpful comments to this paper.

Funding

This study was supported by 2014 Research Grant from Kangwon National University (No. 12014-0239).

Availability of data and materials

Please contact the corresponding author for data requests.

Authors' contributions

HL conducted the field observation and collection and wrote the manuscript. HK examined the manuscript and provided critical suggestions. WGP is involved in the field observation and collection and examined the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Received: 29 January 2018 Accepted: 23 March 2018
Published online: 27 April 2018

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