JSE Journal of Systematics and Evolution

Journal of Systematics and Evolution 49 (4): 330–338 (2011) doi: 10.1111/j.1759-6831.2011.00125.x

Research Article

Pollination systems, biogeography, and divergence times of three allopatric species of *Schisandra* in North America, China, and Japan

[1,2]Jian-Hua FAN* [3]Leonard B. THIEN [1]Yi-Bo LUO

[1](*State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences,* Beijing 100093, China)
[2](*Graduate University of Chinese Academy of Sciences,* Beijing 100049, China)
[3](*Cell and Molecular Biology Department, Tulane University, New Orleans,* Louisiana 70118, USA)

Abstract This study analyses the pollination systems and biogeography of three allopatric species of *Schisandra* (Section *Euschisandra*) consisting of *S. glabra* (North America), *S. bicolor* (China), and *S. repanda* (Japan); the clade is delimited in a phylogenetic tree of Schisandraceae constructed with nuclear and plastid genes. The male and female flowers of these species have similar floral structures, but exhibit different pollination systems. At the base of the clade, *S. glabra* is pollinated by a wide variety of beetles and flies in a generalist pollination system that also includes floral heat and the use of male and female flowers as brood sites for insects. In Asia, however, *S. bicolor* and *S. repanda* are pollinated exclusively by one or two different species of gall midges (*Resseliella* spp.) in a specialist pollination system. In this system only female, pollen-eating gall midges pollinate the flowers and breed on nearby spiderwebs. The gall midge pollination system is specialized and derived from the generalist system in *S. glabra*, and basal in the clade. Pollen is the main floral resource, and we hypothesize it is exploited to enrich eggs, and as a result species of gall midges could increase reproductive fitness by feeding on a single dependable food source. Subsequently the life cycles of the plants and insects evolved into a tight association in old stable plant communities in the Sino-Japanese flora. Divergence times for the plant species are presented and correlated with past distributions and migration routes.

Key words biogeography, Diptera, phylogeny, pollination, *Resseliella*, *Schisandra*.

The family Schisandraceae (Austrobaileyales), positioned on the third branch of the flowering plant phylogenetic tree, contains three genera: *Kadsura* (22 species), *Schisandra* (25 species), and *Illicium* (42 species) (Smith, 1947; APG, 2009). In Schisandraceae, *Kadsura* and *Schisandra* species have unisexual flowers (Smith, 1947; Saunders, 1998, 2000; Xia et al., 2009), and *Illicium* species bear bisexual flowers (Smith, 1947; Xia & Saunders, 2009). The scandent woody vines of *Kadsura* and *Schisandra* occur in the New World and in Asia; however, only one species, *Schisandra glabra* (Brickell) Rehder occurs in North America, with the bulk of the species occurring throughout Asia, with a center of diversity in China (Smith, 1947; Saunders, 1998, 2000). The shrubs and small trees of *Illicium* species are found in the New World (five species) with the remaining 37 species distributed across southeastern Asia (Smith, 1947).

Traditionally, the shape of the torus, and fruit type were used to distinguish the genera *Kadsura* and *Schisandra* (Smith, 1947; Saunders, 1998, 2000). Phylogenetic trees of Schisandraceae constructed with nucleic acids, however, do not support the traditional relationships constructed with morphological characters. Morphological characters, such as habit (deciduous vs. evergreen or semi-evergreen), fruits with aggregate free berries separated on an elongated receptacle versus closely compressed berries on an ellipsoid or clavate receptacle, arrangement of flowers, and other characters have evolved more than once in Schisandraceae. The only morphological characters that correlate in the morphology-based versus DNA-based phylogenetic trees concern androecial types, some of which show great variation in apical appendages, especially in *Kadsura* (Smith, 1947; Saunders, 1998). The androecium type "B" is found in the male flowers of *Schisandra glabra* (New World), *Schisandra bicolor* W.-C. Cheng (China), and in *Schisandra repanda* (Siebold & Zuccarini) Radlkofer (Japan); two of these species were shown to form a clade (*S. repanda* was not included) in DNA-based phylogenetic trees of Schisandraceae

Received: 9 November 2010 Accepted: 30 December 2010
* Author for correspondence. E-mail: jianhuafan@yahoo.com; Tel.:86-10-62836909; Fax: 86-10-62590843.

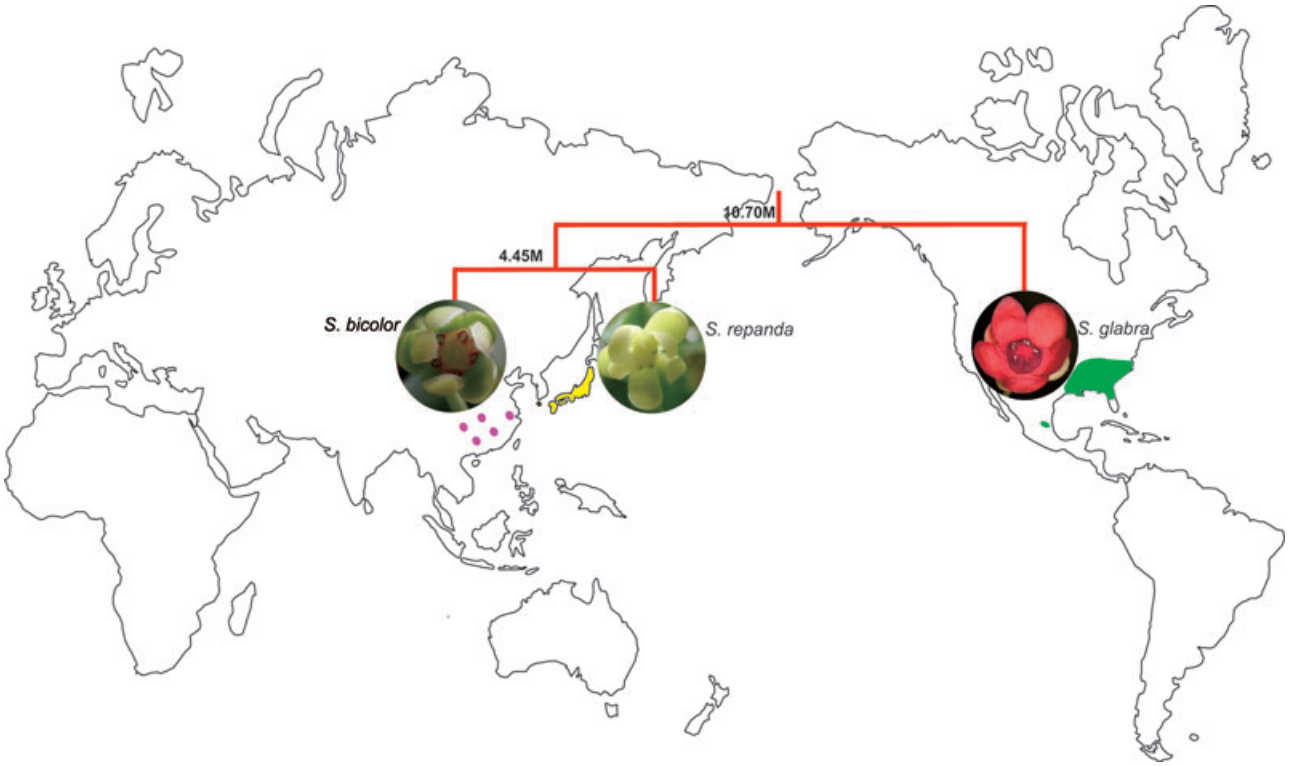

Fig. 1. Morphology and distribution maps of *Schisandra bicolor* (purple shaded areas), *S. repanda*, (yellow), and *S. glabra* (green). The distribution range was modified from Saunders (2000). The numbers on each node indicate the split time inferred from the molecular clock.

(Liu et al., 2006). The androecium type "B" can be viewed as an open upside umbrella with the stamens embedded in the top (Fig. 1).

Recent pollination studies of *Schisandra henryi* Clarke subspecies *henryi* and *Kadsura longipedunculata* Finet & Gagnepain, show both species are pollinated by *Megommata* spp. (Cecidomyiidae: Diptera) in central China (Yuan et al., 2007, 2008). Previous observations of pollination in *S. glabra* in Louisiana (USA) showed a wide variety of insect pollinators including flies and beetles (Liu et al., 2006).

This study presents a molecular phylogenetic study of *Schisandra* and *Kadsura*, and concentrates on a clade containing *S. glabra* (North America), *S. bicolor* (China), and *S. repanda* (Japan). These three species show the same basic floral morphology, yet have two different pollination systems, that is, *S. glabra* a generalist pollination system with a wide variety of insect pollinators, whereas *S. bicolor* and *S. repanda* have a specialist pollination system based on one or two different species of female pollen-eating gall midges, as pollinators. We wanted to determine and/or answer three questions: (i) to determine the molecular relationships of the three *Schisandra* species using nucleic acids; (ii) to determine the divergence times and possible migration routes of the three plant species; and (iii) to compare the different pollination systems in terms of pollinators, fruit set, and evolution of these pollination systems.

1 Material and methods

We sequenced six genomic regions, including nuclear ribosomal internal transcribed spacer (ITS), plastid *matK*, *psbA-trnH*, *rbcL*, *rpl16*, and *trnL-F*, for 21 species of *Schisandra* and *Kadsura*. The outgroups for the plant phylogenetic tree were *Austrobaileya scandens* C.T. White (Austrobaileyaceae) and *Illicium floridanum* Ellis. The program ClustalX 2.012 (Larkin et al., 2007) was used for optimal alignment of *rbcL*, *matK*, *trnG-trnS*, *trnL-F*, *psbA-trnH* spacer, and ITS. We carried out phylogenetic analyses of Schisandraceae using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) methods for each segment separately. Because no strongly supported conflicting topologies were found among molecular datasets, we combined the segments for further analyses.

Parsimony analyses were carried out with PAUP* 4.0b10 (Swofford, 2002); The six plant genes

contained 5829 total characters of which 5055 were constant, yielding 258 informative characters for parsimony analysis. Heuristic searches with 1000 random addition sequence replicates were carried out, using tree−bisection−reconnection (TBR) branch swapping, and MulTrees in effect, with steepest descent off. Gaps were treated as missing data. Bootstrap analyses with 1000 replicates (Felsenstein, 1985) were carried out to estimate internal branch support.

Maximum likelihood was carried out using PHYML 3.0 (Guindon & Gascuel, 2003). Modeltest 3.8 (Posada & Crandall, 2001) was used to determine the best models of sequence evolution for each dataset. For Schisandraceae, the TIM+I+G model was suggested by Akaike information criterion (AIC) as the best-fit model for the combined sequence data among 56 models. Gamma shape parameter = 0.8169 and proportion of invariable sites = 0.5357. Likelihood analysis was carried out in PHYML using a gamma substitution (Yang, 1994) model with invariant sites and additional among site rate variation modeled as a discrete gamma distribution. The ML parameter values were then optimized, with a BIONJ tree as a starting point (Gascuel, 1997) with the appropriate parameters. Nodal robustness on the ML tree was estimated by the non-parametric bootstrap with 1000 replicates.

For Bayesian analyses (Ronquist & Huelsenbeck, 2003) of Schisandraceae, the data were divided into six partitions (*matK*, *psbA-trnH* spacer, *rbcL*, *rpl16*, *trnL-F*, and ITS) to improve the fit of the substitution mode to the data. We used AIC as implemented in Modeltest 3.8 to determine the appropriate model of evolution for each partition (TVM+G, K81uf+G, HKY+I+G, K81uf+G, TIM+G, and GTR+G). Bayesian analyses of the partitioned data were carried out using Metropolis coupled Markov Chain Monte Carlo as implemented in MrBayes version 3.1.2. We ran four chains of the Markov Chain Monte Carlo, sampling one tree every 100 generations for 1000000 generations commencing with a random tree. The number of generations needed to reach the stationary phase was determined by plotting likelihood scores against generations.

Species determination of members of *Megommata*, is based on morphological features of males including genitalia, antennal segments, and head capsular characters (Harris, 1967). However, some males were captured on the flowers of *Kadsura longipedunculata* attempting to mate with females (they normally breed on the edge of old spiderwebs). Thus, the female gall midges that pollinate the flowers of *Kadsura* and *Schisandra* in China and Japan have been identified as members of the genus *Resseliella* and are probably new species. Apparently adult members of *Megommata* and *Resseliella* are similar in appearance and size.

To determine species of gall midge pollinators, the mitochondrial *CO1* gene (428 bp) was sequenced in five female individuals selected at random from the flowers of five plants (different populations) for *S. repanda* and *S. bicolor*. The primers used were C1-J-1718 5′-GGAGGATTTGGAAATTGATTAGTTCC-3′ and C1-N-2191 5′-CCCGGTAAAATTAAAATATAAACTTC-3′. We then assigned a number to each putative species based upon the DNA sequences. Hebert et al. (2003) suggested that segments of the *CO1* gene were diagnostic at species-level in animal phyla. All specimens of plants and animals mentioned in this study are stored at the PE (Herbarium, Institute of Botany, Chinese Academy of Sciences, Beijing, China).

1.1 Fossil calibration and divergence time estimation

The earliest pollen fossils of Schisandraceae, from the western San Joaquin Valley, California, USA dated to ca. 65.5 Mya (Chmura, 1973) and seed fossils of *Schisandra oregonensis* S.R. Manchester were used to calibrate points for divergence time estimations. *Schisandra oregonensis* was described from seeds found in the Clarno Formation of Oregon with an estimated age of 43.76 Mya (Manchester, 1994). To place the fossil *S. oregonensis* in the phylogenetic tree with modern taxa, we scored a total of 13 morphological characters from seed structures for 13 *Schisandra* and seven *Kadsura* species. The coding of these characters was according to Denk & Oh (2006). Taxa were coded as polymorphic when more than one character state was observed for a character. Unknown character states of fossil species were scored as missing. These 13 characters were added to the molecular matrix for further analysis. We carried out MP analyses on the combined molecular and morphological data, heuristic searches with 1000 random addition sequence replicates were carried out, using TBR branch swapping, and MulTrees in effect, with steepest descent off. Bootstrap analyses with 1000 replicates (Felsenstein, 1985) were carried out to estimate internal branch support. The position of *S. oregonensis* was resolved; it is sister to clade I (*Schisandra* clade) with 60% bootstrap support (Fig. 2). The root node (*Illicium* + *Schisandra* + *Kadsura* + *Austrobaileya*) was constrained to a maximum of 132 Mya, the earliest-known occurrence of angiosperm pollen in the fossil record (Brenner, 1996), and to a minimum of 65.5 Mya, the earliest fossil of *Schisandra* and *Kadsura*. The most logical position for *S. oregonensis* is the node between clade I and clade II, along the stem lineage to clade I.

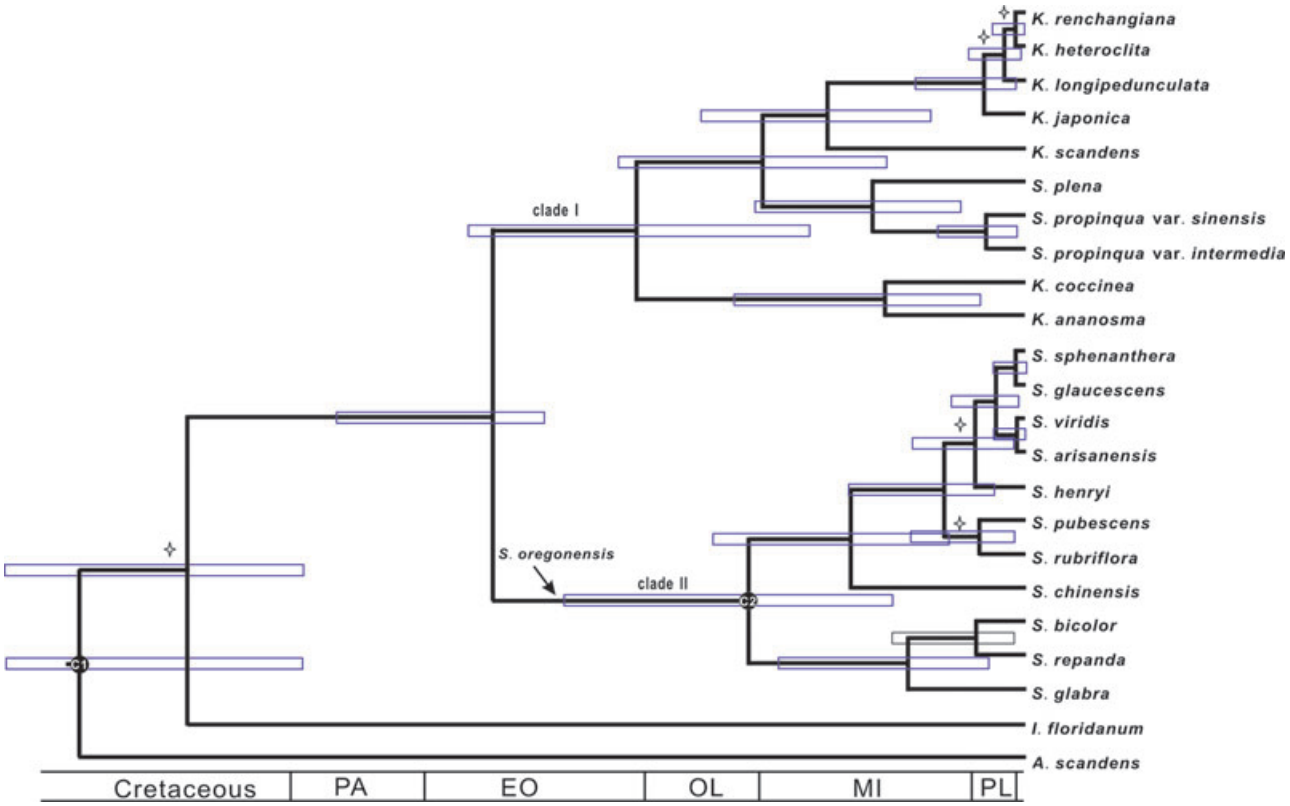

Fig. 2. Chronogram based on BEAST analysis of combined internal transcribed spacer (ITS), *rbcL*, *trnL-F*, *matK*, *psbA-trnH*, and *rpl16* data for *Kadsura*, *Schisandra*, *Illicium*, and *Austrobaileya* using three fossil constraints. C1, maximum constraint of 132 Mya and minimum constraint of 65.5 Mya; C2, maximum constraint of 65.5 Mya and minimum constraint of 43.76 Mya. EO, Eocene; MI, Miocene; OL, Oligocene; PA, Paleocene; PL, Pliocene. Stars indicate nodes with Bayesian inference <95%, maximum likelihood <70, and maximum parsimony <70%.

Currently it is not feasible to place *S. oregonensis* along the clade I stem lineage, and we placed it at the node of clade I to limit the maximum age of the crown group of clade I.

1.2 Pollination

Field studies of *S. reppanda* were carried out in two populations at Mountain Rokko (N 34°45′ E 135°13′), Hyogo Prefecture of Japan, and three populations of *S. bicolor* on Mountain Ziyun (N 26°21′ E 110°45′), Hunan Province of China, from 2005 to 2007. Only two sites were chosen for field observation because large natural populations of these two species in the wild are rare.

1.2.1 Phenology The flowering period of *S. bicolor* was recorded using 100 flowers on 10 plants (50 males and 50 females) in populations I, II, and III, all of which were located at a mixed coniferous broad-leaved forest at ca. 1000 m. Population II was located ca. 2 km southeast of population I, and population III was located ca. 1.5 km northeast of population I. For *S. repanda*, 50 flowers on five plants (25 males and 25 females) in populations I and II, both of which were located in evergreen broad-leaved forest with a distance of ca. 2.5 km. The flowers were observed with a 10× hand lens, and the following traits were recorded for both species: tepal movement; presence or absence of floral odor; time of stigma and stamen secretions (if present); and anther dehiscence. Stigmas were considered to be receptive if they displayed a white, glassy, and moist appearance. The number of flowers opening daily on five plants were selected at random in the population and recorded throughout the flowering season.

1.2.2 Floral thermogenesis and odor In *S. bicolor* and *S. repanda*, floral and ambient temperatures were measured using a Teflon-coated contact sensor (1.2 mm diameter, 0.6 m length), connected to a portable battery powered TR-52 Thermo Recorder (accurate to ±0.1°C; T&D, Matsumoto, Japan). The sensor was inserted between the inner tepals and the base of the torus to test the floral temperature, and the ambient temperature was measured by placing the sensor in the air, ca. 1 cm

from the flower. Floral and ambient temperatures were recorded every 30 min for both male and female flowers throughout their lifespan. Excess temperature for each flower was calculated as the difference between flower and ambient temperature. In *S. bicolor*, five male flowers and five females from five plants of two populations were selected at random; in *S. repanda*, five male flowers and five females from four plants of two populations were selected randomly; mean excess flower temperature during this period was calculated and graphed. Presence or absence of floral scent was determined by smelling the flowers.

1.2.3 Insect pollination Insects visiting the flowers of *S. bicolor* and *S. repanda* and their behaviors were recorded throughout the day and night. The former species were observed more than 200 h and the latter more than 100 h. In addition, two plants of each population were selected for continuous observation of flowers for 3 days. The frequency of insect visits, as well as the length of time they remained in the flower, was recorded. In addition, insects were collected using jars containing ethyl acetate. Vouchers were deposited at the herbarium of Institute of Botany, Chinese Academy of Sciences, Beijing, China.

1.2.4 Wind pollination To test whether the pollen of *S. bicolor* and *S. repanda* is dispersed by wind, one individual plant for each species was chosen and microscope slides covered with petroleum jelly were placed around this plant at 1 m intervals, 1–2 m above the ground, for 10 m. The slides were removed after two days, and the pollen grains were counted using a compound light microscope.

1.2.5 Hand pollinations Fruit set in plants of *S. bicolor* was analyzed in populations I and II, with reference to cross- and self-pollinations. The experiments were carried out on sunny days from June 8 to 11, 2006. Nylon mesh bags were used to cover 100 female and male flower buds in populations I and II ($n =$ 5 plants). Four different controlled-pollination treatments were carried out to determine the breeding system: (i) to test xenogamy, 60 previously bagged flowers were artificially pollinated with pollen from flowers of a different individual located 50–500 m from the recipient experimental plants previously bagged; (ii) to test autogamy, 20 female flowers were bagged but not artificially pollinated; and (iii) 20 control flowers were left unbagged to be freely pollinated. Fruits were examined early in September and the number of fruiting carpels per flower was recorded. The average percentage fruit set of the various crosses and the control were calculated. In *S. repanda*, 20 female flowers were tested for autogamy (bagged) but not artificially pollinated.

2 Results

The family Schisandraceae is monophyletic and well identified with 100% bootstrap support, however, neither *Schisandra* nor *Kadsura* is monophyletic as *S. propinqua* A.C. Smith and *S. plena* (Wallich) Baillon are nested in the *Kadsura* clade (Fig. 2). In *Schisandra*, three species consisting of *S. glabra*, *S. bicolor*, and *S. repanda* form a clade with 100% bootstrap support in which *S. bicolor* and *S. repanda* are sister species pairs with 99% bootstrap support and *S. glabra* is sister to the clade comprised of *S. repanda* and *S. bicolor*.

The age of the crown group of Schisandraceae is estimated to be 48.3 (62.4–43.8) Mya, and the crown group of clade I approximately 25.2 (41.9–12.2) Mya, 10.1 Mya younger than clade II. The clade containing *S. glabra*, *S. bicolor*, and *S. repanda* divided from its sister clade 25.2 Mya. *Schisandra glabra* diverged from two Asian species pairs at ca. 10.7 (22.4–3.3) Mya. The Chinese species *S. bicolor* separated from its sister species *S. repanda* approximately 4.5 (12.1–1.0) Mya.

2.1 Floral structure and flowering

The flowers of *S. glabra*, *S. bicolor*, and *S. repanda* differ in color but are similar in structure, with male flowers all possessing the same "B" type androecium (Fig. 1). Table 1 shows the main character differences of the three species in the clade (Sect. *Euschisandra)*. The three species occur at the same altitudes in the various geographical areas in the Sino-Japanese flora and in North America. Based on our field observations, flower color is variable at the infra-species level and fruit color varies. The tepals of the various species, in both male and female flowers, are light green and some are partially red spotted or entirely red, whereas androecia vary from red to light green. The female flowers are obviously larger than male flowers and have thicker inner tepals. The flowers of *S. repanda* produce yellowish flowers, and tepals in both males and females are similar. The color of androecia is always yellowish (Fig. 1, Table 1).

Schisandra repanda started flowering on 24 June, 2007 and finished in one week. The plants of *S. repanda* are dioecious with male and female flowers opening at ca. 19:00; the male flowers are completely open at 04:00 the following day, 1–3 h earlier than female flowers. All flowers are pendulous. Tepals of male flowers drop with the androecium in the morning of the third day; unpollinated female flowers drop 2–3 days later. In population I there were four individuals that did not produce any flowers in 2007. The male flowers in population I were more abundant than female flowers. The torus (receptacle) of male flowers of *S. repanda* usually produces small liquid droplets as do the tepals of female flowers.

Table 1 Comparisons of living habits, morphological characters, and pollinators among *Schisandra glabra*, *S. bicolor*, and *S. repanda*

Taxon	Distribution	Habit	Altitude (m)	Sexuality	Deciduous/ evergreen	Flower color	Fruit color	Chromosome number	Pollinators	Reference
S. glabra	USA and Mexico	Stream, steep slope	500–1000	Monoecious	Deciduous	Red	Red	$n = 13$,	Diptera, Coleoptera	Saunders, 2000; Liu et al., 2006
S. bicolor	China	Sparse woodlands	750–1300	Monoecious	Deciduous	Red to greenish	Black to purple	$2n = 28$	Cecidomyiidae, sp. 1 and sp. 2	Saunders, 2000; This study
S. repanda	Japan and Korea	Dense forest	600–1300	Dioecious	Deciduous	Yellowish	Blackish	$2n = 28$	Cecidomyiidae, sp. 3	Saunders, 2000; 2000;This study

Note: Sexuality, flower color, and pollinators of *S. bicolor* and *S. repanda* are from this study.

In *S. bicolor*, the first flower opened on 29 June, 2006. Flowers are unisexual and all individuals are monoecious. Both male and female flowers start to open at 17:00–18:30 and are completely open at ca. 19:30; all flowers are pendulous. Tepals of male flowers drop with the androecia during the next 1–2 days. Female flowers that were not pollinated drop throughout the third day. No liquid droplets were produced by the tepals or torus of the flowers of *S. bicolor*.

The male and female flowers of *S. repanda* produce a weak, sweet odor shortly after the tepals unfolded. The fragrance continued throughout the lifespan of the flower. In *S. bicolor*, a strong sweet odor was emitted just after the male and female flowers opened and from 19:00 to 22:30 the floral odor gradually increased and then decreased after 22:30; no odor could be detected after 06:00 the following day. Female flowers resumed fragrance production at the same time on the second night but only if the tepals were still attached (the torus and tepals produce the odor).

The flowers of *S. bicolor* and *S. repanda* do not produce heat. During the lifespan of the flowers, no significant differences were detected between the ambient temperature and the temperature of male and female flowers.

2.2 Insect pollination

In northern Louisiana, *S. glabra* (plants can be monoecious or dioecious, depending upon the year) is pollinated by a wide variety of insects including beetles (Chrysomelidae, Helodidae, and Anthribidae), Diptera (Chironomidae and Ceratopogonidae) in a generalist pollination system that also includes floral thermogensis and brood sites on female and male flowers (LB Thien, pers. obs.).

In Asia, however, the pollination systems of *S. repanda* (Japan) and *S. bicolor* (China) are both pollinated exclusively by gall midges in the genus *Resseliella*. The flowers of *S. bicolor* are pollinated by two species of *Resseliella* designated sp. 1 and sp. 2 and *S. repanda* by sp. 3 (Table 1). In this specialized pollination system, only female pollen-eating *Resseliella* species pollinate the flowers and breed on spiderwebs located near the *Schisandra* plants. As *S. glabra* occurs at the base of the three species clade (Fig. 2), the gall midge pollination system is specialized and derived from a generalized system (beetle and fly pollinated) in *S. glabra*. Gall midges are the most frequently collected insects on the flowers and leaves of *S. repanda* and *S. bicolor*. We seldom found flies active during the day on *S. repanda* and *S. bicolor*.

During the anthesis of *S. repanda*, only flies and gall midges visit flowers and leaves. A few flies visit both male and female flowers from 10:00 to 16:00. The flies usually suck the liquid droplets emitted from the torus of male flowers and tepals of female flowers. The flies usually walk on the torus of male flowers and tepals of female flowers and eat the tiny secreted droplets. We seldom observed flies on the carpels, as they usually stayed inside the flowers for only a few seconds and thus are not considered pollinators, but liquid thieves.

The activities of gall midges on *S. repanda* are quite different from that of the flies. The first gall midges arrived at ca. 04:00 in male flowers. From 04:00 to 12:00 the number of gall midges gradually increased, but after 12:00 few gall midges were found. The first gall midges found in female flowers arrived at ca. 07:00 and left at ca. 12:00. Gall midges usually land on the leaves first then fly or quickly crawl to the inside of flowers. Upon entering the flowers they actively walk on the tepals, torus, and carpels. After a few minutes, they become less active and probe the open anthers of and eat pollen. In the process, pollen adheres to all parts of the gall midges. Some gall midges stay for a few seconds, but others up to 30 min.

In *S. bicolor*, gall midges visited both male and female flowers during a short period from 18:00 to 23:00, which correlates with peak fragrance production. At ca. 22:30 the gall midges began to leave and after 23:00 few gall midges were found inside flowers. Gall midges were not found during the day. Flies walk on the androecia, carpels, and tepals. The flies emerge from 08:00 to 14:00, walk on the torus, and quickly leave. During this period, the anthers are usually empty. No adult male gall midges were observed or collected on flowers of

S. repanda or *S. bicolor*. The pollination regime described for *S. repanda* and *S. bicolor* correlate with the descriptions of pollination of *K. longipedunculata* and *S. henryi* by *Megommata* sp. (now *Resseliella*) in which hundreds of female gall midges swarm over the flowers and were shown to be efficient pollinators.

2.3 Wind pollination

Glass microscope slides (14 cm^2) coated with petroleum jelly for pollen traps captured an average of 0.20 pollen grains of *S. bicolor* and 0.13 of *S. repanda* at a distance of 0.5 m from the source. Females of *S. repanda* and *S. bicolor* are all pendulous and carpels are enclosed by the thick inner tepals. Wind pollination plays no role in either *S. repanda* or *S. bicolor*.

3 Discussion

The order Austrobaileyales, comprised of Austrobaileyaceae, Trimeniaceae, and Schisandraceae are pollinated by a variety of flies and beetles (Endress, 2001; Bernhardt et al., 2003; Thien et al., 2009). Luo et al. (2010) hypothesized that early species of Schisandraceae were pollinated by flies and/or beetles, and that gall midge pollination systems originated several times in the family. Gall midges (*Clinodiplosis* spp.) pollinate *Illicium dunnianum* and *I. tsangii* in China in which the flowers produce floral heat and also function as a brood site for the insects (Luo et al., 2010). In this scenario, floral heat enhances floral odor to attract pollinators (Luo et al., 2010). In another gall midge pollination system in China and Japan, species of *Kadsura* and *Schisandra* are pollinated by female pollen-eating gall midges (*Resseliella* spp.) (Yuan et al., 2007, 2008). In the New World, *S. glabra* exhibits a generalist system of pollination based on flies and small beetles that also includes species of gall midges (Liu et al., 2006), whereas *S. bicolor* in China and *S. repanda* in Japan are pollinated by one or two different species of gall midges in a specialized pollination system (a pollination switch within the clade); yet all three species exhibit the same type of androecial structure (type "B") and female floral structure.

One species of *Schisandra* is always pollinated by at most two gall midges species, probably caused by differences in the life cycles of the gall midges. Pollen was initially exploited to enrich eggs when gall midges leave spiderwebs after mating. Feeding on a single dependable food source would be more helpful for gall midges to survive. Different gall midges are active at different periods to limit pollen for the specific gall midge species and to deter the attraction of other insects. In addition, gall midges become efficient pollinators when the life cycle of the plants and insects evolved into a tight association in old stable plant communities in the Sino-Japanese flora.

Both Schisandraceae and Cecidomyiidae were present in the Cretaceous (Grimaldi & Engel, 2005). As pollen-eating gall midges are rare in extant Cecidomyiidae and their occurrence in old relictual forest communities, the current *Schisandra* pollination system is probably a remnant of a once more widespread system. Pollination studies on more species of *Kadsura*, *Schisandra*, and *Illicium* occurring in the New World and the Sino-Himalayan region would be helpful to clarify pollination systems in the Austrobaileyales.

Palynological data indicate Schisandraceae species were widely distributed in the northern hemisphere until the Pliocene (Saunders, 1998, 2000). The disappearance of species of Schisandraceae in western North America and Europe was probably caused by drying climates in the Miocene and Pliocene and the Quaternary glaciations (Tiffney, 1985; Tiffney & Manchester, 2001). China was also affected by global climatic change in the later Tertiary and Quaternary, however, the east–west orientation of the mountains created different ecological habitats and protected it from glaciations (Hsu, 1983).

Saunders (2000) strongly suggests the extant *Schisandra* species with North American–east Asian disjunction distribution resulted from contraction of the range of the species. He maintains that distribution contractions and extinctions were main factors, but not long-range dispersal events. The split between North American (*S. glabra*) and East Asian (*S. bicolor* and S. *repanda*) species ranges from 3.3 to 22.4 Mya, and this date is high in comparison with similar Asian species in morphology and habits. The ancestors of these three species are supposed to have been widely distributed in North America and East Asia, with the Bering Land Bridge a possible migration route. Paratropical taxa *Illicium*, fossils of which are known from southern Alaska (Wolfe, 1977), share the overlapped distribution pattern and habits with those of *Schisandra* and *Kadsura*, furthering the suggestion that the Bering Land Bridge also served as the immigration route for *Schisandra* and *Kadsura* in the Miocene. The split of this disjunction was possibly caused by the unviability of the Bering Land Bridge from the late Miocene when the climate become cooler (Tiffney & Manchester, 2001).

In Asia, *S. repanda* separated from *S. bicolor* in the Pliocene. The paleomagnetic evidence indicates that Japan separated from the eastern Eurasian continent ca. 15 Mya, then separated into the northeast and southwest Japan arcs (Otofuji et al., 1991, 1994). The

proto-Japanese Islands became an archipelago driven by drastic submergence in the following period. The early formation of the Japanese Islands occurred 5 Mya. The Japanese Sea between the Asian continent and the Japanese Islands has probably provided an adequate barrier to gene flow between previously widespread ancestors of modern *S. repanda* and *S. bicolor*.

Acknowledgements This work was supported by the Ministry of Science and Technology of China (Grant No. 2007CBI411600) and the National Natural Science Foundation of China (Grant No. NSFC30570106). The authors thank Mr. Zhongchun LUO, Dr. Baoqing REN, Prof. Junichi YUKAWA, and Dr. Shinsuke SATO for their kind support in fieldwork, and the Forestry Department of Xinning County, Hunan Province, China for kindly permitting us to enter the study sites. The authors also thank Dr. Richard M. K. SAUNDERS for providing the material used in this study and John GWALTNEY for permission to use the photograph of *Schisandra glabra*.

References

APG. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Botanical Journal of the Linnean Society 161: 105–121.

Bernhardt P, Sage T, Weston P, Azuma H, Lam M, Thien LB, Bruhl J. 2003. The pollination of *Trimenia moorei* (Trimeniaceae): Floral volatiles, insect/wind pollen vectors and stigmatic self-incompatibility in a basal angiosperm. Annals of Botany 92: 445–458.

Brenner GJ. 1996. Evidence for the earliest stage of angiosperm pollen evolution: A paleoequatorial section from Israel. In: Taylor DW, Hickey L eds. Angiosperm origin, evolution, and phylogeny. London: Springer. 91–115.

Chmura CA. 1973. Upper Cretaceous (Campanian–Maastrichtian) angiosperm pollen from the Western San Joaquin Valley, California, USA. Palaeontographica Abteilung B 141: 89–171.

Denk T, Oh IC. 2006. Phylogeny of Schisandraceae based on morphological data: Evidence from modern plants and the fossil record. Plant Systematics and Evolution 256: 113–145.

Endress PK. 2001. The flowers in extant basal angiosperms and inferences on ancestral flowers. International Journal of Plant Sciences 162: 1111–1140.

Felsenstein J. 1985. Confidence-limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791.

Gascuel O. 1997. BIONJ: An improved version of the NJ algorithm based on a simple model of sequence data. Molecular Biology and Evolution 14: 685–695.

Grimaldi D, Engel MS. 2005. Evolution of the insects. New York: Cambridge University Press. 755.

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52: 696–704.

Harris KM. 1967. A systematic revision and biological review of the cecidomyiid predators (Diptera: Cecidomyiidae) on world Coccoidea (Hemiptera–Homoptera). Transactions of the Royal Entomological Society of London 119: 401–488.

Hebert PDN, Ratnasingham S, de Waard JR. 2003. Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society Series B: Biological Sciences 270: S96–S99.

Hsu J. 1983. Late Cretaceous and Cenozoic vegetation in China, emphasizing their connections with North America. Annals of the Missouri Botanical Garden 70: 490–508.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007. ClustalW and ClustalX version 2.0. Bioinformatics 23: 2947–2948.

Liu Z, Hao G, Luo Y-B, Thien LB, Rosso SW, Lu A-M, Chen Z-D. 2006. Phylogeny and androecial evolution in Schisandraceae, inferred from sequences of nuclear ribosomal DNA ITS and chloroplast DNA *trn*L-F regions. International Journal of Plant Sciences 167: 539–550.

Luo S-X, Chaw S-M, Zhang D, Renner S. 2010. Flower heating following anthesis and the evolution of gall midge pollination in Schisandraceae. American Journal of Botany 97: 1220–1228.

Manchester SR. 1994. Fruits and seeds of the Middle Eocene Nut Beds Flora, Clarno Formation, Oregon. Palaeontographica Americana 1–205.

Otofuji Y, Itaya T, Matsuda T. 1991. Rapid rotation of southwest Japan – paleomagnetism and K-Ar age of Miocene volcanic rocks of southwest Japan. Geophysical Journal International 105: 397–405.

Otofuji Y, Kambara A, Matsuda T, Nohada S. 1994. Counterclockwise rotation of northwest Japan: Paleomagnetic evidence for regional extent and timing of rotation. Earth Planet Science Letters 121: 503–518.

Posada D, Crandall KA. 2001. Selecting the best-fit model of nucleotide substitution. Systematic Biology 50: 580–601.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

Saunders RMK. 1998. Monograph of *Kadsura* (Schisandraceae). Systematic Botany Monographs 54: 1–106.

Saunders RMK. 2000. Monograph of *Schisandra* (Schisandraceae). Systematic Botany Monographs 58: 1–146.

Smith AC. 1947. The families Illiciaceae and Schisandraceae. Sargentia 7: 1–224.

Swofford DL. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0 b10. Sunderland: Sinauer Associates.

Thien LB, Bernhardt P, Devall MS, Chen Z-D, Luo Y-B, Fan J-H, Yuan L-C, Williams JH. 2009. Pollination biology of basal angiosperms (ANITA grade). American Journal of Botany 96: 166–182.

Tiffney BH. 1985. The Eocene north Atlantic land bridge: Its importance in Tertiary and modern phytogeography of the northern hemisphere. Journal of the Arnold Arboretum 66: 243–273.

Tiffney BH, Manchester SR. 2001. The use of geological and paleontological evidence in evaluating plant phylogeographic hypotheses in the Northern Hemisphere tertiary. International Journal of Plant Sciences 162: S3–S17.

Wolfe JA. 1977. Paleogene floras from the Gulf of Alaska region. United States Geological Survey Professional Paper 997: 1–108.

Xia N-H, Saunders RMK. 2009. Illiciaceae. In: Wu ZY, Raven PH eds. Floral of China. Beijing: Science Press; St. Louis: Missouri Botanical Garden Press. 7: 32–38.

Xia N-H, Liu Y-H, Saunders RMK. 2009. Schisandraceae. In: Wu ZY, Raven PH eds. Floral of China. Beijing: Science Press; St. Louis: Missouri Botanical Garden Press. 7: 39–47.

Yang Z-H. 1994. Maximum-likelihood phylogenetic estimation from DNA sequences with variable rates over sites: Approximate methods. Journal of Molecular Evolution 39: 306–314.

Yuan L-C, Luo Y-B, Thien LB, Fan J-H, Xu H-L, Chen Z-D. 2007. Pollination of *Schisandra henryi* (Schisandraceae) by female, pollen-eating *Megommata* species (Cecidomyiidae, Diptera) in south-central China. Annals of Botany 99: 451–460.

Yuan L-C, Luo Y-B, Thien LB, Fan J-H, Xu H-L, Yukawa J, Chen Z-D. 2008. Pollination of *Kadsura longipedunculata* (Schisandraceae), a monoecious basal angiosperm, by female, pollen-eating *Megommata* sp. (Cecidomyiidae: Diptera) in China. Biological Journal of the Linnean Society 93: 523–536.