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RESEARCH ARTICLE

Adaptive function of duodichogamy: Why do chestnut trees have two pollen emission phases?

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Abstract

Premise: Intersexual mating facilitation in flowering plants has been largely underexplored. Duodichogamy is a rare flowering system in which individual plants flower in the sequence male-female-male. We studied the adaptive advantages of this flowering system using chestnuts (*Castanea* spp., Fagaceae) as models. These insect-pollinated trees produce many unisexual male catkins responsible for a first staminate phase and a few bisexual catkins responsible for a second staminate phase. We hypothesized that duodichogamy increases female mating success by facilitating pollen deposition on stigmas of the rewardless female flowers through their proximity with attractive male flowers responsible for the minor staminate phase.

Methods: We monitored insect visits to 11 chestnut trees during the entire flowering period and explored reproductive traits of all known duodichogamous species using published evidence.

Results: In chestnuts, insects visited trees more frequently during the first staminate phase but visited female flowers more frequently during the second staminate phase. All 21 animal-pollinated duodichogamous species identified are mass-flowering woody plants at high risk of self-pollination. In 20 of 21 cases, gynoecia (female flower parts) are located close to androecia (male flower parts), typically those responsible for the second minor staminate phase, whereas androecia are often distant from gynoecia.

Conclusions: Our results suggest that duodichogamy increases female mating success by facilitating pollen deposition on stigmas by means of the attractiveness of the associated male flowers while effectively limiting self-pollination.

KEYWORDS

calyptrate flies, *Castanea*, Fagaceae, female mating success, geitonogamy, insect pollination, mating facilitation, monoecious, self-pollination, soldier beetle

The idea that the combination of male and female organs within hermaphrodite flowers facilitates female mating is not new (Grant, 1951; Baker and Hurd, 1968; Bawa and Beach, 1981; Bertin, 1982; Charlesworth, 2006). As pointed out by Lloyd (1982), if pollen is the only reward to pollinators, then sex association (i.e., the close grouping of androecia and gynoecia in flowers or in inflorescences) will be critical to female mating success. The close proximity of pistils to stamens in space as well as in time might be advantageous in securing enough pollen brought by pollinators to stigmas, but it will come at the cost of increased risk of self-pollination. Self-pollination can be highly detrimental to female mating success for at least three reasons. First, self-pollen can clog stigmas (Bawa and Opler, 1975; Bawa, 1980). Second, self-pollen tubes can disable ovules (Barrett, 2002; Aizen and Harder, 2007; Duffy et al., 2013; Larue et al., 2022). Third, self-fertilization can usurp ovules by producing selfed offspring that suffer from inbreeding depression. Hence, animal-pollinated outcrossing plants have evolved strategies that minimize self-pollination caused by the close spatiotemporal coupling of sexual organs. The strategies that limit self-pollination

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include spatial separation (herkogamy, monoecy, or dioecy) and temporal separation (dichogamy) (Baker and Hurd, 1968; Lloyd and Webb, 1986). However, even a combination of these strategies might not suffice to completely eliminate self-pollination in large mass-flowering plants such as trees or shrubs (Petit and Hampe, 2006; Harder and Prusinkiewicz, 2013; Wang et al., 2020).

Strategies of temporal separation of pollen emission and receipt to limit self-pollination in cosexual plants include protandry (male-female flowering sequence) and protogyny (female-male flowering sequence). However, other more complex dichogamous systems exist (Lloyd and Webb, 1986). One of the most intriguing of these systems was called duodichogamy by Stout (1928), following his work on chestnuts (genus *Castanea*). In this very rare flowering system, individual plants have a synchronized one-and-a-half flowering sequence, with two distinct phases of pollen emission, separated by a female phase of pollen receipt (male-female-male flowering sequence; Luo et al., 2007; Lee et al., 2018). To ensure effective cross-fertilization, all flowers of a plant must bloom synchronously but the individuals in the population must flower asynchronously (Luo et al., 2007). The adaptive value of this strategy, characterized by an additional pollen emission phase, is not well established. Based on their knowledge of dichogamy and on observations of a few duodichogamous species, Lloyd and Webb (1986) tentatively proposed a “male strategy” hypothesis for the origin of duodichogamy. Citing Bateman (1948), they recall that “maternal fitness tends to be limited by the quality (average fitness) of offspring and paternal fitness by their quantity.” They argue that under strong inter-male competition, adaptations that increase male fecundity should be selected for, even if they come at the cost of increased self-pollination. One such male strategy would be the production of another staminate phase extending the duration of pollen presentation, thereby increasing male fitness (Lloyd and Webb, 1986). Luo et al. (2007) also support this model, pointing out that most duodichogamous species tend to have high pollen-ovule ratios. Duodichogamy would thus appear to be a male strategy.

The idea that duodichogamy evolved to increase male fitness seems, at first glance, quite logical (Lloyd and Webb, 1986; Luo et al., 2007). However, it neglects the fact that, in animal-pollinated plants, increased maleness will generally increase attractiveness to pollinators and could indirectly facilitate female fitness by bringing pollinators close to stigmas through the physical coupling of sexual organs. In many plant species, including in most families of basal angiosperms, pollen, a protein-rich resource, is the primary insect reward (Baker and Hurd, 1968; van der Pijl, 1978; Bernhardt and Thien, 1987). Hence, the association of stamens and pistil should enhance pollen deposition on stigmas by pollinators attracted by pollen (Baker and Hurd, 1968; Duffy and Johnson, 2011). Actually, sexual selection should favor male attractiveness regardless of the reward involved because male fitness often continues to increase with additional pollinator visits, whereas female fitness is quickly saturated, given that a few pollinator visits generally suffice to fertilize all ovules (Bell, 1985; Delph et al., 1996; Carlson and Harms, 2006; Huang et al., 2006; Paterno et al., 2020). Female fitness could passively benefit from male attractiveness as a result of the spatial association of gynoecia with androecia. This association needs to be studied at the flower scale and at the inflorescence scale, because either flowers or inflorescences can be the relevant pollinator attraction units in flowering plants (Baker and Hurd, 1968; Bawa and Beach, 1981). Studies of andromonoecious plants, characterized by hermaphrodite and male flowers, illustrate this point. Although considered a male strategy, andromonoecy has been shown to increase female fitness in some plant species because hermaphrodite flowers surrounded by male flowers receive more pollen than hermaphrodite flowers not surrounded by male flowers (Bertin, 1982; Podolsky, 1992; Vallejo-Marín and Rausher, 2007; Tomaszewski et al., 2018; but see Schlessman et al., 2004).

Focusing on animal-pollinated species, we used chestnuts (Fagaceae: *Castanea* spp.)—large trees that are widely distributed in the Northern Hemisphere, with numerous unisexual male catkins that flower first and sparser bisexual inflorescences that flower later—as model species to explore the potential advantage of duodichogamy for female fitness. Compared to Lloyd and Webb's (1986) “male hypothesis,” our “female hypothesis” states that in duodichogamous species, the existence of two staminate phases accrues benefits mostly to female fitness. We tested this hypothesis by estimating the number of insect visits to female flowers (a proxy for female mating success) during each of the two staminate phases. We predicted that insect visits to female flowers will increase during the second, smaller staminate phase, due to spatial proximity of the corresponding male catkin with the female flowers, thereby ensuring pollen deposition while minimizing self-pollination. We then investigated sex association in all animal-pollinated duodichogamous species identified to date. We predicted that sexes would be asymmetrically coupled, with gynoecia typically located close to attractive androecia, whereas androecia would not necessarily be located close to gynoecia. We also predicted that self-pollination would be minimized through preferential association of gynoecia with androecia responsible for the minor staminate phase. To test these predictions, we relied on published descriptions of duodichogamous species.

MATERIALS AND METHODS

Study species

Castanea is a monoecious insect-pollinated genus of trees with numerous unisexual (male) inflorescences and ~30 times fewer bisexual inflorescences (Petit and Larue, 2022; Figure 1). Pollen production is massive. Combined with the

FIGURE 1 Chestnut (*Castanea* spp.) bisexual inflorescences. For each of the two bisexual inflorescences visible in the picture, there is a group of three female flowers at the base (with white styles surrounded by green bracts that will produce the spiny bur) and a long male catkin with numerous white stamens. Notice the red soldier beetle (*Rhagonycha fulva*) on the lower bisexual inflorescence.

tiny size of pollen grains (i.e., 10 times smaller than those of wind-dispersed oaks), this results in an average pollen-ovule ratio for chestnuts of about 20 million, a record for angiosperms (Larue et al., 2021a). The flowering sequence of chestnuts is male-female-male, with significant overlap between phases (Hasegawa et al., 2017). The first staminate phase corresponds to the flowering of purely male inflorescences (unisexual catkins). In male-fertile (i.e., monoecious) trees, it is responsible for ~97% of pollen production (Larue et al., 2021a). The next phase corresponds to the receptivity of the rewardless pistillate flowers. It is followed by the second staminate phase, which corresponds to the anthesis of the male flowers from the few scattered bisexual inflorescences; this staminate phase is responsible for only 3% of pollen production, but this pollen is as fertile as that produced by unisexual inflorescences (Silva et al., 2020). Interestingly, in European chestnut (*Castanea sativa*), individuals showing various degrees of male sterility coexist with monoecious individuals (Larue and Petit, 2022 [preprint]; Larue et al., 2022). In these male-sterile (female) individuals, the sterile male catkins still produce nectar, thereby continuing to play a role in insect attraction (Larue and Petit, 2022 [preprint]). The negative effects of self-pollination are considerable in this self-incompatible species characterized by a late-acting (ovarian) self-incompatibility: nearly half of the female flowers abort under natural conditions because of self-pollination (Larue and Petit, 2022 [preprint]; Larue et al., 2022).

Study site and plant material

We surveyed flowering phenology and insect visits to flowering chestnut trees in 2021 in a large chestnut germplasm collection located in southwestern France (Larue et al., 2021b). This collection consists of two nearby experimental plots: the first (plot A) was planted in 1970, and the second (plot E) was planted in 1990. We selected four *C. sativa* × *C. crenata* hybrid clones, including two female clones, “Bouche de Bétizac” (called “Bétizac” below) and CA120, and two partly male-fertile monoecious clones, “Marlhac” and “Maridonne.” We monitored two ramets (clonal copies) of each clone in plot E and one additional ramet of “Bétizac,” “Maridonne,” and “Marlhac” in plot A. For each clone, we chose ramets that were as far apart as possible in the study site (Appendix S1).

Phenology monitoring

For each of the eight trees from plot E, we selected 25–30 easily accessible branches with as many bisexual catkins as possible. We monitored them throughout the flowering season, by estimating the percentage of mature male flowers as well as the percentage of receptive female flowers per branch, following Hasegawa et al. (2017). For male flowers involved in each staminate phase, we visually determined the proportions of open and wilted male flowers. For the female flowers, we examined whether the stigmas had emerged and whether they had become brownish, marking the beginning and end of the period of receptivity. We counted the total numbers of undeveloped, open, and wilted female flowers on each of the 30 branches. For the three remaining trees located in plot A, we used a more rapid method based on the assessment of phenological flowering stages at the tree level, which also depends on the proportion of open and wilted male flowers (Larue et al., 2021c). Two teams of at least two observers monitored all trees twice a week during six weeks in spring 2021, between 08:30 and 16:00 hours (CEST).

Insect observations on trees and on female flowers

We systematically monitored insect visits on all 11 trees throughout the flowering season. The inventories were always performed immediately after monitoring phenology. We used a nondestructive sampling method that combines direct field determination of insect visitors with macrophotography. We inventoried insects on the accessible parts of a tree (≤2 m) during 10 min to estimate the abundance of insect visitors on each chestnut tree. We counted the insects on both fertile parts (flowers) and sterile parts (branches and leaves). Many insect visitors readily move from sterile to fertile parts of the tree, so including all insects was more straightforward and resulted in larger, and presumably more precise and more representative, inventories. We photographed insects to confirm identification. We used digital cameras (Nikon D850, Nikon D7200, and Fujifilm X-T3) equipped with a macro lens objective (AF-S VR Micro-Nikkor 105 mm f/2.8 G and Fujinon XF 80 mm f/2.8

R LM OIS W Macro). We then identified all photographed insects to morpho-species by relying mostly on two websites (Anonymous, 2002; Mathieu and Mathieu, 2014). We also monitored insects visiting female flowers, during periods of 10 min of observation entirely dedicated to that task, to identify chestnut pollinators and to estimate pollen receipt. To increase the number of observations of such rare events, we recorded additional cases of interactions of insects with female flowers during the other phases of monitoring of each tree. For most insect visits to female inflorescences, we also noted the phenological stage of the associated male catkin (Figure 1).

Phenological phases

To characterize flowering phenology throughout the flowering season, we estimated for each tree inventory the percentage of open, non-wilted male flowers per branch for each of 25–30 branches. Similarly, we estimated the percentage of receptive female flowers per branch as the percentage of receptive, non-wilted female flowers. We used the threshold of 10% of open male flowers of unisexual catkins to indicate the onset of flowering of a tree. Symmetrically, we used the threshold of 90% of wilted male flowers of bisexual catkins to indicate the end of flowering. We performed a linear interpolation to estimate the Julian day of the beginning and end of flowering for the two staminate phases, accounting for the fact that in three of eight trees (E69D, E48F, E50P), the second staminate phase was not fully finished at the end of the survey. Because of the large flowering overlap of the two types of male catkins, we opted for a conservative approach to define the second staminate phase. We conservatively assigned an inventory to this second staminate phase only if >90% of male flowers from unisexual catkins were wilted or had fallen, thus omitting cases where male flowers from bisexual catkins were already flowering. Indeed, our goal was to test whether insect visits to female flowers increased during this second staminate phase because of the sole presence of male flowers from bisexual catkins, taking into account the fact that these bisexual catkins are much rarer than unisexual catkins. To be even more conservative, we also reduced the window of the first staminate phase by transposing the threshold for the onset of the second staminate phase, thereby discarding the earliest inventories (see Figure 2). We applied this approach to each tree independently in plot E. For trees in plot A, we relied on standard phenological stages assessed at tree level (Larue et al., 2021c). The first staminate phase corresponds to phenological scores for male catkins of unisexual inflorescences ranging from 64 (≥40% of flowers open) to 67 (≥50% of wilted flowers). The second staminate phase corresponds to scores of 69 (≥50% of fallen catkins) for

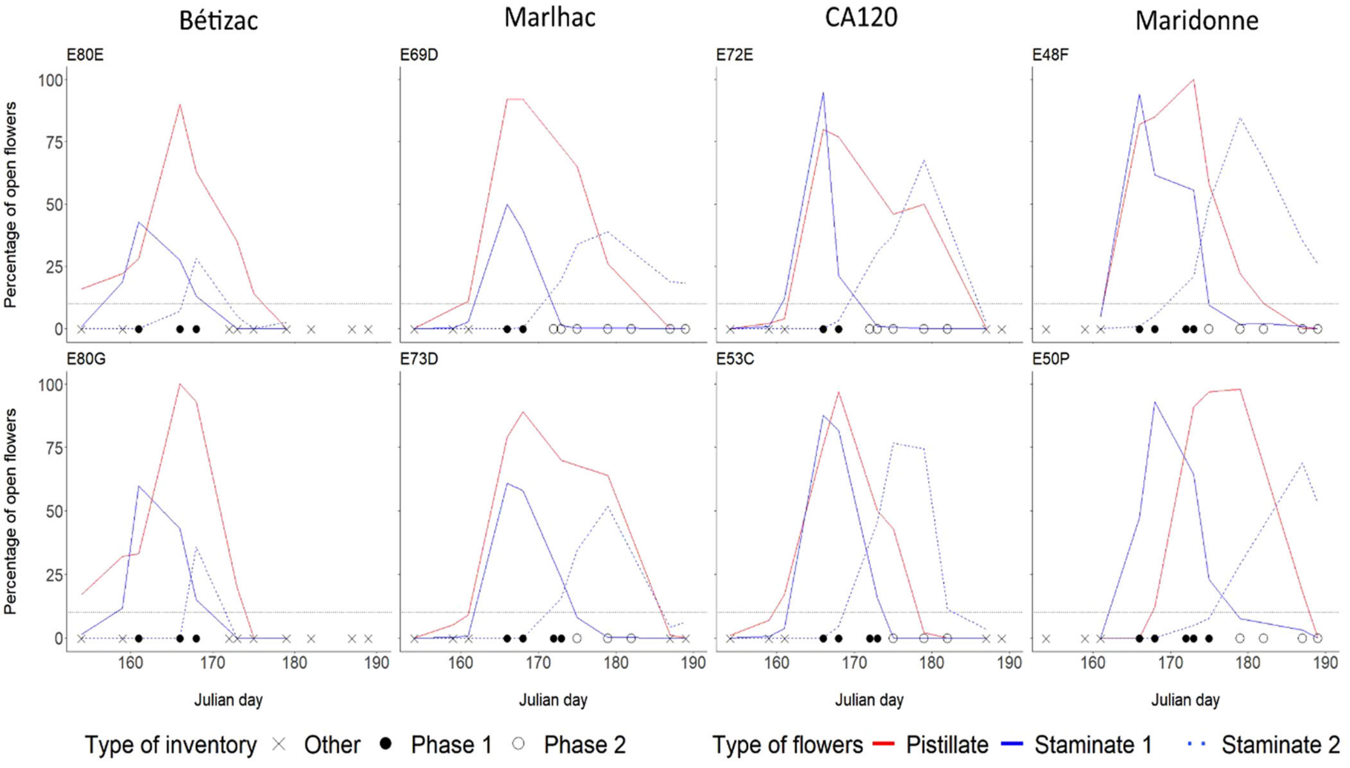

FIGURE 2 Flowering phenology of eight monitored chestnut trees (*Castanea* spp.) in plot E. The two clonal copies of each of the four clones are represented one above the other. Inventories performed during staminate phases 1 and 2 are represented by circles; inventories corresponding to nonflowering trees are represented by crosses. The 10% flowering threshold is indicated by horizontal black dotted lines. Blue lines correspond to percentage of open male flowers from unisexual catkins (solid) and bisexual catkins (dotted). Red lines correspond to percentage of open (i.e., receptive) female flowers.

male catkins of unisexual inflorescences and to scores of 64 to 67 for male catkins of bisexual inflorescences. To assess the phenology of male catkins from bisexual inflorescences whose female flowers had been visited by insects, we directly evaluated the percentage of open and wilted flowers of the corresponding male catkins.

Insect taxa considered

We considered five taxonomic groups of insects for the subsequent analyses: the red soldier beetle (*Rhagonycha fulva*, Coleoptera: Cantharidae), "other beetles" (Coleoptera other than the soldier beetle, mostly Coccinellidae and Tenebrionidae), calyptrate flies (Diptera, section Schizophora, subsection Calyptratae, mostly Anthomyiidae, Calliphoridae, Muscidae, Rhinophoridae, Sarcophagidae, and Tachinidae), hoverflies (Diptera, section Aschiza: Syrphidae), and bees (Hymenoptera: Anthophila, mostly Apidae and Halictidae). We did not consider in these analyses other rare arthropod visitors mostly observed on leaves or previously ruled out as important pollinators of chestnuts (Larue et al., 2021a). We distinguished the red soldier beetle from the other beetles because of its abundance in our study site and its sedentary behavior involving long foraging bouts on flowering branches (Larue et al., 2021a). We also distinguished hoverflies (Syrphidae) from the other flies because they have distinct ecology and behavior. Hoverflies have precise hovering flight that enables accurate visits of rewarding male catkins and thus reduces foraging behavior on flowering branches (Dunn et al., 2020). We also distinguished calyptrate flies from other flies. According to Larson et al. (2001), "the calyptrates are generally larger and hairier than the acalyptrates, so they are more effective as pollen vectors and pollinators.… They generally have lapping labella suited for obtaining nectar from open flowers and capitula." We studied the abundance of these insects on trees and the number of visits to female flowers, contrasting their abundances between the two staminate phases.

Sex association in duodichogamous species

We used web literature searches on Google scholar and Web of Science with the keyword "duodichogam*" to compile a first list of duodichogamous taxa. We then retrieved other duodichogamous taxa cited in the corresponding articles. The definition of duodichogamy was not consistent across studies (Luo et al., 2007; Endress, 2020). Luo et al. (2007) define duodichogamy as a flowering system in which plants are successively male, female, and male at the whole-plant level. In contrast, Endress (2020) defines duodichogamy as a flowering system in which inflorescences, but not necessarily the entire plant, flower in this sequence. In our study, we decided to follow the definition proposed by Luo et al. (2007). We also report plants with a female-male-female sequence for comparison. For each of these taxa, we searched for additional information on the species' life form and pollination system in scientific papers or on referenced web sites. To describe sex association within flowers or inflorescences, we relied on botanical descriptions obtained from the literature. If a plant had hermaphrodite flowers or bisexual inflorescences, we considered the sexes to be associated. We assessed the symmetry of this sex association by evaluating whether gynoecia are surrounded by androecia and vice versa. For instance, in andromonoecious species, there is systematic association of gynoecia with androecia (in hermaphrodite flowers) but the reciprocal is not true, due to the existence of male flowers, so we considered the sex organization asymmetric. We used the same reasoning at the inflorescence level in monoecious species. When there was more than one species per genus, we included only one species for each unique type of flowering system. To determine potential risks of self-pollination, we searched for information on pollen production during each staminate phase, as indicated by flowering duration or by the number of male flowers involved. We considered that the two staminate phases differ in terms of pollen emission when one pollen emission phase is at least twice as important as the other one. We then examined whether androecia responsible for the minor staminate phase are located closer to gynoecia than androecia responsible for the major staminate phase. If so, we considered that the evidence supported our prediction regarding minimization of self-pollination

Statistical analyses

We built all graphical representations with R studio version 4.1.2, using the packages ggplot2 version 3.4 (Wickham et al., 2016) and gridExtra version 2.3 (Auguie and Antonov, 2017). To establish the limits of the two staminate phases through extrapolation, we used the package Hmisc version 4.7 (Harrell, 2022). To test the difference in insect abundance between staminate phases on trees and on female flowers, we selected chestnut clones with at least two copies in the experimental plot. To facilitate comparisons between staminate phases, we selected those trees for which we had performed at least two inventories during each phase. For each insect taxon, we performed a negative binomial family generalized linear mixed model (GLMM) to account for overdispersion in the data. We considered two fixed effects on insect abundance, staminate phase effect and clone effect, and a random effect corresponding to the tree effect. We also calculated a marginal R^2 and conditional R^2 using the trigamma function (Barton, 2022) for each GLMM. We used package lme4 version 1.1-28 (Bates et al., 2015) and MuMIn version 1.46.0 (Barton, 2022) for this purpose.

RESULTS

Flowering and insect monitoring on chestnut trees

Phenology monitoring revealed that the overall duration of flowering time greatly differs across chestnut clones, lasting from 13 to 32 days. Trees (ramets) of the same clone had very similar flowering phenologies (Figure 2). There was considerable overlap between pistil receptivity and pollen emission phases. The male-sterile clone "Bétizac" had a very short second staminate phase, during which we did not manage to perform enough insect inventories for a relevant comparison of the two staminate phases.

In 2021, we made 105 inventories of insects on trees and on female flowers, corresponding to >35 h of insect monitoring. In total, we counted 4061 insects on trees and observed 239 insects visiting female flowers (Pauly et al., 2023). These insects belong to nine orders, the most important being Coleoptera, Diptera, and Hymenoptera. After removing trees corresponding to the "Bétizac" clone and inventories made outside the flowering period (see above), there were 62 inventories left for comparison of the two staminate phases, 28 performed during the first staminate phase and 34 during the second staminate phase. These inventories included 2792 insect observations on trees and 196 on female flowers. For the five insect taxa considered, there were 1412 insects seen on trees during the first staminate phase (50.4 individuals/inventory) and 1143 during the second staminate phase (33.6 individuals/inventory). The most abundant insect taxon on trees was red soldier beetle (on average 21.0 individuals/inventory), followed by calyptrate flies (10.2) and "other beetles" (7.2). Bees (2.3) and hoverflies (1.2) were much less abundant. Three taxa were significantly more abundant during the first staminate phase than during the second one: red soldier beetle (27.3 vs. 15.8 individuals/inventory, $P < 0.001$), hoverflies (2.3 vs. 0.21, $P < 0.001$), and bees (4.2 vs. 0.8, $P < 0.005$) (Table 1, Figure 2). However, the abundance of "other beetles" did not differ significantly between the first and second staminate phases (9.1 vs. 5.5 individuals/inventory, $P > 0.05$). Finally, the abundance of calyptrate flies increased slightly during the second staminate phase (7.5 vs. 11.3 individuals/inventory, $P < 0.05$).

For the five taxa combined, we observed 193 visits to female flowers during the 62 inventories. The insects observed most frequently on female flowers were red soldier beetles (2.1 individuals/inventory), followed by calyptrate flies (0.6) and "other beetles" (0.4). Bees visited female flowers only exceptionally (two observations). Hoverflies were never seen visiting female flowers. Overall, we observed 61 insects (32%) on female flowers during the first staminate phase (2.3 visits/inventory) and 132 (68%) during the second staminate phase (3.9 visits/inventory). For calyptrate flies, the number of observations on female flowers increased significantly during the second staminate phase, from 0.1 to 1.0 individuals/inventory ($P < 0.001$) (Table 2, Figure 3). For red soldier beetle, there was no change to visits to female flowers between the two staminate phases: 1.6 visits/inventory in phase 1 vs. 2.5 in phase 2 ($P > 0.05$). This was also the case for "other beetles": 0.4 visits/inventory during phase 1 vs. 0.4 visits/inventory during phase 2 ($P > 0.05$).

Altogether, we found that both red soldier beetle and calyptrate flies (the two most abundant insect taxa) are more likely to visit female flowers during the second staminate phase than during the first one. For the soldier beetle, there was a significant decrease in the number of insects on trees during the second staminate phase associated with a nonsignificant increase in visits to female flowers, resulting

TABLE 1 Results of generalized linear mixed model (GLMM) analysis of differences in abundance of taxa on trees according to staminate phase and clone.

Variable explained	Fixed factor[a]	Chisq	df	$Pr(>\chi^2)$[b]	Marginal R^2	Conditional R^2[c]
Red soldier beetle	Phase	11.4	1	***	0.36	0.71
	Clone	6.1	2	*		
Calyptrate flies	Phase	5.4	1	*	0.15	/
	Clone	7.8	2	*		
Other beetles	Phase	2.0	1	ns	0.28	0.52
	Clone	6.7	2	*		
Bees	Phase	10.4	1	**	0.38	/
	Clone	7.7	2	*		
Hoverflies	Phase	25.6	1	***	0.44	/
	Clone	0.5	2	ns		

[a] Each model was run with the tree factor set as random effect.

[b] ns = nonsignificant; * $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$.

[c] Slash indicates that the random effect is too small to be evaluated.

TABLE 2 Results of generalized linear mixed model (GLMM) analysis of differences in abundance of taxa on female flowers according to staminate phase and clone.

Variable explained	Fixed factor[a]	Chisq	df	Pr(>χ^2)[b]	Marginal R^2	Conditional R^2[c]
Red soldier beetle	Phase	3.0	1	ns	0.27	/
	Clone	25.5	2	***		
Calyptrate flies	Phase	10.6	1	***	0.12	/
	Clone	0.0	2	ns		
Other beetles	Phase	0.4	1	ns	0.05	0.13
	Clone	2.3	2	ns		

[a] Each model was run with the tree factor set as random effect.

[b] ns = nonsignificant; * $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$.

[c] Slash indicates that the random effect is too small to be computed.

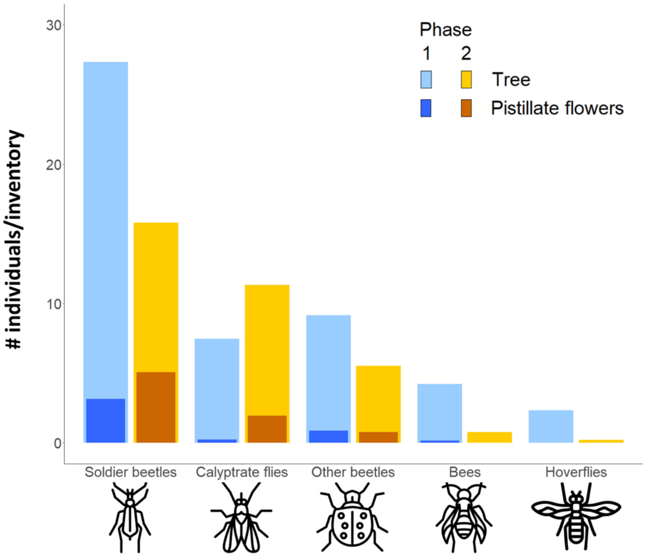

FIGURE 3 Number of insect individuals per inventory observed on chestnut trees and twice the number of insect individuals per inventory observed on pistillate flowers during the first and second staminate phases (see definition in the text). Insect drawings modified from https://www.flaticon.com.

in a threefold increase across staminate phases in the probability of an insect visiting a female flower. For calyptrate flies, there was a slight but significant increase in the number of insects on trees during the second staminate phase, along with a considerable increase in visits to female flowers, resulting in a ninefold increase across staminate phases in the probability of an insect visiting a female flower (Figure 3). These are conservative estimates; male catkins from bisexual catkins start to flower before the end of the first staminate phase. In fact, among the 61 insect visits to female flowers that took place during the first staminate phase, the associated male catkin was flowering in ≥21 cases, implying that <21% of visited female flowers took place before the associated male catkins had started to flower. We provide further details of the mean abundance of these insects on trees and on female flowers across clones in Appendix S2.

Sex distribution in duodichogamous species

We selected from the literature 26 species in 24 genera and nine families (Apiaceae, Araliaceae, Cyperaceae, Ecediocolaceae, Fagaceae, Juglandaceae, Meliaceae, Phyllanthaceae, and Sapindaceae) that matched our definition (i.e., duodichogamous at the whole-plant level). Two of these species are wind pollinated. The 24 remaining species are animal pollinated, and 23 have a male-female-male flowering sequence. Among these 23 species, all are woody plants: 18 trees or treelets, three shrubs, and two lianas. We were unable to establish the floral architecture for two of them, due to insufficient information in the literature. Among the 21 remaining species, 15 are monoecious and six are andromonoecious; 14 have bisexual inflorescences, six have male and bisexual inflorescences, and one has both male and female inflorescences (for more details, see Appendix S3). Altogether, 20 have gynoecia positioned next to androecia at either the flower or the inflorescence level. For 11 of these species, the sex organization is fundamentally asymmetric, due to the presence of a combination of male and bisexual flowers (=andromonoecy) or male and bisexual inflorescences (Table 3). There were no duodichogamous species with only hermaphrodite flowers, with both hermaphrodite and female flowers (gynomonoecy), or with both female and bisexual inflorescences. Many of these species were polymorphic, having also unisexual individuals (seven cases) or flowering in different sequences than duodichogamy (eight cases). However, duodichogamous individuals were typically predominant among the species listed.

Role of each staminate phase

Among the 21 selected duodichogamous species, we found only six taxa (including chestnuts) with enough information to test our hypothesis of a preferential coupling of gynoecia with androecia involved in the minor staminate phase (Table 4). One species, *Bridelia retusa* (Phyllanthaceae), has two staminate phases of similar importance and independent

TABLE 3 Floral architecture of 21 duodichogamous animal-pollinated taxa and support for preferential placement of gynoecia close to androecia.

	Inflorescences			
Flowers	**Male and bisexual**	**Bisexual**	**Male and female**	**Female and bisexual**
Male and bisexual	**2**	**4**	/	/
Male and female	**4**	10	1	0
Female and bisexual	/	0	/	0

Notes: Bold type indicates that the hypothesis is supported; roman type indicates that the hypothesis is not ruled out; underlined type indicates that the hypothesis is falsified; slash indicates that the corresponding sex arrangements are impossible.

TABLE 4 Spatial and temporal arrangements of the two types of male flowers in duodichogamous taxa and support for the asymmetric sex association hypothesis.

			Staminate phase[b]					
Family	**Species**	**Sexual system[a]**	**Smaller**	**Shorter**	**Closer in space**	**Closer in time**	**Support[c]**	**References**
Apiaceae	*Dorema aucheri*	A	1	=	1	1	Yes	Ajani and Claßen-Bockhoff (2021)
Fagaceae	*Castanea* spp.	M	2	=	2	=	Yes	Larue et al. (2021a)
Juglandaceae	*Platycarya strobilacea*	M	2	=	2	2	Yes	Fukuhara and Tokumaru (2014)
Phyllanthaceae	*Bridelia retusa*	M	/	1	=	=	No	Dias and Ratnayake (2021)
Sapindaceae	*Acer oblongum*	M	2	/	/	2	Yes	Yadav et al. (2016)
Sapindaceae	*Cupania guatemalensis*	M	2	=	2	2	Yes	Bawa (1977)

[a] A = andromonoecious; M = monoecious.

[b] Comparison of the two staminate phases. 1 = first staminate phase; 2 = second staminate phase; equal sign indicates "indistinguishable"; slash indicates "no information found."

[c] Support for the asymmetric sex association hypothesis.

male and female inflorescences, thus supporting Lloyd and Webb's (1986) male hypothesis rather than our female hypothesis. Among the five remaining cases, one (*Dorema aucheri*, Apiaceae) has andromonoecious flowers. The hermaphrodite flowers, which flower first, are protandrous and are less numerous than the male flowers (Ajani and Claßen-Bockhoff, 2021), thus supporting our female hypothesis. The four other species, including chestnuts, are monoecious and produce less pollen during the second staminate phase. The male flowers of the second staminate phase are spatially (and sometimes temporally) closer to the female flowers than the male flowers of the first staminate phase, thus also supporting our female hypothesis (Table 4). Hence, all five cases are compatible with our prediction of a role for the less productive staminate phase in promoting female mating success while mitigating the negative effects of self-pollination

DISCUSSION

To evaluate whether duodichogamy can be beneficial to female fitness, we studied chestnut pollination. We precisely followed the flowering phenology of eight trees. We detected two distinct pollen emission phases that overlapped with each other and with the pollen receipt phase, confirming that duodichogamy only partly limits the risk of self-pollination in chestnuts (Hasegawa et al., 2017). Moreover, there was considerable variation among clones in the timing and duration of flowering phases and much less variation between ramets from the same clone, suggesting that flowering phenology is heritable and can quickly evolve in response to selection. Phenology has been shown previously to be highly heritable in chestnuts (e.g., Furones-Pérez and Fernández-López, 2009). Beetles and calyptrate flies visit chestnut female flowers, but bees and hoverflies do not (Larue et al., 2021a). As expected, we found that insect visitors were more abundant during the first staminate phase, when chestnut trees produce an estimated 97% of the overall rewards (pollen and nectar). However, the difference in insect abundance between the two phases was small, considering the 30-fold imbalance in terms of pollen and nectar production between phases: only 20% more insects were observed during the first staminate phase than during the second staminate phase. This might indicate pollinator saturation during the first massive staminate phase in these monospecific stands (Ohashi and Yahara, 2002). Red soldier beetle and (especially) calyptrate flies, the two most abundant pollinators in the study site, increased their visits to female flowers during the second minor staminate phase, at a time when female receptivity is at its maximum (Shimura et al., 1971). Several insect visits to female flowers, although assigned to the first staminate phase, actually took place when the male catkin associated with the female inflorescence was already flowering. This confirms the crucial role of the associated male catkin in triggering visits to female flowers. The increased visitation rates to female flowers during the second staminate phase is probably also due to the reduced density of male flowers at that time, thus further concentrating pollinators close to female flowers. We observed the same trends in all three chestnut clones, one of which is male-sterile, indicating that pollen production is not required to attract insects to female flowers.

Overall, the results fit well with the intuitive prediction by van der Pijl (1978) that, in entomophilous Fagaceae, "sexual aggregation … ensures the pollination of the unattractive pistillate flowers." The results also show the relevance of exploring sex association at the inflorescence level. Inflorescences can evolve into bisexual units well adapted to animal pollination, called pseudanthia, some of which closely resemble hermaphrodite flowers (Classen-Bockhoff, 1990). Our results for chestnuts, as well as previously published results for andromonoecious species, show that all types of bisexual inflorescences, not only those resembling flowers, can increase female mating success (Solomon, 1987; Podolsky, 1992; Vallejo-Marín and Rausher, 2007; Tomaszewski et al., 2018; but see Schlessman et al., 2004). In a previous study on chestnuts, we found that removing male-sterile catkins decreased fruit set (Larue and Petit, 2022 [preprint]), showing that male-sterile but nectar-producing catkins promote pollination of nearby female flowers. In the future, it would be interesting to directly test whether removing male catkins from bisexual inflorescences reduces insect visits to female flowers. The delayed flowering of female flowers and their spatial separation from the numerous unisexual catkins should minimize self-pollination, thus further increasing female fitness. This interpretation is supported by partial-emasculation experiments showing that, in male-fertile chestnut trees, self-pollen interference is mostly attributable to unisexual catkins (Larue and Petit, 2022 [preprint]).

To explore further the female hypothesis of the origin of duodichogamy, we examined flower traits of all duodichogamous species described to date. We found more duodichogamous taxa than in previous reviews (Renner, 2014; Lee et al., 2018; Endress, 2020). Most of these species are insect-pollinated woody plants (especially trees) with massive synchronous flowering. In large and long-lived organisms, risks of pollinator-assisted self-pollination among flowers of the same individual (geitonogamy) and the consequences of inbreeding depression are magnified (Petit and Hampe, 2006; Harder and Prusinkiewicz, 2013; Wang et al., 2020). However, a majority of these species, in addition to being duodichogamous (temporal separation of the sexes), are also monoecious (spatial separation of the sexes), thus somewhat limiting self-pollination risks.

We identified only one duodichogamous species in which female flowers were fully separated from male flowers (*Bridelia retusa*, Phyllanthaceae; Dias and Ratnayake, 2021). This floral organization should rule out any positive effect of male flower attractiveness on female mating success. In this tree species, female flowers are as attractive as male flowers, showing that female flowers must invest in pollinator attractiveness when they are located away from male flowers. However, in all other duodichogamous species identified (20 of 21), gynoecia are located close to androecia. In 11 of these 20 cases, the association between sexes is asymmetric: gynoecia are systematically located next to androecia, whereas the opposite is not true. In the nine remaining species, which are all monoecious with bisexual inflorescences, flower sex ratios are male-biased, which is the typical situation in monecious species (Gross, 2005), so gynoecia are also more likely to be closely surrounded by androecia than vice versa. The generally asymmetric sex organization reported here is consistent with female mating success often benefiting from male attractiveness, even if other non–mutually exclusive interpretations are possible (e.g., Miller and Diggle, 2007).

In outcrossing plants, selection for the association of gynoecia with androecia to facilitate pollen receipt will be opposed by selection to limit self-pollination (Bertin, 1993; de Jong et al., 2008; Harder and Prusinkiewicz, 2013). In addition to the case of *B. retusa* already discussed, we found precise descriptions of the two staminate phases in only five duodichogamous taxa. In all five cases, the two staminate phases differ in pollen production. Furthermore, gynoecia are always located closer to androecia producing the smaller staminate phase (Bawa, 1977; Fukuhara and Tokumaru, 2014; Yadav et al., 2016; Ajani and Claßen-Bockhoff, 2021; Larue et al., 2021a), likely reducing risks of self-pollination. In four of these cases, the corresponding androecia are involved in the second staminate phase, further minimizing risks of self-pollination, because female flowers are then receptive before the male flowers of the associated catkin start to release pollen (Bawa and Beach, 1981; Bertin, 1993). Hence, duodichogamy seems to balance female mating facilitation with self-pollination avoidance, thus maximizing the benefit of close sex association for pollen deposition while minimizing self-pollination.

At this point, our interpretation of the adaptive role of duodichogamy relies mostly on evidence from chestnuts. Careful descriptions and observations of other duodichogamous species should be performed, ideally completed by experiments manipulating floral rewards and measuring self-pollination rates. Our female hypothesis is the only one that considers intersexual mating facilitation. It differs from a previous hypothesis that assumed that the existence of two staminate phases reflects intense male selection (e.g., Lloyd and Webb, 1986; Luo et al., 2007). Based on an exploration of insect pollination in chestnuts and on an examination of the flowering architecture of other animal-pollinated duodichogamous species, we have highlighted the advantage of an additional attractive male phase for pollen capture on stigmas and for outcrossing. Pollination mode has profound consequences on plant sexual systems, as shown early on by

Bawa and Beach (1981). In animal-pollinated plants, sexual selection should lead to high "male sex appeal" to pollinators. Different cost-effective strategies to secure pollen to stigmas build on this greater male attractiveness. Beyond the peculiar case of duodichogamy, we argue that female mating facilitation by attractive male flower parts deserves more consideration in evolutionary studies of the structure of hermaphrodite flowers and of bisexual inflorescences.

AUTHOR CONTRIBUTIONS

G.P.: conceptualization, investigation, data curation, formal analysis, visualization, writing. C.L.: conceptualization, investigation, formal analysis, visualization, writing, funding acquisition. R.J.P.: conceptualization, investigation, formal analysis, writing, funding acquisition, supervision.

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DATA AVAILABILITY STATEMENT

R script and all data used for figures and models are available at https://entrepot.recherche.data.gouv.fr/dataset.xhtml?persistentId=doi:10.57745/PELMZJ.

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REFERENCES

Aizen, M. A., and L. D. Harder. 2007. Expanding the limits of the pollen-limitation concept: Effects of pollen quantity and quality. *Ecology* 88: 271–281.

Ajani, Y., and R. Claßen-Bockhoff. 2021. The unique inflorescence structure of *Dorema aucheri* (Apiaceae): An adaptation to the arid environment. *Journal of Arid Environments* 184: 104194.

Anonymous. 2002. Galerie du monde des insectes. Website: https://www.galerie-insecte.org/galerie/fichier.php [accessed 3 June 2021].

Auguie, B., and A. Antonov. 2017. gridExtra: miscellaneous functions for" Grid" graphics. R package version 2.3. [*Computer software*]. Website: https://CRAN.R-project.org/package=gridExtra

Baker, H., and P. D. J. Hurd. 1968. Intrafloral ecology. *Annual Review of Entomology* 13: 385–414.

Barrett, S. C. H. 2002. The evolution of plant sexual diversity. *Nature Reviews Genetics* 3: 274–284.

Barton, K. 2022. Package 'MuMIn'. *Version 1.47.1* 1: 439.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila. Heredity* 2: 349–368.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1–48.

Bawa, K. S. 1980. Evolution of dioecy in flowering plants. *Annual Review of Ecology and Systematics* 11: 15–39.

Bawa, K. S. 1977. The reproductive biology of *Cupania guatemalensis* Radlk. (Sapindaceae). *Evolution* 31: 52–63.

Bawa, K. S., and J. H. Beach. 1981. Evolution of sexual systems in flowering plants. *Annals of the Missouri Botanical Garden* 68: 254–274.

Bawa, K. S., and P. A. Opler. 1975. Dioecism in tropical forest trees. *Evolution* 29: 167.

Bell, G. 1985. On the function of flowers. *Proceedings of the Royal Society of London. Series B. Biological Sciences* 224: 223–265.

Bernhardt, P., and L. B. Thien. 1987. Self-isolation and insect pollination in the primitive angiosperms: New evaluations of older hypotheses. *Plant Systematics and Evolution* 156: 159–176.

Bertin, R. 1993. Incidence of monoecy and dichogamy in relation to self-fertilization in angiosperms. *American Journal of Botany* 80: 557–560.

Bertin, R. 1982. The evolution and maintenance of andromonoecy. *Evolutionary Theory* 6: 25–32.

Carlson, J. E., and K. E. Harms. 2006. The evolution of gender-biased nectar production in hermaphroditic plants. *Botanical Review* 72: 179–205.

Charlesworth, D. 2006. Evolution of plant breeding systems. *Current Biology* 16: R726–R735.

Classen-Bockhoff, R. 1990. Pattern analysis in pseudanthia. *Plant Systematics and Evolution* 171: 57–88.

Delph, L. F., L. F. Galloway, and M. L. Stanton. 1996. Sexual dimorphism in flower size. *American Naturalist* 148: 299–320.

Dias, M. A., and R. M. C. S. Ratnayake. 2021. Variation in dichogamy and myophily in two dioecious *Bridelia* species (Phyllanthaceae). *Australian Journal of Botany* 69: 301.

Duffy, K. J., and S. D. Johnson. 2011. Effects of pollen reward removal on fecundity in a self-incompatible hermaphrodite plant: Pollen reward removal and plant fecundity. *Plant Biology* 13: 556–560.

Duffy, K. J., K. L. Patrick, and S. D. Johnson. 2013. Emasculation increases seed set in the bird-pollinated hermaphrodite *Kniphofia linearifolia* (Xanthorrhoeaceae): Evidence for sexual conflict? *American Journal of Botany* 100: 622–627.

Dunn, L., M. Lequerica, C. R. Reid, and T. Latty. 2020. Dual ecosystem services of syrphid flies (Diptera: Syrphidae): Pollinators and biological control agents. *Pest Management Science* 76: 1973–1979.

Endress, P. K. 2020. Structural and temporal modes of heterodichogamy and similar patterns across angiosperms. *Botanical Journal of the Linnean Society* 193: 5–18.

Fukuhara, T., and S. Tokumaru. 2014. Inflorescence dimorphism, heterodichogamy and thrips pollination in *Platycarya strobilacea* (Juglandaceae). *Annals of Botany* 113: 467–476.

Furones-Pérez, P., and J. Fernández-López. 2009. Morphological and phenological description of 38 sweet chestnut cultivars (*Castanea sativa* Miller) in a contemporary collection. *Spanish Journal of Agricultural Research* 7: 829–843.

Grant, V. 1951. The fertilization of flowers. *Scientific American* 184: 52–56.

Gross, C. L. 2005. A comparison of the sexual systems in the trees from the Australian tropics with other tropical biomes–more monoecy but why? *American Journal of Botany* 92: 907–919.

Harder, L. D., and P. Prusinkiewicz. 2013. The interplay between inflorescence development and function as the crucible of architectural diversity. *Annals of Botany* 112: 1477–1493.

Harrell, Jr. F. E. 2022. Package 'Hmisc'. *Version 4.7-2* 2019: 235–236.

Hasegawa, Y., Y. Suyama, K. Seiwa, and others. 2017. Flowering phenology of a duodichogamous self-incompatible tree species, *Castanea crenata. Japanese Journal of Ecology* 67: 31–39.

Huang, S., L. Tang, J. Sun, and Y. Lu. 2006. Pollinator response to female and male floral display in a monoecious species and its implications for the evolution of floral dimorphism. *New Phytologist* 171: 417–424.

de Jong, T. J., A. Shmida, and F. Thuijsman. 2008. Sex allocation in plants and the evolution of monoecy. *Evolutionary Ecology Research* 10: 1087–1109.

Larson, B. M. H., P. G. Kevan, and D. W. Inouye. 2001. Flies and flowers: Taxonomic diversity of anthophiles and pollinators. *Canadian Entomologist* 133: 439–465.

Larue, C., E. Austruy, G. Basset, and R. J. Petit. 2021a. Revisiting pollination mode in chestnut (*Castanea* spp.): An integrated approach. *Botany Letters* 168: 348–372.

Larue, C., T. Barreneche, and R. J. Petit. 2021b. An intensive study plot to investigate chestnut tree reproduction. *Annals of Forest Science* 78: 90.

Larue, C., T. Barreneche, and R. J. Petit. 2021c. Efficient monitoring of phenology in chestnuts. *Scientia Horticulturae* 281: 109958.

Larue, C., E. K. Klein, and R. J. Petit. 2022. Sexual interference revealed by joint study of male and female pollination success in chestnut. *Molecular Ecology* 32: 1211–1228.

Larue, C., and R. J. Petit. 2022. Self-interference and female advantage in chestnut. *bioRxiv* https://doi.org/10.1101/2022.08.01.502348 [preprint].

Lee, H., H. Kang, and W.-G. Park. 2018. A rare duodichogamous flowering system in monoecious *Toona sinensis* (Meliaceae). *Journal of Ecology and Environment* 42: 7.

Lloyd, D. G. 1982. Selection of combined versus separate sexes in seed plants. *American Naturalist* 120: 571–585.

Lloyd, D. G., and C. J. Webb. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms I. Dichogamy. *New Zealand Journal of Botany* 24: 135–162.

Luo, S., D. Zhang, and S. S. Renner. 2007. Duodichogamy and androdioecy in the Chinese Phyllanthaceae *Bridelia tomentosa*. *American Journal of Botany* 94: 260–265.

Mathieu, M., and F. Mathieu. 2014. Quel est cet animal? Website: https://www.quelestcetanimal.com [accessed 25 April 2023].

Miller, J. S., and P. K. Diggle. 2007. Correlated evolution of fruit size and sexual expression in andromonoecious *Solanum* sections *Acanthophora* and *Lasiocarpa* (Solanaceae). *American Journal of Botany* 94: 1706–1715.

Ohashi, K., and T. Yahara. 2002. Visit larger displays but probe proportionally fewer flowers: counterintuitive behaviour of nectar-collecting bumble bees achieves an ideal free distribution. *Functional Ecology* 16: 492–503.

Paterno, G. B., C. L. Silveira, J. Kollmann, M. Westoby, and C. R. Fonseca. 2020. The maleness of larger angiosperm flowers. *Proceedings of the National Academy of Sciences* 117: 10921–10926.

Pauly, G., C. Larue, and R. J. Petit. 2023. Adaptive function of duodichogamy: Why do chestnut trees have two pollen emission peaks? (V1) [Data set]. Recherche Data Gouv. Website: https://doi.org/10.57745/YOCXKZ

Petit, R. J., and A. Hampe. 2006. Some evolutionary consequences of being a tree. *Annual Review of Ecology, Evolution, and Systematics* 37: 187–214.

Petit, R. J., and C. Larue. 2022. Confirmation that chestnuts are insect-pollinated. *Botany Letters* 169: 370–374.

van der Pijl, L. 1978. Reproductive integration and sexual disharmony in floral functions. *In* A. J. Richards [ed.], The pollination of flowers by insects, Linnean Society Symposium Series, 79–88. Academic Press, London.

Podolsky, R. D. 1992. Strange floral attractors: Pollinator attraction and the evolution of plant sexual systems. *Science* 258: 791–793.

Renner, S. S. 2014. The relative and absolute frequencies of angiosperm sexual systems: dioecy, monoecy, gynodioecy, and an updated online database. *American Journal of Botany* 101: 1588–1596.

Schlessman, M. A., N. Underwood, T. I. M. Watkins, L. M. Graceffa, and D. Cordray. 2004. Functions of staminate flowers in andromonoecious *Pseudocymopterus montanus* (Apiaceae, Apioideae). *Plant Species Biology* 19: 1–12.

Shimura, I., M. Yasuno, and C. Otomo. 1971. Studies on the breeding behaviors of several characters in chestnuts, *Castanea* spp.: II. Effects of the pollination time on the number of nuts in the burr. *Japanese Journal of Breeding* 21: 77–80.

Silva, D. M., C. R. Zambon, V. H. Techio, and R. Pio. 2020. Floral characterization and pollen germination protocol for *Castanea crenata* Siebold & Zucc. *South African Journal of Botany* 130: 389–395.

Solomon, B. 1987. The role of male flowers in *Solanum carolinense*: Pollen donors or pollinator attractors? *Evolutionary Trends in Plants* 1: 89–93.

Stout, A. B. 1928. Dichogamy in flowering plants. *Bulletin of the Torrey Botanical Club* 55: 141.

Tomaszewski, C. E., M. W. Kulbaba, and L. D. Harder. 2018. Mating consequences of contrasting hermaphroditic plant sexual systems. *Evolution* 72: 2114–2128.

Vallejo-Marín, M., and M. D. Rausher. 2007. Selection through female fitness helps to explain the maintenance of male flowers. *American Naturalist* 169: 563–568.

Wang, Y., T. Lyu, N. Shrestha, L. Lyu, Y. Li, B. Schmid, R. P. Freckleton, et al. 2020. Drivers of large-scale geographical variation in sexual systems of woody plants. *Global Ecology and Biogeography* 29: 546–557.

Wickham, H., W. Chang, L. Henry, T. Lin Pederson, K. Takahashi, C. Wilke, and K. Woo. 2016. ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics. *Version 3.4.0* 2: 1–189.

Yadav, N., A. K. Pandey, and A. K. Bhatnagar. 2016. Cryptic monoecy and floral morph types in *Acer oblongum* (Sapindaceae): An endangered taxon. *Flora* 224: 183–190.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Location of the studied trees.

Appendix S2. Mean number of insects observed on trees and on female flowers.

Appendix S3. Main traits of duodichogamous taxa.

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