# Evidence that male sex appeal enhances female fitness in animal-pollinated plants

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# Introduction

In angiosperms, most species have bisexual flowers (Tree of Sex Consortium, 2014). Starting with this ancestral arrangement, an amazing diversity of sexual systems have evolved (Renner & Müller, 2022; Specht & Bartlett, 2009). A major selective force driving the evolution of sexual systems in plants is avoidance of self-pollination (Baker & Hurd, 1968; Barrett & Harder, 2017; Charlesworth, 2006; Cronk, 2022; Henry et al., 2018; Lloyd & Webb, 1986). It would combine with climate, evolutionary age, and mature plant height to explain the present diversity of plant sexual systems (Barrett & Harder, 2017; Wang et al., 2020, 2021). According to Bawa & Beach (1981), pollination mode could have also played an important role in the diversification of sexual systems in angiosperms. After all, the angiosperm hermaphroditic flower itself has evolved in response to selection for effective pollen import and export by animals (Baker & Hurd, 1968).

A number of authors have portrayed the coupling of sexual organs in the angiosperm flower as a result of selection to mutualise efforts for pollinator attraction, resulting in equal benefits for male and female fitness (Charnov et al., 1976; Maynard-Smith, 1978). However, this is misleading. The coupling of sexes benefits more to female fitness than to male fitness (Baker & Hurd, 1968; Bertin, 1982). Indeed, protein-rich pollen was likely the original reward for pollinators and remains a major reward, together with nectar, resulting in asymmetric selection pressures for the juxtaposition of the two sexes, stronger for the female sex (Bawa & Beach, 1981; Charlesworth, 2006). Experiments have clearly shown that stamens juxtaposed with pistils enhance female fitness (Duffy & Johnson, 2011). Similarly, many female flowers retain non-functional stamens called staminodes to lure pollinators and ensure pollen import, while male flowers only rarely maintain a pistillode (Ashman, 2000; Pontes et al., 2022; Willson & Ågren, 1989).

Another mechanism responsible for the placement of carpels close to the stamens to optimise pollen import is sexual selection (Bateman, 1948; Bawa & Beach, 1981; Lloyd, 1979). According to Bawa & Beach (1981), who transposed Bateman's principle (1948) to plants, the «*paternal reproductive success is limited by a plant's ability to disperse pollen to conspecific stigmas, whereas maternal success is usually limited by the amount of nutritional resources available for developing seed*». As a consequence, to achieve optimal fitness, a plant needs more pollinators visits to export its pollen from its stamens than to import pollen to its stigmas (Bell, 1985; Huang et al., 2006). Selection for male fitness thus incurs strong

selection on plants to increase pollinator attraction, by developing visual and chemical signals such as coloured petals or perfumes and by providing different types of rewards (Delph et al., 1996; Huang et al., 2006; Paterno et al., 2020). Female fitness can benefit from male sex appeal of a plant (i.e. its attractiveness to pollinators) in different ways. First, female flowers, especially deceptive ones, can imitate male flowers, representing cases of intersexual Bakerian mimicry (Baker, 1976; Grant et al., 2021; Larue, Austruy, et al., 2021). Second, the gynoecia (i.e. the female organs) can be associated with the androecia (i.e. the male organs) to favour pollen import brought about by animals attracted by male structures. Both options can be potentially effective resource-saving strategies for seed production, as shown in other contexts (Dafni, 1984; Huang et al., 2006).

The study of specific sexual systems could help to evaluate these issues. Andromonecy is a well-investigated sexual system in which male and hermaphrodite flowers coexist on the same plant. It is often viewed as a "male" strategy, i.e. an adaptation to increase pollen export and hence male fitness, in situations where male-male competition is high (Vallejo-Marin & Rausher, 2007). However, experiments on andromonoecious plants have revealed that male flowers can enhance not only pollen export but also pollen import (Tomaszewski et al., 2018; Vallejo-Marin & Rausher, 2007). This supports the "pollinator attraction hypothesis", which suggests that male flowers of andromonoecious plants can serve to attract pollinators to enhance seed set (Schlessman et al., 2004; Solomon, 1986). It could be interesting to investigate other sexual systems classically described as "male" strategies. For instance, duodichogamy is a very rare sexual system with two distinct phases of pollen production separated by a female phase (male-female-male flowering sequence). The origin of the second staminate phase is generally considered as an adaptation to maximize male fitness (Lloyd & Webb, 1986; Luo et al., 2007). Alternatively, this second phase could also enhance pollen import and female fitness, as proposed in some recent studies (Ajani & Claβen–Bockhoff 2021; Yadav et al., 2016).

Here, we build on this "pollinator attraction hypothesis" to propose a new more general framework that we call the "female hitchhiking hypothesis". We use this expression to introduce the idea that, in animal-pollinated plants, female fitness often benefits one way or another from male attractiveness. Focusing on duodichogamous taxa, which provide an intriguing case study, we use two complementary approaches to test this hypothesis. First, we rely on detailed morphological descriptions of duodichogamous animal-pollinated plant species. We extract information on the two phases of pollen production, exploring their

spatiotemporal overlap with the female phase. We predict asymmetric phases of pollen production, with the androecia involved in the production of the smallest pollen emission phase located close to the gynoecium to enhance pollinator visits and seed set while limiting self-pollination. Second, we study in the field the possible effect on female fitness of each of the two staminate phases, using chestnut trees as model. We quantify the number of insect visits to female flowers, as a proxy for female fitness, during each of the two staminate phases. We predict that insect visits to female flowers will increase during the smaller staminate phase, which is closer to the female flowers. We finally discuss the perspectives offered by our new hypothesis to understand better the diversity of sexual systems in angiosperms.

## Materials and methods

### Literature review and trait analysis

I used web literature searches on Google scholar and Web of Science with the keyword "duodichogam*" to compile a first list of duodichogamous taxa. I then retrieved other duodichogamous taxa cited in the corresponding articles. However, the definition of duodichogamy was not consistent across studies (contrast e.g. Luo et al., 2007 and Endress, 2020). I decided to keep only those taxa that are duodichogamous at the whole plant level, not at the inflorescence level, in accordance with most treatments of duodichogamy to date (e.g. Luo et al., 2007). For each of these taxa, I searched for additional information on its life form and pollination system in scientific papers or on referenced web sites.

To explore the role of the two pollen production phases, I restricted this study to well-described animal-pollinated taxa with a male-female-male sequence. I searched for information on relative pollen production of the two staminate phases as indicated by number of flowers or the duration of each phase. I considered that the two phases are asymmetric if one phase is at least twice as big as the other. I also searched for information on the spatial and temporal proximity of the two sexes during each staminate phase. I considered that a given taxon concurred with our hypothesis if the androecia producing the small staminate phase were close in space or time to the gynoecia and closer to it than large staminate phase. Otherwise, I considered that it refuted our hypothesis.

# Flowering and insect monitoring of chestnut tree

## Study species

Chestnut is a monoecious insect-pollinated tree genus with both male and bisexual inflorescences. Duodichogamy was first described in chestnut (Stout, 1928), in which the flowering sequence is male-female-male (Hasegawa et al., 2017). The first staminate phase corresponds to the massive flowering of purely male inflorescences. It represents on average 97% of the pollen emitted. The second staminate phase corresponds to the flowering of the male flowers present in the bisexual inflorescences. It represents only about 3% of the total amount of emitted pollen (Larue, Austruy, et al., 2021). Interestingly, in European chestnut (*Castanea sativa*), male individuals with various degree of sterility coexist with bisexual individuals. In male-sterile individuals, the sterile male flowers continue to play a role in insect attraction, suggesting that the attractive power of male flowers benefit to female fitness (Larue, 2021).

## Study site and plant material

We surveyed phenology and insect visitors to chestnut trees in 2021 in the INRAE chestnut genetic resource collection located in south-western France (Larue, Barreneche, et al., 2021a). There are two distinct orchards, one planted in 1970 (A) and another located close to the first one and planted in 1990 (E). In orchard E, we selected two clonal copies of four *C. sativa × C. crenata* hybrid varieties, three male-sterile varieties, 'Bouche de Bétizac' (called 'Bétizac' below), 'Marlhac' and CA120, and one male-fertile variety, 'Maridonne'. In orchard A, we monitored three trees belonging to the varieties 'Maridonne', 'Bétizac' and 'Marlhac'. We chose clonal copies that were as distant as possible from each other in the orchard.

## Phenology monitoring

To study chestnut flowering phenology, we randomly selected 30 branches from eight trees growing in orchard E. We monitored them in spring to estimate the percentage of mature male flowers as well as the percentage of receptive female flowers, following Hasegawa et al. (2017). For male flowers of each phase, we visually determined the proportion of open and wilted male flowers. For the female flowers, we examined if the stigmas had emerged and if they had become brownish, marking the end of the period of receptivity. We counted the total number of undeveloped, open and wilted female flowers on each of the 30 branches. We also used a method described in a recent study based on phenological flowering stages (Larue, Barreneche et al., 2021b) on these eight trees and on the three trees studied in orchard A. The phenological stages for male flowers depend on the proportion of open and wilted male flowers. During six weeks in spring 2021, we carried out two measurements per week between 8:30 and 16:00. Two

teams of at least two observers carried out these measurements. We used the same organization for the other observations (see below).

## Insect observations on trees and on female flowers

We monitored insect visits on the eight trees of orchard E and on the three trees of orchard A immediately after monitoring their phenology. We used a non-destructive sampling method that combines direct field determination of insect visitors with a posteriori determination on photographs, following a protocol adapted from the French citizen science programme SPIPOLL (Deguines et al., 2012). We inventoried insect on the flowers, leaves and branches on the accessible parts of a tree ($\leq$ 2m) during 10 min. We photographed insects that we did not readily identified in the field for later identification. We used an APS-C camera (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). All insects photographed were then identified to morpho-species by relying mostly on two websites (*Galerie-Insecte*, 2002; Mathieu & Mathieu, n.d). In the same way, we monitored insects visiting female flowers during 10 min of observation entirely dedicated to that task. To increase the number of observations for this comparatively rare event, we recorded extra-cases of interaction of insects with female flowers during the other phases of monitoring of each tree leading to 45 minutes of observations.

## Data curation and statistical analyses

### Phenology monitoring

To estimate the percentage of mature male flowers emitting pollen during the two staminate phases, I calculated the average percentage of open male flowers minus the percentage of wilted male flowers for each tree. Similarly, I estimated the percentage of receptive female flowers per tree by subtracting the number of open flowers by the number of wilted flowers, divided by the total number of flowers. For missing or inconsistent data such as a backward step in the flowering process, I corrected as far as possible the information or deleted it.

For the trees in plot E, I used the threshold of 10% of open flowers to indicate the onset and end of flowering. I performed a linear interpolation to estimate the Julian day of the beginning and end of flowering for the two staminate phases. For three out of eight trees (E69D, E48F, E50P), the second staminate phase was not fully complete at the end of the survey. To estimate the date of the end of flowering period, I performed a linear extrapolation using the last two phenological measurements. To account for the frequent overlap of the second staminate phase

with the much more massive first staminate phase, I decided to consider only those inventories taking place during the second staminate phase but after the end of the first staminate phase (<10% of pollen-emitting purely male inflorescences). To allow a strict comparison between the two staminate phases, I also reduced the window of the first staminate phase by transposing the resulting window of the second staminate phase. I applied this approach to each tree independently. For trees in orchard A, I estimated staminate anthesis using the phenological stages (Larue, Barreneche, et al., 2021b). The first staminate phase corresponds to scores of purely male inflorescences higher than 63 (>30% of flowers open) and lower than 69 (>50% of inflorescences fallen). The second staminate phase corresponds to scores higher than 67 (>50% wilted flowers) for flowers of purely male inflorescences and between 63 and 69 for male flower of bisexual inflorescences.

### Insect observations on trees and on female flowers

I considered five taxonomic groups of insects for the subsequent analyses: the red soldier beetle (*Rhagonycha fulva*, Coleoptera: Cantharidae)*,* "other beetles" (Coleoptera without the soldier beetle, mostly Coccinellidae, Cleridae, Oedemeridae), hoverflies (Diptera: Syrphidae), "other flies" (Diptera without Syrphidae) and bees (Hymenoptera: Apidae). I did not consider in these analyses other rare insect taxa previously ruled out as regular visitors of chestnut (Larue, Austruy, et al., 2021). I distinguished the red soldier beetle from the other beetles because of its abundance and original behaviour (Larue, Austruy, et al., 2021). I distinguished also hoverflies (Syrphidae) from the other flies because they have specific ecology and behaviour (Dunn et al., 2020). The proposed phenological approach allowed me to contrast the abundance of these taxa between the two staminate phases on trees and on female flowers.

### Statistical analyses

I built all graphs with R studio (v4.1.2) with the packages ggplot2 (v3.3.5, Wickham, 2016) and gridExtra (v2.3, Auguie & Antonov, 2017). To test the difference in insect abundance between staminate phases on trees and on female flowers, I selected varieties with at least two clonal copies, with the condition that there are at least two measurements for each clonal copy performed during each staminate phase. For each insect taxon, I performed a negative binomial family GLMM considering overdispersion of the data. I considered two fixed effects, the staminate phase and the variety, and a random effect corresponding to the tree effect. I also calculated a marginal $R^2$ and conditional $R^2$ using *trigamma* function (Barton, 2022) for each GLMM. I used package lme4 (v1.1-28, Bates et al., 2012) and MuMIn (v1.46.0, Barton, 2022) to realise it.

# Results

## Literature review

I found 24 duodichogamous genera from nine families (Apiaceae, Araliaceae, Cyperaceae, Ecediocolaceae, Fagaceae, Juglandaceae, Meliaceae, Phyllanthaceae and Sapindaceae) that matched with our definition (duodichogamous at the whole plant level). Two genera are wind-pollinated and two have a female-male-female flowering sequence (one of them wind-pollinated). The 21 remaining genera are animal-pollinated with a male-female-male flowering sequence. Most of them are woody plants, especially trees or large shrubs (see **Table S1** for more details).

**Table 1.** Spatial and temporal arrangement of the two types of male flowers in duodichogamous taxa and support for our female hypothesis

| Family | Species | Sexual system | Smallest phase | Shortest phase | Closest to gynoecia in space | Closest to gynoecia in time | Support for hypothesis | References |
|--------|---------|---------------|----------------|----------------|------------------------------|-----------------------------|------------------------|------------|
| Apiaceae | *Dorema aucheri* | A | 1 | = | 1 | 1 | + | Ajani & Claβen–Bockhoff, 2021 |
| Fagaceae | *Castanea spp.* | M | 2 | = | 2 | = | + | Larue, Austruy, et al., 2021 |
| Juglandaceae | *Platycarya strobilaceae* | M | 2 | = | 2 | 2 | + | Fukuhara & Tokumaru, 2014 |
| Phyllanthaceae | *Bridelia retusa* | M | / | 1 | = | = | - | Dias & Ratnayake, 2021 |
| Sapindaceae | *Acer oblongum* | M | 2 | / | / | 2 | + | Yadav et al., 2016 |
| Sapindaceae | *Cupania guatemalensis* | M | 2 | = | 2 | 2 | + | Bawa, 1977 |

[1] 'Smallest phase' = Smallest staminate phase; 'Shortest phase' = idem; 'Closest to gynoecia in space' = Closet staminate phase to female; 'Closest to gynoecia in time' = Idem; 'Support for hypothesis' = Support for female hypothesis.
[2] 'A'= Andromonoecious ; 'M' = Monoecious; '1' = first staminate phase; '2' = second staminate phase; '=' = Indistinguishable; "/" = No information '+' = validate female hitchhiking hypothesis; '-' = invalidate female hitchhiking hypothesis

I found six species with enough details on the two staminate phases to test our predictions on the two staminate phases in relation to the gynoecium (**Table 1**). In *Dorema aucheri* (Apiaceae), the flowers are andromonoecious. The bisexual flowers are protandrous and precede the anthesis of the more numerous male flowers (Ajani & Claβen–Bockhoff, 2021). In the remaining five species, the plants are monoecious. In four of these five species, the second staminate phase is smaller than the first staminate phase. In these four species, the male flowers of the second staminate phase are spatially or temporally closer to the female flowers than the other phase. In contrast, in *Bridelia retusa* (Phyllanthaceae), there is a gap of several weeks between each flowering phase, so the plant is fully temporally dioecious (Dias & Ratnayake, 2021). Hence, five out of six cases support our prediction for a role of one of the two pollen phases in promoting female fitness.

## Flowering and insect monitoring on chestnut trees

### Phenology monitoring

All eight trees of plot E flower in the sequence male-female-male, with considerable overlap of the female phase with both staminate phases (**Figure 1**). The duration of flowering greatly differs across varieties (from 12.7 to 32.4 days), with very similar flowering phenologies for the trees of the same variety. In the male sterile variety 'Bétizac', due to a much shorter second staminate phase and a large overlap between both staminate phases, we had no inventories during the second staminate phase in orchard E. Consequently, I removed all inventories performed on this variety for this study.
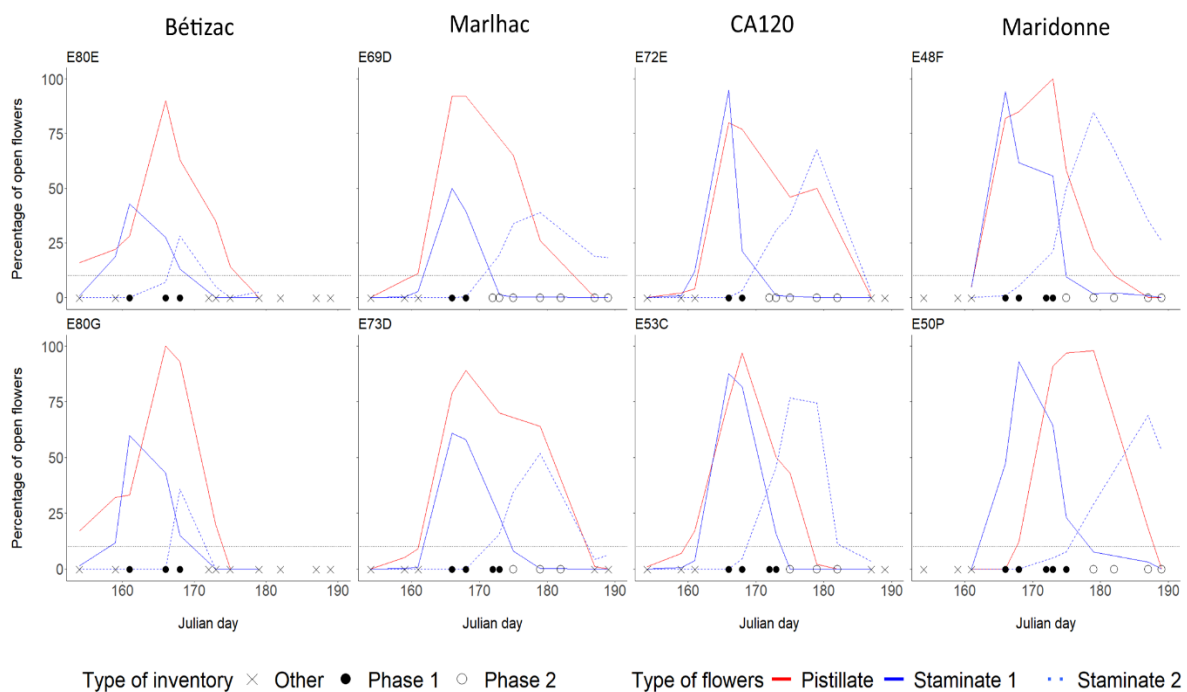


**Figure 1.** Flowering phenology of the eight monitored trees in orchard E. The two clonal copies of each of the four varieties are represented one above the other. The inventories of phase 1 and 2 correspond to inventories during staminate phases 1 and 2. The other correspond to out-of-phase inventories. The 10% flowering threshold is symbolised by a horizontal black dotted line. The blue lines correspond to the percentage of open male flowers of phase 1 (solid) and 2 (dotted). The red lines correspond to the percentage of open female flowers.

### Insect observations on tree and on female flowers

In 2021, we made 105 inventories of insects on trees and on female flowers, corresponding to more than 35 h of insect monitoring. In total, we counted 4061 insects on trees and 239 on

female flowers. These animals belong to nine different orders, mainly diptera, coleoptera and hymenoptera. After removing 'Bétizac', there were 62 inventories left to compare the two staminate phases, including 2792 insect observations on trees and 204 on female flowers. The most abundant insect taxon on trees were the red soldier beetle (on average 21.0 individuals/inventory), followed by "other flies" (10.7) and "other beetles" (7.2). Bees (2.2) and hoverflies (1.2) were much less abundant. On trees, three taxa (red soldier beetle, bees and syrphid flies) were significantly more abundant during the first staminate phase than during the second one ($p < 0.001$) (**Table 2 and Figure 2**). In contrast, the abundance of "other flies" and "other beetles" did not differ between the two staminate phases ($p > 0.05$).

**Table 2.** Results of generalized linear mixed model (GLMM) analysis of difference in taxa abundance on tree according to pollen phases and varieties

| Variable explained | [1]Fixed factor | *F value* | Pr(>Chisq) | Marginal R² | Conditional R² |
|---|---|---|---|---|---|
| **Soldier beetles** | Phases | 11.0 | [2]***<0.05 | 0.36 | 0.71 |
| | Variety | 3.0 | *<0.05 | | |
| **Other flies** | Phases | 1.4 | 0.24 | 0.09 | [3]/ |
| | Variety | 2.9 | 0.06 | | |
| **Other beetles** | Phases | 2.0 | 0.15 | 0.28 | 0.52 |
| | Variety | 3.4 | *<0.05 | | |
| **Bees** | Phases | 12.8 | ***<0.05 | 0.38 | / |
| | Variety | 5.4 | **<0.05 | | |
| **Hoverflies** | Phases | 25.7 | ***<0.05 | 0.44 | / |
| | Variety | 0.2 | 0.80 | | |

[1] Each model was run with the tree variable, representing each individual, as a random factor
[2] * <0.05 ; **<0.005 ; ***<0.001
[3] In these models the random effect is too small

For the five taxa combined, we observed 196 visits to the female flowers during the two staminate phases of the three selected varieties. Visits to female flowers (0.6 visit/inventory) were much rarer than visits to trees (8.5 individuals/inventory) despite the greater time allocated for observations on female flowers (**Figure 2**). The insects observed most frequently on female flowers were red soldier beetles (2.1 individuals/inventory), followed by "other flies" (0.6) and "other beetles" (0.4). Hoverflies and bees rarely visited female flowers (two observations in each case). The number of observations on female flowers increased during the second staminate phase for "other flies" ($p < 0.001$) (**Table 3 and Figure2**). For red solider beetles and "other beetles", I did not observe any change in the visits to female flowers between the two staminate phases ($p > 0.05$). Due to the too low numbers of interactions with female flowers, I did not test that effect for bees and hoverflies. Contrasting the number of visits to female flowers and to trees during the two staminate phases, we note a clear increase of insects visit to female flowers during the second staminate phase for red soldier beetle and "other flies". For soldier beetles, the female/tree visits ratio is 0.06 during the first staminate phase and 0.16 during the

second staminate phase. For the other flies, the ratio is 0.02 during the first phase and 0.82 during the second. For other beetles, the difference is less marked, with a ratio of 0.05 during the first phase and 0.07 during the second (**Figure 2**). Other factors that affect insect abundance include chestnut variety and the random effect of the clonal copy of the tree (**Table 2 and Table 3**).
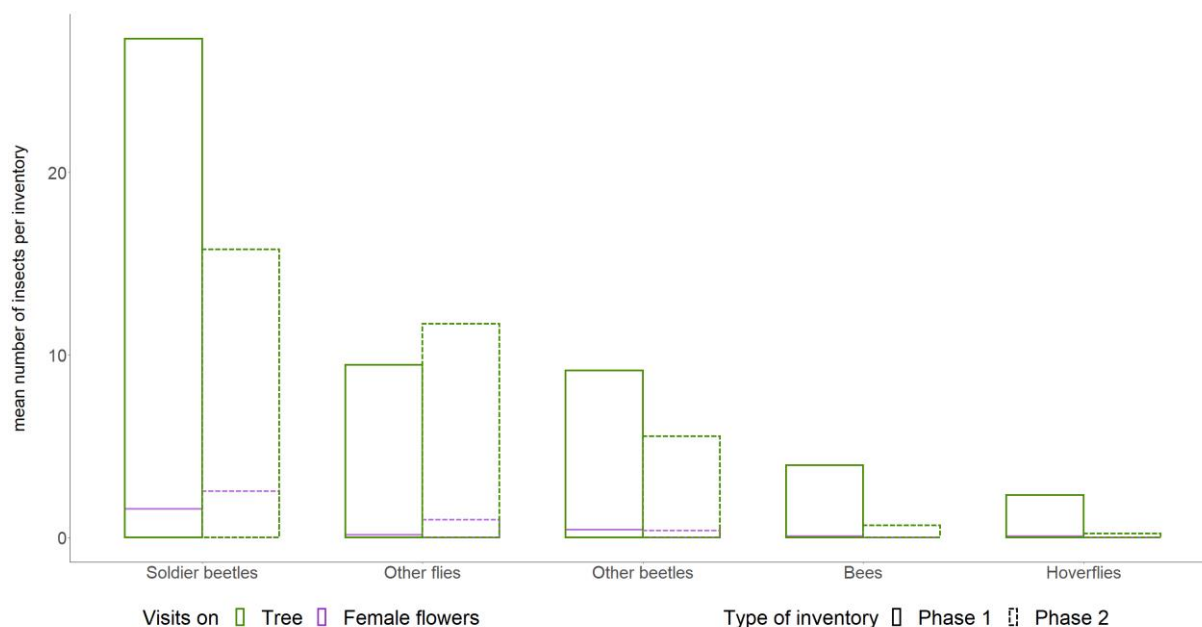


**Figure 2.** Mean number of insects per inventory observed on chestnut trees and its female flowers during anthesis of male flowers from purely male inflorescences (phase 1) and from bisexual inflorescences (phase 2).

**Table 3.** Results of generalized linear mixed model (GLMM) analysis of difference in taxa abundance on female flowers according to phases and variety.

| Variable explained | [1]Fixed factor | *F value* | Pr(>Chisq) | Marginal R² | Conditional R² |
|---|---|---|---|---|---|
| **Soldier beetles** | Phases | 2.7 | 0.09 | 0.27 | / |
| | Variety | 12.8 | [2]***<0.05 | | |
| **Other flies** | Phases | 11.3 | ***<0.05 | 0.12 | / |
| | Variety | 0.0 | 1.00 | | |
| **Other beetles** | Phases | 0.40 | 0.55 | 0.05 | 0.13 |
| | Variety | 1.05 | 0.32 | | |

[1] Each model was run with the tree variable, representing each individual, as a random factor
[2] * <0.05 ; **<0.005 ; ***<0.001
[3]In these models the random effect is too small

# Discussion

Our results on duodichogamous taxa support the female hitchhiking hypothesis. According to the definition of Luo et al. (2007) I identified more duodichogamous genera than previous reports (Endress, 2020; Renner, 2014), even if the total number remains small. Most species have a male-female-male flowering sequence (Lloyd & Webb, 1986; Luo et al., 2007). They are insect-pollinated trees or shrubs that experience a high risk of self-pollination due to their large size and massive synchronous flowering (Harder & Prusinkiewicz, 2013; Wang et al., 2020). I identified six well-described duodichogamous species from five different families. The authors often assume that the apparition of a second pollen emission phase evolved to increase male fitness (Lloyd & Webb, 1986). However, I identified only one monoecious species (*Bridelia retusa*) that seems to comply with this hypothesis. Its female flowers are fully separated in time from the male flowers and are as attractive as the male flowers (Dias & Ratnayake, 2021), illustrating the need for female flowers to invest in flower attractiveness when gynoecia is disjoint from the male function. In contrast, five species fully support predictions of the "female hitchhiking hypothesis". First, they have asymmetric staminate phases. Second, the smaller staminate phase corresponds to pollen produced by stamens located close to the pistil in space or in time. In addition, based on the descriptions of the authors, it appears that the female flowers are less attractive than the male flowers (Bawa, 1977; Fukuhara & Tokumaru, 2014; Larue, Austruy, et al., 2021; Yadav et al., 2016). Ajani & Claβen–Bockhoff (2021) and Yadav et al. (2016) both argue that the small pollen phase could enhance female fitness in their respective model species. However, direct evidence for such an effect is still missing.

Our results on chestnut fill this gap. We identified three insect groups involved in chestnut pollination, confirming a previous study (Larue, Austruy, et al., 2021). We then showed that visits of pollinating insects to female flowers increase during the second pollen phase, most likely due to the greater spatial proximity of the corresponding male flowers with the female flowers. These results are consistent with those of a previous study on male-sterile chestnut trees. Following the experimental removal of the sterile but attractive male flowers, fruit set significantly decreased in two of the three studied varieties, suggesting that the attractive "male" structure benefits to female fitness (Larue, 2021). Our observations and the previously reported experiment support the female hitchhiking hypothesis but do not exclude a role for this second pollen phase in male fitness (Lloyd & Webb, 1986; Luo et al., 2007).

The benefit of juxtaposition of the gynoecium with the androecium for female fitness is particularly striking when we consider another selective force that tends to decouple both sexual functions, the avoidance of self-pollination (Bertin, 1993; de Jong et al., 2008; Harder & Prusinkiewicz, 2013). The deleterious effects of coupling can be important for both sexual functions, resulting in either pollen discounting or ovule discounting (Barrett, 2002a), considered to be especially important in trees (Harder & Prusinkiewicz, 2013; Wang et al., 2020). In plants with an abiotic mode of pollination, these two antagonistic forces do not co-exist, only the selective force of self-pollination exerts selective pressure on sexual arrangement. This commonly results in sexual segregation (Harder & Prusinkiewicz, 2013), as shown by the greater prevalence of monoecious and dioecious plants in abiotic-pollinated plants than in animal-pollinated plants (Barrett, 2002b; de Jong et al., 2008; Renner & Ricklefs, 1995). In the same way, a regional study has shown that gynomonoecy is more frequent in abiotic-pollinated species (de Jong et al., 2008), probably because there is no more selective pressure by pollinators for the juxtaposition of gynoecia with androecia. The Asteraceae, which are mostly animal-pollinated, represent an exception to this trend, as gynomonoecy is common in this family. In fact, Asteraceae are characterized by a single bisexual pollinator attraction unit (Cronk, 2022; Lloyd, 1979), retaining the advantage of the juxtaposition of both functions. Similarly, some monoecious animal-pollinated species exhibit bisexual units of attraction (Baker & Hurd, 1968), which tend to benefit to female fitness, as demonstrated in chestnut.

In animal-pollinated plant, there seems to be an optimal balance between the two antagonistic selection forces, so that the attractiveness of the androecia benefits to the juxtaposed gynoecia while limiting self-pollination. Other sexual strategies considered to have evolved to limit self-pollination, including herkogamy, dichogamy or prezygotic self-incompatibility (Barrett, 1998, 2003; Lloyd & Webb, 1986), could in fact also result from the action of these antagonistic selective forces. A similar trade-off has been found for inflorescence size, with large inflorescence attracting more pollinators and small inflorescences limiting self-pollination (Harder & Prusinkiewicz, 2013).

## Conclusion

Our results on duodichogamous species confirm those obtained on andromonoecious species (Tomaszewski et al., 2018; Vallejo-Marin & Rausher, 2007). These two sexual systems, considered so far to be "male" strategies, turn out to be important for female fitness. We argue that the importance of androecia for pollen import and not only export could be an important

driver in the evolution of the sex distribution in plants and should be more widely included in evolutionary studies of plant sexual systems.

In the future, to confirm our female hitchhiking hypothesis, reviewing sex distribution in plants both at the flower and at the inflorescence level would be useful to gain a better understanding of the forces underlying sex distribution across species. In animal-pollinated plants, focusing on pollinator attraction units (Bell, 1985) would be particularly relevant to test the hypothesis. Another sexual strategy that could be revisited is dichogamy (Lloyd & Webb, 1986). Wind-pollinated plants are often protogynous, whereas animal-pollinated plants tend to be protandrous. Considering that protogyny is sometimes described as the best adaptation to avoid self-pollination, the prevalence of protandry among animal-pollinated dichogamous plants (Mallick, 2001) might indicate an advantage of producing attractive male structures first, as a signal to pollinators. This could facilitate pollen import later when the plant is in its female phase, by taking advantage of the cognitive power of pollinators (Van der Ham, 1990).

Overall, our study shows that pollination mode is relevant to our understanding of sex distribution in plants, as argued early on by Bawa & Beach (1981). In animal-pollinated plants, avoidance of self-pollination will favour separate sexes, whereas female hitchhiking will tend to keep them coupled. Together, these two antagonistic forces could have driven the remarkable diversification of sexual strategies in angiosperms, as predicted by theory when sexual selection and conflict coexist (Bonduriansky, 2011).

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## Appendix

**Table S1: List of duodichogamous taxa with main traits.**

| [1]Family | *Genus* | L-F | P | Sq | References |
|---|---|---|---|---|---|
| Apiaceae | *Dorema* | [2]S | Bi | (MF)M | Ajani & Claβen–Bockhoff, 2021 |
| Araliaceae | *Delarbrea* | H | Bi | (MF)M | Schlessman et al., 1990 |
| Araliaceae | *Myodocarpus* | T | Bi | MFM | Schlessman et al., 1990 |
| Araliaceae | *Schefflera* | T | Bi | (MF)M | Schlessman et al., 1990 |
| Cyperaceae | *Cladium* | H | Ab | M(FM) | Snyder & Richards, 2005 |
| Ecdeiocoleaceae | *Ecdeiocolea* | H | Ab | FMF | Briggs & Tinker, 2014 |
| Fagaceae | *Castanea* | T | Bi | MFM | Stout 1928; Larue, Austruy, et al., 2021 |
| Juglandaceae | *Platycarya* | T | Bi | MFM | Fukuhara & Tokumaru, 2014 |
| Meliaceae | *Toona* | T | Bi | MFM | Lee et al., 2018 |
| Phyllanthaceae | *Bridelia* | T | Bi | MFM | Dias & Ratnayake, 2021, Singapore Government, 2022 |
| Phyllanthaceae | *Cleistanthus* | T | Bi | MFM | Li et al., 2014 |
| Sapindaceae | *Acer* | T | Bi | MFM | De Jong, 1976; Yadav et al., 2016 |
| Sapindaceae | *Aesculus* | T | Bi | MFM | De Jong, 1976 |
| Sapindaceae | *Cupania* | T | Bi | MFM | Bawa, 1977 |
| Sapindaceae | *Deinbollia* | T | Bi | MFM | De Jong, 1976 |
| Sapindaceae | *Diatenopteryx* | T | Bi | MFM | Acevedo-Rodríguez et al., 2017 |
| Sapindaceae | *Dipteronia* | T | Bi | MFM | De Jong, 1976 |
| Sapindaceae | *Guindilia* | T | Bi | MFM | Acevedo-Rodríguez et al., 2017 |
| Sapindaceae | *Guioa* | T | Bi | MFM | Van Welzen, 1989 |
| Sapindaceae | *Koelreuteria* | T | Bi | FMF | De Jong 1976; Avalos et al., 2019 |
| Sapindaceae | *Paullinia* | S | Bi | MFM | De Lima et al., 2016 |
| Sapindaceae | *Sapindus* | T | Bi | MFM | Mahar et al., 2011 |
| Sapindaceae | *Serjania* | S | Bi | MFM | Ferrucci & Steinmann, 2019 |
| Sapindaceae | *Urvillea* | L | Bi | MFM | Zapata & Arroyo, 1978 |

[1]Heading : L-F = Life form ; P = Pollination system ; Sq = Sequence of duodichogamy.
[2]H = Herbaceous; S = Shrub, T= Tree; Bi = Biotic ; Ab = Abiotic; ()= Same flower; MFM = Male-Female-Male ; FMF = Femelle-Male-Femelle.

## Contributions

## Remerciements

# Summary

The diversity of spatial arrangements of sexes in angiosperms is impressive. In this study, I explore the possible role of pollination mode on plant sexual system diversification, focusing on the contrasted investments for pollinators attraction needed to optimize male versus female fitness. I propose the "female hitchhiking hypothesis" to emphasize that the asymmetric rewards (pollen) and consequences of sexual selection for male and fitness success will favour sexual arrangements in which pollen import passively benefits from male attractiveness. To test this hypothesis, I used two complementary approaches, focusing on duodichogamous taxa. Duodichogamy is a rare sexual system in which plants present two pollen emission phases separated by a phase of pollen reception (i.e. male-female-male flowering sequence). First, I explore the potential role of each of the two staminate phases on female fitness by analysing the sexual arrangement of duodichogamous species described in the literature. I find evidence for an association of one of the two staminate phases with female structures in five out of six duodichogamous species investigated, suggesting that it could play a role in female fitness. Second, to test further this hypothesis, we performed field studies on chestnut tree, a classic model for duodichogamy. We monitored insect visits to deceptive female flowers during each of the two staminate phases. I find increased visitation rates to chestnut female flowers during the second much smaller staminate phase. I argue that this staminate phase enhances pollen import while limiting self-pollination. Overall, my results suggest that selective forces exist that maintain gynoecia close to androecia, despite the opposite selection pressure caused by the necessity to limit self-pollination. Hence, it is important to consider these two antagonistic selective forces to understand the distribution and evolution of sexual systems in animal-pollinated plants.