

Impact of plant flowering phenology on the cost/benefit balance in a nursery pollination mutualism, with honest males and cheating females

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Abstract

This study documents the flowering phenology and its potential consequences on a nursery pollination mutualism between a dioecious plant, in which honest male plants, but not cheating females, allow the specific pollinator to reproduce within inflorescences. Very few pollinators were found to emerge during plant anthesis, leading to a low (if any) potential benefit through pollen dispersal. This opens the question why male plants do not also cheat their pollinators. Female plants flowered late in the season, when many males had just achieved their own anthesis, which increased the efficiency of pollen transfer. Finally, some late-flowering males reached their anthesis simultaneously with females, which open the possibility for pollinator to choose between honest males and cheating females. Nevertheless, female plants were found to produce fruits, even though fruit production was limited by pollen (and pollinator) supply, meaning that cheating was not entirely retaliated by the mutualistic partner.

Introduction

Mutualisms are reciprocal exploitations in which each partner is selected for increasing its own benefits and decreasing its costs (Leigh & Rowell, 1995; Maynard Smith & Szathmáry, 1995). This opens ample opportunity for individuals to exploit resources without providing any reward in return. Such cheating strategy can be facultative, with individuals that may either be 'cheater' or be 'honest', according to the circumstances (Tyre & Addicott 1993; Addicott & Tyre, 1995; Bshary & Grutter, 2002). In other cases, cheating behaviour varies either at the intra-specific (Bao & Addicott, 1998) or at the inter-specific level (Pellmyr *et al.*, 1996; Després & Jaeger, 1999). A cheating strategy, genotype or species can be 'punished' if the partner can selectively direct benefits towards cooperators or selectively finishing interactions with cheaters. However, if cheating invades, this may

cause a breakdown of mutualism, driving the interaction either towards parasitism or towards extinction (Axelrod & Hamilton, 1981; Soberon & Martinez del Rio, 1985; Bull & Rice, 1991; Bronstein, 1994a; Pellmyr & Hutch, 1994; Doebeli & Knowlton, 1998; Herre *et al.*, 1999; West *et al.*, 2002; Kiers & Denison, 2008). One major question about the dynamics of inter-specific interactions thus concerns the factors that cause the shift in the cost/benefit balance for both partners, along the continuum between parasitism and mutualism.

Nursery pollination mutualisms, between a plant and a specific pollinator that reproduces within inflorescences, have proved to be highly instructive for understanding the evolutionary processes acting on mutualistic partners and cheating dynamics. Because at least one of the partners often pays a high fitness cost (inflorescences can be destroyed by pollinator larvae, and/or pollinator larvae can be killed by some individual host plants), selection for cheating is expected to be strong (for a review see Dufay & Anstett, 2003). In these interactions, the timing at which partners encounter and interact, i.e. the precise time at which plants produce flowers, compared to pollinator phenology, seems to be crucial

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for the cost/benefit balance and for the evolutionary stability of the interaction. For example, in fig/fig wasp mutualisms, protogyny within fig inflorescences allows female pollinators to lay eggs within receptive ovules, and larvae to complete their development just before the male phase, ensuring that pollen is dispersed by the pollinators' offspring. In this case, the high cost paid by the female function is thought to be counterbalanced by the benefit for male function, through pollen dispersal (reviewed in Anstett *et al.*, 1997). In contrast, in some other nursery pollination mutualisms, the cost of having inflorescences destroyed by pollinator larvae seems not to be compensated and nursery pollinators act almost as cheaters (e.g. in *Silene/Hadena*, reviewed in Kephart *et al.*, 2006). Finally, in *Yucca/Yucca* moths and globe-flower/globeflower flies mutualisms, some of the insect species are specialist cheaters since they emerge later than pollinators and lay eggs directly into fruits, leading to high costs and no benefit (Pellmyr *et al.*, 1996; Desprès & Jaeger, 1999). The timing of flower production by the plant and of emergence of pollinators from the inflorescences seem thus to often define the global outcome of the interactions.

The present work aims to study a nursery pollination mutualism between the dioecious dwarf palm *Chamaerops humilis* and its obligatory specific nursery pollinator, the weevil *Derelomus chamaeropsis*, in which cheating occurs within the plant species but differs between males and females. While male plants provide pollinators a brood-site, female plants cheat by destroying pollinators eggs laid within their inflorescences (Dufay & Anstett, 2004). The killing of pollinator larvae by female plants might function to defend their rachises, which, in contrast to rachises of male plants, must support the weight of developing fruits and provide the vascular supply to fuel their development. Direct retaliation of cheating females by pollinators is not possible *sensu stricto*: because larval destruction occurs after pollinators have left the plant, pollinators have no possibility to learn to avoid deceptive female plants or to leave their inflorescences earlier (Dufay & Anstett, 2004). However, any innate preference for male plants should be strongly selected for, but such pollinator behaviour could lead to a rupture of pollen flow. Thus, if cheating female plants were 'punished' by their pollinators, this could ultimately lead to both species extinction. In a previous study, females have been shown to be less often visited than males (Dufay & Anstett, 2004), but nothing is known about the consequences of this difference for pollination efficiency in females.

This study attempts to investigate the evolutionary causes and consequences of sex-specific cheating within the palm/weevil mutualism, with a particular interest for flowering phenology. The focus on flowering phenology had two main reasons. First, withholding a brood-site to pollinators could confer to male plants a benefit through greater pollen dispersal by pollinator offspring, if these

offspring emerge synchronously with pollen production, and explain why male plants do not cheat their pollinating partner. Second, because any opportunity to choose the sex of their host plant (and 'punish' females), should be selected for in pollinators, the flowering phenologies of male and female plants, as whether or not they are synchronous, are of critical importance. In particular, I ask the following questions:

- (1) Do new pollinators emerge from male plants during anthesis, potentially providing a benefit of pollen dispersal to their host plants ?
- (2) Are male and female plants asynchronous in their flowering, or is there an overlap between their anthesis, enabling pollinators to choose the sex of their host plant, and consequently avoid visiting deceptive females ?
- (3) If so, do female plants nevertheless produce fruits ? Is fruit production limited by the availability of pollen and of pollinators ?

I then discuss these results in the light of their consequences in terms of the evolution of cheating and consequences for mutualism stability.

Material and methods

Study species

The dwarf palm (*Chamaerops humilis* L., Arecaceae) is a dioecious palm growing on the Mediterranean coasts of Europe and North Africa. It is a shrub-like clumping palm, with several stems growing from a single base. The stems (or trunks) grow slowly and often tightly together, eventually reaching 2–5 m height. Flowers are borne in dense inflorescences that develop within a single enlarged bract. This prophyll covers the flowers until the sexual phase (anthesis) and then splits open apically into two triangular lobes. The number of flowers per inflorescence is highly variable for both male and female plants, depending on the size of the inflorescence. Female flowers are tri-ovulate.

The dwarf palm is engaged in a nursery pollination mutualism with a specific weevil (*Derelomus chamaeropsis* F., Curculionidae). At anthesis, both male and female plants attract pollinators with floral-like chemical compounds that are released by leaves, and not flowers (Dufay *et al.*, 2003). Once pollinating weevils have found a plant (either male or female), they typically stay on the same plant until the end of its anthesis, finding shelter, egg-laying sites and food within inflorescences. When the host plant reaches the end of anthesis, weevils leave the plant and forage to find a new host plant, either male or female. Larval development occurs within rachises of inflorescences of male plants during autumn and winter. At the beginning of the next flowering period, adult weevils emerge from the dry and brittle stems of old inflorescences of the previous year of male plants. Weevils were showed to lay eggs within female

inflorescences, but as soon as seeds start to develop, eggs or larvae are destructed (Anstett, 1999; Dufay & Anstett, 2004).

Flowering phenology and fruit production

This study was performed in a natural population of *C. humilis*, in the *Parque Natural de Garraf*, south of Barcelona (Spain) during spring 2000. In this population, 49 male plants (97 trunks) and 59 female plants (104 trunks) were marked and followed for their flowering phenology. From 15 March to 2 May 2000, flowering phenology was checked every 2 days. For each plant, the number of trunks bearing inflorescences and the number of inflorescences per trunk were recorded. Each inflorescence was individually marked and recorded for its phenology every 2 days, according to three phenological stages: (i) Closed floral bracts: inflorescences have emerged but are still enclosed within floral bracts and weevils cannot enter them; (ii) Anthesis: floral bracts are opened; male flowers produce pollen, and female flowers are receptive; (iii) End of flowering: flowers of male plants are faded and their inflorescence rachises are starting to dry; stigmas of female flowers turn brown and their ovaries become swollen and green. On 24 May, the number of fruits and seeds was counted on all inflorescences of female plants. Because inflorescences are very dense, it was impossible to reliably measure the exact number of (tri-ovulate) flowers, and thus of ovules per inflorescence, during anthesis. Seed set (as the seeds/ovules ratio) could thus not be directly estimated in this study. Female mating success and an estimate of the efficiency of pollination were measured by recording the total number of seeds, as well as the number and the proportion of fruits containing one, two or three seeds.

Plant investment in flowering (number of flowering trunks and number of inflorescences), as well as plant flowering phenology (duration of plant anthesis, first day and last day of plant anthesis) was compared between sexes, using a linear model (proc GLM, SAS). I also analysed the duration of anthesis at the inflorescence level, testing for a sex effect and for a plant effect that was nested within sex. For this analysis, sex was coded as a fixed factor, and plant was coded as a random factor. Regarding the timing of phenology, seven plants (3 males and 4 females) were excluded from the dataset because of their low vigour (e.g. destroyed or heavily damaged by herbivores). Finally, the number of seeds, as well as the number and the proportions of fruits containing one, two and three seeds, was analysed using proc GLM (SAS), the proportions being arcsin-square root transformed to achieve a normal distribution. I analysed whether fruit and seed production depended on plant investment in flowering (i.e. number of flowering trunks and of inflorescences) and on its flowering phenology (duration, first day and last day of anthesis). For all analyses, I checked for normality of residuals

(Kolmogorov-Smirnov, $P > 0.15$ for all tests presented in this study).

Weevil emergence from old inflorescences of male plants

Forty of 49 marked male plants bore old dry rachises from the previous year's inflorescences. On 15 March, all these rachises were cut at their base and enclosed in a mesh bag. Each bag containing all the old rachises of a given trunk was put on the soil, less than 30 cm under the usual location of the rachises. Thus, enclosed rachises experienced temperature and humidity conditions similar to those they would have experienced if they had still been on the trunk. From this date on, every 2 days, the bags were opened, and weevils that had emerged from the old rachises were collected and counted. On 28 April, at the end of the phenological observations, all rachises were dissected. Adult weevils, larvae and pupae still within the rachises were collected and counted.

The proportion of weevils that emerged from the old rachises at the different phenological stages of their host plant was analysed and compared between each other, using a logistic regression (binomial distribution and logit link function, proc GENMOD, SAS; overdispersion of data was corrected for using the dscale option). For this analysis, only plants that hosted at least one weevil and for which floral phenology had been entirely recorded were investigated ($N = 29$). Weevil emergence and male host plants' phenology were analysed as a factor of climatic conditions, average temperature per day and precipitations for the study period, which were provided by the meteorological station located in the *Parque Natural de Garraf*.

Pollen supplementation

Among the female plants followed for their flowering phenology, we chose 20 individual plants that bore more than one inflorescence, with all inflorescences at early phenological stages. On each of the 20 plants, two inflorescences of similar size and phenology were marked just before they reached anthesis. From 14 April to 28 April, as soon as the inflorescences emerged from their prophyll, one of the marked inflorescences was hand-pollinated with a mixture of pollen grains, collected on 10 male plants in the population. Inflorescences were hand-pollinated every 2 days, until the stigmas turned brown. The other marked inflorescence was used as a control. Fruit production (number of seeds, number and proportion of fruits containing one, two and three seeds) was then compared between manipulated and control inflorescences, using nonparametric pairwise tests (Wilcoxon signed-rank test). In *C. humilis*, the size of inflorescences is strongly correlated with the number of flowers (M.-C. Anstett, unpublished). To minimize the effect of variation in the number of flowers per

inflorescence on seed number, I chose one control and one manipulated inflorescence of the same size on each focus plant.

Significant differences in seed production between hand-pollinated and control inflorescences on the same plant could be because of a compensation for seed production, a classical source of bias in pollen supplementation experiments (Ashman *et al.*, 2004). To investigate this possible mechanism, I analysed variation in seed production on all inflorescences that were followed in the population that reached anthesis during the same period as manipulated inflorescences (from 14 April to 28 April). Inflorescences were then classified into the following four categories: (i) hand-pollinated inflorescence; (ii) control inflorescence on the same trunk as a manipulated inflorescence; (iii) control inflorescence on the same plant, but on a different trunk as a manipulated inflorescence; (iv) control inflorescence on a nonmanipulated plant. Fruit production was compared among these different categories, testing as well for an effect of the first day of anthesis, to remove any bias because of an overall difference in flowering phenology.

Results

Weevil emergence from old male inflorescences

Of the 40 male plants with old rachises that had been bagged, 30 had rachises that hosted weevil pollinators. The rachises in which no weevils were found could either have hosted no weevils during winter (either because no eggs were laid in the rachises during the previous spring or because eggs that were laid did not develop) or have hosted weevils that emerged before this experiment began. A total of 540 adult weevils were collected in the mesh bags during this experiment; 17 additional individuals (3 adults, 10 larvae and 4 pupae) were collected on 28 April during dissections of rachises. From 1 to 114 weevils were recorded per male plant (13.9 ± 20.2). Because weevil emergence apparently started before the beginning of the survey, the number of weevils hosted by male plants was probably under-estimated. For this reason, I decided to not perform statistical analyses on this variable.

Weevil emergence from the old rachises varied according to the host plant phenology (logistic regression: $\chi^2_{2,84} = 1147$, $P < 0.0001$). The probability of weevil emergence from old rachises significantly decreased between the first stage (all new inflorescences enclosed in floral bracts) and anthesis and again between anthesis and the end of flowering (contrast analyses, $P < 0.0001$ for all comparisons). This analysis is based on 516 weevils; one plant on which 24 weevils were collected was excluded from the analysis because the day on which its floral bracts opened was not recorded. Of all 516 weevils, 484 (94%) emerged before the new inflorescences of their host plant had opened their floral

bracts. The average delay between emergence of these weevils and the day when the first new inflorescence of the host plant reached anthesis was 23 days. Only 31 weevils emerged from the rachises at a moment when at least one inflorescence of their host plant was at anthesis. Emergence of some pollinators during anthesis was observed on 10 male plants, which were not found to bear significantly more or less trunks or old rachises than the other followed male plants (proc GLM, $P > 0.2$). Finally, the 31 weevils that emerged during their host plant's anthesis represented only 15% on average of the total number of weevils hosted by these 10 host plants.

At the population level, the number of weevils collected per field session (every 2 days) globally decreased over time, but eight increases in weevil emergence were recorded (i.e. for eight sessions, the number of collected weevils exceeded the number of weevils collected at the previous session, Fig. 1A). Each time rainfall occurred the day before a session of weevil collection, I observed such an increase in weevil emergence (two-tailed Fisher exact test: $P < 0.0001$; Fig. 1A), probably because humidity renders the tissues of old rachises of inflorescences more flexible, facilitating emergence of the insects. Among the 30 male plants that

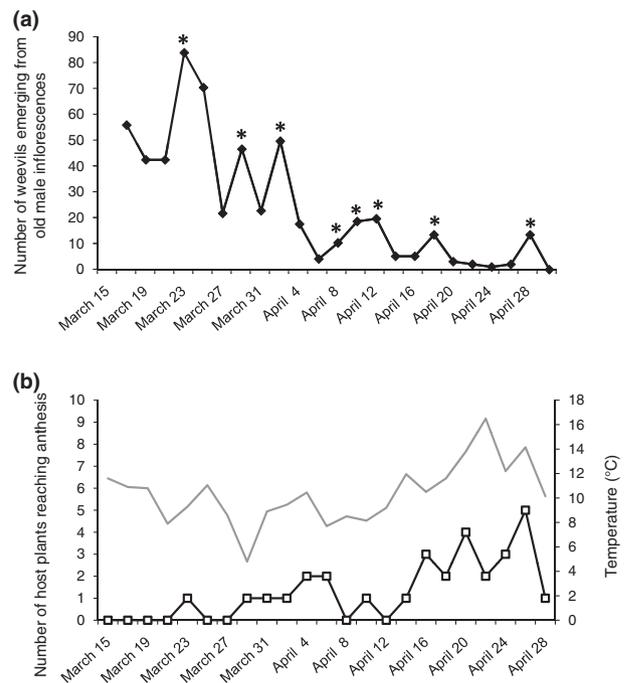


Fig. 1 Asynchrony between weevil emergence and their host plants' phenology. (a) the number of weevil that emerged on each date from old male inflorescences on the 30 surveyed male plants. (*) indicates that rain was recorded the day before the field session. (b) the flowering phenology of the 30 male host plants; open black squares indicate the number of male host plants that reached anthesis on each date; the grey line shows average day temperature.

hosted weevil larvae, the proportion of plants that started their anthesis at a given field session did not depend on the occurrence of rainfall the same day nor the day before ($P > 0.1$). However, this proportion of male plants starting their anthesis was positively affected by temperature (proc GENMOD, $\chi^2_{1,20} = 10.07$; $P = 0.0015$; Fig. 1B).

Flowering phenology of male and female plants

Chamaerops humilis plants had 1–7 flowering trunks and produced from 1 to 38 inflorescences. Male plants did not have significantly more trunks (Proc GLM, $P > 0.1$) but did produce significantly more inflorescences than female plants (average number of inflorescences \pm SD in male and female plants: 7.24 ± 5.6 and 4.47 ± 3.6 ; $F_{1,107} = 9.7$; $P = 0.0024$). At the inflorescence level, anthesis lasted from 2 to 12 days; this duration significantly varied among individuals (plant effect, as a random factor nested within sex: $F_{95,461} = 8$; $P < 0.0001$) but did not differ between sexes ($F_{1,461} = 1.06$; $P > 0.1$). At the plant level, however, male plants were at anthesis (i.e. carried at least one inflorescence with open flowers) for a significantly longer period of time than females (9.6 ± 4.8 days in male plants compared to 7.4 ± 3.6 days in female plants; $F_{1,100} = 5.46$; $P = 0.02$). This difference was because of the fact that male plants bore a larger number of inflorescences (cf. above), which did not reach anthesis simultaneously. Finally, at the plant level, male plants reached anthesis significantly earlier than female plants ($F_{1,100} = 16.31$; $P < 0.0001$). No significant sex difference was found, however, for the timing of the end of anthesis ($F_{1,100} = 2.32$; $P = 0.13$), indicating that the occurrence of flowering males within the population lasted longer than the occurrence of flowering females (Fig. 2A).

Male plants showed very high inter-individual variability for the timing of anthesis. The first day of anthesis varied from before March 14th to April 30th. Two peaks of flowering in male plants were recorded, the first one on April 4th and a second one on April 26th (Fig. 2). Among the 46 followed male plants, 20 individuals (11 early flowering and 9 late-flowering) had been qualitatively recorded for their flowering phenology in 1997, during preliminary observations. I classified these plants into two groups, according to their phenology in 1997: (i) anthesis before the end of March 1997; (ii) anthesis at the beginning of April 1997. Plants that flowered early in 1997 (group 1) were found to belong significantly more often to the early flowering category in 2000, compared to those that flowered later in 1997 ($\chi^2 = 5.3$; $df = 1$; $P = 0.02$). Male plants also showed great variation for the date at which anthesis ended, the last day of pollen production varying from 27 March to 2 May. Female plants were more synchronous in their flowering phenology. Female flowering showed a simple unimodal distribution (Fig. 2A); the first day of anthesis in females

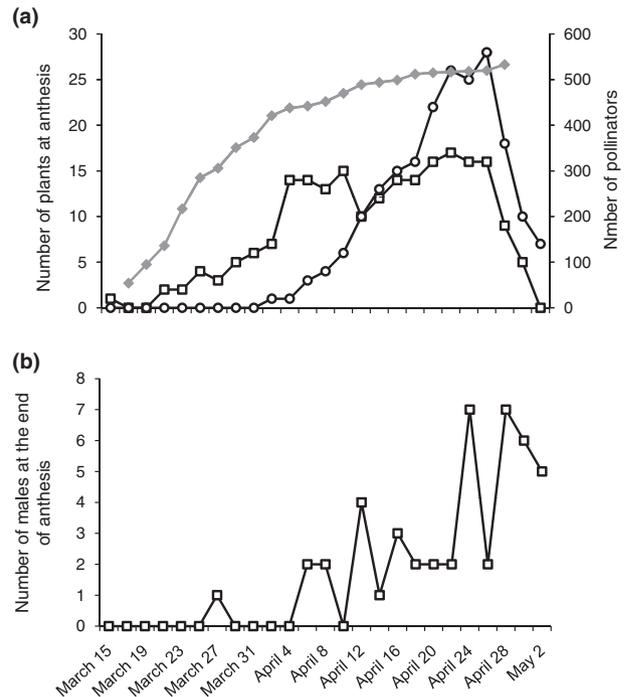


Fig. 2 Flowering phenology of male and female plants. (a) the number of male (open square symbols) and female plant (open circles) at anthesis at each date; as well as the number of pollinator weevils within the population (grey diamond symbols), estimated as the cumulated number of weevil that have emerged from old male inflorescences. (b) the number of male plants that reached the end of their anthesis at each date, estimating the number of male plants that released pollinators covered with pollen.

varied from 8 April to 2 May, with a peak of flowering occurring on 26 April.

Seed production by female plants

Nonmanipulated female inflorescences produced from none to 91 fruits, which represented from 0 to 203 seeds (52.6 ± 35.5 seeds per inflorescence). As the number of inflorescences per plant was highly variable within the population, the total seed production also showed an important inter-individual variation (from 0 to 1191 per plant; average seed production = 235 ± 244). Proportions of these one-seeded, two-seeded and three-seeded fruits within an inflorescence were highly variable as well (from 0 to 83%; from 0 to 77% and from 0 to 100% of fruits containing one, two and three seeds, respectively).

None of the factors linked with plant size (total number of inflorescences and number of flowering trunks) had an effect on the average number of fruits per inflorescence, nor on the average numbers and proportions of fruits containing one, two and three seeds. Neither the day at which females reached anthesis, nor

the functional sex ratio (ratio of males vs. females at anthesis), this same day affected these different estimates of seed production. The number of males that ended their pollen production, thus potentially exporting pollinators covered by pollen, was recorded each day. Thus, each group of female plants that started their anthesis on a given day could be associated with such a number of males, finishing their own anthesis. This variable was found to significantly decrease the average proportion of fruits containing one seed ($F_{1,51} = 4.23$; $P = 0.04$), while increasing the average proportion of fruits with three seeds ($F_{1,51} = 4.31$; $P = 0.04$). This number of males that reached the end of their anthesis was highest on April 12th, April 24th and from April 28th to May 2nd (Fig. 2B), all these dates belonging to the main peak of female flowering.

Pollen supplementation

Hand-pollinated inflorescences produced significantly more seeds than their paired control inflorescences (94.05 ± 40.21 against 69.23 ± 38.97 ; Wilcoxon signed-rank test: $N = 20$; $T = 2.8$; $P = 0.01$). Same results were found for the number of fruits containing three seeds and two seeds (Wilcoxon signed-rank test, 15.3 fruits with three seeds in hand-pollinated inflorescences vs. 10.8 in controls, $N = 20$; $T = 2.7$; $P = 0.01$; 17.2 fruits with two seeds in hand-pollinated inflorescences vs. 12.7 in controls, $N = 20$; $T = 1.8$; $P = 0.02$), and a marginal difference was found for the proportion of fruits containing three seeds (Wilcoxon signed-rank test: 34.07% of fruits contained three seeds in hand-pollinated vs. 30.13% in controls, $N = 20$; $T = 1.5$; $P = 0.09$).

By including in the analyses, all inflorescences having reached anthesis between April 14th and 28th, I found that the number of seeds varied with inflorescence category ($F_{3,207} = 6.87$; $P = 0.0002$): hand-pollinated inflorescences (category 1) produce significantly more seeds than all other types of inflorescences (Tukey test, $P < 0.05$), whereas other categories of inflorescences [i.e. (ii): control inflorescence on the same trunk as a manipulated inflorescence; (iii) control inflorescence on the same plant, but on a different trunk as a manipulated inflorescence; (iv) control inflorescence on a nonmanipulated plant] were not significantly different from each other (Tukey test, $P > 0.1$). Similar results were found for the number of fruits containing three seeds and two seeds, as well as for the proportion of fruits containing three seeds and one seed. It is thus unlikely that control inflorescences to which hand-pollinated inflorescences were previously compared, suffered from a lower resources allocation for their seed production (Fig. 3).

Discussion

This study addressed three questions about the flowering phenologies of plants and insects, which should have

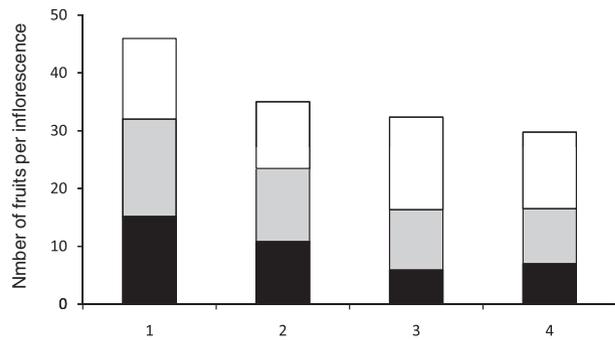


Fig. 3 Fruit production by the four categories of inflorescences: (1) hand-pollinated inflorescences; (2) control inflorescences on the same flowering trunk; (3) control inflorescences on another trunk from the same plant; (4) control inflorescences on a nonmanipulated plant. Histograms show the average number of fruit for each category, detailing the number of fruits that contained 3 seeds (black), 2 seeds (grey) and one seed (white).

important consequences on the evolutionary dynamics of the nursery pollination mutualism. The first question was about the timing of pollinator emergence compared to pollen production by host plants, and this study showed evidence for an imperfect synchrony these two events, which may lower the benefit for males of hosting pollinator larvae. The second concern was about the synchrony of male and female flowering. About this, the fact that females plants flowered late in the season, when many males had just achieved their own anthesis, apparently increases the efficiency of pollen transfer. However, the fact that some late-flowering males reached their anthesis simultaneously with females opens the possibility for pollinator to choose between male plants and cheating female ones. The last question concerned the consequences of cheating for female pollination success: I found that female plants could nevertheless attract (and deceive) pollinators and produced fruits, even though fruit production was limited by pollen (and pollinator) supply, meaning that cheating was not entirely retaliated by the mutualistic partner.

Male plants do not always benefit from rearing their pollinator larvae

In this study, I showed that a vast majority of the weevils emerged from the old male inflorescences before their host plant reached anthesis. It was not possible to follow weevils individually and directly assess their behaviour after emergence. However, a previous study (Dufay & Anstett, 2004) showed (i) that virtually no pollinators could be found on male plants before anthesis within this particular population, the same year and (ii) that pollinators artificially deposited on male plants in controlled conditions during plant anthesis were leaving the plant as soon as no food was available (i.e. just at the end of anthesis). This strongly suggests that weevils have the

behaviour to leave a plant that cannot provide them with food or shelter, as it is the case before anthesis. This is particularly expected since the average delay between weevil emergence and plant anthesis was extremely long (23 days).

As a result, only 32% of followed male plants had some adult insects emerging from their rachis during their anthesis and could benefit from the visit by insects they had reared during winter. Besides, even for males that flowered soon enough to entail such mutualistic reward, the number of potential visitors was low, compared to the number of pollinators hosted within inflorescences. Moreover, because the survey missed the first emergences, the data presented here probably under-estimate the proportion of weevils that left their host plant without visiting its inflorescences. The fact that weevil emergence and plant anthesis were apparently facilitated by distinct climatic factors, rain and temperature, respectively, prevented a better synchrony between the two events. This suggests that the benefits of mutualism for both partners could vary with locations and years. The importance of external factors on the cost/benefit balance in mutualism and their consequences in terms of varying outcomes of the interaction has already been stressed for several plant/insect mutualisms (Bronstein, 1994b).

Why do plants pay the cost of hosting pollinator larvae is one major question posed by nursery pollination mutualisms (Dufay & Anstett, 2003). Of course, any plant (cheating) strategy able to remove this cost (thus benefitting from pollination while providing no reward in return) is expected to be strongly selected for, possibly leading to pollinator extinction, and ultimately to plant extinction, in case of species-specific interactions. One striking example is the case *Yucca baccata*, in which 70% of individual plants 'kill' their pollinator larvae, and which pollination entirely relies on the 30% honest individuals that allow larval development within their inflorescences (Bao & Addicott, 1998). Such polymorphism may represent a transient state of a population doomed to extinction. Because we only know about mutualisms that still exist, we observe the interactions in which no such cheating had evolved, for some reasons. In the case of fig/fig wasp mutualism, fig trees entirely achieve their male function through rearing pollinator larvae, which are the only possible pollen dispersers of a fig (Anstett *et al.*, 1997). Killing the pollinators would entirely prevent any pollen dispersal and should be counter-selected at the individual level. At the other extreme of mutualism continuum (Bronstein, 1994b), in interactions such those between *Silene* species and *Hadena* moths, pollinators act as parasites by consuming a high number of seeds and exit the inflorescences before the next production of pollen by the host plant; host plants are viewed as unable to remove or regulate the cost of interaction (for a review, see Dufay & Anstett, 2003). In this regard, the palm/weevil interaction

appears to be an intermediate case. On the one hand, because female plants do destroy pollinator eggs or larvae, it seems unlikely that male plants are unable to do the same. On the other hand, male plants may benefit from rearing pollinator larvae but this benefit, when it occurs, seems to be low and could vary according to location and year. Moreover, contrarily to the case of fig/fig wasps, a male palm plant that would prevent pollinator development should be anyhow able to attract other pollinators and achieve its pollen dispersal. In that case, the low cost of having larvae developing at the expense of old and dry male inflorescences may have played an important role by lowering the benefit for male plants of setting up defences against pollinators and thus allowing the mutualism to persist.

Imperfect asynchrony of male and female flowering, consequences for the mutualism

This study showed a clear difference in the timing of anthesis between male and female plants. On average, males carried more inflorescences, they flowered earlier and for a longer time than females. In dioecious species, although males and females show a remarkable flowering synchrony in some species (Armstrong, 1997; Patel & McKey, 1998; Meagher & Delph, 2001; Moog *et al.*, 2002; Wright & Meagher, 2003), the general pattern seems to be that males begin to flower earlier than females (Lloyd & Webb, 1977; Willson, 1979; Beach, 1981; Patel & McKey, 1998). In *Aucuba japonica*, Abe (2001) proposed that such sex difference was because of an extensively long flowering period in males, which function would be to reduce the risk of pollination failure. This suggests the occurrence of a stronger selective pressure to attract pollinators on males, as a result of sexual selection (Charnov, 1979; Willson, 1979). Similar processes can be invoked in *C. humilis*, according to the present study.

Besides, because *D. chamaeropsis* do not leave the plants before the end of their anthesis (entailing visits that last several days on average, Dufay & Anstett, 2004), it is tempting to hypothesize that such flowering asynchrony between sexes increases the efficiency of pollen transfer from male plants to female plants. Experimentally assessing the consequences of flowering phenology on plant fitness is extremely difficult, if not impossible, and this requires cautiousness. However, this study showed that the female plants that started their anthesis at the time many males were ending their own anthesis produced the highest proportions of three-seeded fruits, suggesting a better pollination. Similar results have been found in many other species, in which female fitness depends on the availability of pollen producers (e.g. Mendez & Diaz, 2001). In this particular case, the peak of female flowering occurred when the number of male plants that had just ended their anthesis was maximum. The particular timing of flowering documented here may illustrate an adaptation of *C. humilis* to the behaviour of

its obligate-specific pollinator, which stays several days on the same flowering plant.

Such asynchrony between males and females can be compared to the flowering phenology of the temperate fig species, *Ficus carica*. In this species, two distinct peaks of flowering occur, with functionally male fig trees flowering first, attracting wasps that lay eggs within figs, and female figs reaching their receptive and attractive period 1 month later, when male figs 'export' wasp offspring, covered with pollen (Kjellberg *et al.*, 1987). Besides its efficiency for pollen transfer from male to female plants, this flowering asynchrony is often viewed as an important trait that stabilizes the fig/fig wasp mutualism, by preventing pollinating wasps to choose to visit male figs rather than females, in which they cannot reproduce (reviewed in Anstett *et al.*, 1997 and in Dufay & Anstett, 2003). Similarly, in *C. humilis*, female plants cheat their pollinators, by preventing pollinator larvae to develop within rachises. This study, however, revealed one major difference between *C. humilis* and *F. carica*: in *C. humilis*, the flowering periods of male and female palm plants overlapped, females flowering simultaneously with late-flowering males. There is thus a period of time during which weevil pollinators can either visit deceptive females, or rewarding male plants. Any innate preference for male plants within the weevil population would thus have the opportunity to be selected for. Thus, contrarily to the mutualism fig/fig wasp, the flowering phenology of the plant species does not perfectly protect mutualism stability. The fact that pollinators still (although not optimately) visit deceptive female plants (see below) may be because of other plant traits, such as the similarity of olfactory signals released by male and female plants (Dufay *et al.*, 2004). Finally, even in case weevils would not have any innate preference for male plants, the fact that more weevils could be found on male plants, compared with females, in this population (Dufay & Anstett, 2004) can be explained by the higher quantities of attractive volatile compounds released by these male plants (Dufay *et al.*, 2004). This may (at least partly) explain why female plants do not benefit from enough visits to achieve a maximum fruit production (see below).

The particularly long period of time during which male plants were at anthesis was partially because of the occurrence of two groups of males, with either an early or a late-flowering phenology. Such pattern has two remarkable and very different consequences for the mutualism. One, already noted, is that deceptive, less attractive female plants flower in the same time as late-flowering males. Another is that reliable egg-laying sites are available even in the late dates of the season, which probably has a positive feedback for the pollinator population, and ultimately for the plant population. One possible cause of such spread in flowering phenology may be the occurrence of two antagonistic factors that must affect the optimal phenology in terms

of male reproductive success. On the one hand, the number of freshly emerged weevils was found to strongly decrease with time (Fig. 1A), suggesting a higher availability of pollinators for early flowering individuals. In *C. humilis*, as in many other species (reviewed in Elzinga *et al.*, 2007), flowering early may thus be a good strategy to attract a lot of pollinators. On the other hand, the availability in mates, estimated as the number of receptive females when a male reaches the end of its anthesis (thus 'releasing' pollinators covered with pollen), was highly variable among dates (from 0 on 27 March to 28 on 26 April) and was globally much higher for the late-flowering males (Fig. 2). These two contradictory patterns may have thus led to the emergence of two flowering strategies, which seem to be consistent over time for a given individual plant.

Seed production and pollen limitation in female plants: are cheating females partially punished?

In this nursery pollination mutualism, deceptive female plants (i) compete with rewarding male plants that release a higher quantity of olfactory signals (Dufay *et al.*, 2004) and (ii) were shown to indeed attract a lower number of pollinators than males in natural conditions (Dufay & Anstett, 2004). The current study showed that most of female plants in this natural population were pollinated, although fruit production was limited by pollen supply. This has been showed in many different insect-pollinated plant species (for reviews, see Larson & Barrett, 2000; Knight *et al.*, 2005), contradicting somehow the view that male fitness is limited by pollinator availability, whereas female fitness is mainly limited by resources. In the case of *C. humilis*, pollen limitation challenges the fact that female plants apparently invest less in flowering and in pollinator attraction than males (Dufay *et al.*, 2003). It is thus not clear why female individuals that would release higher quantities of attractive chemicals had not been selected for. In such a context, it would be particularly useful to know about pollinator availability and about the limiting factors of female fitness in other populations, to assess whether these results constitute a general pattern in *C. humilis*. If pollen limitation of seed production was the rule, it could be explained by the particular nature of the pollination ecology of the dwarf palm. Indeed, weevil insects not only pollinate but also breed within palm inflorescences. Although female plants prevent larval development, nothing is known about neither the cost of the involved defences nor the cost of wounding performed by weevil on rachises, while attempting to lay eggs or for their feeding behaviour. To this regard, the lower attractive power of females could result from a trade-off between increasing pollination and limiting wounding and damage by pollinating weevils.

Conclusion

In such an interaction, in which life cycles of both partners are tightly linked and interwoven, phenological patterns play an important role for the costs/benefits balance and may affect the mutualistic/parasitic nature of the interaction. Compared to the fig/fig wasp system, the timing of pollinator emergence and plant flowering in dwarf palm/weevil seems to be not as optimized and may be less beneficial for the plant species: male plants do not always benefit from rearing pollinator larvae, and female plants face the competition with more attractive, nondeceptive male host plants. This study also opens many questions that should be investigated in future studies. In particular, more direct measurements of male fitness and of possible costs ensured by female plants when they host pollinators within inflorescences would interestingly complete the results of the current study and help in understanding how the global cost/benefit outcome of the interaction may vary among individuals within the plant species.

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