Empty flowers as a pollination-enhancement strategy

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ABSTRACT

Question: Can the reproductive benefits gained by mitigating the costs of self-pollination drive the evolution of nectarless flowers?

Features of model: Complementary analytical and simulation models determined the optimal proportion of nectarless flowers ('nectar phenotype') to maximize male reproductive success. Models considered a range of self-pollination costs and pollinator abundances. In the analytical model, equal numbers of each nectar phenotype were present. Pollinators used simple rules of behaviour, related to their current host plant's perceived nectar status, to decide whether to stay on that plant or to move to a new plant. In the simulation model, pollinators used more sophisticated departure rules, comparing the current host plant's perceived nectar status to the population mean. Plants with different proportions of nectarless flowers competed for successful pollination over multiple seasons.

Ranges of key variables: Relative cost of self-pollination (0.5–1); number of pollinators acting on a plant population per season (1–101); and proportion of nectarless flowers per plant (0–1).

Conclusions: Enhanced pollination success can drive the evolution of empty flowers in plants that are reliant on vector-mediated pollination. When the costs of selfing are low, an inflorescence with a low proportion of nectarless flowers is optimal, because pollination success is primarily determined by pollen removal. When the costs of selfing are high, an inflorescence with mostly nectarless flowers is optimal, because pollination success is primarily determined by outcrossing. Low pollinator abundances lead to a decreased optimal proportion of empty flowers to mitigate pollinator limitation.

Keywords: cross-pollination, geitonogamy, inbreeding depression, male function, nectar, nectarless flowers, outcrossing, reward, self-pollination.

INTRODUCTION

Nectar plays an important ecological role in plant reproduction as the primary floral reward offered to pollinators (Neiland and Wilcock, 1998). However, nectarless flowers appear repeatedly within angiosperms and occur in three forms: (1) entirely nectarless species [e.g. one-third of all orchids (Dressler, 1981)]; (2) nectarless individuals within an otherwise nectar-producing

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species [e.g. *Prosopis grandulosa* (Golubov *et al.*, 1999)]; and (3) nectarless flowers within nectar-producing inflorescences (Feinsinger, 1978), hereafter referred to as empty flowers. Field surveys of plant communities in Costa Rica, India, and western Canada show that species with empty flowers are relatively common (Feinsinger, 1978; Thakar *et al.*, 2003; Tindall, 2006). Of the 90 species examined in these studies, 71 (79%) contained some percentage of empty flowers, ranging from 1% [*Zigadenus venenosus* (Tindall, 2006)] to 68% [*Latana camara* (Thakar *et al.*, 2003)].

Two non-exclusive hypotheses have been proposed to explain the function of empty flowers, both of which assume that pollinators cannot discriminate between empty flowers and flowers that contain nectar. The first hypothesis suggests that empty flowers are an energy-saving strategy that enables the plant to save resources normally allocated to nectar production (Bell, 1986). The second hypothesis proposes that empty flowers enhance pollination by manipulating pollinator behaviour to increase outcrossing and minimize self-pollination (Smithson and Gigord, 2003). Inflorescences with many flowers typically experience greater pollen removal by pollinators, but can suffer reduced reproductive success from increased geitonogamy [self-pollination between flowers on the same plant (Harder and Barrett, 1995)]. Self-pollination can reduce the number or fitness of offspring as a consequence of inbreeding depression [the expression of deleterious recessive alleles and/or decreased heterozygosity at loci with heterozygote advantage (Charlesworth and Charlesworth, 1987, 1990)] and/or pollen discounting [the wastage of pollen in selfing that could otherwise be used in outcrossing (Nagylaki, 1976; Holsinger *et al.*, 1984)]. Furthermore, there is generally a positive but decelerating relationship between the amount of pollen removed by a single pollinator and the fraction of that pollen that is successfully deposited on another plant (Lloyd, 1984; Harder and Thomson, 1989). These diminishing returns for male function make it advantageous for plants to restrict the amount of pollen removed by individual pollinators (Harder and Thomson, 1989).

Empty flowers could alleviate the problems of geitonogamy and diminishing returns by encouraging pollinators to visit fewer flowers per plant (Harder *et al.*, 2001). The positive correlation between nectar reward and the number of flowers visited per plant is well documented (e.g. Zimmerman, 1983; Irwin and Brody, 1998; Johnson and Nilsson, 1999; Smithson, 2002), and low-yield and empty flowers can encourage visitors to leave plants sooner than they would have otherwise (Gill and Wolf, 1977; Hodges, 1985; Maloof and Inouye, 2000; Jersakova and Johnson, 2006). By encouraging early departures from the plant, empty flowers may reduce geitonogamy and pollen discounting, thus increasing the amount of pollen available for successful deposition on compatible stigmas, hereafter referred to as 'export' (Johnson and Nilsson, 1999; Harder *et al.*, 2001).

The pollination-enhancement hypothesis suggests that there should be some optimal proportion of empty flowers that maximizes pollination success by balancing self-pollen deposition and pollen export. This proportion is expected to increase as the benefits of empty flowers increase and/or the potential risks decrease. The pollination benefit of empty flowers depends primarily on the costs of self-pollination. Conceptually, these can be divided into genetic costs, largely determined by the degree of self-incompatibility and the severity of inbreeding depression, and the cost of lost outcrossing opportunities caused by pollen, ovule, and seed discounting. Therefore, the higher the cost of selfing, the greater the proportion of empty flowers we would expect to be optimal. Conversely, if there is no cost to selfing (i.e. self and outcross pollen and seeds are equally successful), there is no anti-selfing benefit to having empty flowers. In fact, costs of selfing below 0.5 are outweighed by

the benefit of contributing two gametes to a single offspring, in which case one might expect plants to evolve mechanisms for autonomous, rather than pollinator-mediated, selfing (Lloyd, 1992). The risk associated with empty flowers depends on pollinator abundance. Given unlimited pollinator visitation, inflorescences could maintain a high proportion of empty flowers for maximal outcrossing and still achieve complete pollen removal. Conversely, if pollinators are scarce, plants with many empty flowers risk not having all of their pollen removed (Harder and Thomson, 1989; Harder and Wilson, 1994). When the risk of not being pollinated is high, plants are expected to encourage pollinators to visit all the flowers on an inflorescence by having few or no empty flowers, despite a potential increase in self-pollination.

In this paper, we use two complementary approaches, an analytical model and a simulation model, to explore the pollination-enhancement hypothesis of empty flower evolution. Previous attempts to model the proportion of empty flowers in otherwise reward-ing inflorescences have assumed that empty flowers are an energy-saving strategy, and have modelled the costs of nectar production (Bell, 1986; Sakai, 1993; Smithson and Gigord, 2001; Thakar *et al.*, 2003). By explicitly considering the potential benefits from enhanced cross-pollination, our model allows us to develop an alternative hypothesis about the evolution of empty flowers. Here we examine how the optimal proportion of empty flowers that maximizes male fitness is affected by (1) pollinator abundance and (2) the genetic cost of selfing. We present two models describing a single, closed population in which plants are identical except for their number of empty flowers. Plants compete for bee-mediated deposition of both self and outcross pollen. Bee behaviour is modified by the number of empty flowers on a plant such that bees visit fewer flowers on plants that have a higher proportion of empty flowers. We systematically adjust pollinator abundance and the cost of selfing to determine how these parameters affect which empty flower strategy is the most successful. Finally, we discuss the implications of our results for the evolution of empty flowers.

METHODS

Description of system

We investigated the importance of empty flowers in modulating pollination success by modelling pollen transfer in a single population of monocarpic plants with non-overlapping generations (i.e. annuals). The total plant population size was assumed to be fixed through time, with no long-term seed bank or dispersal.

Each plant in the population had a single inflorescence with F flowers (Table 1). All the flowers on every plant were open simultaneously, completely overlapping with the presence of nectar-collecting bees (the pollinators), and each flower had the same initial amount of pollen (θ_0). Flowers within a plant's inflorescence were either nectarless ('empty') or 'full'. Nectar was assumed to have negligible production cost to the plant and the rate of refill after a pollinator visit was instantaneous. These assumptions allowed us to test the hypothesis that empty flowers could result purely from outcrossing considerations, without having to invoke a cost of nectar production. Moreover, a flower's nectar status was cryptic to pollinators, which could not determine whether a flower was nectarless until visitation. Other than the flowers' nectar status, all flowers were identical.

Given that each plant had F flowers, there were $F + 1$ possible plant phenotypes with unique proportions of empty flowers (e). These phenotypes ranged from no empty flowers ($e = 0$) to all empty flowers ($e = 1$) in increments of $1/F$. We did not consider the effects of

Table 1. Parameter definitions and values used (footnoted papers gave a range of values from which listed values were selected)

Parameter	Definition	Values used
B	Number of bees in a plant population per season	1–101
N_g	Number of plants per genotype in a population	5
F	Number of flowers per plant	10
θ_0	Initial amount of pollen in a flower	1000 grains
e	Proportion of empty flowers on a plant	0–1
V_f	Total number of flowers visited by a bee in a season	50
t	Number of empty flower encounters that cause a bee to switch plants (analytical model)	3
a	Proportion of pollen removed during a single flower visit	0.5, 0.8[a,b,c,d]
l	Proportion of pollen lost from bee between flowers on the same plant	0.5[e]
s	Proportion of carried self-pollen deposited on a flower	0.04
x	Proportion of pollen successfully exported to other plants	0.01, 0.02[a,f]
Ψ	Genetic cost of selfing	0.5–1
V_p	Total number of bee visits a plant will experience in a season (analytical model)	Equation-based
$OX(e)$	Total amount of successful outcrossed pollen from a single plant in a season	Equation-based
$SE(e)$	Total amount of successful self-pollen from a single plant in a season	Equation-based
R	Total amount of successful pollen from an individual plant in a season	Equation-based

[a] Harder and Thomson (1989); [b] Thomson and Thomson (1989); [c] Harder (1990a); [d] Thomson and Goodell (2001); [e] Rademaker *et al.* (1997); [f] Harder *et al.* (2000).

inflorescence architecture, but rather assumed that the spatial arrangement of flowers on a plant was inconsequential to the order in which the flowers were visited.

Each bee visited V_f flowers during a single season (Table 1). The distribution of these visits among plants was determined according to the bee behaviour rules built into the models. During each flower visit, the bees removed a fixed proportion (a) of the remaining pollen on that flower. Bees lost a proportion (l) of the pollen they carried when moving between two flowers on the same plant (as a consequence of passive loss and active grooming). When arriving at a new flower on the same plant, bees deposited a certain proportion (s) of the pollen they carried onto the flower's stigma. Of this self pollen, a proportion (Ψ) was unsuccessful in pollination because of the genetic cost of selfing (e.g. inbreeding depression and self-incompatibility). For bees moving between two flowers on different plants, the proportion of pollen that was not lost between flowers and that was subsequently successfully deposited on the flowers of new plants was x. By analogy, x was to inter-plant movement as the combination of $1 - l$ and s was to intra-plant movement. This means that the value of x compared with $s(1 - l)$ affects the relative efficiency of outcrossing versus geitonogamy, in terms of pollen deposition. To explore how altering this relative efficiency affects selection for empty flowers, we varied x while holding s and l constant. We considered the total 'successful' pollen of a plant to be its outcrossed pollen plus a proportion $(1 - \Psi)$ of its self pollen. The fitness values of plants were assumed to be correlated with the proportion of their pollen that was successful. We considered only male fitness, hereafter referred to as 'fitness'.

Analytical model

We constructed an analytical model to calculate the average amount of 'successful' pollen for all possible plant phenotypes in the described system. We considered the optimal proportion of empty flowers given that all possible empty flower phenotypes were present in the population. We examined the simple case where there were equal numbers of each phenotype.

The number of flowers a bee visits within a plant is dependent on the number of empty flowers encountered, so each phenotype influenced bee behaviour differently. When arriving on a new plant, each bee made a minimum of t visits to randomly chosen flowers, unless this caused the pollinator to exceed its seasonal maximum number of visits (V_f). After the pollinator visited its tth flower on a plant, and after every flower visited thereafter, the pollinator could either move to a different randomly chosen flower on the same plant, or move to a flower on a different plant. The pollinator left for a new plant if (a) it had already made F visits to flowers on the current plant, such that each flower on the plant had received one visit on average, or (b) it had already visited t empty flowers on the current plant. If both conditions were false, the pollinator randomly chose a different flower on the same plant. Thus, pollinators only used information about the current plant to decide whether to leave or stay.

With these foraging rules, the number of flowers visited during a single plant visit was the result of a modified hypergeometric random process. The modification comes from the requirement that the final flower a bee visits before leaving a plant must be empty. Using this distribution we calculated $f(e)$, the expected number of flowers an individual bee visits on a single visit to a plant with a proportion e empty flowers [see Appendix for further explanation of $f(e)$].

There were B bees, each making V_f flower visits per season. The total number of times a single plant in a population with N_g plants of each genotype was visited in a season was

$$V_p = \frac{B \cdot V_f}{\sum_{n=0}^{F} \left(f\left(e = \frac{n}{F}\right) \cdot N_g \right)} .$$ (1)

Using sums of geometric series, we define total successful outcrossed pollen from an individual plant over one season as

$$OX(e) = xa\theta_0 \left(\frac{1 - \alpha^{f(e)}}{1 - \alpha} \right) \left(\frac{1 - \beta^{V_p}}{1 - \beta} \right),$$ (2)

and total successful selfed pollen from an individual plant over one season as

$$SE(e) = (1 - \Psi) a\theta_0 \left(\frac{1 - \beta^{V_p}}{1 - \beta} \right) \frac{(1 - l)s}{1 - \alpha} \left(f(e) - \frac{1 - \alpha^{f(e)}}{1 - \alpha} \right),$$ (3)

where $\alpha = (1 - s)(1 - l)$ and $\beta = \frac{F - af(e)}{F}$. [See Appendix for further explanation of the function $f(e)$ and derivation of equations (2) and (3).] Thus, the total successful pollen from an individual plant in one season is

$$R = OX(e) + SE(e),$$ (4)

which we used as a measure of relative fitness.

By calculating R over all possible values of e, we found the optimal proportion of empty flowers at which fitness is maximized over a range of pollinator abundances (1 to 101), selfing costs (0.5 to 1), two levels of a (0.5 and 0.8), and two levels of x (0.01 and 0.02) (see Table 1). We limited our model to consider cost of selfing (Ψ) values above 0.5, because values lower than this should promote the evolution of autonomous self-fertilization (Lloyd, 1992) and so are not applicable to our model of a purely vector-mediated pollination system.

Simulation model

A simulation model was constructed to describe the same basic system as in our analytical model. This allowed us to explore the effects of alternative bee behaviours and variable bee abundances. The simulation model tracked the amount of pollen in each flower in each inflorescence in the population and described a distribution of pollinator behaviours for a given value of e. We initially ran the simulation model with simplistic bee behaviour rules similar to those used in the analytical model. These results were qualitatively similar, with more noise in the simulation model results due to its stochastic components.

We then modified the bee behaviour rules so that bees could use limited population-level and plant-level information to decide to leave a plant if they perceived it to have a below-average nectar reward. On each new plant, a pollinator visited a minimum of three flowers unless this caused the pollinator to exceed its total number of seasonal flower visits. There-after, the pollinator left for a new plant if (a) it had already made F visits to flowers on the current plant, or (b) the current proportion of visited flowers on the plant that were empty exceeded the average proportion of empty flowers of all the plants in the population. If conditions (a) and (b) were both false, the pollinator randomly chose a different flower on the same plant. Note that pollinators occasionally 'made mistakes' by leaving a plant early that actually provided a better-than-average nectar reward. These mistakes seem to be consistent with pollinator behaviour in natural systems (J.R. Tindall, personal observations), and reflect the fact that pollinators must make foraging decisions based on incomplete information.

This model was run for 1000 simulated seasons and pollinator abundance was initially assumed to be constant over each season. Because pollinator abundance can be highly unpredictable, we also considered an alternative scenario in which each season's pollinator abundance was randomly drawn from a Poisson distribution. However, this had no appreciable effect on our results.

To find the optimal proportion of empty flowers produced by plants under a range of pollinator abundances, costs of selfing, and other mating system and pollinator traits, we used an approach similar to a genetic algorithm. We assumed that the $F + 1$ phenotypes directly corresponded to $F + 1$ genotypes (i.e. F alleles at a single locus). This scenario was not meant to reflect the actual genetic control of empty flowers; rather, it was chosen to allow phenotypes to compete in the arena of our simulation.

At the beginning of each run, there were N_g individuals of each genotype for a total of $N_g \cdot (F + 1)$ individuals. The number of offspring each individual plant contributed to the next season was determined randomly, but was, on average, in direct proportion to the total successful pollen it produced in the current season. To avoid having the winning genotype dependent on initial or transient abundances, we introduced rare mutations. Each season, every potential offspring had a probability of μ (set at 1/500) of mutating to a different

randomly chosen genotype. Thus, a genotype that became abundant early in each model run was repeatedly confronted with rare 'invaders'. We ran the simulation model for the same range of parameters as the analytical model using increments of 0.05 for cost of selfing and increments of 5 for mean pollinator abundance. We defined the 'winning genotype' for each suite of model conditions as the genotype with the greatest average abundance between seasons 500 and 1000.

RESULTS

Qualitatively, the results of the analytical and simulation models were very similar despite the difference in bee behaviour (compare Fig. 1 and Fig. 2). The cost of selfing (Ψ) was important in determining the optimal proportion of empty flowers. Relatively large Ψ favoured plants with all empty flowers and relatively small Ψ favoured plants with all full flowers (Fig. 1 and Fig. 2); however, an intermediate proportion of empty flowers was often the optimal genotype (Fig. 1 and Fig. 2).

The way in which cost of selfing (Ψ) and bee abundance influenced the optimal proportion of empty flowers was affected by the proportion of outcrossed pollen deposited (x) and proportion of pollen removed (a). The value of Ψ at which the optimal proportion of empty flowers became non-zero decreased as the relative efficiency of outcrossing versus geitonogamy increased (x: compare top and bottom rows of panels in Fig. 1 and Fig. 2). Also, there was a general increase in the optimal proportion of empty flowers as the proportion of pollen removed (a) increased (compare the left and right columns of panels in Fig. 1 and Fig. 2). However, when the relative efficiency of outcrossing versus geitonogamy was low ($x = 0.01$), this increase in the optimal proportion of empty flowers was apparent only for high levels of cost of selfing ($\Psi > 0.75$ in Fig. 1 and Fig. 2, panels i and ii).

Increasing bee abundance had a positive effect on the optimal proportion of empty flowers. Again, when the relative efficiency of outcrossing versus geitonogamy was low ($x = 0.01$), this positive effect was apparent only for high levels of genetic cost of selfing ($\Psi > 0.75$ in Fig. 1 and Fig. 2, panels i and ii).

Example model runs emphasize the stochastic nature of the simulation outcomes and the effect of the cost of selfing (Ψ) and bee abundance (B) on the optimal proportion of empty flowers (Fig. 3). These runs show that the most successful genotype changed over time, especially during the early stages of the run. Runs of the simulation model occasionally resulted in the dominance of plants with an intermediate proportion of empty flowers, outside the range of conditions predicted by the analytical model (Fig. 1 and Fig. 2). This was due to stochasticity in both realized bee behaviour and plant reproduction in the simulation model (Fig. 3).

DISCUSSION

Our models support the hypothesis that nectarless flowers could evolve as a response to the genetic cost of self-pollination, even when there is no energetic cost to producing nectar. As the genetic cost of selfing increases, the difference in fitness between selfed and outcrossed offspring increases and there is stronger selection on plants to promote pollinators to leave an inflorescence before all the flowers have been visited. In this case, an inflorescence of
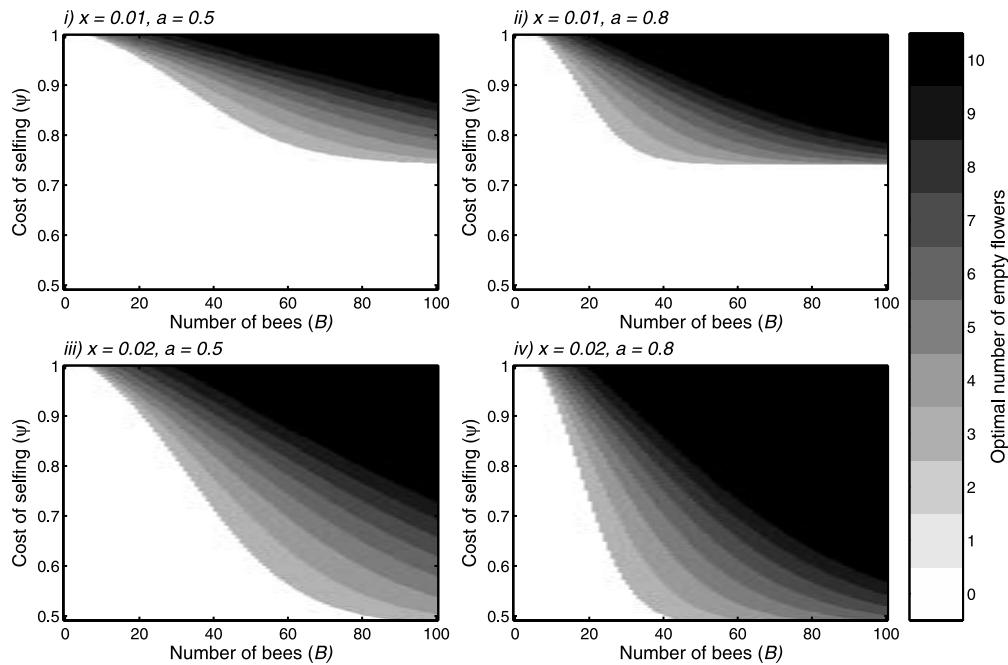
Fig. 1. The optimal number of empty flowers (0–10, shading) as a function of the cost of selfing and number of bees for the analytical model. The model was run at four different combinations of the proportion of pollen successfully exported to other plants (x) and the proportion of pollen removed from a flower after a bee's visit (a): (i) $x = 0.01$, $a = 0.5$; (ii) $x = 0.01$, $a = 0.8$; (iii) $x = 0.02$, $a = 0.5$; (iv) $x = 0.02$, $a = 0.8$. Other parameter values are given in Table 1.

mostly empty flowers is the optimal strategy for maximizing male reproductive success. Conversely, an inflorescence with few empty flowers is the optimal strategy when the genetic cost of selfing is low. The optimal proportion of empty flowers was often either 0 or 1, but an intermediate proportion of empty flowers was the optimal strategy for a range of selfing costs and bee abundances.

Results from both the analytical and simulation models show that bee abundance has an important effect on the optimal proportion of empty flowers on a plant. The greater the bee abundance, the higher the proportion of empty flowers that maximizes a plant's pollen export. As bee abundance increases, pollen export becomes less limited by the number of bee visits a plant receives. Plants can then maximize their pollen export by encouraging bees to leave with pollen from fewer flowers, thus minimizing the costs associated with selfing (Iwasa *et al.*, 1995; Harder *et al.*, 2001). When bees are scarce, plants maximize their pollen export by fully exploiting the bee visits they receive. Having few or no empty flowers encourages a bee to remain on the plant and remove a larger proportion of pollen. When bees are limiting, the export benefit of this increased removal outweighs the cost of pollen lost to selfing. Similar results are obtained by modifying the number of flowers visited per bee per season (not shown). Unfortunately, neither pollinator abundance nor visitation rates have been quantified in the context of empty flower research, though their importance is not only highlighted in our model, but has been identified in other studies (Harder, 1990a; Klinkhamer *et al.*, 1994; Thakar *et al.*, 2003; Biernaskie and Cartar, 2004).
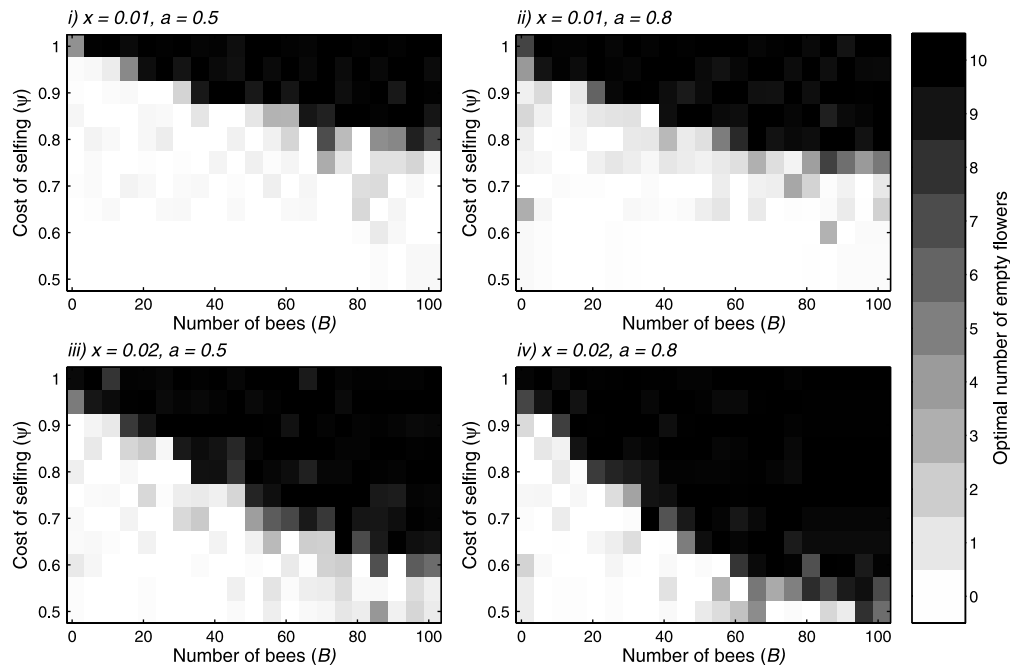
Fig. 2. The optimal number of empty flowers (0–10, shading) as a function of the cost of selfing and number of bees for the simulation model. The model was run at four different combinations of the proportion of pollen successfully exported to other plants (x) and the proportion of pollen removed from a flower after a bee's visit (a): (i) $x = 0.01$, $a = 0.5$; (ii) $x = 0.01$, $a = 0.8$; (iii) $x = 0.02$, $a = 0.5$; (iv) $x = 0.02$, $a = 0.8$. Other parameter values are given in Table 1.

The optimal proportion of empty flowers changes with the proportion of pollen a bee removes from a flower (a) and this relationship is modified by changes in bee abundance. There are several reproductive costs associated with high values of a, which include pollen discounting as in our model (Klinkhamer *et al.*, 1994; Harder and Wilson, 1997), and also pollen lost to increased pollen layering and pollinator grooming (Harder, 1990a; Harder *et al.*, 2001). Thus when a is relatively small, a low proportion of empty flowers enables plants to increase their pollen export by encouraging pollinators to visit more flowers without suffering the reproductive costs usually associated with increased visitation. The difference between the optimal proportion of empty flowers when a is low versus high is particularly evident under conditions of low bee abundance (compare Fig. 1, panels i and ii), when the total pollen removed from a plant is most severely limited by the absolute number of visits it receives. Conversely, when bee abundance is high, the proportion of pollen removed by a bee no longer limits total plant pollen removal and the main factor determining the optimal proportion of empty flowers in our model is the genetic cost of selfing.

Our models build on previous work exploring the relationships between the cost of selfing, geitonogamy, and pollinator abundance. Early mating-system models that did not account for pollen discounting and the probability of successful outcrossing consistently predicted disruptive selection for pure selfing when inbreeding depression (δ, equivalent to our Ψ for self-compatible plants) < 0.5 and pure outcrossing when $\delta > 0.5$ (reviewed in Harder and Wilson, 1998; but see Holsinger, 1991, for an exception). Models by Harder and Wilson (1998) clarified that
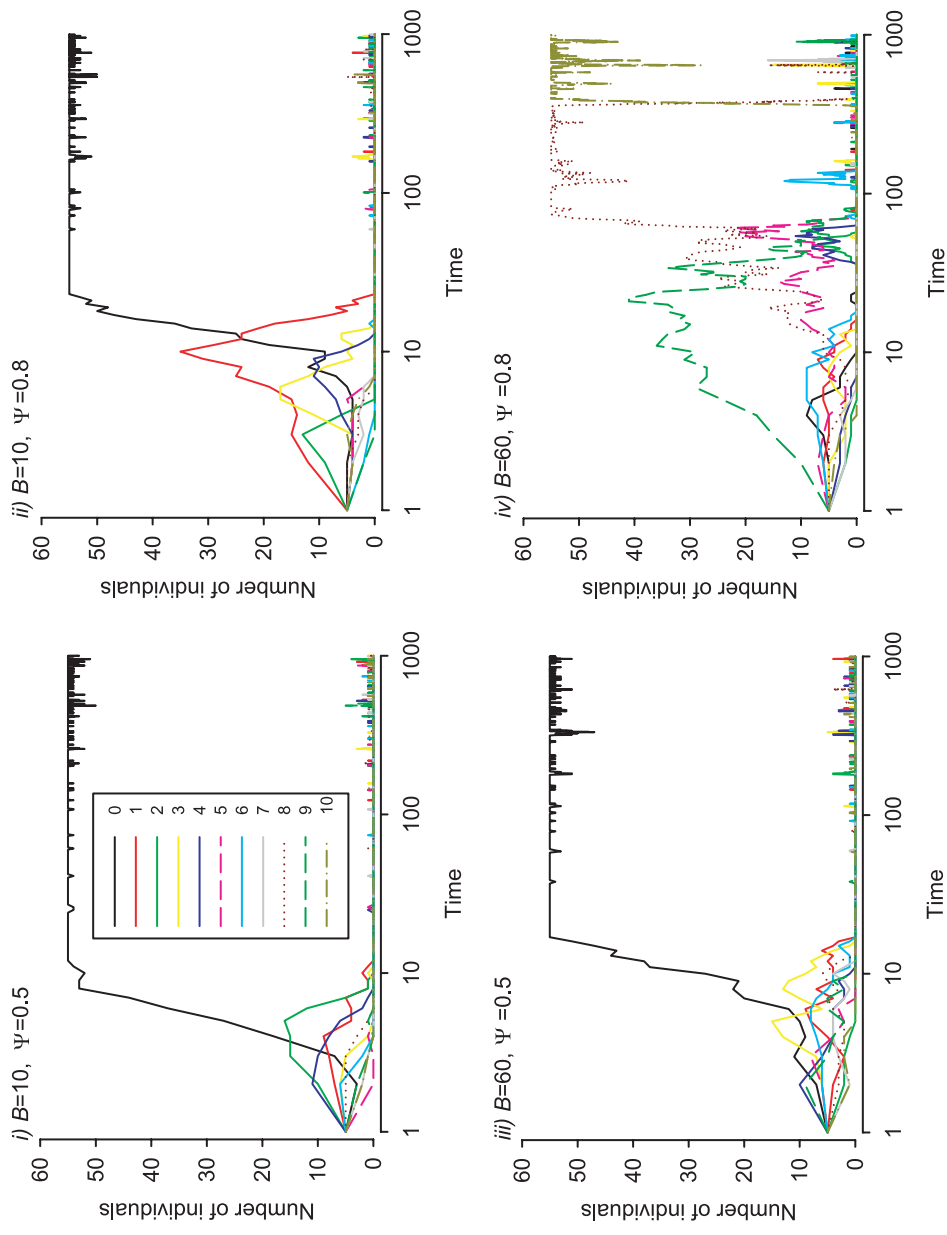
Fig. 3. Four example time-series for the simulation model, indicating the abundance of each of the 11 genotypes at different combinations of the number of bees (*B*) and the cost of selfing (**Ψ**): (i) *B* = 10, **Ψ** = 0.5; (ii) *B* = 10, **Ψ** = 0.8; (iii) *B* = 60, **Ψ** = 0.5; (iv) *B* = 60, **Ψ** = 0.8. In each case, *a* = 0.8 and *x* = 0.01. All other parameters are given in Table 1. The inset legend indicates the genotype represented by each line, from 0 to 10 empty flowers.

this conclusion holds even when pollen discounting is accounted for, as long as discounting is equal among phenotypes. Our models explore scenarios in which pollen discounting is not equal among phenotypes, but rather varies according to the degree of geitonogamy phenotypes experience due to their proportion of empty flowers. In such circumstances, intermediate proportions of empty flowers (and consequently intermediate levels of selfing) can be selected under a range of selfing costs (Figs. 1 and 2). This apparent selection for geitonogamy (the only mode of selfing allowed in our models) is largely the indirect result of selection for increased outcrossing; as pollinators visit additional flowers per plant they pick up more pollen, which is subsequently available for outcross pollination. This is most obvious when $\Psi = 1$ (i.e. self-pollen grains make no contribution to fitness), and the all-nectarful phenotype experiences the highest fitness (Fig. 1; pollination abundance < 10). The fitness benefit of increased pollen export per pollinator is mediated by pollinator abundance, as discussed above; when pollinators are abundant, it is outweighed by greater pollen diversion to geitonogamy, but when pollinators are very scarce, the outcrossing benefit of fewer empty flowers is sufficient to confer a fitness advantage (Figs. 1 and 2). This conclusion is consistent with models by Harder *et al.* (2001), who found that at low pollinator abundances, plants exported the most pollen when pollinators visited a high proportion of flowers per plant, which in our model is equivalent to phenotypes with low proportions of empty flowers. Similarly, Holsinger (1991) found that when the probability of successful outcross pollen export was low (this occurs when pollinator abundance is low in our model), genotypes that experienced higher degrees of selfing could increase in frequency, and mixed mating systems of selfing and outcrossing became evolutionarily stable.

In addition to indirect selection for geitonogamy, our model allows geitonogamy to confer a direct fitness advantage, which contrasts with previous models in which geiton-ogamy is never advantageous (Lloyd, 1992). Lloyd's pioneering work on selection for selfing proposed that geitonogamy necessarily results in complete pollen and ovule discounting, because it involves the same processes as outcrossing (Lloyd, 1988, 1992). In our model, this is approximated when $x \approx s(1 - l)$, although an exact equivalence cannot be calculated as self-pollination can occur multiple times whereas we model outcrossing as a single event. However, when pollinator behaviour differs between inter- and intra-plant movements, such as when bees groom more when flying between plants than when moving between flowers on the same plant (Harder, 1990b), geitonogamy does not necessarily result in complete discounting, and can be relatively more efficient in terms of pollen transfer than outcrossing (Harder *et al.*, 2001). Our model differs from Lloyd's (1992) as it allows geitonogamous pollen transfer to be more efficient numerically than outcross pollen transfer. When the efficiency of outcross pollen deposition is decreased relative to that of geitonogamous deposition (i.e. x is reduced while holding s and l constant), empty flowers become less advantageous, as long as self pollen makes some fitness contribution (compare Fig. 1, panels iii and iv with Fig. 2, panels iii and iv when $\Psi < 1$).

Our model tested two sets of pollinator behaviour rules. Alternative bee behaviour rules did not appreciably affect our model results; however, flowers can be visited by many species of animals that differ more drastically in morphology and behaviour (Wilson and Thomson, 1991). Although there may be general patterns of pollinator behaviour rules, such as pollen removal increasing with the amount of available pollen per flower (Conner *et al.*, 1995), pollinator species, and even individuals within species, can show significant differences in their response to variation in floral traits. These responses may directly affect selfing patterns and pollen transfer among plants (Thompson, 2001; Mitchell *et al.*, 2004). It is reasonable to assume that

these behavioural differences may also occur in response to empty flowers. Also, the primary pollinator for a particular plant population may change, as pollinator abundance is highly variable between years (e.g. Rush *et al.*, 1995). Future research on the function of empty flowers that considers these differences would help illuminate the generality of pollinator–empty flower interactions. There is also a need for studies that simultaneously measure the occurrence of empty flowers, pollinator abundance, and the costs of self-pollination and nectar. This will allow for empirical testing of our models' prediction that pollination enhancement can drive the evolution of empty flowers.

Our models predict the evolution of entirely nectarless plants under a range of conditions, but surveys of nectar volumes have found nectarless individuals to be uncommon (Feinsinger, 1978; Thakar *et al.*, 2003; Biernaskie and Cartar, 2004; Tindall, 2006). This seemingly unrealistic model prediction occurs because the model environment lacks meta-population and community structure, and pollinators must forage within the model patch. As a result, there is no cost to being entirely nectarless, since our pollinators cannot choose to avoid nectarless patches or species. In a more realistic environment, non-rewarding patches or species would eventually be discriminated against in favour of those that offer reward (Cartar, 2004). For example, *Dactlyorozhea sambucina*, a nectarless orchid, relies on naïve bumblebee-queen pollinators, because experienced queens learn to avoid it (Nilsson, 1980). Furthermore, the more common a deceptive plant is in a community, the more rapidly pollinators will stop visiting that community (Ferdy *et al.*, 1998). Thus, the structure of the plant community, especially with respect to alternate foraging opportunities for pollinators, as well as the frequency of deceptive individuals, may influence the evolution of empty flowers. Although entirely nectarless species have evolved [e.g. one-third of all orchids (Dressler, 1981)], these species have also evolved complex adaptations to ensure pollination by deception [e.g. food deception, floral mimicry, and sexual deception (Jersakova and Johnson, 2006)].

Much of the previous work exploring the function of empty flowers has focused on the energetic cost of nectar production (Bell, 1986; Gilbert *et al.*, 1991; Sakai, 1993). The energy-saving hypothesis for the function of empty flowers is based on the explicit assumption that nectar production represents a significant energetic cost for plants (Feinsinger, 1978; Bell, 1986; Gilbert *et al.*, 1991), and the implicit assumption that increased nectar production reduces the resources available for other plant functions, such as female reproduction (Pyke, 1991). The energetic cost of nectar varies greatly among species. Although nectar is costly for some species [30% of floral energy and up to 37% of daily plant photosynthate (Pleasants and Chaplin, 1983; Southwick, 1984)], such costs seem to be unusually high (reviewed in Harder and Barrett, 1992), and other research has found nectar cost to be low [3% of floral tissue (Harder and Barrett, 1992) and as low as 4% of daily plant photosynthate (Southwick, 1984)] or undetectable (Leiss *et al.*, 2004). Although reducing the cost of nectar may be important, our results suggest that pollination enhancement could also drive the evolution of empty flowers.

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APPENDIX

The simple foraging rules used in the analytical model work such that the number of flowers visited during a single plant visit is the result of a modified hypergeometric process. The modification comes from the requirement that the final flower a bee visits before leaving a plant must be empty. The expected number of flowers visited by a bee visiting a plant with eF empty flowers is

$$f(e) = \sum_{n=t}^{F-1} \left[n \cdot \frac{C(eF, t-1)C((1-e)F, n-t)}{C(F, t-1)} \cdot \frac{eF-(t-1)}{F-(n-1)} \right], \tag{A1}$$

where F is the total number of flowers on a plant, t is the number of empty flowers encountered after which a bee will leave a plant, n is the number of flowers visited, and $C(x,y)$ represents 'x choose y' – the binomial coefficient function. This equation is the sum of each possible value of n weighted by the probability of a bee encountering t empty flowers out of n, where the last encountered flower is empty.

There were B bees, each making V_f flower visits per season. The total number of times a single plant in a population with N_g plants of each genotype was visited in a season was

$$V_p = \frac{B \cdot V_f}{\sum_{n=0}^{F} \left(f\left(e = \frac{n}{F}\right) \cdot N_g \right)}. \tag{A2}$$

All flowers initially contain the same amount of pollen, θ_0. A bee visiting a plant removes a proportion (a) of pollen from $f(e)$ flowers out of the F flowers on the plant. The second bee to visit that same plant will then experience some flowers with θ_0 pollen and some with $(1-a)\theta_0$ pollen. The average amount of pollen in each flower on that plant on the second bee visit is

$$\theta(v=2) = \theta_0 \left(\frac{F - a \cdot f}{F} \right).$$

On the third bee visit, the average amount of pollen in each flower on that plant is

$$\theta(v=3) = \theta(v=2)\left(\frac{F - a \cdot f}{F}\right)$$

$$= \theta_0 \left(\frac{F - a \cdot f}{F}\right)^2 .$$

Thus, the expected amount of pollen in each flower on a given plant on the vth bee visit is

$$\theta(v) = \theta_0 \left(\frac{F - a \cdot f}{F}\right)^{v-1} . \tag{A3}$$

We define the amount of pollen successfully outcrossed from a single plant on the vth visit to that plant, where the bee visits f flowers, as $OX_{singlevisit}(f,v)$. A bee visiting any given plant picks up $a\theta(v)$ from each flower it visits. Between visiting the first, second, and future consecutive flowers on a plant, a bee loses a proportion l of the pollen collected on its body due to passive loss and grooming. While visiting the second and any subsequent flowers on that plant, a bee deposits a proportion (s) of the pollen collected on its body onto that flower. This pollen is considered selfed pollen.

If a bee visits only one, two or three flowers on a plant ($f = 1, 2, 3$) on the vth bee visit to that plant and then leaves, the amount of successfully outcrossed pollen is

$$OX_{singlevisit}(f=1,v) = xa\theta(v) ,$$

$$OX_{singlevisit}(f=2,v) = x[a\theta(v)(1-l)(1-s) + a\theta(v)] ,$$

$$OX_{singlevisit}(f=3,v) = x[(a\theta(v)(1-l)(1-s) + a\theta(v))(1-l)(1-s) + a\theta(v)] .$$

By extension, the amount of successfully outcrossed pollen from a single plant on the vth bee visit, where the number of flowers visited is $f(e)$, can be described by the summed geometric series:

$$OX_{singlevisit}(f(e),v) = xa\theta(v)\sum_{i=1}^{f(e)} [(1-l)(1-s)]^{i-1}$$

$$\therefore OX_{singlevisit}(f(e),v) = xa\theta(v)\frac{1 - [(1-l)(1-s)]^{f(e)}}{1 - (1-l)(1-s)} . \tag{A4}$$

The total amount of pollen successfully outcrossed from a single plant over the entire season (V_p plant visits) is

$$OX(e) = \sum_{v=1}^{V_p} OX_{singlevisit}(f(e),v)$$

$$= \sum_{v=1}^{V_p} \left(xa\theta(v)\frac{1 - [(1-l)(1-s)]^{f(e)}}{1 - (1-l)(1-s)}\right)$$

$$= xa\frac{1 - [(1-l)(1-s)]^{f(e)}}{1 - (1-l)(1-s)} \cdot \sum_{v=1}^{V_p} \theta(v) ,$$

where $\sum_{v=1}^{V_p} \theta(v)$ can be expressed as the sum of a geometric series:

$$\sum_{v=1}^{V_p} \theta(v) = \sum_{v=1}^{V_p} \theta_0 \left(\frac{F - a \cdot f}{F}\right)^v$$

$$= \theta_0 \frac{1 - \left(\frac{F - a \cdot f(e)}{F}\right)^{V_p}}{1 - \left(\frac{F - a \cdot f(e)}{F}\right)}$$

Therefore,

$$OX(e) = xa\theta_0 \frac{1 - [(1 - l)(1 - s)]^{f(e)}}{1 - (1 - l)(1 - s)} \cdot \frac{1 - \left(\frac{F - a \cdot f(e)}{F}\right)^{V_p}}{1 - \left(\frac{F - a \cdot f(e)}{F}\right)}.$$

For simplicity, we let $\alpha = (1 - l)(1 - s)$ and $\beta = \frac{F - a \cdot f(e)}{F}$, such that

$$OX(e) = xa\theta_0 \left(\frac{1 - \alpha^{f(e)}}{1 - \alpha}\right) \cdot \left(\frac{1 - \beta^{V_p}}{1 - \beta}\right). \tag{A5}$$

We define the amount of pollen successfully selfed on a single plant on the vth visit to that plant as $SE_{singlevisit}(f, v)$, where the bee visits f flowers. If a bee visits only one, two or three flowers on a plant ($f = 1, 2, 3$) on the vth bee visit to that plant and then leaves, the amount of successfully selfed pollen is

$SE_{singlevisit}(f = 1, v) = 0$,

$SE_{singlevisit}(f = 2, v) = (1 - \Psi)a\theta(v)(1 - l)s$,

$SE_{singlevisit}(f = 3, v) = (1 - \Psi)[a\theta(v)(1 - l)s + (a\theta(v)(1 - l)(1 - s) + a\theta(v))(1 - l)s]$,

where $1 - \Psi$ is the proportion of self-deposited pollen that is reproductively successful.

Thus, the amount of successfully selfed pollen on a single plant on the vth bee visit, where the number of flowers visited is $f(e)$, can be described by the sum of the summed geometric series:

$$SE_{singlevisit}(f(e), v) = (1 - \Psi)a\theta(v)(1 - l)s \sum_{n=2}^{f(e)} \sum_{i=2}^{n} [(1 - l)(1 - s)]^{i-2}$$

$$= (1 - \Psi)a\theta(v) \frac{(1 - l)s}{1 - (1 - l)(1 - s)} \left(f(e) - \frac{1 - [(1 - l)(1 - s)]^{f(e)}}{1 - (1 - l)(1 - s)}\right). \tag{A6}$$

The total amount of pollen successfully selfed from a single plant over the entire season (V_p plant visits) is

$$SE(e) = \sum_{v=1}^{V_p} SE_{singlevisit}(f(e), v)$$

$$= (1 - \Psi)a\theta_0 \left(\frac{1 - \left(\frac{F - a \cdot f(e)}{F} \right)^{V_p}}{1 - \left(\frac{F - a \cdot f(e)}{F} \right)} \right) \frac{(1 - l)s}{1 - (1 - l)(1 - s)} \left(f(e) - \frac{1 - [(1 - l)(1 - s)]^{f(e)}}{1 - (1 - l)(1 - s)} \right).$$

Substituting in α and β, the equation simplifies to

$$SE(e) = (1 - \Psi)a\theta_0 \left(\frac{1 - \beta^{V_p}}{1 - \beta} \right) \frac{(1 - l)s}{1 - \alpha} \left(f(e) - \frac{1 - \alpha^{f(e)}}{1 - \alpha} \right). \tag{A7}$$