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ORIGINAL PAPER

Nectar robbing improves male reproductive success of the endangered *Aconitum napellus* ssp. *lusitanicum*

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Abstract Nectar robbery is usually thought to impact negatively on the reproductive success of plants, but also neutral or even positive effects have been reported. Very few studies have investigated the effects of nectar robbing on the behaviour of legitimate pollinators so far. Such behavioural changes may lead to the reduction of geitonogamy or to increased pollen movement. We simulated nectar robbing in experimental sites as well as in natural populations of *Aconitum napellus* ssp. *lusitanicum*, a rare plant pollinated by long-tongued bumblebees. In an experimental setup, we removed the nectaries of 40 % of the flowers, which is similar to rates of robbing observed in wild populations. Patches of plants with experimentally robbed flowers were compared with control patches containing plants with untreated flowers. We observed pollinator behaviour, mimicked male reproductive success (pollen dispersal) using fluorescent dye, and measured female reproductive success (seed set). The main legitimate visitors were bumblebees while honeybees were often observed robbing nectar. They did so by "base working", i.e. sliding between tepals. Bumblebees tended to visit fewer flowers per plant and spent less time per single flower when these had been experimentally robbed. This change in behaviour consequently increased the proportion of flowers visited by bumblebees in patches with robbed flowers. Fluorescent dye mimicking pollen flow was dispersed larger distances after pollinators had visited patches with robbed flowers compared to control patches. Average seed set per plant was not affected by nectar robbing. Our results demonstrated that *A. napellus* does not suffer from nectar robbery but may rather benefit via improved pollen dispersal and thus, male reproductive success. Knowledge on such combined effects of behavioural changes of pollinators due to nectar robbery is important to understand the evolutionary significance of exploiters of such mutualistic relationships between plants and their pollinators.

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Introduction

Plants and their pollinators usually maintain a mutualistic relationship where pollinators benefit from resources, such as nectar or pollen and flowers receive outcross pollen in return to fertilise ovules and insure reproductive success (Proctor et al. 1996; Bronstein 2001). Exploiters of this mutualism are also widespread in nature, either plants that seduce pollinators to visit their flowers but offer no rewards (Schiestl 2005; Jersáková et al. 2006) or animals that steal resources from flowers without delivering pollen to stigmas (Maloof and Inouye 2000; Bronstein 2001). “Cheaters” among flower visitors have been considered detrimental to the reproductive success of plants for long time, dating back to Darwin (1872). In contrast to nectar thieves, whose activities do not harm floral tissues, nectar robbers are defined as illegitimate flower visitors that destructively force their way to nectar (Inouye 1980). Hence, nectar robbing directly impacts on flowers not only through nectar depletion, but also through floral damage or shortening of the floral life span. This has been considered to reduce attractiveness to pollinators and plant reproductive success (Traveset et al. 1998; Navarro 2001; González-Gómez and Valdivia 2005; Zhang et al. 2007). However, recent reviews and meta-analyses show that effects of nectar robbing might be neutral, when experimental protection from robbery did not increase the reproductive success of a plant (e.g. Morris 1996; Stout et al. 2000; Maloof 2001). Even positive effects on the reproductive success of plants have been observed (Navarro 2000; Maloof and Inouye 2000; Irwin et al. 2010).

Nectar robbing may furthermore change the behaviour of legitimate pollinators, since these often visit fewer numbers of flowers per robbed patch or inflorescence, spend less time on robbed flowers, or increase flight distances (Zimmerman and Cook 1985; Irwin and Brody 1998; Maloof 2001; Dohzono et al. 2008). It could therefore cause indirect positive effects on fruit and seed set and subsequent female reproductive success, by increasing the number of flowers visited per foraging bout and reducing geitonogamy (Irwin et al. 2010). This could be of special advantage for plant species that suffer from inbreeding depression (Harder and Barrett 1995). Longer foraging distances may further translate into pollen dispersal over larger ranges and resultant increased pollen flow, cross-pollination and genetic exchange (Fenster 1991; Barrett 2003).

Such indirect effects of nectar robbery on pollinator behaviour and the consequences for female reproductive success have only rarely been investigated (Roubik 1982; Irwin and Brody 1998). Even fewer studies focus on male reproductive success such as pollen dispersal. Only two studies have investigated pollinator flight distances (Zimmerman and Cook 1985; Maloof 2001), which increased after visits to low-rewarding patches with robbed flowers conforming to theoretical predictions of optimal foraging (Pyke 1984). Hardly any experimental work has been conducted and no evidence has been found to support the idea that nectar robbery may lead to enhanced pollen flow (Maloof and Inouye 2000; Irwin et al. 2010). In this study, we used fluorescent dye as pollen analogue to determine pollen dispersal distances. This has been demonstrated to successfully track pollinator movements (Campagne et al. 2009; Van Geert et al. 2010; Mayer et al. 2012).

The three existing studies mimicking pollen dispersal from robbed to unrobbed patches of flowers with fluorescent dye showed contradictory results (Irwin 2003; Richardson 2004; Castro et al. 2008). Combined studies about the interacting effects of nectar robbing on pollinator behaviour as well as female and male reproductive success remain scarce. A better understanding of positive or negative impacts of nectar robbing on plant fitness via pollinator behaviour and pollen flow is necessary to evaluate costs and benefits in these mutualistic networks and to understand evolutionary significance of nectar robbers for plant reproduction.

In our experiments, we aimed at illuminating the effects of nectar robbing on pollinator behaviour and possible consequences for male and female reproductive success of plants. We chose *Aconitum napellus* ssp. *lusitanicum* Rouy (Common monkshood, Ranunculaceae, Fig. 1) as the model plant because it is a species that has become threatened in Europe due to destruction of the wet biotopes it inhabits (Lambinon and Verloove 2012). Le Cadre et al. (2008) showed that *A. napellus* ssp. *lusitanicum* suffers from Allee effects showing decreased individual plant fitness in small populations. Though the species is self-compatible, seed set significantly benefits from outcrossing (Le Cadre et al. 2008; Rigo 2013). During a previous study (Cawoy et al. 2012), we had observed that the helmet-shaped flowers of this bumblebee-pollinated plant species were heavily robbed by honeybees that slipped between tepals from the side thereby avoiding contact with reproductive organs (Fig. 1). This kind of robbery has been described as base working to

Fig. 1 A honeybee slips between the tepals of *A. napellus* ssp. *lusitanicum* to steal nectar from the nectaries that are hidden in the upper part of the helmet (base working), photo by C. Dehon

distinguish it from the destructive way of robbing where insects chew holes into corollas (Inouye 1980). We artificially simulated flower robbing in experimental and natural plant populations to investigate the following hypotheses: (1) Pollinators visit fewer flowers, spend less time per flower or plant in robbed patches compared to unrobbed ones, and (2) they fly further when leaving patches with robbed flowers consequently increasing pollen flow and male reproductive success. (3) These changes in pollinator behaviour positively influence female reproductive success of *A. napellus* ssp. *lusitanicum*.

Materials and methods

Plant species

Aconitum napellus ssp. *lusitanicum* Rouy (Ranunculaceae, Fig. 1) is an endangered perennial herb growing in wet biotopes, such as fens, marshes and riparian zones along rivers in Southwest Europe (Lambinon and Verloove 2012). The species flowers for <2 weeks between August and September. The main inflorescence bears up to 40 zygomorphic flowers, that gradually open from the base to the apex (Le Cadre et al. 2008). The blue to purple flowers are protrandrous starting with a male phase of 5–6 days followed by the female phase that lasts for further 2–3 days (Dehon 2011). The upper, helmet-shaped petaliferous tepal protects the nectaries that produce about 8 μL per flower.day (Heinrich 1979; Dehon 2011). Nectar production peaks on the third or fourth day and diminishes continuously thereafter (Dehon 2011). Insect visitors have to pass over anthers and stigmas when crawling up into the helmet where they turn around to reach the nectar. *Bombus pascuorum*, a common long-tongued bumblebee, has been recorded as the major pollinator of *A. napellus* in Belgium (Cawoy et al. 2012). *Bombus terrestris* on the other hand, a short-tongued species, has been observed biting holes in the helmet and robbing the flowers in France (Le Cadre 2005). Following successful pollination, single flowers produce two to three follicles, together containing 20.7 ± 6.9 seeds (mean ± SD, N = 66; Naveau 2012).

Study sites

Experimental site

In 2010 and 2011, we set up experimental patches with potted plants on an open lawn next to greenhouses at the Université Catholique de Louvain, Louvain-la-Neuve, in Central Belgium (50°39′55″N; 4°37′11″E). Rhizomes of the species had been collected in three natural populations in 2008 (50 fragments per population) and individually cultivated with peat compost in 5 L pots, each thinned to contain only one plant. We arranged 20 pots with flowering plants in square plots of 3 m length with patches of five pots grouped in each corner. Of these plants, open flowers in their male phase were then artificially robbed: the helmet-shaped tepals were gently bent back to expose the nectaries. These were then cut off with a scalpel to inhibit any nectar production and the helmet moved back to its original position. This rigorous method causes only invisible damage but no changes in flower viability or fertility (pers. obs.). It was therefore preferred to repeatedly removing nectar with capillaries to avoid provoking obvious harm to the upper tepals by frequent manipulation. About 40 % of all open flowers per plant were robbed and marked with green woollen threads, which corresponded to the proportion of robbed flowers observed in natural populations in France (Le Cadre 2005). At about 20 m distance, there was a second plot of 20 plants all with untreated flowers as a control. Plants from the three different populations were always mixed in the patches (and the larger plots) to guarantee outcrossing (Le Cadre et al. 2008).

Natural populations

In 2011 and 2012, investigations took place in the four remaining natural populations in fens in South Belgium. Of these, the nature reserve "Les Abattis" contains the largest and densest population ("Appendix Table 3"). At "Fouches", the second largest population, individuals of *A. napellus* were very scattered over the area and at much lower densities. "Chantemelle" had a discontinuous population consisting of three larger patches (161, 423 and 500 m^2) with relatively low plant and flower densities ("Appendix Table 3"). "Sainte-Marie" had a tiny and patchy population stretching about 150 m along a former railway track.

Pollinator observations

Experimental site

Flower visitors were recorded during 10-min observations during peak flowering on 4 days (19.–22.8) in 2010 and on 3 days (9.–11.8) in 2011 with dry and warm weather conditions. The proportion of male flowers during these days was equally high for the two treatments (MWU: $Z = -1.6$, $P = 0.1$) and gradually declined from 91 to 62 %. Eighteen observations per treatment were conducted 2010, and 24 in 2011. The observation of patches with different treatments was alternated and care was taken to spread observations of different patches evenly over the course of a day. The five plants per patch were observed at the same time. For each visitor we recorded the species, number of robbed and unrobbed flowers visited per plant and patch, time spent per single flower as well as their foraging behaviour (pollen or nectar collection, base working, referred to as "robbing").

Natural populations

We conducted similar 10-min flower observations in the natural populations on 3 days in 2011 (between 30.8 and 3.9) and on 4 days in 2012 (between 21.8 and 27.8). Flowers within 1 m^2 (or about five plants) were observed at the same time. A minimum of ten observations per population were conducted (maximum 16), spread over the entire populations and over different times during the day. As done at the experimental sites, the species' name of each visitor, number of flowers visited per plant and patch, time spent per single flower as well as their foraging behaviour was noted.

Male reproductive success: pollen dispersal

Experimental site

We measured pollen dispersal and flight distances of pollinators with fluorescent dye serving as a pollen analogue. At the experimental site, we arranged 28 potted plants along a transect of about 30 m long in 2010 (31.8), and 41 and 49 plants along two transects of 50 m in 2011 (15. and 28.8). Pots were placed up to 1.5 m apart. At each end of a transect, three pots were grouped together to act as pollen or dye source. The dye source on one end of the transect was then artificially robbed as described above (i.e. the nectaries of ~40 % of the flowers were cut). Early in the morning, pink fluorescent powdered dye (Radiant Color Corp., Serie Radglo© R) was applied with toothpicks to dehiscing anthers of all available flowers on these source plants. On the other end of each transect, orange fluorescent dye was put on anthers of the three grouped plants with untreated flowers as control. One day later, we collected receptive stigmas of up to seven flowers from all plants along the transect ($N_{total} = 134$ in 2010; $N_{total} = 207$ and 127 in 2011). Stigmas were transported on ice in separate containers to the laboratory for further examination as described below.

Natural population

In 2012 (22.8), we measured dye dispersal in one natural population ("Les Abattis"). With its large extension and continuous plant cover, it is the only suitable population for this experiment. Two groups of three plants about 100 m apart were chosen as dye sources. As described above, 40 % of all open flowers of one dye source were artificially robbed (marked with pink dye) while the other dye source was left untreated as a control (marked with orange dye). One day after the dye application, recipient stigmas of up to seven flowers from 27 plants growing between the two sources were collected ($N_{total} = 109$). All sampled plants were mapped using a 50 m tape measure. According to flower and plant availability, distance between subsequent individuals ranged from 1 to 11 m with a mean of 3.9 m. The distance of recipient plants to dye source ranged from 0.5 to 91.5 m.

Dye observations

Collected stigmas were embedded in glycerine jelly on microscope slides (Van Rossum 2010) for examination at 250 × under a fluorescence microscope (Nikon, Eclipse E400). Presence or absence of dye of both colours was recorded for each stigma. The proportion of stigmas with dye was calculated for each recipient plant as an average weighted by the number of stigmas collected per plant (Sokal and Rohlf 2000).

Female reproductive success: seed set

In all 3 years of observation, on average five fruits (±2.0; SD) from 12 to 20 plants (13 ± 3.2) were collected at the end of September from the experimental site. Ovules and seeds were counted to calculate seed set as number of viable seeds per total number of ovules. Seeds were classified according to their size and form in 'plump' or 'aborted', the latter being about half the size and flattened, appearing empty. Viability within the resulting two groups was verified for 15 seeds each by staining them with a solution of 1 % Tetrazolium following the protocol of Kearns and Inouye (1993).

Statistical analyses

Pollinator behaviour

Data from the experimental site were pooled for 2010 and 2011 per pollinator type (honeybees and bumblebees, see below) and analysed with generalised linear mixed models (GLMM). We defined treatment (robbing or control) as influencing (fixed) effect and included year and floral display (i.e. number of open flowers per patch) as random factors. Response variables were the average number of flowers visited per plant and patch, the proportion of flowers visited per patch (i.e. number of flowers visited divided by floral display, which was then not included as random factor) as well as the average time spent by individual foragers per single flower. To identify possible differences of time spent per flower between the two treatments, we compared time spent on robbed and unrobbed flowers within robbed patches separately with time per flower in control patches. For all analyses on time, only nectar collecting bumblebee individuals were considered, since the duration of pollen collection was not affected by nectar availability in a flower. The data on nectar collecting honeybees were too few to perform any analyses (only three individuals per treatment). To see whether pollinators would respond to the lack of rewards prior to visitation or would avoid manipulated flowers, we compared the number of visits of individual foragers to robbed and unrobbed flowers within robbed patches only. For this analysis, the number of robbed flowers visited was multiplied with 0.6 and that of unrobbed flowers with 0.4 to adjust the abundance of both flower types (the abundance of unrobbed flowers was higher since only 0 % of the open flowers had been artificially robbed).

For count data, we calculated GLMMs using either a poisson error distribution with a log-link function and residual penalized or Laplace likelihood approximation (for number of flowers visited per plant and proportion of flowers visited per patch), or a negative binomial distribution with residual penalized likelihood approximation (for number of flowers visited per patch and all time analyses), which effectively reduced overdispersion in variance of the data. For proportional data (seed set), best model adaptation was achieved by using a β-distribution with a logit-link function and residual penalized likelihood approximation (Ferrari and Cribari-Neto 2004). GLMMs were performed with SAS 9.2 (‘‘Proc GLIMMIX’’; SAS Institute Inc., Cary, NC, USA) where significance is determined with “Type III Tests of Fixed Effects”.

Pollen dispersal

Pollen dispersal generally follows a leptokurtic distribution, where most pollen is deposited within short distance from the source and rarely over longer ranges (Thomson and Plowright 1980; Hardy et al. 2004). We characterised the shape of the dye dispersal distributions with the best-fitting parameter β of the dye dispersal kernel, an exponential power function where the proportion of stigmas carrying dye was used to fit the function (for more details, see Van Rossum et al. 2011). The dispersal kernel is fat-tailed when $\beta < 1$ (leptokurtic distribution), and thin-tailed when $\beta > 1$ (exponential distribution). We further described the relationship between the distance to the dye source and the proportion of stigmas with dye per recipient plant with Gamma (Γ) correlations (Mayer et al. 2012). To detect differences in dispersal patterns between robbed and control transects, we pooled the data for different years and trials and tested the homogeneity of the slopes of the two types of transects. For this purpose, we first calculated logit-link models with the proportion of stigmas carrying dye related to the distance to the dye source (independent variable), that was nested in the transect (to account for different dates and years). We then analysed for significant differences between robbed and unrobbed models with a likelihood ratio Chi square test of nested models (“lrtest”, R-Package lmtest version 0.9-30, R Development Core Team 2009).

Results

Pollinator behaviour

Experimental site

During 360 min of flower observations in 2010, we observed 192 insect individuals and 195 during 480 min in 2011. At the experimental site, *Bombus pascuorum* was the most abundant species observed in both years of study (70 and 82 % of all individuals in 2010 and 2011), followed by *Apis mellifera* (17 % in both years). Three individuals of the short-tongued *B. terrestris* s.l. were observed legitimately probing flowers for nectar. Individuals of *B. pascuorum* predominantly collected nectar (94 and 95 % of all flower visits for 2010 and 2011) and hardly any pollen (in 3 and 4 %). Bumblebee individuals occasionally robbed flowers, 3 % (2010) and 1 % (2011) of all visits. Honeybees on the other hand largely visited flowers for pollen collection (81 and 96 % of all visits) and much less for nectar (in 10 and 3 % or all flower visits). In 2010, honeybees were robbing flowers (8 %, but only 1 % in 2011). Flowers were robbed exclusively by entering from the side, sliding between the tepals (Fig. 1, i.e. base working). We never observed that visitors chewed holes to access nectar. About 10 % of all bumblebee individuals and 12 % of honeybees switched from legitimate nectar or pollen collection to nectar robbing (and back) during single foraging bouts.

Bumblebee individuals showed a tendency to visit fewer flowers per plant in patches where flowers had been artificially robbed compared to control patches (Table 1). Within the robbed patches though, bumblebees did not approach untreated flowers more frequently than robbed flowers ($F_{1,185} = 1.86$, $P > 0.1$). Looking at the average number of flowers visited per patch, we found no difference between robbed and control patches (Table 1). The proportion of flowers visited by individual bumblebees per patch was significantly higher in robbed patches compared to control patches (Table 1). Individual bumblebees spent significantly less time per flower for nectar collection in robbed patches than in control patches (Table 1). In fact, they spent much less time on a robbed flower in a robbed patch than on an unrobbed flower in a control patch ($F_{1,175} = 69.50$, $P < 0.001$). There was no difference in time spent per flower for unrobbed flowers in robbed and control patches ($F_{1,199} = 1.51$, $P = 0.23$).

Honeybees on the other hand made no difference; neither in the number of flowers visited per plant in control and robbed patches nor in the number or proportion of flowers visited per patch (Table 1).

Natural populations

During 530 min of flower observations in 2011 and 400 min in 2012, we recorded 108 and 110 flower visitors. In natural populations as well, bumblebees (*B. pascuorum* and *B. hortorum*) and honeybees were the major flower visitors, though their numbers varied considerably among populations and years (Table 2). Similar to the experimental sites, bumblebees almost exclusively collected nectar and hardly ever visited flowers for pollen or robbed nectar (Table 2). Contrarily to observations from the experimental site, honeybees were mainly robbing nectar and visited only few flowers legitimately (Table 2). Honeybees collecting pollen were hardly recorded.

Table 1 Pollinator behaviour (number of flowers visited per plant and patch, proportion of visited flowers per patch and time spent per single flower) in control and robbed patches at the experimental site (Louvain-la-Neuve)

	Bombus pascuorum		GLMM			*Apis mellifera*		GLMM		
	Control	Robbed	df	F	*P*	Control	Robbed	df	F	*P*
Flowers per plant	3.0 ± 2.3	2.5 ± 1.4	1,290	3.37	0.06	2.4 ± 1.9	2.2 ± 1.2	1,62	0.11	0.75
Flowers per patch	16.8 ± 18.6	19.6 ± 29.2	1,212	0.45	0.51	32.8 ± 36.5	6.9 ± 9.6	1,28	1.91	0.18
Proportion of visited flowers	0.19 ± 0.23	0.34 ± 0.53	1,290	5.54	**0.02**	0.46 ± 0.58	0.16 ± 0.21	1,62	0.58	0.45
Time per flower (s)	12.5 ± 7.0	10.6 ± 5.0	1,272	20.13	**0.001**	*too few*	*data*	–	–	–

Mean values ± SD and test statistics of GLMMs are given for each comparison between treatments with significant *P* values in bold

Table 2 Foraging behaviour of pollinators (i.e. total number of flowers visited and proportion of flowers visited for nectar legitimately, pollen or robbed) in four natural populations in South Belgium during two years of observation

Population	Species	Year	No of individuals	Number of flowers visited	Proportion (%) of flowers visited for		
					Nectar	Pollen	Robbed
Les Abattis ($2,970\ m^2$, 1,330)	*A. mellifera*	2011	43	158	8	1	91
		2012	19	65	26	–	74
	Bombus	2011	2	7	100	–	–
		2012	21	196	100	–	–
Fouches ($1,788\ m^2$, 176)	*Bombus*	2011	11	89	100	–	–
		2012	10	143	99	–	1
Chantemelle ($1,084\ m^2$, 179)	*A. mellifera*	2011	6	27	89	–	11
	Bombus	2011	7	47	100	–	–
		2012	31	401	98	1	1
Sainte-Marie ($300\ m^2$, 152)	*A. mellifera*	2011	9	40	35	–	65
	Bombus	2011	11	72	99	1	–
		2012	12	100	100	–	–
Total	*A. mellifera*		78	291	23	1	76
	Bombus		105	1,065	98	1	1

Populations are sorted from large to small with surface cover and mean number of flowers per m^2 in brackets

Male reproductive success: pollen dispersal

Experimental site

Dye was dispersed over the entire transect length at the experimental site (Fig. 2a). Most dye was transferred within the first ten meters following a fat-tailed or leptokurtic distribution, as the best-fitting β parameter describing the shape of the distribution curves was <1 (Fig. 2a). The proportion of stigmas carrying dye declined significantly faster in the control transects ($\Gamma = -0.51$, $P < 0.001$) than in the robbed transects where it remained almost stable at 100 % ($\Gamma = -0.001$, $P < 0.001$; test of homogeneity of the slopes: $\chi^2 = 45.29$, $P < 0.001$).

Natural population

Similar differences were found in the natural population. In the control transect dye was dispersed <60 m ($\Gamma = -0.85$, $P < 0.001$; Fig. 2b). Comparing the two types of transects, we found that in the robbed transect fluorescent dye was transported over significantly larger distances (>90 m) than in the control transect (test of homogeneity of the slopes: $\chi^2 = 4.70$, $P < 0.001$). In the robbed transect, the proportion of stigmas with dye declined at a slower rate than predicted by a letpokurtic distribution ($\beta = 2.09$, $\Gamma = -0.69$, $P < 0.001$).

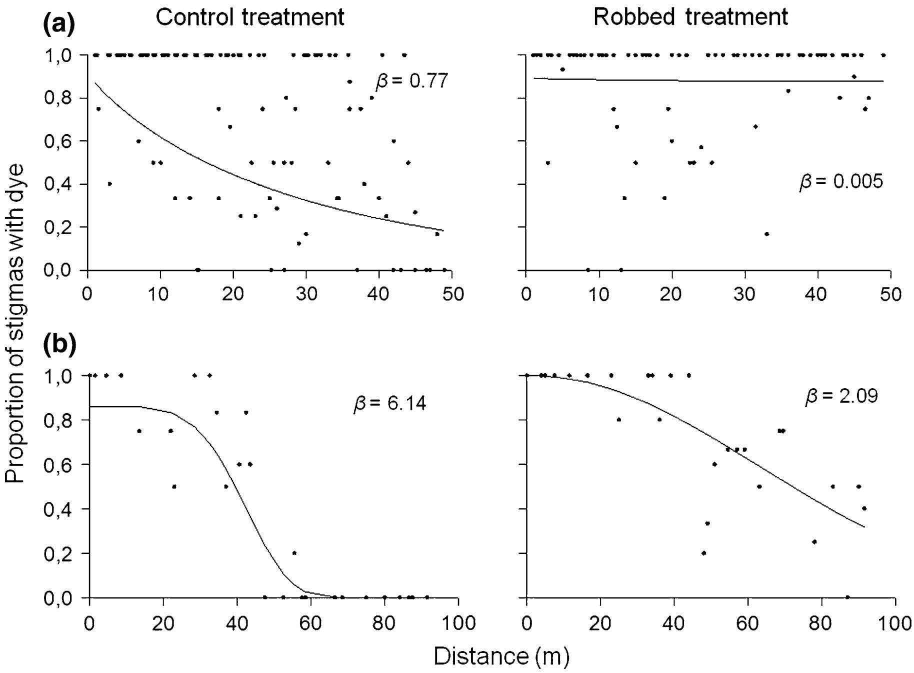

Fig. 2 Proportions of stigmas with dye as a function of the distance to dye source at the experimental site (**a**) and in natural population (**b**). Control transects on the left and robbed transects on the right side. The line represents the distribution expected under the exponential power dispersal model for best-fitting parameter β of the dye dispersal kernel

Female reproductive success: seed set

Experimental site

Average seed set per plant did not differ between robbed (78.1 ± 10.4) and unrobbed patches (81.1 ± 7.2, $F_{1,59} = 1.78$, $P = 0.19$).

Discussion

Pollinator behaviour

All recorded robbers stole nectar by base working, i.e. slipping between the tepals from the side (Fig. 1). This is probably owed to the fact that the majority of individuals that were robbing nectar were honeybees (*A. mellifera*), especially in the natural populations where about three quarters of their visits were illegitimate. Honeybees however are incapable of chewing robbery holes (Delaplane and Mayer 2000). Surprisingly and contrarily to observations from Northern France made by Le Cadre (2005), we did not recognise any insect visitor biting holes in the helmet, neither on the experimental sites with potted plants in Central Belgium (Louvain-la-Neuve), nor in the natural populations in South Belgium. We hardly saw the short-tongued *B. terrestris* visiting flowers (only three individuals at the experimental site in Central Belgium), which were responsible for nectar robbery in the study by Le Cadre (2005). In the natural populations, the long-tongued *B. hortorum* that is able to reach nectar legitimately was the most common flower visitor.

In our study, we could see a trend in bumblebees visiting fewer flowers per plant in robbed patches as already observed by other authors (e.g. Irwin and Brody 1998; Maloof and Inouye 2000; Maloof 2001; González-Gómez and Valdivia 2005). It has been suggested that bumblebees are capable of avoiding nectarless flowers, maybe through detection of scent marks left by previous visitors (Goulson et al. 1998; Williams 1998). Within the robbed patches however, they visited similar numbers of robbed and unrobbed flowers. Thus, it seems that bumblebees in our study did not have knowledge about the reward in flowers prior to visitation and could obviously not distinguish treated (nectar-free) from control flowers. It was most likely that the lack of resources encountered while probing a flower changed bumblebee behaviour. Pollinators that visit fewer flowers per inflorescence are supposed to reduce geitonogamy (Maloof and Inouye 2000). This would be advantageous for *A. napellus* that produces more seeds with outcross pollen (Le Cadre et al. 2008; Rigo 2013). The number of flowers visited per patch was equal for the two treatments. The proportion of flowers visited was even higher in robbed patches, which we explain with the shorter time bumblebees spent on robbed flowers (see also Zimmerman and Cook 1985; Richardson 2004; Zhang et al. 2007). Consequently, bumblebees probed more flowers per unit time in robbed patches. This behaviour is also considered to increase pollen flow (Maloof and Inouye 2000). Besides, reduced time per flower may diminish pollen deposition and limit ovule fertilisation (but see below). Honeybees on the other hand, showed similar behaviour in robbed and unrobbed patches. They principally collected pollen, which does not depend on nectar quantities.

Quite some individuals (10 %) mixed legitimate foraging (either pollen or nectar collection) with exploiting behaviour during single foraging bouts. In fact, such mixed foraging strategies seem to be quite common (Rust 1979; Morris 1996; Newman and Thomson 2005; Richardson and Bronstein 2012 and references therein) rendering the predictability of pollinator efficiency rather impossible. Plants face the risk of losing their pollinators when trying to deter robbers. Traditionally, it is agreed that nectar robbers impose evolutionary pressure on plants' traits that would in turn evolve defence strategies such as thicker corollas, production of large inflorescences or toxic nectar (Roubik 1982; Adler 2000; Adler and Irwin 2005; Irwin and Adler 2006; Irwin et al. 2008). *Aconitum* plants contain toxic alkaloids, but their concentrations in the nectar have been found to be extremely low (Gosselin et al. 2013). Almost all of the works on nectar robbery were based on primary robbers, seldom considering secondary robbing and never base working (Maloof and Inouye 2000; Irwin et al. 2010). Inouye (1980) suggested that base workers would, similarly to nectar thieves, probably not greatly influence the activities of pollinators. Nectar thieves are considered to be small insects that leave nectar behind. Base workers however, are often honeybees or bumblebees that pollinate the same plant species they rob (Weaver 1956; Torvik et al. 1998). They could be able to deplete nectar completely and thus, theoretically have similar effects on plant traits as primary or secondary robbing (apart from thickening floral tissue). It is further assumed that cheating is the more efficient behaviour for a flower visitor (Dedej and Delaplane 2005; Leadbeater and Chittka 2008). If so, why would they then not always cheat and never visit flowers legitimately? More work on such mixed foraging strategies is needed to examine their evolutionary consequences as well as the triggers for a particular behaviour (Irwin et al. 2010; Richardson and Bronstein 2012).

Male reproductive success: pollen dispersal

Two studies noted that pollinators increased flight distance after visits to robbed flowers and suggested that this behavioural change would lead to farther pollen dispersal (Zimmerman and Cook 1985; Maloof 2001). Contrary to such theoretical predictions, the very few investigations that simulated pollen dispersal with fluorescent dye found mainly neutral (Irwin 2003; Richardson 2004) or even negative effects (Castro et al. 2008) of nectar robbery on the distance of pollen flow. Our results clearly indicated that pollinators dispersed the pollen to further distances after visits to robbed compared to unrobbed sources. This was true not only at the experimental site, were other floral resources were scarce, but even more so in the natural population where the distance of dye dispersal even doubled. Though dye transfer cannot always be translated into pollen transfer (Thomson 1986; Waser 1988), several studies have shown that it is often a good proxy for pollen dispersal distance (Mitchell 1993; Townsend and Levey 2005; Van Rossum et al. 2011). Our observations suggest that, at least for *A. napellus*, nectar robbing could positively affect male reproductive success via changes in pollinator behaviour leading to enhanced pollen flow within populations. At the same time, this should increase outcrossing which in turn is supposed to improve female reproductive success (Barrett and Harder 1996). From the pollinator point of view, it remains to be explored whether these might face energy losses and reduced fitness through increased foraging times and larger distances travelled in plant populations with high occurrence of nectar robbery.

Female reproductive success: seed set

It has often been shown that nectar robbery reduces female reproductive success of plants through limited fruit or seed set (Maloof and Inouye 2000; Burkle et al. 2007; Brody et al. 2008; Castro et al. 2008). Fruit set per plant was high (>90 %) for *A. napellus* ssp. *lusitanicum* and not affected by the robbing treatment (pers. obs.). Our results from the experimental site further revealed no effect of (artificial) nectar robbing on average seed set per plant, similar to other studies (Zimmerman and Cook 1985; Stout et al. 2000; Maloof 2001). Therefore, though pollinators left robbed flowers much quicker than unrobbed ones, this obviously did not affect reproductive success. The common assumption that time spent per flower improves pollen deposition on stigmas and thus, ovule fertilisation and resulting seed set (Thomson and Plowright 1980; Galen and Plowright 1985) does not really apply to *A. napellus* flowers. While probing nectar hidden in the upper part of the helmet, bees are usually not in contact with anthers or stigmas situated in the lower part (Fig. 1).

Our experimental and naturally observed levels of nectar robbing of *A. napellus* may be moderate (~40 %, Le Cadre 2005) compared to other plant species reaching 96 and even 200 % with multiple robber holes (e.g. Stout et al. 2000; Irwin and Maloof 2002). The modest levels of nectar robbery may explain why we did not find any negative effects of nectar robbery on female reproductive success. The complete suppression of nectar replenishment caused in our experiments may not reflect a natural distribution of nectar levels under robbing. However, no nectar in robbed flowers and unknown (maybe robbed) nectar levels among the untreated flowers generated a similar situation as in other experimental setups used by Irwin (2003) for example. Here, flowers were protected from robbing (i.e. completely replenished nectar) and compared to naturally robbed flowers (of unknown levels). From our findings we may conclude that *A. napellus* ssp. *lusitanicum* indirectly benefited from nectar robbing via changes in pollinator behaviour. These changes led to more flower visits per unit time causing a trend where bees visited fewer flowers per plant. This could limit geitonogamy and improve female reproductive success. On the other hand, changed pollinator behaviour led to increased dispersal of pollen, which possibly improves male reproductive success. Female reproductive success of *A. napellus* plants was not negatively impacted. Evidence gathered during this study does not support the hypothesis that a simple antagonistic relationship exists between a plant species and nectar robbers. More detailed observations of other robbed plant species without apparent defence strategies could reveal similar relationships. It has been questioned how mutualisms facing exploitation can persist over an evolutionary time scale. Maybe we need to rethink the definition of nectar robbery being exclusively exploitative.

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Appendix

See Table 3.

Table 3 Characteristics of four remnant natural populations of *Acontium napellus* ssp. *lusitanicum* in South Belgium

Population	Coordinates	Surface covered (m^2)	Plants per m^2	Flowers per m^2
Les Abattis	49°40′50″N; 5°32′59″E	2,970	15.5 ± 8.9	1,330 ± 1,030
Fouches	49°4′8″N; 5°43′06″E	1,788	3.9 ± 2.7	176 ± 177
Chantemelle	49°39′37″N; 5°39′37″E	1,084	4.7 ± 3.3	179 ± 125
Sainte-Marie	49°40′06″N; 5°32′56″E	292	6.0 ± 6.2	152 ± 163

Mean values ± SD are given for plant and flower density, sampled from 10 m^2 in each population in 2012

References

Adler LS (2000) The ecological significance of toxic nectar. Oikos 91:409–420. doi:10.1034/j.1600-0706.2000.910301.x

Adler LS, Irwin RE (2005) Ecological costs and benefits of defenses in nectar. Ecology 86:2968–2978. doi:10.1890/05-0118

Barrett SCH (2003) Mating strategies in flowering plants: the outcrossing–selfing paradigm and beyond. Philos Trans R Soc Lond B Biol Sci 358:991–1004. doi:10.1098/rstb.2003.1301

Barrett SCH, Harder LD (1996) Ecology and evolution of plant mating. Trends Ecol Evol 11:73–79. doi:10.1016/0169-5347(96)81046-9

Brody AK, Irwin RE, McCutcheon ML, Parsons EC (2008) Interactions between nectar robbers and seed predators mediated by a shared host plant, Ipomopsis aggregata. Oecologia 155:75–84. doi:10.1007/s00442-007-0879-8

Bronstein JL (2001) The costs of mutualism. Am Zool 41:825–839. doi:10.2307/3884527

Burkle LA, Irwin RE, Newman DA (2007) Predicting the effects of nectar robbing on plant reproduction: implications of pollen limitation and plant mating system. Am J Bot 94:1935–1943. doi:10.3732/ajb.94.12.1935

Campagne P, Affre L, Baumel A et al (2009) Fine-scale response to landscape structure in *Primula vulgaris* Huds.: does hedgerow network connectedness ensure connectivity through gene flow? Popul Ecol 51:209–219. doi:10.1007/s10144-008-0124-2

Castro S, Silveira P, Navarro L (2008) Consequences of nectar robbing for the fitness of a threatened plant species. Plant Ecol 199:201–208. doi:10.1007/s11258-008-9424-z

Cawoy V, Jonard M, Mayer C, Jacquemart A-L (2012) Do abundance and proximity of the alien *Impatiens glandulifera* affect pollination and reproductive success of two sympatric co-flowering native species? J Pollinat Ecol 10:130–139. http://www.pollinationecology.org/index.php?journal=jpe&page=article&op=view&path[]=203

Darwin C (1872) Effects of cross and self fertilisation in the vegetable kingdom. Murray, London

Dedej S, Delaplane KS (2005) Net energetic advantage drives honey bees (*Apis mellifera* L.) to nectar larceny in *Vaccinium ashei* Reade. Behav Ecol Sociobiol 57:398–403. doi:10.1007/s00265-004-0852-z

Dehon C (2011) Etude de l'impact du vol de nectar sur *Aconitum napellus* spp. *lusitanicum* et sur le comportement de ses pollinisateurs. Master thesis, Université catholique de Louvain

Delaplane KS, Mayer DF (2000) Crop pollination by bees. CABI, New York

Dohzono I, Kunitake YK, Yokoyama J, Goka K (2008) Alien bumble bee affects native plant reproduction through interactions with native bumble bees. Ecology 89:3082–3092. doi:10.1890/07-1491.1

Fenster CB (1991) Gene flow in *Chamaecrista fasciculata* (Leguminosae) I. Gene dispersal. Evolution 45:398–409. doi:10.2307/2409673

Ferrari S, Cribari-Neto F (2004) Beta regression for modelling rates and proportions. J Appl Statist 31:799–815. doi:10.1080/0266476042000214501

Galen C, Plowright RC (1985) The effects of nectar level and flower development on pollen carry-over in inflorescences of fireweed (*Epilobium angustifolium*) (Onagraceae). Can J Bot 63:488–491. doi:10.1139/b85-060

González-Gómez PL, Valdivia CE (2005) Direct and indirect effects of nectar robbing on the pollinating behavior of *Patagona gigas* (Trochilidae). Biotropica 37:693–696. doi:10.1111/j.1744-7429.2005.00088.x

Gosselin M, Michez D, Vanderplanck M et al (2013) Does *Aconitum septentrionale* chemically protect floral rewards to the advantage of specialist bumblebees? Ecol Entomol 38:400–407. doi:10.1111/een.12032

Goulson D, Hawson SA, Stout JC (1998) Foraging bumblebees avoid flowers already visited by conspecifics or by other bumblebee species. Anim Behav 55:199–206. doi:10.1006/anbe.1997.0570

Harder LD, Barrett SCH (1995) Mating cost of large floral displays in hermaphrodite plants. Nature 373:512–515. doi:10.1038/373512a0

Hardy OJ, González-Martínez SC, Fréville H et al (2004) Fine-scale genetic structure and gene dispersal in *Centaurea corymbosa* (Asteraceae) I. Pattern of pollen dispersal. J Evol Biol 17:795–806. doi:10.1111/j.1420-9101.2004.00713.x

Heinrich B (1979) Resource heterogeneity and patterns of movement in foraging bumblebees. Oecologia 40:235–245. doi:10.1007/BF00345321

Inouye DW (1980) The terminology of floral larceny. Ecology 61:1251–1253. doi:10.2307/1936841

Irwin RE (2003) Impact of nectar robbing on estimates of pollen flow: conceptual predictions and empirical outcomes. Ecology 84:485–495. doi:10.1890/0012-9658(2003)084[0485:IONROE]2.0.CO;2

Irwin RE, Adler LS (2006) Correlations among traits associated with herbivore resistance and pollination: implications for pollination and nectar robbing in a distylous plant. Am J Bot 93:64–72. doi:10.3732/ajb.93.1.64

Irwin RE, Brody AK (1998) Nectar robbing in *Ipomopsis aggregata*: effects on pollinator behavior and plant fitness. Oecologia 116:519–527. doi:10.1007/s004420050617

Irwin R, Maloof J (2002) Variation in nectar robbing over time, space, and species. Oecologia 133:525–533. doi:10.1007/s00442-002-1060-z

Irwin RE, Galen C, Rabenold JJ et al (2008) Mechanisms of tolerance to floral larceny in two wildflower species. Ecology 89:3093–3104. doi:10.1890/08-0081.1

Irwin RE, Bronstein JL, Manson JS, Richardson L (2010) Nectar robbing: ecological and evolutionary perspectives. Annu Rev Ecol Evol Syst 41:271–292. doi:10.1146/annurev.ecolsys.110308.120330

Jersáková J, Johnson SD, Kindlmann P (2006) Mechanisms and evolution of deceptive pollination in orchids. Biol Rev Camb Philos Soc 81:219–235. doi:10.1017/S1464793105006986

Kearns CA, Inouye DW (1993) Techniques for pollination biologists. University Press of Colorado, Niwot

Lambinon J, Verloove F (2012) Nouvelle Flore de la Belgique, du Grand-Duché de Luxembourg, du Nord de la France et des Régions voisines, 6th edn. Jardin botanique national de Belgique, Meise

Le Cadre S (2005) Allee effects on plants. The case of *Aconitum napellus* subsp. *lusitanicum*. PhD Thesis, Université Pierre et Marie Curie (Paris VI)

Le Cadre S, Tully T, Mazer SJ et al (2008) Allee effects within small populations of *Aconitum napellus* ssp. *lusitanicum*, a protected subspecies in northern France. New Phytol 179:1171–1182. doi:10.1111/j.1469-8137.2008.02529.x

Leadbeater E, Chittka L (2008) Social transmission of nectar-robbing behaviour in bumble-bees. Proc R Soc B 275:1669–1674. doi:10.1098/rspb.2008.0270

Maloof JE (2001) The effects of a bumble bee nectar robber on plant reproductive success and pollinator behavior. Am J Bot 88:1960–1965

Maloof JE, Inouye DW (2000) Are nectar robbers cheaters or mutualists? Ecology 81:2651–2661. doi:10.1890/0012-9658(2000)081[2651:ANRCOM]2.0.CO;2

Mayer C, Van Rossum F, Jacquemart A-L (2012) Evaluating pollen flow indicators for an insect-pollinated plant species. Basic Appl Ecol 13:690–697. doi:10.1016/j.baae.2012.09.012

Mitchell RJ (1993) Adaptive significance of *Ipomopsis aggregata* nectar production: observation and experiment in the field. Evolution 47:25–35. doi:10.2307/2410115

Morris WF (1996) Mutualism denied? Nectar-robbing bumble bees do not reduce female or male success of bluebells. Ecology 77:1451–1462. doi:10.2307/2265542

Navarro L (2000) Pollination ecology of *Anthyllis vulneraria* subsp. *vulgaris* (Fabaceae): nectar robbers as pollinators. Am J Bot 87:980–985

Navarro L (2001) Reproductive biology and effect of nectar robbing on fruit production in *Macleania bullata* (Ericaceae). Plant Ecol 152:59–65. doi:10.1023/A:1011463520398

Naveau O (2012) Etude de l'impact du vol de nectar sur *Aconitum napellus* spp. *lusitanicum* et sur le comportement de ses pollinisateurs. Master thesis, Université catholique de Louvain

Newman DA, Thomson JD (2005) Interactions among nectar robbing, floral herbivory, and ant protection in *Linaria vulgaris*. Oikos 110:497–506. doi:10.1111/j.0030-1299.2005.13885.x

Proctor M, Yeo P, Lack A (1996) The natural history of pollination. Harper Collins Publishers (The New Naturalist), London

Pyke GH (1984) Optimal foraging theory: a critical review. Annu Rev Ecol Syst 15:523–575. doi:10.1146/annurev.es.15.110184.002515

R Development Core Team (2009) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Richardson S (2004) Are nectar-robbers mutualists or antagonists? Oecologia 139:246–254. doi:10.1007/s00442-004-1504-8

Richardson L, Bronstein JL (2012) Reproductive biology of pointleaf manzanita (*Arctostaphylos pungens*) and the pollinator-nectar robber spectrum. J Pollinat Ecol 9:115–123. http://www.pollinationecology.org/index.php?journal=jpe&page=article&op=view&path[]=177

Rigo C (2013) Etude de l'impact du vol de nectar sur *Aconitum napellus* spp. *lusitanicum* et sur le comportement de ses pollinisateurs. Master thesis, Université catholique de Louvain

Roubik DW (1982) The ecological impact of nectar-robbing bees and pollinating hummingbirds on a tropical shrub. Ecology 63:354–360. doi:10.2307/1938953

Rust RW (1979) Pollination of *Impatiens capensis*: pollinators and nectar robbers. J Kansas Entomol Soc 52:297–308. doi:10.2307/25083907

Schiestl FP (2005) On the success of a swindle: pollination by deception in orchids. Naturwissenschaften 92:255–264. doi:10.1007/s00114-005-0636-y

Sokal RR, Rohlf FJ (2000) Biometry: the principles and practices of statistics in biological research, 3rd edn. Freeman and Company, New York

Stout JC, Allen JA, Goulson D (2000) Nectar robbing, forager efficiency and seed set: bumblebees foraging on the self incompatible plant *Linaria vulgaris* (Scrophulariaceae). Acta Oecol 21:277–283. doi:10.1016/S1146-609X(00)01085-7

Thomson JD (1986) Pollen transport and deposition by bumble bees in *Erythronium*: influences of floral nectar and bee grooming. J Ecol 74:329–341. doi:10.2307/2260258

Thomson JD, Plowright RC (1980) Pollen carryover, nectar rewards, and pollinator behavior with special reference to *Diervilla lonicera*. Oecologia 46:68–74. doi:10.1007/BF00346968

Torvik SE, Borgen L, Berg RY (1998) Aspects of reproduction in *Pulsatilla pratensis* in Norway. Nord J Bot 18:385–391. doi:10.1111/j.1756-1051.1998.tb01515.x

Townsend PA, Levey DJ (2005) An experimental test of whether habitat corridors affect pollen transfer. Ecology 86:466–475. doi:10.1890/03-0607

Traveset A, Willson MF, Sabag C (1998) Effect of nectar-robbing birds on fruit set of *Fuchsia magellanica* in Tierra Del Fuego: a disrupted mutualism. Funct Ecol 12:459–464. doi:10.1046/j.1365-2435.1998.00212.x

Van Geert A, Van Rossum F, Triest L (2010) Do linear landscape elements in farmland act as biological corridors for pollen dispersal? J Ecol 98:178–187. doi:10.1111/j.1365-2745.2009.01600.x

Van Rossum F (2010) Reproductive success and pollen dispersal in urban populations of an insect-pollinated hay-meadow herb. Perspect Plant Ecol 12:21–29. doi:10.1016/j.ppees.2009.08.002

Van Rossum F, Stiers I, Van Geert A et al (2011) Fluorescent dye particles as pollen analogues for measuring pollen dispersal in an insect-pollinated forest herb. Oecologia 165:663–674. doi:10.1007/s00442-010-1745-7Waser

Waser NM (1988) Comparative pollen and dye transfer by pollinators of *Delphinium nelsonii*. Funct Ecol 2:41–48. doi:10.2307/2389458

Weaver N (1956) The foraging behavior of honeybees on hairy vetch foraging methods and learning to forage. Insectes Soc 3:537–549. doi:10.1007/BF02226457

Williams GS (1998) The identity of the previous visitor influences flower rejection by nectar-collecting bees. Anim Behav 56:673–681. doi:10.1006/anbe.1998.0794

Zhang Y-W, Robert GW, Wang Y, Guo Y-H (2007) Nectar robbing of a carpenter bee and its effects on the reproductive fitness of *Glechoma longituba* (Lamiaceae). Plant Ecol 193:1–13. doi:10.1007/s11258-006-9244-y

Zimmerman M, Cook S (1985) Pollinator foraging, experimental nectar-robbing and plant fitness in *Impatiens capensis*. Am Midl Nat 113:84–91. doi:10.2307/2425350