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Does local conspecific density influence fruit set via pollinator visitation in *Orchis militaris*?

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Abstract:	Plant density varies naturally, from isolated plants to clumped individuals, and can influence pollinator foraging behaviour and plant reproductive success. The effect of conspecific density could depend on the pollination system, and deceptive species differ from rewarding ones in this regard. In our study, we aimed to determine how local conspecific density affects fruit set in *Orchis militaris* (Orchidaceae) through changes in pollinator visitation. We measured fruit set in a natural population and recorded pollinator abundance and foraging behaviour within plots of different *O. militaris* density. We also took into account the concurrent effect of the number of flowers per plant (floral display size). Detailed data were recorded for the most abundant potential pollinators of *O. militaris*, i.e. solitary bees. Floral display size was negatively correlated to fruit set in medium-density plots, but uncorrelated in low- and high-density plots. Plot density had no effect on solitary bee abundance and visitation, which may be due to low pollinator abundance within the study site. The proportion of visited flowers per inflorescence was negatively influenced by floral display size, which is in line with previous studies. In addition, solitary bees spent decreasing time in successive flowers within an inflorescence, and the time spent per flower was negatively affected by ambient temperature. Our results suggest that pollinator behaviour during visitation is poorly linked to pollen deposition and reproductive success in *O. militaris*.

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Abstract

Plant density varies naturally, from isolated plants to clumped individuals, and can influence pollinator foraging behaviour and plant reproductive success. The effect of conspecific density could depend on the pollination system, and deceptive species differ from rewarding ones in this regard. In our study, we aimed to determine how local conspecific density affects fruit set in *Orchis militaris* (Orchidaceae) through changes in pollinator visitation. We measured fruit set in a natural population and recorded pollinator abundance and foraging behaviour within plots of different *O. militaris* density. We also took into account the concurrent effect of the number of flowers per plant (floral display size). Detailed data were recorded for the most abundant potential pollinators of *O. militaris*, i.e. solitary bees. Floral display size was negatively correlated to fruit set in medium-density plots, but uncorrelated in low- and high-density plots. Plot density had no effect on solitary bee abundance and visitation, which may be due to low pollinator abundance within the study site. The proportion of visited flowers per inflorescence was negatively influenced by floral display size, which is in line with previous studies. In addition, solitary bees spent decreasing time in successive flowers within an inflorescence, and the time spent per flower was negatively affected by ambient temperature. Our results suggest that pollinator behaviour during visitation is poorly linked to pollen deposition and reproductive success in *O. militaris*.

Keywords: conspecific density, floral display size, fruit set, Orchidaceae, *Orchis militaris*, solitary bees

Introduction

In natural populations, plant density is highly variable, both at the inter- and intraspecific level. Individuals may grow isolated at some sites, whereas at others they are clumped in dense patches. In flowering plants, conspecific density can affect pollen movement or visitation rates by potential pollinators (e.g. Rathcke 1983, Bosch and Waser 1999, Duffy and Stout 2008, 2011), and thus can have an effect on fruit or seed production (Elliott and Irwin 2009). Flower-rich patches tend to attract more pollinators (Ghazoul 2005) since they provide more floral resources (nectar, pollen, etc.) (Totland 1994, Feldman 2006). As such, high density can favour outcrossing (Karron et al. 1995) whereas at low density, plants may be less visited by pollinators (see references in Feldman 2006, 2008) and receive more potentially undesirable self-pollen (Bosch and Waser 2001, Ashman et al. 2004). On the other hand, pollinators can become a limiting factor at high plant density. For example, individual flowers in dense plots may receive fewer visits due to handling time limitations (Feldman 2006). Either facilitative or competitive interactions (Rathcke 1983) may thus impact pollination success.

In addition to the ecological context, floral or plant traits can also influence pollinator behaviour and reproductive success. According to Makino et al. (2007), floral display size and density may similarly influence pollinator attraction. Within a given patch, larger floral displays attract more insects and more flowers per plant are probed than in smaller displays (Ohashi and Yahara 2001). In addition, increased visit duration on an inflorescence is documented in plants with a larger floral display (Ishii 2006, Routley and Husband 2003). Such a preference of pollinators for larger displays (Ohara and Higashi 1994, Miyake and Sakai 2005) could be due to a reduction of flight costs or better detection (Brody and Mitchell 1997, Ohashi and Yahara 2001). Floral display size has been shown to be under positive pollinator-mediated selection in several species

(Sandring and Ågren 2009, Sletvold and Ågren 2009, Sletvold et al. 2010) and fruit production is positively affected by floral display size in numerous Angiosperm species (e.g. Willson and Rathcke 1974, Udovic 1981, Bartkowska and Johnston 2014).

Floral signals used by plants to attract pollinators usually advertise rewards (Schiestl and Johnson 2013). However, some species do not produce any reward while displaying attractive floral signals such as showy flower colours or spurs (Jersáková et al. 2009). These plant species are called deceptive (Renner 2006), and deceptive pollination occurs in about 30-40% of orchids (Johnson and Schiestl 2016). Among the latter, most species attract pollinators by exploiting their food-seeking behaviour (Jersáková et al. 2006). The impact of conspecific density on fruit set has been investigated in several deceptive orchid species (e.g. Fritz and Nilsson 1996, Meléndez-Ackerman and Ackerman 2001, Juillet et al. 2007). Its negative effect on fruit set (e.g. Gumbert and Kunze 2001, Internicola et al. 2006) is hypothesised to be due to learning avoidance (Anderson and Johnson 2006, but see Juillet et al. 2011), which lasts at least in the short term (Hobbhahn et al. 2017). In addition, deceptive small floral displays can experience reduced pollination success or fruit production compared to larger displays (e.g. Aragón and Ackerman 2001, Sletvold and Ågren 2011). In deceptive species, larger displays are likely to increase the frequency of pollinator visits (Suetsugu et al. 2015); on the contrary, the number of flowers visited per inflorescence by individual pollinators is unlikely to be related to display size since they usually visit only one or a few flowers per inflorescence before leaving (Smithson 2002, Suetsugu et al. 2015).

In this study, we aimed to test how density affects fruit set through changes in pollinator visitation in natural plant populations. We investigated the impact of local *Orchis militaris* density on pollinator abundance and behaviour and fruit set, taking into account the simultaneous

effect of floral display size. According to the results of Henneresse et al. (2017), we expected fruit set to be positively correlated with floral display size in low-density plots but not in high-density plots. Since fruit set could increase in response to repeated pollinator visits (Lammi et al. 2003), we hypothesised a higher frequency of pollinator visits to larger displays in low-density plots. Although plant density probably affects pollinator behaviour (Mustajärvi et al. 2001, Ghazoul 2005) – and visitation rate or time spent per flower might in turn affect plant reproductive success (Gigord et al. 2002) –, pollinators rapidly leave deceptive inflorescences after a few probes. Therefore, the number of flowers visited per inflorescence and the time spent per flower and per inflorescence are not expected to be affected by density or floral display size. A decrease in the time spent per flower could occur along individual foraging bouts due to the rewardless nature of these flowers. To test these hypotheses, we conducted pollinator observations in 2017 in a single population in Belgium, using the natural density variation to establish plots of varying density.

Material and methods

Study species and sites

Orchis militaris is a perennial herb with a Eurasian-Mediterranean distribution (Kretzschmar et al. 2007). The species ranges from sea-level to more than 2,000 m and grows in chalk grasslands, wet meadows or open woodlands. In Western Europe, flowering takes place from April to June (Delforge 2012). The height of the flowering stem varies between 20 and 45 cm and it bears ten to forty hermaphroditic flowers (Delforge 2012, Farrell 1985). *O. militaris* is assumed to be self-compatible (Metsare et al. 2015, Neiland and Wilcock 1998) and non-autogamous (Delforge 2012). It is hypothesised to depend on generalised food deception to attract pollinators

(Henneresse et al. 2017). Hymenopterans from the genera *Andrena* (Andrenidae) and *Halictus* (Halictidae) are thought to be confirmed pollinators (Vöth 1987). In addition, several potential pollinator species are also known (bumblebees, honeybees, solitary bees and one beetle species – Henneresse and Tyteca 2016). Low levels of fruit set (< 30%) are common (Claessens and Kleynen 2011, Henneresse et al. 2017).

The study was conducted from May to July 2017 in a large Belgian population located in Andenne (50°29′60″N 5°03′30″E, altitude: ≈ 175 m). The site was an open woodland dominated by *Betula pendula*.

Study plots and fruit production

Using natural density variation, we established twenty 1 m × 1 m low-density plots, eight medium-density plots and four high-density plots. We considered the following *O. militaris* densities: one (low density), four (medium density) and eight (high density) inflorescences. Each plot was given a unique identifier (plot ID) and marked with bamboo sticks; plots were similar in terms of light levels. Other flowers and inflorescences and vegetation were clipped at 5-10 cm from the ground to make the plots homogenous. The eight 1 m × 1 m squares surrounding each plot ('buffer zone') were similarly cleared of plants, except *O. militaris*, to homogenise the local-scale landscape. Within the buffer zone, the number of *O. militaris* inflorescences was counted (buffer density). Within each plot, every *O. militaris* inflorescence was individually marked using bamboo canes and paper tags. Nearest neighbour (not necessarily found within the plot) distance was measured for each individual to evaluate plant spatial distribution, which may not be systematically correlated with plot density (Meléndez-Ackerman and Ackerman 2001). Plots were interspersed within the population and there was no overlap between buffer zones.

About one month after the end of the flowering period, the total number of flowers (floral display size) and the number of fruits per tagged inflorescence were counted in order to quantify fruit set by dividing the number of fruits by the total number of flowers.

Insect visitation

Insect observations took place during days without rain or strong wind, between 9.00 a.m. and 5.00 p.m., between 21 and 29 May. Plot visitation order was randomized and plots were studied during 15-min censuses. We made 43.0 total hours (172 15-min censuses) of observation: 58 censuses in low-density plots, 57 censuses in medium-density plots and 57 censuses in high-density plots. Before each census, we recorded air temperature using a thermometer and we counted the number of *O. militaris* open flowers per plant (number of flowers per inflorescence) and within the plot (total number of flowers). We used a portable digital voice recorder to record all aspects of the visitor behaviour. During the censuses, we counted the number of potential pollinators (bumblebees, honeybees and solitary bees) entering the plot. For each insect, we recorded its behaviour after a first approach (as defined by Gumbert and Kunze 2001 – visit or departure from the plot), the number of flowers visited per inflorescence (bout length) and the time spent per flower and per inflorescence. The proportion of flowers visited (per 15-min census) was calculated by dividing the number of flowers probed by an insect on an inflorescence by the number of open flowers on this inflorescence. The order of visit of the flowers in a given inflorescence ('sequence position', from 1 to x) was noted. A visit was confirmed when the insect landed on the labellum and/or probed the spur. Plots were monitored at a distance of 0.5-1 m to allow insect observation without disturbing their foraging activity.

151 ***Statistical analyses***

152

153 We only considered the data collected for solitary bees, since honeybees and bumblebees were
154 very rare.

155

156 We used linear mixed models (LMM) to determine if nearest neighbour distance was influenced
157 by plot density; plot ID was included as a random effect. Since distances were not independent,
158 repeated measures were considered only once. Data were log-transformed prior to analysis to
159 meet the normality and homoscedasticity assumptions. A similar model (log-transformed data)
160 was used to check if the total number of flowers per plot was influenced by plot density. A LMM
161 was also used for analysis of the time spent per inflorescence and per flower (log-transformed).
162 Plot density, floral display size, sequence position, temperature, buffer density and the
163 interactions between plot density and floral display size and between plot density and sequence
164 position were included as fixed effects. Plot ID and insect identity (each insect was given a
165 unique identifier) were included as random effects. We used generalised linear mixed models
166 (GLMMs) to analyse how plot density, floral display size and their interaction affected fruit set
167 (binomial error, logit link function) and the number of potential pollinators visiting at least one
168 flower per inflorescence (Poisson error, log link function); plot ID was included as a random
169 effect. We also used a GLMM to analyse if the number of flowers per inflorescence (measured
170 about one month after the end of the flowering period) was influenced by plot density (negative
171 binomial error, log link function); plot ID was included as a random effect. We used GLMMs to
172 analyse how the proportion of flowers visited (binomial error, logit link function) and bout
173 length (negative binomial error, log link function) were affected by plot density, buffer density,
174 floral display size, the quadratic effect of the latter, sequence position, temperature and the
175 interactions between plot density and floral display size; plot ID and insect identity were

considered as random effects. All statistical analyses were performed in R ver. 3.5.1 (R Development Core Team 2018). The statistical significance threshold was set at 0.05.

Results

Flower number, plant spatial distribution and fruit production

Plot density had a statistically significant effect on the total number of flowers produced per plot (Table 1). It was logically much higher in high-density plots than in medium- and low-density plots (Table 2). Plot density also had a statistically significant effect on the distance to the nearest neighbour (Table 1). The latter was obviously higher in low-density plots than in medium- and high-density plots (Table 2). The difference between medium- and high-density plots was also statistically significant (contrast, $\chi^2 = 4.83$, d.f. = 1, $p = 0.03$). The number of flowers per inflorescence significantly differed between plot density conditions (GLMM: $\chi^2 = 26.5$, d.f. = 2, $p = 1.74.10^{-6}$): it was higher in low- and medium-density plots than in high-density plots; there was no statistically significant difference between low- and medium-density plots (Table 3). Fruit set varied from 0 to 0.50 (on average, 0.19 ± 0.02), and inflorescences developed 6 to 47 flowers (on average, 20.5 ± 1.2) (Table 2). Plot density, floral display size and their interaction had a statistically significant effect on fruit set (Table 4). Fruit set was negatively influenced by floral display size in medium-density plots, and unaffected by display size in low- and high-density plots (Figure 1).

199 ***Insect visitation***

200

201 A total of 66 hymenopterans were observed approaching plots, 34 of which visited at least one
202 flower and 52 flower visit events were recorded. Solitary bees, honeybees and bumblebees
203 accounted respectively for 94.2, 3.9 and 1.9 % of the visitation events. Per plot per 15-min
204 census, 0.38 ± 0.05 solitary bees (n = 172) approached a plot and 0.20 ± 0.03 visited at least one
205 flower (n = 172). They spent 7.1 ± 1.4 s (n = 172) per inflorescence and 4.6 ± 0.4 s per flower (n
206 = 46). The proportion of flowers visited (per inflorescence) was 0.11 ± 0.01 (n = 38), and they
207 visited 1.3 ± 0.2 flowers (bout length, n = 38) per inflorescence (Table 5). None of the tested
208 variables had a statistically significant effect on the number of visiting potential pollinators
209 (Table 4) and bout length (Table 6). The proportion of flowers visited was significantly
210 negatively influenced by floral display size (Table 6; Figure 2). The (log-transformed) time spent
211 per flower was significantly affected by sequence position and temperature (Table 6). Within an
212 inflorescence, solitary bees spent decreasing time in successive flowers (Figure 3), and the time
213 spent per flower was negatively influenced by temperature (Figure 4).

214

215 **Discussion**

216

217 ***Foraging behaviour of potential pollinators***

218

219 Although dense plots and large floral displays often attract more pollinators due to higher
220 potential food production (Pyke 1984, Totland 1994), better detection and reduced travel time
221 between plants (Ohashi and Yahara 2001, Elliott and Irwin 2009), the number of visiting
222 pollinators per *O. militaris* plant was not affected by plot density and its interaction with floral
223 display size. The absence of density effects could be due to the low number of pollinators within

the study site and its influence on visitation rates (Field et al. 2005). The study site was a woodland and the abundance of bees is generally lower in closed biotopes than in open ones (Grundel et al. 2010, Fisher et al. 2017). The effect of floral display size was nearly statistically significant and larger displays tended to be visited by more solitary bees than smaller displays, which is consistent with previous studies (Ishii and Sakai 2001 and references therein).

In rewarding plant species, bout length is predicted to increase with increasing floral display size (Ohashi and Yahara 2001), potentially because of increased mean reward value of flowers due to lower odds of revisiting a flower on large displays (Mitchell et al. 2004). In deceptive species however, larger display size likely does not lead to an increased bout length since visitors detect the deception and leave after a few visits (Smithson and Gigord 2003). In *O. militaris*, bout length was actually not influenced by floral display size. In addition, decreasing bout length with increasing plant density would suggest a tendency for flight costs (associated to movements between plants) to decrease in dense plots (Nattero et al. 2011). The proportion of flowers visited per plant decreased with floral display size. Such type of correlation is common in various plant-pollinator associations (Ohashi and Yahara 2001, Mitchell et al. 2004) and makes sense in our case since only a few flowers are visited per inflorescence, no matter the display size.

Although a positive correlation is expected between the time spent on a flower and the distance between flowers (Inouye et al. 2015), density had no effect on handling time per flower. Similar results were obtained for various insect groups (Conner and Rush 1996, Ghazoul 2005, Akter et al. 2017). They seemingly visit individual flowers in a constant manner irrespective of the number of open flowers (Akter et al. 2017). The time spent per flower was negatively influenced by its position in the visitation sequence. Over successive visits on an inflorescence, solitary bees

spent less time per flower, probably due to the absence of nectar and increasing experience. A decrease in handling time (or 'discovery time') over successive visits may also arise due to improvement of flower-handling skills (e.g. Laverty and Plowright 1988, Lewis 1986). This statistically significant effect must however be considered with caution since the majority of solitary bees visited only one flower per inflorescence. A similar pattern was observed in other deceptive species (e.g. Ayasse et al. 1997). The time spent per flower was also negatively influenced by temperature. In solitary bees, handling time per flower (Szabo and Smith 1972) and per inflorescence (Ginsberg 1984) tend to be lower at high temperature. Our result could simply be explained by a positive effect of temperature on bee activity, bees being ectothermic most of the time (endothermic mechanisms are known – Willmer and Stone 2004). Alternatively, thoracic temperature of solitary bees visiting *O. militaris* may have been close to the upper critical temperature which ranges between 45 and 50 °C in most bees (Willmer and Stone 2004). For example, the thoracic temperature of foraging solitary bees *Centris pallida* frequently exceeds 40 °C throughout the day when ambient temperature ranges between 25 and 40 °C (Chappell 1984). This temperature excess may be further exacerbated by the capture of solar radiation by flowers which can lead to their warming (or the warming of specific floral parts), depending on pigmentation, structure and heliotropism (Harrap et al. 2017). In taxa with zygomorphic flowers (the same symmetry as in *O. militaris*) studied by Harrap et al. (2017), the difference between ambient temperature and hotter parts of the flowers is on average (± SE) –4.6 ± 0.8 °C, and the difference between ambient temperature and coldest parts is –2.3 ± 0.6 °C (n = 22). Within-flower temperature is thus often 2 to 5 °C hotter than ambient temperature. As a result, visits may be brief to avoid extended exposure to high temperatures (causing heat stress) within sunlit flowers, and insect temperature may decrease due to thermoregulatory cooling flights when solitary bees leave a flower (Corbet and Huang 2016).

Fruit set and pollinator abundance and behaviour

Plot density and floral display size interacted to influence fruit set. In other studies, the effect of local density of conspecifics on pollinator visitation (Grindeland et al. 2005, Jing et al. 2013, Weber and Kolb 2013) and fruit set (Ruane et al. 2014, Henneresse et al. 2017) has also been reported to be contingent upon floral display size. According to the results of Henneresse et al. (2017), we expected fruit set to be positively correlated with floral display size in low-density plots. In the latter, showy inflorescences often attract more pollinators than smaller ones (Ohashi and Yahara 2001, Jing et al. 2013), leading to higher pollen deposition and potentially higher relative fruit production (Lammi et al. 2003). We also expected fruit set to be negatively correlated with floral display size in high-density plots, although discrimination between small and large floral displays may not be crucial in dense plots because of low flight costs associated with interplant movements (Jing et al. 2013, Weber and Kolb 2013). Contrary to our expectations, there was no significant correlation between floral display size and fruit set in low- and high-density plots; a significant negative correlation was detected in medium-density plots. The factors causing these patterns are unclear, especially as plot density, floral display size and their interaction had nearly no influence on solitary bee abundance and foraging behaviour. Such inconsistency was also noted by Mustajärvi et al. (2001), who concluded that reproductive success in *Silene viscaria* (L.) Jess. was probably determined more by available resources than available pollen. Plot density may be correlated with environmental parameters, such as resource availability, which could lead to confounding conclusions (Knight 2003, Johnson et al. 2012). In our case, individual floral display size was affected by plot density: resource availability could be higher in low- and medium-density plots than in high-density plots. This result suggests that resource competition can influence floral display size (Caruso 1999).

The link between pollination (and thus plant reproductive success) and quantitative data on visitation or visitor abundance is either supported (Vázquez et al. 2005, Hegland et al. 2010) or criticised (Duffy et al. 2013, King et al. 2013) and could depend on the studied species. For example, pollinia removal (and deposition) is likely to be positively affected by visit (pseudocopulation) duration in sexually deceptive orchids (de Jager and Peakall, in press). In several non-orchid species however, there is no clear relationship between visit duration and pollen deposition (King et al. 2013). In our study, pollinators are probably an important intermediate causal link between plot density and fruit set. However, the individual foraging responses we measured may be poorly related to pollen deposition in *O. militaris* and pollinator efficacy (*sensu* Freitas 2013) should be evaluated to address this point. In addition, the potentially low abundance of pollinators in the study site could explain why the number of visiting pollinators per plant could not be indirectly linked to fruit set. Although fieldwork allowed us to describe natural patterns, this approach is not sufficient to fully understand the precise mechanisms of density effects on fruit set in *O. militaris*. In addition, using natural patches can lead to methodological challenges such as spatial autocorrelation in patch parameters (Johnson et al. 2012). *In situ* manipulation of conspecific density with controlled edaphic conditions could provide an appropriate alternative solution. Moreover, even if fruit production is not related to pollinator visitation rates, female fitness can increase with increasing visitation rates (Real and Rathcke 1991) and seed production and quality should also be investigated.

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Figure 1. Fruit set in *O. militaris* in relation to floral display size and plot density. Black = high density, dark grey = medium density, light grey = low density. Symbols correspond to plot IDs (8 symbols for the 8 medium-density plots and 4 symbols for the 4 medium-density plots). P-values are given for the null hypothesis that the slope is equal to zero.

Figure 2. Relationship between floral display size and the proportion of flowers visited by solitary bees. The blue line is a reference line representing the minimum possible value for the proportion (1/floral display size).

Figure 3. Time spent per flower (Y axis is logarithmic) in solitary bees in relation to its position in a sequence of visited flowers.

Figure 4. Relationship between temperature and the time spent per flower (Y axis is logarithmic) in solitary bees.

530 Table 1. Results of the LMMs showing the effects of plot density on the total number of flowers
531 produced per plot and on nearest neighbour distance.
532

Effect	χ^2	df	p-value
Total number of flowers per plot			
Plot density	124.85	2	$< 2.2.10^{-16}$
Nearest neighbour distance			
Plot density	130.03	2	$< 2.2.10^{-16}$

533

534 Table 2. Flower density, nearest neighbour distance (NND) and fruit set averaged (± SE) over
535 plot densities.
536

Plot density	Number of flowers per plot	Number of flowers per inflorescence	NND (cm)	Fruit set
Low	21.8 ± 1.2 (n = 58)	27.2 ± 2.0 (n = 18)	138.3 ± 20.2 (n = 19)	0.27 ± 0.03 (n = 18)
Medium	57.4 ± 1.9 (n = 56)	21.2 ± 1.9 (n = 22)	27.7 ± 3.1 (n = 19)	0.14 ± 0.03 (n = 22)
High	105.6 ± 2.6 (n = 33)	15.8 ± 1.6 (n = 28)	18.5 ± 2.4 (n = 20)	0.18 ± 0.03 (n = 28)

537

538 Table 3. Results of the LMMs showing the effects of plot density on the total number of flowers
539 produced per plot and on nearest neighbour distance.
540

Value	χ^2	df	p-value
High vs.low	20.37	1	$1.91.10^{-5}$
High vs. medium	6.73	1	0.03
Low vs. medium	2.45	1	0.35

541

542 Table 4. Results of the GLMMs showing the effect of plot density, floral display size (i.e. total
543 number of flowers per inflorescence) and their interaction on fruit set and the number of visiting
544 potential pollinators.
545

Effect	χ^2	df	p-value
Fruit set			
Plot density	21.44	2	$2.21.10^{-5}$
Floral display size	4.93	1	0.03
Plot density × floral display size	9.29	2	$9.62.10^{-3}$
Number of visiting pollinators			
Plot density	2.51	2	0.29
Floral display size	3.73	1	0.053
Plot density × floral display size	0.69	2	0.71

547 Table 5. Number of visiting pollinators per plant (NVP), proportion of flowers visited, bout length (all per 15-min census), time spent per
548 inflorescence and time spent per flower averaged (± SE) over plot densities.
549

Plot density	NVP	Proportion visited	Bout length	Time per inflorescence (s)	Time per flower (s)
Low	0.14 ± 0.05 (n = 58)	0.07 ± 0.03 (n = 6)	2.0 ± 0.8 (n = 6)	3.9 ± 0.8 (n = 5)	3.8 ± 0.6 (n = 11)
Medium	0.21 ± 0.06 (n = 57)	0.10 ± 0.01 (n = 12)	1.0 ± 0.0 (n = 12)	4.4 ± 0.7 (n = 12)	4.4 ± 0.7 (n = 12)
High	0.25 ± 0.07 (n = 57)	0.12 ± 0.02 (n = 20)	1.3 ± 0.1 (n = 20)	6.0 ± 0.9 (n = 19)	5.1 ± 0.6 (n = 23)

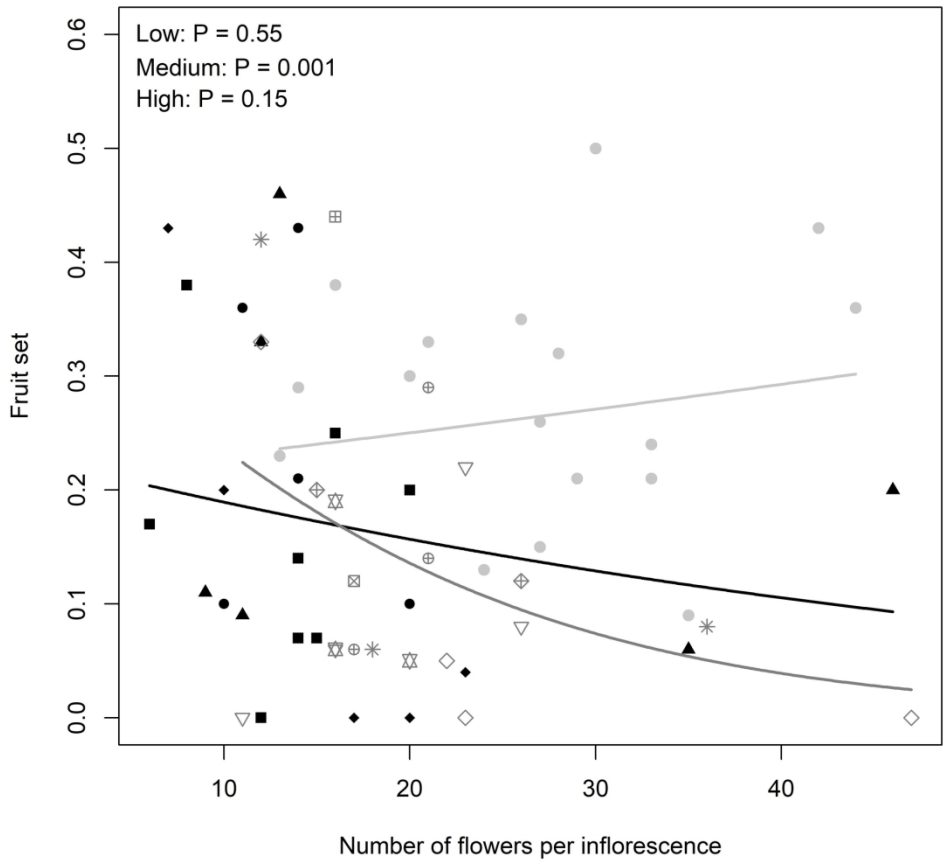
551 Table 6. Results of the GLMMs showing the effect of fixed variables and their interactions on parameters of insect visitation: proportion of

552 flowers visited, bout length, time spent per inflorescence and time spent per flower.

553

Effect	Proportion visited			Bout length			Time per inflorescence			Time per flower		
	χ^2	df	p-value	χ^2	df	p-value	χ^2	df	p-value	χ^2	df	p-value
Plot density	2.54	2	0.28	2.33	2	0.31	1.24	2	0.54	0.52	2	0.77
Floral display size	6.38	1	0.01	0.12	1	0.73	2.33	1	0.13	1.91	1	0.17
Floral display size²	0.26	1	0.61	na	na	na	na	na	na	na	na	na
Sequence	0.03	1	0.87	0.001	1	0.98	2.46	1	0.12	9.88	1	0.002
Temperature	0.45	1	0.50	0.67	1	0.41	1.00	1	0.32	4.55	1	0.03
Buffer density	0.22	1	0.64	0.27	1	0.60	1.09	1	0.30	1.09	1	0.30
Plot density × display size	0.55	2	0.76	0.88	2	0.64	0.11	2	0.95	0.43	2	0.80
Plot density × sequence	na	na	na	na	na	na	na	na	na	2.11	2	0.35

555



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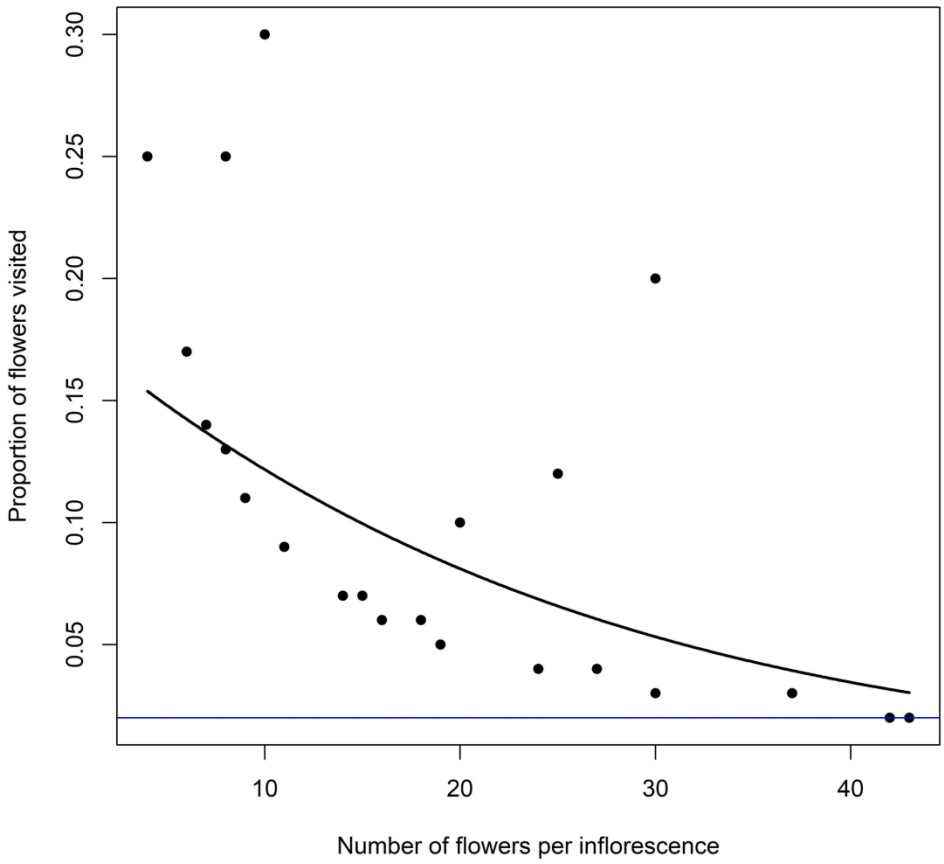

Figure 2. Relationship between floral display size and the proportion of flowers visited by solitary bees. The blue line is a reference line representing the minimum possible value for the proportion (1/floral display size).

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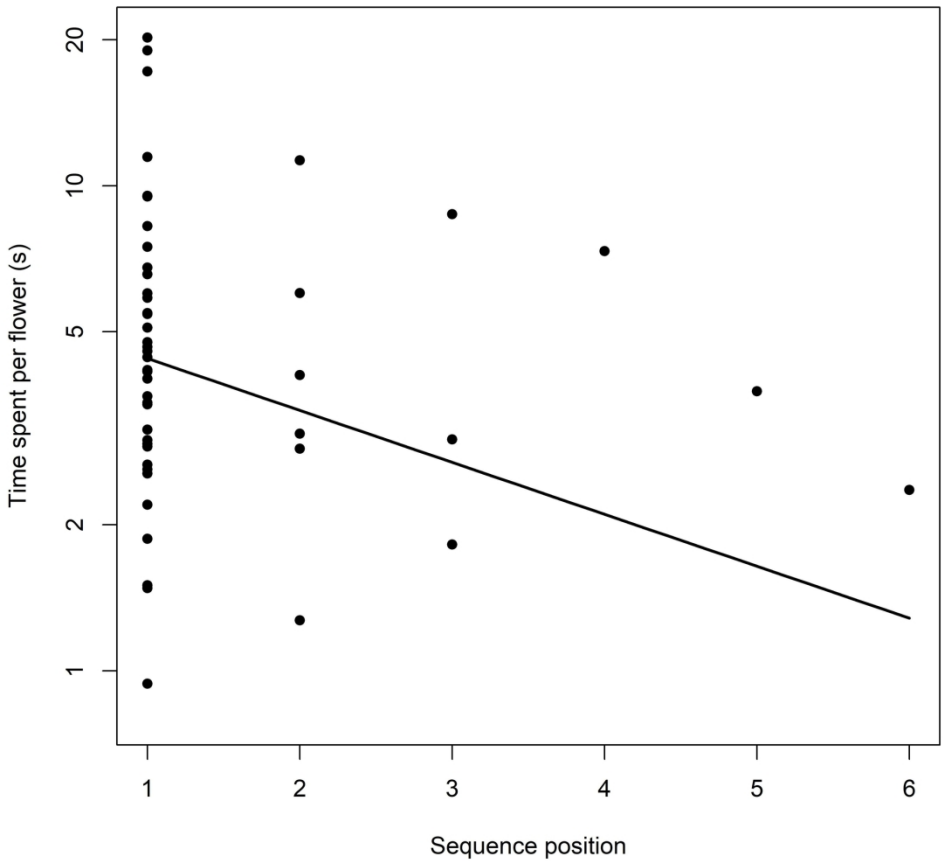

Figure 3. Time spent per flower (Y axis is logarithmic) in solitary bees in relation to its position in a sequence of visited flowers.

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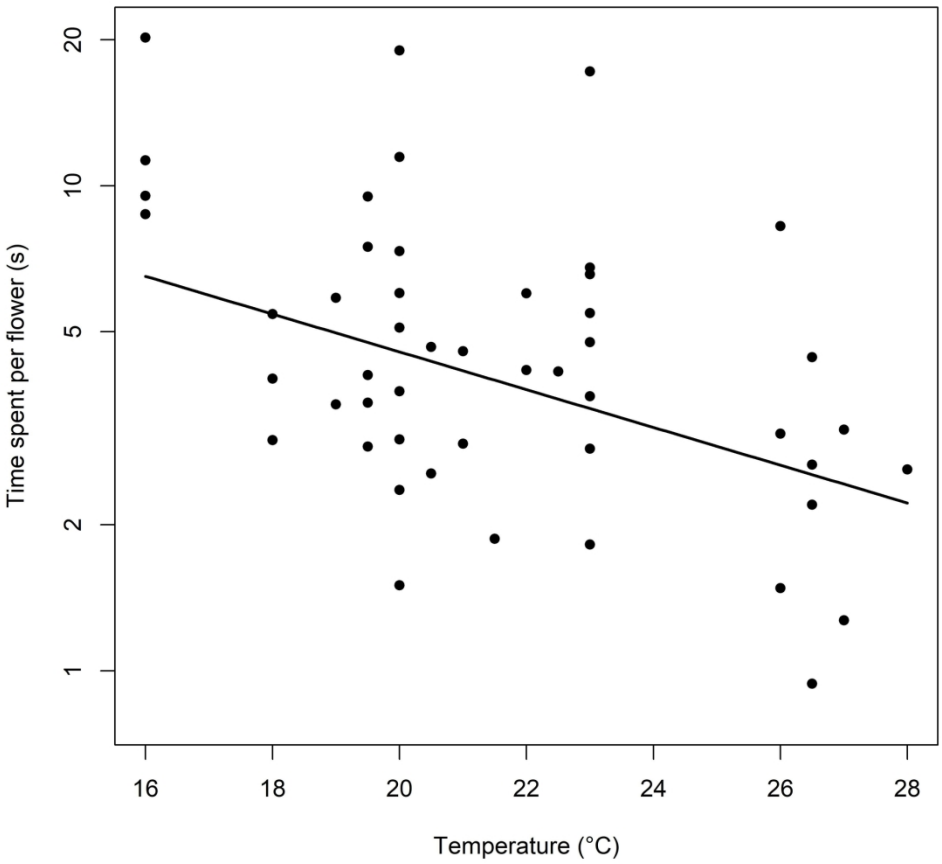

Figure 4. Relationship between temperature and the time spent per flower (Y axis is logarithmic) in solitary bees.

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