Effects of floral display, conspecific density and rewarding species on fruit set in the deceptive orchid *Orchis militaris* (Orchidaceae)

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Background and aims – About one third of orchid species do not produce any floral reward but have developed various strategies to attract pollinators. The most common system is food deception, which takes place through two types of attraction mechanisms: Batesian floral mimicry and generalised food deception. In rewardless species, fruit set is generally low and various factors, such as floral display size or density, are known to positively or negatively influence fruit set. This study aimed to clarify the deceptive pollination system in *Orchis militaris* and to investigate the effect of individual, population and environmental factors on fruit set in this orchid species.

Methods – The effects of floral display size, con- and heterospecific density and rewarding species diversity were studied both at the scale of the individual plant (fourteen sites) and of the population (22 sites). Generalised linear (mixed) models and multivariate analysis were performed to examine which factors affect fruit set.

Key Results – At the individual scale, the probability of setting fruit was influenced by several factors and their interaction, including floral display size, conspecific density, and heterospecific total density and richness. Fruit set was higher in medium-height vegetation than in short vegetation. *Globularia bisnagarica* was the only species whose density was significantly (and negatively) correlated with fruit set. At the population scale, none of the studied factors was significantly correlated to fruit set.

Conclusions – In *Orchis militaris*, generalised food deception is the most likely strategy of pollinator attraction. This orchid species may benefit from the proximity of rewarding plants through the magnet-species effect, depending on the size of its floral display. The effect of con- and heterospecific density and rewarding species richness seem scale-dependent.

Key words – Conspecific density, deceptive orchid, food deception, fruit set, generalised food deception, Orchidaceae, *Orchis militaris*, pollination facilitation, reward, species richness.

INTRODUCTION

Globally, around 88 % of flowering plant species rely on biotic pollen vectors for sexual reproduction (Ollerton et al. 2011). Usually, plants attract pollinators with floral signals (e.g. colour, pattern, shape, scent) that advertise rewards (Schiestl & Johnson 2013). Pollinators are able to learn to associate floral traits with the abundance or quality of rewards, typically nectar and pollen, but also floral oils and liquid fragrances (Renner 2006), and usually forage constantly after learning (Juillet & Scopece 2010, Gaskett 2014). Some plant species also display attractive floral signals, but do not provide any reward and other species produce a low or unsuitable reward (Jersáková et al. 2009); they are called deceptive species (Renner 2006). Note that a rewardless plant species is not necessarily deceptive (Johnson & Schiestl 2016). Deceptive pollination is found in at least 146 genera from 33 plant families (Jersáková et al. 2009), including Apocynaceae, Aristolochiaceae, Iridaceae, and Orchidaceae (Renner 2006). It occurs in about 30 % of orchids (Jersáková et al. 2006, Claessens & Kleynen 2011), one of the most diversified angiosperm families (more than 27 000 accepted names – The Plant List 2013).

The main mechanisms responsible for the maintenance and evolution of deceptive pollination systems are still debated. Among the Orchidaceae, the commonest deceptive systems involve the exploitation of the food-seeking behaviour of the pollinators (Jersáková et al. 2006). It takes place through two types of pollinator attraction mechanisms (Nils-

son 1992): Batesian floral mimicry (BFM) and generalised food deception (GFD). Batesian mimics exploit specialised relationships between specific rewarding plant species (models) and their pollinators (operators) through a rather precise mimicry of the size, shape and/or colour of the floral display of models (Peter & Johnson 2008). BFM can become established as occurring in a system of two or more similar species when (1) the species have strongly overlapping flowering periods; (2) they have strongly overlapping distribution areas; (3) the model, but not the mimic, provides the operator with a reward; (4) they share the same pollinator species and the same individual operators can move freely between the species; (5) the similarity between the mimic's signals and those of the model is so high that the operators are unable to discriminate between them (but see de Jager et al. 2016); (6) the mimics are infrequent; and (7) the similarity positively influences the mimic's fitness (Roy & Widmer 1999, Johnson 2000, Jersáková et al. 2006, 2009, 2012). In these mimetic systems, visual signals are probably more important than floral fragrances to attract pollinators. In fact, several studies have reported weakly scented or scentless mimics (Dafni 1984, Peter & Johnson 2008, Schlüter & Schiestl 2008). On the contrary, in GFD, no specific model is involved. The deceptive species exploit the innate foraging behaviour of pollinators through general floral signals (typical for rewarding plants) such as showy flower colours, spurs, fragrances or nectar guides (Jersáková et al. 2009). The display of strong floral signals (i.e. large inflorescence or colours matching the innate colour preferences of pollinators) elicits high spontaneous attraction of pollinators. Through an innate measure of attractiveness, the pollinators evaluate the quality of floral signals and visit the most attractive species (Gumbert & Kunze 2001). In practice, the opposition between GFD and BFM is less obvious and would be an oversimplification. Indeed, each food-deceptive species may occupy a point along a continuum "no specific model – mimic of a particular species" (Jersáková et al. 2012). Selection might favour deceptive floral phenotypes that resemble (in a strongly mimetic way or not) a co-occurring rewarding species (Peter & Johnson 2008).

In deceptive species, several factors are known to influence fruit set and pollination success: amongst others, plant height or inflorescence size (Fritz & Nilsson 1996, Kropf & Renner 2005, Suetsugu et al. 2015), population size (Fritz & Nilsson 1994, Tremblay et al. 2005), vegetation height (Toräng et al. 2006, Sletvold et al. 2013), and abundance or density of con- and heterospecific flowering plants (Lammi & Kuitunen 1995, Fritz & Nilsson 1996, Sabat & Ackerman 1996, Gumbert & Kunze 2001, Johnson et al. 2003, Kropf & Renner 2005, Internicola et al. 2006, Juillet et al. 2007). A positive effect of plant or inflorescence size, population size, heterospecific plant density, and a negative effect of con-specific density on fruit set is generally (but not systematically) observed. Pollination facilitation could explain the positive effect of rewarding flowering plant abundance and diversity on the reproductive success of deceptive species. Bi- or multidirectional facilitation can occur via an aggregative response when floral density increases in an accelerating fashion, leading to increased visitation rate in one or several plant species (Rathcke 1983, Feldman et al. 2004). It has also

been hypothesised that, flower abundance being constant, reproductive success of plants in species-rich plots is higher than in less diverse plots through greater pollinator attraction (Ghazoul 2006). Morphological similarity and floral synchrony of species could interact to influence the relationship, leading to competition rather than facilitation (Bizecki Robson 2013). Besides bi- or multidirectional facilitation, unidirectional facilitation is illustrated by the 'magnet-species effect' (Peter & Johnson 2008). Rewarding (magnet) plants attract pollinators and non-rewarding individuals flowering nearby benefit from chance visits and pollination events (Jersáková et al. 2009). Other factors like colour similarity between deceptive plants and neighbouring rewarding species (Internicola et al. 2007) or pollinator abundance (Ye et al. 2014) could also play a role in indirect interactions for pollination.

In the genus *Orchis* (Orchidaceae), none of the species produce nectar and the majority of the species depends on food deception to attract pollinators. Despite this assertion, only few studies have focused on the relationship between the characteristics of the co-flowering insect-pollinated species and *Orchis* fruit set (Scopece et al. 2014). Research on *O. spitzelii* Saut. ex W.D.J.Koch (Fritz 1990), *O. mascula* (L.) L. (Nilsson 1983), and *O. pallens* L. (Vöth 1982), belonging to the subgenus *Masculae*, has shown that the pollination of *O. mascula* and *O. spitzelii* does not depend on the presence of a specific rewarding model, while *O. pallens* may rely on the presence of *Lathyrus vernus* (L.) Bernh. for its reproduction. Other works have investigated entomophily and fruit set in *Orchis* spp. and related species (e.g. Nilsson 1980, Dafni & Ivri 1981, Bino et al. 1982, Dafni 1987, Vöth 1987, Jacquemyn et al. 2002, 2006, Schatz 2006, Dormont et al. 2010, Claessens & Kleynen 2011).

In this study, we present data on *Orchis militaris* L., a species belonging to subgenus *Orchis* known to be well differentiated from subgenus *Masculae* in morphology, mycorrhizal associations, and pollinator assemblages (Tyteca et al. 2012). Our aim was to test the impact of several factors on *O. militaris* fruit set, and therefore to investigate its deceptive pollination mechanism. We hypothesise that total (but not species-specific) plant density and richness of rewarding co-flowering insect-pollinated species will positively affect fruit set as a result of pollination facilitation (Rathcke 1983, Ghazoul 2006). We also expect a positive relationship between *O. militaris* inflorescence length and fruit set because of the higher pollinator response (more insects per unit of time and more flowers probed per plant per insect) to increased floral display size (Ohashi & Yahara 2001). On the contrary, fruit set should decrease with increasing *O. militaris* flowering plant density because of its deceptive pollination system (Tremblay et al. 2005): pollinators will rapidly leave patches with no or low reward (Internicola et al. 2007). Vegetation height is hypothesised to interact with floral display size to influence fruit set: large floral displays in short vegetation should be more visible and thus experience higher fruit set than small floral displays in tall-sized vegetation. To test these hypotheses, we assessed the effects of density and richness of co-flowering species, vegetation height, and *O. militaris* density and inflorescence size on *O. militaris* fruit set in 24 populations in Western Europe. To get the most

Table 1 – Individual- and population-scale studies: population and site characteristics. Abbreviations: BE = Belgium; FR = France; GE = Germany; LU = Luxembourg; NL = Netherlands; n_i = sample size for individual-scale study; n_p = sample size for population-scale study. Sample size and population size are given in number of inflorescences.

No.	Study site	Year	Geographical coordinates	Altitude (m)	n_i	n_p	Pop. size
1	Freudenburg (GE)	2014	49°33′55″N 6°32′16″E	425–430		100	400
2	Voerendaal (NL)	2015	50°50′57″N 5°54′44″E	145–150	43	150	180
3	Niederanven (LU)	2014	49°40′52″N 6°15′14″E	≈ 325		40	43
4	Rumelange (LU)	2014	49°28′56″N 6°02′25″E	≈ 335		100	105
5	Visé (BE)	2014	50°46′41″N 5°40′46″E	80–90		108	140
6	Andenne (BE)	2015	50°29′60″N 5°03′30″E	≈ 175	33	122	400
7	Musson (BE)	2015	49°33′08″N 5°42′59″E	≈ 290	30	125	250
8	Saint-Thomas (FR)	2014	49°30′09″N 3°49′14″E	185–190		32	35
9	Charency-Vezin (FR)	2014	49°29′38″N 5°30′45″E	260–275	40	91	165
10	Nixéville-Blercourt (FR)	2015	49°06′22″N 5°15′22″E	310–325	22	122	150
11	Saint-Julien-lès-Gorze (FR)	2015	49°01′20″N 5°54′49″E	≈ 240	18	35	80
12	Waville (FR)	2015	49°01′17″N 5°56′59″E	295–305	27	50	70
13	Dompcevrin (FR)	2015	48°56′16″N 5°28′51″E	270–280	36	133	160
14	Bacourt (FR)	2015	48°55′15″N 6°24′41″E	345–350	36	125	220
15	Longchamps-sur-Aire (FR)	2015	48°55′04″N 5°17′24″E	250–260	28	100	150
16	Jezainville (FR)	2015	48°51′34″N 6°02′50″E	195–215	15		70
17	Jeandelaincourt (FR)	2015	48°50′24″N 6°13′31″E	360–375	20	66	105
18	Salmagne (FR)	2015	48°46′09″N 5°20′06″E	305–320	20		80
19	Troussey (FR)	2015	48°42′29″N 5°43′19″E	295–305	29	100	160
20	Rochefort-Samson (FR)	2014	44°58′01″N 5°10′27″E	470–480		97	100
21	Léoncel (FR)	2014	44°54′53″N 5°11′47″E	920–925		43	50
22	Le Chaffal (FR)	2014	44°51′52″N 5°10′23″E	995–1000		9	25
23	Ispagnac (FR)	2014	44°25′42″N 3°29′44″E	≈ 980		93	180
24	Laval-du-Tarn (FR)	2014	44°20′48″N 3°21′04″E	820–825		68	110

comprehensive view of the impact of pollinator interactions on fruit set (Gunton & Kunin 2007, Spigler & Chang 2008), and since insect response to rewarding plant species density may vary with spatial scale (Johnson et al. 2003), all investigations were conducted at two spatial scales: the individual (or microhabitat) scale (1 m² surrounding each of the studied plants) and the population scale (an area of 1 500 m² surrounding a group of studied plants).

MATERIAL AND METHODS

Study species

Orchis militaris is a perennial herb restricted to calcareous soils, occurring in sunny or partially shaded habitats (Farrell 1985). The species shows a Eurasian-Mediterranean distribution (Kretzschmar et al. 2007), and ranges from sea level to 2 200 m. It can be found in chalk or calcareous grasslands, wet meadows, scrub communities or open woodlands (Farrell 1985). Plants consist of a rosette of leaves with a single inflorescence (10–)20 to 45(–65) cm high, bearing ten to forty hermaphroditic flowers (Farrell 1985, Delforge 2012). In Western Europe, it flowers from April to June(–July)

(Delforge 2012). *Orchis militaris* is assumed to be self-compatible (Neiland & Wilcock 1998, Metsare et al. 2015) and non-autogamous (Delforge 2012), and to depend on the food deception strategy to attract pollinators (Claessens & Kleynen 2011). Low levels of fruit set (< 30 %) are common (Tremblay et al. 2005, Claessens & Kleynen 2011). According to Vöth (1987), confirmed pollinators are short-tongued hymenopterans from the genera *Andrena* (Andrenidae) and *Halictus* (Halictidae). A number of potential pollinator species are also known (non-exhaustive list in Henneresse & Tyteca 2016): e.g. *Anthophora borealis*, *Apis mellifera*, *Bombus pratorum* (Apidae), *Osmia bicolor* (Megachilidae), and *Sphecodes ferruginatus* (Halictidae).

Study sites

In total 24 study sites located in Western Europe were investigated, ranging from the Netherlands to the south of France (fig. 1, table 1). The individual-scale (as defined below) study was carried out from May to July 2015 in fourteen populations, and the population-scale study from May to July 2014 and 2015 in 22 populations. A population was defined as a group of inflorescences separated from their closest conspe-

cifics by at least 100 m (Alexandersson & Ågren 1996). All populations were at least 2.5 km apart, their geographical coordinates and altitude were recorded with a handheld GPS (Garmin). The biotopes were various: recolonised slag heaps or quarries, semi-dry calcareous grasslands and mesic grasslands more or less colonised by shrubs. In the population-scale study, populations in the south of France were added in order to maximise variation for some variables (especially rewarding species richness, see below).

Data collection

In the individual-scale study, we tagged up to fifty *O. militaris* inflorescences (table 1) in each population at flowering peak using a systematic random sampling method. We set up a plot of 1 m × 1 m centred on each selected (focal) inflorescence and in each plot we counted the number of *O. militaris* inflorescences (conspecific density) and recorded the number of co-flowering plant species offering nectar and/or pollen to visiting insects (with the exception of grasses, trees, and shrubs), hereafter referred to as rewarding species (RSp) richness. To evaluate the total number of floral units (FUs – flowers or inflorescences, depending on the taxon) of rewarding species per plot (RSp density hereafter), we first standardised FUs among species by defining 1 FU as 1 cm² of flowers or inflorescences (after Carvalheiro et al. 2014).
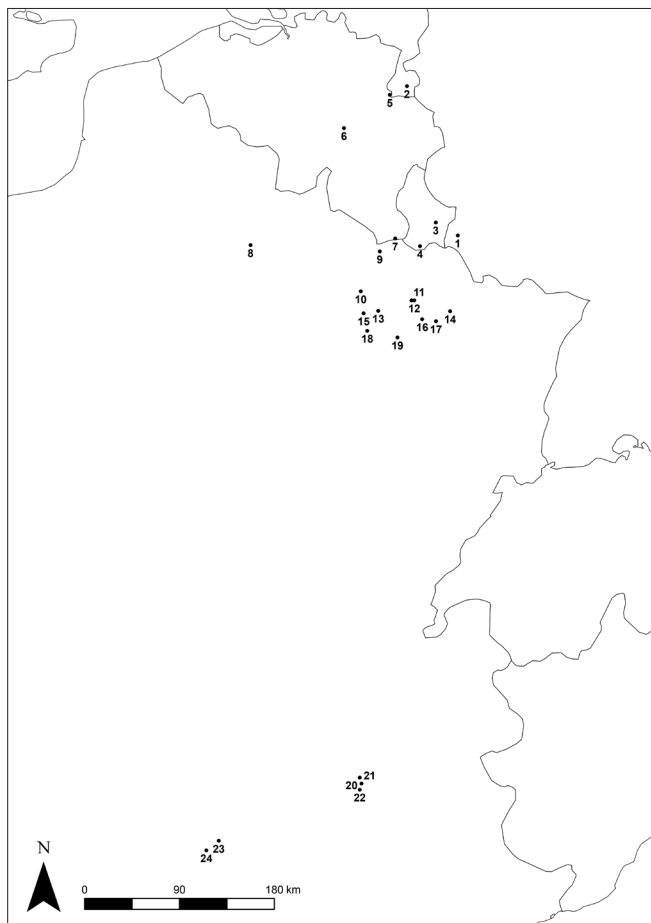

Figure 1 – Study sites location in Germany, The Netherlands, Belgium, Luxembourg and France. Numbers refer to those in table 1.

Then, for each taxon, we multiplied the average area of one FU (mainly obtained from Tutin et al. 1968, 1972, 1976, 1980, 1993) by the number of FUs and summed the products to obtain RSp density (cm²). Different formulas were used for estimating average FU area, with circular ($\pi \times r^2$) or non-circular (length × width) outlines, using the same method as described in Hegland & Boeke (2006). Vegetation height was also measured in each plot, by defining three classes (relative to *O. militaris* plant height; T. Henneresse, unpubl. res., n = 750): low (0–15 cm), medium (> 15–40 cm) and high (> 40 cm). A plot was assigned to one of the three classes if vegetation cover for this class exceeded 75 %. There was no overlap between plots to avoid pseudoreplication. About one month after the end of the flowering period, we measured inflorescence length (in cm) for our focal *O. militaris* plants. The total number of flowers and of swollen ovaries (initiated fruits) per tagged inflorescence was counted in order to quantify fruit set as the proportion of flowers that initiated fruits. This ratio is a direct indicator of pollinator activity in self-compatible and allogamous orchids (Inoue 1986).

For the population-scale study, we set up a rectangle of 30 m × 50 m (after Juillet et al. 2007) in each population, divided into sixty quadrats of 5 m × 5 m. Inside the rectangle, we counted the number of *O. militaris* inflorescences and we marked up to 150 inflorescences (table 1). In ten randomly selected quadrats, we counted the number of RSp and FUs for each RSp to estimate RSp richness and density, respectively. Because of the random sampling method, orchids were not necessarily present in all the quadrats. We also evaluated population size (number of flowering *O. militaris* individuals within the entire population). About one month after the end of the flowering period, we measured inflorescence length and we recorded fruit set of the tagged plants.

Data analysis

We checked for correlation between pairs of continuous variables by calculating the Pearson's correlation coefficient r or the Spearman's rank correlation coefficient ρ if the assumptions of the former were not fulfilled. If two variables were significantly correlated (r or $\rho > 0.7$ or r or $\rho < -0.7$, $p < 0.05$), we excluded one of them to avoid collinearity.

For individual-scale data, we used a generalised linear mixed model (GLMM, binomial error, logit link function) to analyse how the investigated factors affected fruit set. Site was included as a random effect and the following fixed effects (and their two-way interactions) were considered: *O. militaris* inflorescence length, *O. militaris* conspecific density, vegetation height (only low and medium, the high vegetation class was not included in the analysis due to insufficient data), RSp density and RSp richness (table 2). There was a highly significant correlation between inflorescence length and the number of flowers per inflorescence ($\rho = 0.83$, $p = 2.2 \cdot 10^{-16}$) and the former could thus be considered equivalent to floral display size. To determine if a specific RSp density (e.g. the density of *Hippocrepis comosa* L.) could be included in the GLMM, we selected the species found in at least thirty plots. Then, if the relationship between a specific RSp density and fruit set was significant (binomial error, logit link function), this variable was included in the GLMM.

Table 2 – **Variables of the individual-scale study (1 m × 1 m plots around focal plants): fruit set, inflorescence length (mean ± SE, in cm) and conspecific density (number of inflorescences) of *Orchis militaris*, and RSp density (in FUs) and richness (number of species).** Abbreviations: Infl. = inflorescence; Consp. = conspecific.

| Study site	Fruit set	Infl. length	Consp. density	RSp density	RSp richness
Voerendaal	0.17 ± 0.02	6.5 ± 0.4	2.3 ± 0.4	151.4 ± 14.5	2.5 ± 0.1
Andenne	0.11 ± 0.02	6.2 ± 0.4	3.2 ± 0.5	19.9 ± 4.1	0.8 ± 0.1
Musson	0.08 ± 0.01	8.3 ± 0.5	2.1 ± 0.4	24.3 ± 5.7	1.8 ± 0.2
Charency-Vezin	0.14 ± 0.02	7.5 ± 0.4	0.8 ± 0.2	28.0 ± 6.6	1.5 ± 0.2
Nixéville-Blercourt	0.10 ± 0.02	6.8 ± 0.5	1.2 ± 0.3	37.9 ± 10.0	0.8 ± 0.1
Saint-Julien-lès-Gorze	0.21 ± 0.03	8.8 ± 0.8	0.5 ± 0.2	27.9 ± 7.2	0.4 ± 0.1
Waville	0.28 ± 0.04	5.5 ± 0.4	0.3 ± 0.1	123.0 ± 22.1	2.3 ± 0.2
Dompcevrin	0.26 ± 0.02	5.3 ± 0.3	0.3 ± 0.4	52.6 ± 8.8	2.6 ± 0.2
Bacourt	0.20 ± 0.02	6.9 ± 0.3	1.9 ± 0.3	100.3 ± 19.0	2.0 ± 0.1
Longchamps-sur-Aire	0.34 ± 0.05	9.4 ± 0.9	0.7 ± 0.2	14.3 ± 3.9	0.5 ± 0.1
Jezainville	0.24 ± 0.05	5.4 ± 0.3	0.4 ± 0.1	190.1 ± 31.4	1.9 ± 0.2
Jeandelaincourt	0.21 ± 0.03	6.2 ± 0.6	1.1 ± 0.2	23.8 ± 7.1	0.7 ± 0.1
Salmagne	0.11 ± 0.03	7.0 ± 0.6	0.5 ± 0.1	43.3 ± 10.5	1.3 ± 0.2
Troussey	0.20 ± 0.02	5.3 ± 0.3	0.2 ± 0.1	55.4 ± 9.1	2.4 ± 0.2
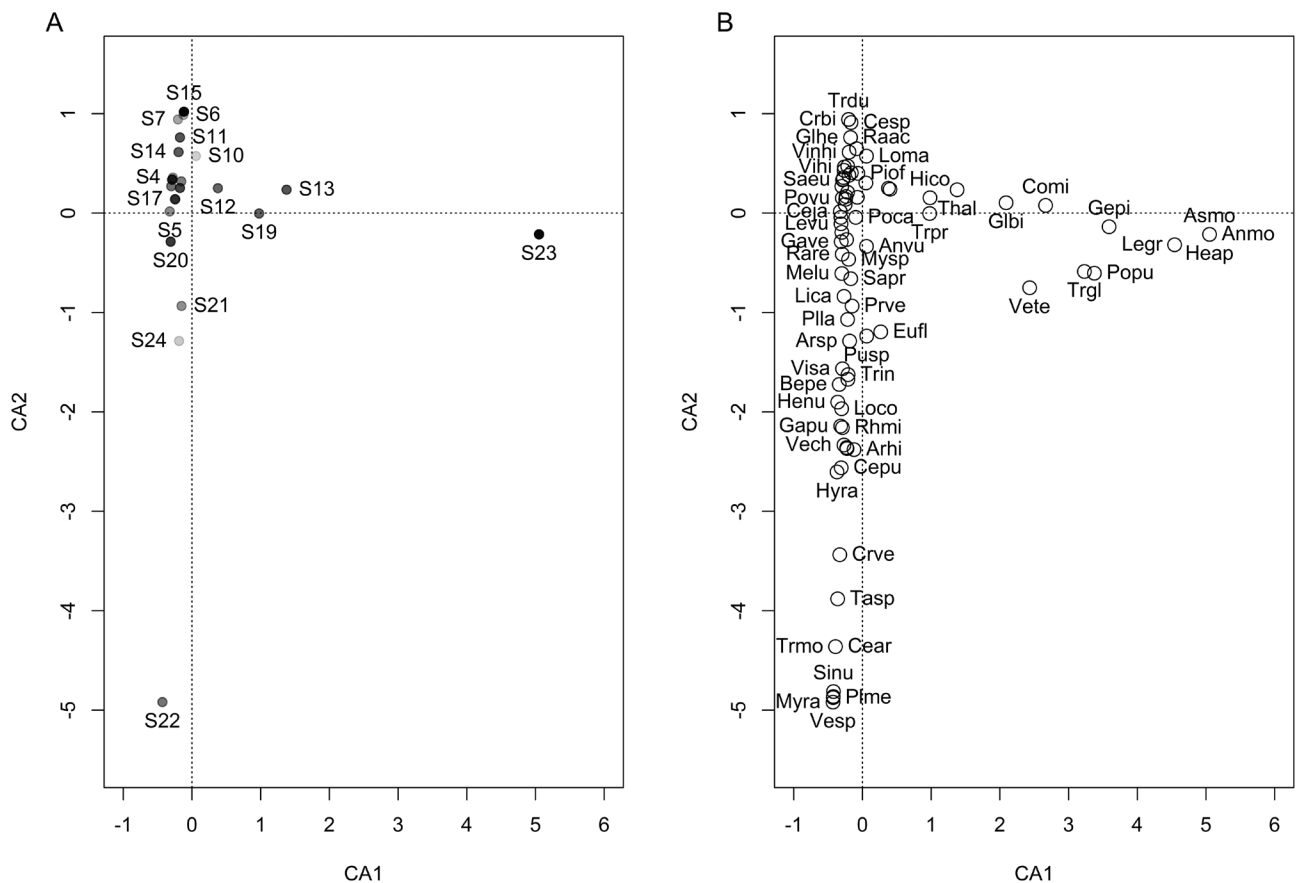
Figure 2 – Correspondence analysis ordination diagram of the population-scale RSp data in the space of the first two axes: A, distribution of site scores with darker points indicating higher fruit set; B, distribution of Syecies scores. To avoid overlap, not all population and species labels are shown. Site numbers refer to those in table 1. Abbreviations of species names refer to those in electronic appendix 2.

Table 3 – **Variables of the population-scale study: fruit set (mean ± SE), inflorescence length (in cm), and conspecific density (number of inflorescences) of *Orchis militaris*, and RSp density (in FUs) and richness (number of species).**
Abbreviations: Infl. = inflorescence; Consp. = conspecific.

Study site	Fruit set	Infl. length	Consp. density	RSp density	RSp richness
Freudenburg	0.22 ± 0.02	6.1 ± 0.3	300	35 703	11
Voerendaal	0.17 ± 0.01	6.1 ± 0.3	225	27 356	9
Niederanven	0.23 ± 0.02	6.5 ± 0.3	41	23 617	10
Rumelange	0.10 ± 0.01	9.0 ± 0.4	140	41 243	9
Visé	0.13 ± 0.02	5.3 ± 0.2	108	18 985	14
Andenne	0.09 ± 0.01	5.7 ± 0.3	245	4 731	10
Musson	0.10 ± 0.01	7.6 ± 0.3	215	5 310	21
Saint-Thomas	0.16 ± 0.02	6.4 ± 0.5	32	109 641	15
Charency-Vezin	0.15 ± 0.01	6.3 ± 0.3	92	44 569	8
Nixéville-Blercourt	0.05 ± 0.01	6.0 ± 0.2	124	7 183	10
Saint-Julien-lès-Gorze	0.18 ± 0.03	7.9 ± 0.8	41	6 034	9
Waville	0.18 ± 0.03	5.4 ± 0.5	52	18 357	15
Dompcevrin	0.20 ± 0.01	4.6 ± 0.1	145	5 026	10
Bacourt	0.20 ± 0.01	6.8 ± 0.2	220	15 401	12
Longchamps-sur-Aire	0.34 ± 0.03	6.3 ± 0.3	125	567	3
Jeandelaincourt	0.31 ± 0.04	6.2 ± 0.4	51	12 912	19
Troussey	0.17 ± 0.02	7.1 ± 0.4	155	10 606	14
Rochefort-Samson	0.25 ± 0.02	6.1 ± 0.2	98	34 683	26
Léoncel	0.13 ± 0.02	5.2 ± 0.2	44	7 905	24
Le Chaffal	0.16 ± 0.05	5.8 ± 0.8	9	15 871	33
Ispagnac	0.34 ± 0.02	5.8 ± 0.2	161	16 938	17
Laval-du-Tarn	0.06 ± 0.01	5.6 ± 0.2	69	22 412	33
Mean	0.17 ± 0.01	6.2 ± 0.1	122 ± 17	22 056 ± 4 941	15 ± 1.7
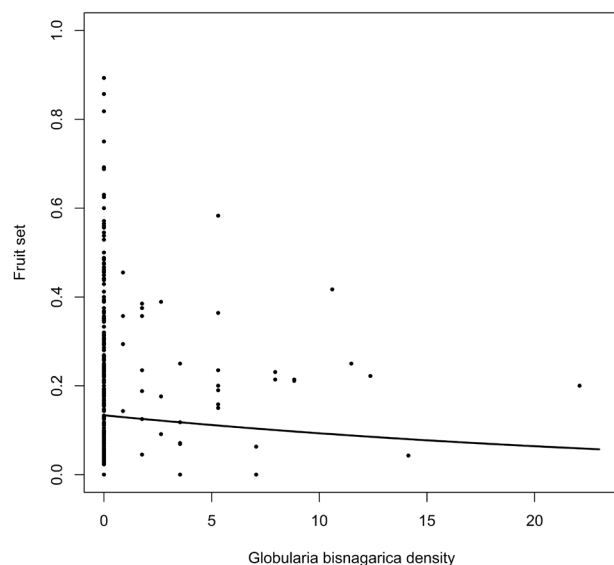
Figure 3 – Relationship between *Orchis militaris* fruit set and the density of *Globularia bisnagarica* at the individual scale.

We applied manually a backward elimination to obtain the final model. We used a graphical visualisation method in order to interpret significant interactions (Lamina et al. 2012).

For population-scale data, correspondence analysis was conducted to analyse RSp composition among study sites and its relationship to fruit set. We used the vegan package of R (Oksanen et al. 2017). There was a significant dependency between sites and RSp (correlation coefficient = 1.95; χ^2 test: $p < 0.001$). According to the centroid principle (ter Braak & Verdonschot 1995), sites that contain a particular RSp have the same coordinates as this species, e.g. site 23 and Anmo. According to the distance rule (ter Braak & Verdonschot 1995), sites close to the species point tend to have a higher abundance than sites far from the species point; e.g. the density of Trmo is maximal in site 22. Species points far apart from each other are considered dissimilar in their distribution across the sites. Sites 22 and 23 are far from the other sites, which form a cluster near the centroid (fig. 2). We used a generalised linear model (GLM, quasi-binomial error) to explore the relationships between mean population fruit set and the following fixed variables: *O. militaris* inflorescence length, RSp density (values averaged for each population), population size, conspecific density, RSp richness, altitude, and latitude (table 3). Owing to insufficient replication, we

Table 4 – **Results of the GLMM (backward manual elimination procedure) showing the effect of fixed variables and their interactions on *Orchis militaris* fruit set at the scale of the individual plant**.

Source of variation	Estimate	SE	d.f.	χ^2	p
Inflorescence length	0.034	0.013	1	16.10	$6.0 \ 10^{-5}$
Conspecific density	0.184	0.041	1	12.46	$4.2 \ 10^{-4}$
Vegetation height	0.227	0.072	1	9.90	0.002
RSp density	0.001	0.001	1	$2 \ 10^{-3}$	0.960
RSp richness	-0.183	0.061	1	4.49	0.034
Globularia bisnagarica density	-0.041	0.019	1	4.81	0.028
Inflorescence length × conspecific density	-0.037	0.006	1	42.50	$7.1 \ 10^{-11}$
Inflorescence length × RSp richness	0.035	0.006	1	25.81	$3.8 \ 10^{-7}$
RSp density × RSp richness	$-4 \ 10^{-4}$	$2 \ 10^{-4}$	1	4.68	0.031

did not include two-way interactions. Because of the highly significant correlation between altitude and latitude ($\rho = -0.73$, $p = 0.0001$), and between conspecific density and population size ($\rho = 0.80$, $p = 9.4 \ 10^{-6}$), we excluded latitude and population size from the GLM analysis. We used the quasi-binomial family to accommodate overdispersion (Ekstrøm 2012). Due to insufficient replication, we did not include year as a random variable. However, no significant difference in mean population fruit set was found between 2014 and 2015 (Kruskal-Wallis test: $\chi^2 = 0.182$, d.f. = 1, $p = 0.670$). To determine if a specific RSp density could be included in the GLM, we selected the species found in at least three populations. Then, if the relationship between a specific RSp density and mean population fruit set was significant (quasi-binomial error, logit link function), this variable was included in the GLM. All statistical analyses were performed in R 3.3.1 (R Development Core Team 2016).

RESULTS

Individual-scale study

In total, we recorded 53 co-flowering rewarding species (RSp richness) in the plots around individual *O. militaris* plants, ranging from four to seventeen taxa per population, with an average of 1.5 ± 0.2 species per plot per population (table 2). Eight taxa were found in more than thirty of 697 plots: *Sanguisorba minor* Scop., *Polygala* gr. *calcarea* F.W.Schultz, *Neottia ovata* (L.) Bluff & Fingerh., *Hippocrepis comosa*, *Euphorbia cyparissias* L., *Globularia bisnagarica* L., *Linum catharticum* L., and *Genista pilosa* L. (electronic appendix 1). Mean individual fruit set of *O. militaris* was 0.19 ± 0.16 (min. = 0.00, max = 0.89, n = 397). Among the species found in more than thirty plots, only *G. bisnagarica* density had a significant ($p = 0.03$) effect on fruit set. When included in the GLMM, this variable remained significant after backward elimination; there was a negative correlation between *G. bisnagarica* density and fruit set (fig. 3). *O. militaris* inflorescence length, conspecific density, RSp richness, vegetation height, and the interactions between inflorescence length and conspecific density, inflorescence length and RSp richness, and RSp density and RSp richness significantly affected fruit set (table 4). Fruit set was higher in medium-height vegetation (0.21 ± 0.01, n = 250) than in short vegetation (0.15 ± 0.01, n = 149). Conspecific density had a positive effect on fruit set for low values of inflorescence length. For increasing values of inflorescence length, the effect of conspecific density on fruit set became negative (fig. 4A). RSp richness had a strong positive effect on fruit set for high values of inflorescence length, but a slightly negative effect for short inflorescences (fig. 4B). RSp richness had a positive effect on fruit set for low values of RSp density; for increasing values of the latter, the effect of RSp richness became strongly negative (fig. 4C).

Population-scale study

In total, we recorded 109 co-flowering rewarding taxa in the 30 × 50 m rectangles including *O. militaris* plants, ranging from three to 33 taxa per population, with an average of 15 ± 1.7 species per population (table 3). More than 30 species were found in at least three of 22 populations (electronic appendix 2). Mean population fruit set of *O. militaris* was 0.18 ± 0.08 (min. = 0.05, max. = 0.35, n = 22). In the correspondence analysis, the eigenvalue of axis 1 was 0.74 and that of axis 2 was 0.63; they explained 19.5 and 16.7 % of the sites-RSp relationship respectively. The first axis separated the sites located in the south of France (S20–24) from the other sites. Most sites with higher fruit set were clustered with those with lower fruit set (fig. 2). In the GLM analysis, among the species found in at least three populations, *Pilosella officinarum* density and *Plantago lanceolata* density had a significant negative effect on mean population fruit set. However, the significant relationship between *P. officinarum* and fruit set was due to a single high leverage data point. When removed, the relationship was no longer significant. When included in the GLM, *P. lanceolata* density was not significant and thus we removed it from the model. The GLM analysis revealed no significant relationship between mean population fruit set and the other explanatory variables (table 5).

DISCUSSION

This study aimed to clarify the deceptive pollination system in *Orchis militaris* and determine the factors influencing its

Table 5 – **Results of the GLM showing the effect of fixed variables on *Orchis militaris* mean population fruit set.**

Source of variation	Estimate	SE	d.f.	χ^2	p
Mean inflorescence length	$-1.17\ 10^{-1}$	$1.20\ 10^{-1}$	1	0.98	0.322
O. militaris density	$-2.53\ 10^{-4}$	$1.66\ 10^{-3}$	1	0.02	0.879
RSp density	$1.0\ 10^{-6}$	$6.84\ 10^{-6}$	1	0.02	0.884
RSp richness	$-3.89\ 10^{-2}$	$2.28\ 10^{-2}$	1	3.01	0.083
Altitude	$1.05\ 10^{-3}$	$6.41\ 10^{-4}$	1	2.58	0.108
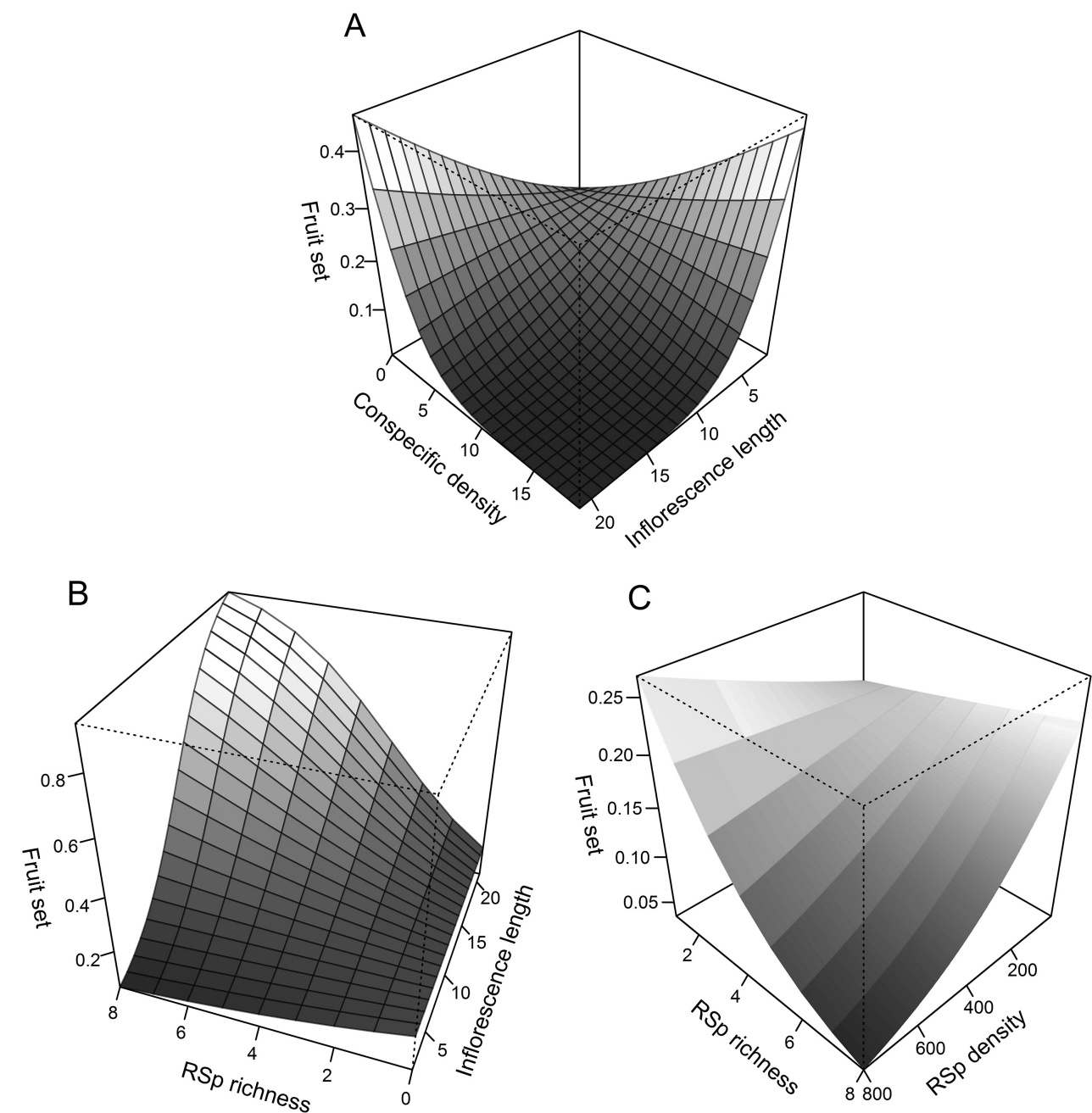
Figure 4 – Interaction surface plots: A, fruit set in *Orchis militaris* in relation to *O. militaris* inflorescence length and conspecific density; B, fruit set in *O. militaris* in relation to *O. militaris* inflorescence length and species richness of co-flowering rewarding species (RSp richness); C, fruit set in *O. militaris* in relation to density of co-flowering rewarding species (RSp density) and RSp richness.

fruit production in natural populations. *Globularia bisnagarica* was the only species whose density was significantly, yet negatively, correlated with *O. militaris* fruit set, at the individual scale. Our results suggest that pollinator attraction implies a mechanism more related to generalised food deception (GFD) than Batesian floral mimicry (BFM). At the microhabitat scale, the probability of setting a fruit was determined by several factors and their interaction, including floral display size, conspecific density, and RSp density and richness. At the population scale however, none of the studied factors was significantly correlated to fruit set.

In a similar study, Juillet et al. (2007) focused on the fly-pollinated (Jersáková et al. 2016) orchid species *Traunsteinera globosa* (L.) Rchb. They found a significant effect of altitude and co-flowering species density on population mean fruit set. The density of *Trifolium pratense* was positively correlated with orchid fruit set. Juillet et al. (2007) hypothesised that this correlation was due to either a magnet-species effect or a more species-specific interaction. According to Jersáková et al. (2016), the mechanism involved in *T. globosa* reproduction may relate to a "loose form of Batesian mimicry". Several models (Caprifoliaceae) selected from the regional pool of fly-pollinated species are thought to be involved in this system.

In our case, at both study scales, none of the co-flowering species had its density significantly positively correlated to *O. militaris* fruit set. If one of the encountered rewarding species and *O. militaris* were part of a mimicry system, any variation in the density of the former should affect the fruit set of the latter in a similar way (Lammi & Kuitunen 1995). In the correspondence analysis, most sites with higher fruit set (e.g. S15 and S17) are close to sites with lower fruit set (e.g. S4, S6 and S7), which do not support a strong relationship between a particular RSp composition and mean population fruit set. GFD rather than (imperfect) BFM can be proposed as a mechanism to explain pollinator attraction in *O. militaris*, like in *O. mascula* (Nilsson 1983) and *O. spitzelii* (Fritz 1990). In other genera, GFD has been demonstrated in a few species: e.g. *Anacamptis papilionacea* (L.) R.M.Bateman, Pridgeon & M.W.Chase, *A. boryi* (Rchb. f.) R.M.Bateman, Pridgeon & M.W.Chase, *Dactylorhiza sambucina* (L.) Soó, and *D. incarnata* (L.) Soó subsp. *incarnata* (see Scopece et al. 2014 for detailed references). In the genus *Orchis*, more specialised cases are also mentioned: *O. pallens* L. may be a mimic of *Lathyrus vernus* (Vöth 1982) but to our knowledge this hypothesis was never tested. In Europe and the Mediterranean Basin, potential BFM cases are rare: *Anacamptis israelitica* (H.Bauman & Dafni) R.M.Bateman, Pridgeon & M.W.Chase and its putative model *Bellevalia flexuosa* Boiss.; *A. collina* (Banks & Sol. ex Russell) R.M.Bateman, Pridgeon & M.W.Chase and *A. coriophora* (L.) R.M.Bateman, Pridgeon & M.W.Chase; or *Cephalanthera rubra* (L.) Rich. and *Campanula persicifolia* L. (Jersáková et al. 2009, Scopece et al. 2014). Other cases are related to sexual deception: *Orchis galilaea* (Bornm. & M.Schulze) Schltr. exclusively attracts males of *Lasioglossum marginatum* (Halictidae) (Bino et al. 1982). Similarly, scent of *O. pauciflora* Ten. contains male bumblebee pheromonal components and its main pollinators are *Bombus terrestris* queens (Valterová et al. 2007).

In our system, at the individual scale, GFD should occur in individuals with a larger floral display (see below) through the magnet-species effect. Although we focused on co-flowering accompanying flora, sequential pollination facilitation may also occur. In other words, an earlier-flowering rewarding species can positively influence the pollination of a later-flowering species through site fidelity of pollinators (Ogilvie & Thomson 2016). A surprising result was the negative correlation between *Globularia bisnagarica* density and fruit set. It seems unlikely that *G. bisnagarica* (nectar-producing species) competed for pollinators with *O. militaris*, since the former is pollinated by butterflies, long-tongued bees, and hoverflies (Honnay et al. 2007) while confirmed pollinators of *O. militaris* are assumed to be short-tongued bees (Vöth 1987). Local density of *G. bisnagarica* may be correlated with some (abiotic) environmental factor that has a negative effect on fruit set in *O. militaris*.

At the individual scale, the effect of inflorescence length on fruit set was contingent upon the density of conspecifics. Other studies have shown that local conspecific density may alter the relationship between floral display size and fruit set or visitation rate (Grindeland et al. 2005, Weber & Kolb 2013, Ruane et al. 2014). For lower values of conspecific density, larger floral displays had an advantage in terms of fruit set compared to smaller ones. In many flowering plant species, fruit production is known to be positively related to floral display size (e.g. Willson & Rathcke 1974, Schemske 1980, Pyke 1981, Udovic 1981, Bartkowska & Johnston 2014, Suetsugu et al. 2015). It has been hypothesised that enhanced fruit set for individuals with larger floral displays is due to the attraction of a higher number of pollinators (Brody & Mitchell 1997). Their preference for larger displays (e.g. Ohara & Higashi 1994, Grindeland et al. 2005, Miyake & Sakai 2005) could be due to better detection (long-distance attraction – Brody & Mitchell 1997) or reduction of flight costs (Ohashi & Yahara 2001). However, pollinators are also expected to probe more flowers on plants with large displays, which could lead to geitonogamy (Ohashi & Yahara 2001) and thus have deleterious effects on reproductive success (de Jong et al. 1993). In *O. militaris*, the cost of geitonogamy should be reduced by the bending movements of pollinaria after removal by an insect. For higher values of conspecific density, fruit set declined with increasing focal inflorescence length (fig. 4A). High levels of conspecific density are known to negatively impact fruit set in several rewardless species (Sabat & Ackerman 1996, Gumbert & Kunze 2001, Internicola et al. 2006). In pollination-limited generalised deceptive species, the negative relationship between density and fruit set should arise from insect avoidance learning (Anderson & Johnson 2006), leading to negative density-dependent selection (Gumbert & Kunze 2001). The development of strong discrimination against a deceptive species may depend on both the abundance of the latter and the abundance of rewarding plants (Tuomi et al. 2015). However, in bees and bumblebees, avoidance learning may not be as important as learning ability (i.e. the ability to learn to focus foraging on rewarding plants) and pollinators could adopt a flexible foraging behaviour instead of strongly avoiding deceptive flowers (Juillet et al. 2011), such as reducing the time spent on rewardless plants or switching to an alternative type of

inflorescence (Smithson & Gigord 2003). At higher conspecific density, the fruit set advantage of larger floral displays disappeared; fruit set was even reduced in individuals with a larger inflorescence. The proportion of flowers visited may decrease faster with floral display size in higher-density plots (Grindeland et al. 2005).

At the local scale, the effect of RSp richness on fruit set depended on both focal inflorescence length and RSp density. Increasing RSp richness positively affected predicted fruit set in plants with a larger inflorescence; on the contrary, it negatively affected fruit set for decreasing values of inflorescence length (fig. 4B). For lower values of RSp density, increasing RSp richness had a positive effect on fruit set; however, for higher values of RSp density, the effect was strongly reversed (fig. 4C). Since co-flowering species often share pollinators, individual reproductive success can be influenced by community composition (Hanoteaux et al. 2013). On the one hand, at a local scale, the frequency of pollinator visits can be positively correlated to flowering plant species richness (Ebeling et al. 2008). On the other hand, higher-density patches of rewarding species may generally be more attractive to pollinators (e.g. Hegland et al. 2009, Dauber et al. 2010), which may exhibit higher visitation rates (Seifan et al. 2014). Specifically, in several deceptive species, the proximity or the abundance of a particular rewarding species is known to positively affect fruit set (Laverty 1992, Johnson et al. 2003, Juillet et al. 2007). The underlying process of pollinator-mediated facilitation has been demonstrated by several studies (Sargent & Ackerly 2008, Morales & Traveset 2009). We hypothesise that facilitation (through increased RSp richness) would only take place in *O. militaris* individuals with a larger floral display, whereas strong competition with rewarding species for pollinator attraction would lead to a decrease of fruit set in smaller floral displays. A strong competition may also occur in dense and RSp-rich plots, leading to low fruit set.

A significantly higher fruit set in medium-height than in short vegetation could be related to the fact that vegetation height has been documented to be positively correlated with abundance and diversity of bees (Kruess & Tscharntke 2002, Pardee & Philpott 2014). However, opposite results were obtained in another study (Latus 2014). Moreover, according to Sletvold et al. (2013), a higher rate of pollinator visitation is expected in short vegetation. A higher vegetation height could be correlated with higher RSp richness or RSp density, but it was not the case in our study. A better detection probability of *O. militaris* inflorescences would be expected in shorter vegetation and our results thus seem counterintuitive. At the population scale, none of the measured variables was significantly correlated with fruit set. Our failure to detect any significant effects could result from insufficient statistical power. Additionally, the effect of some variables, like floral resource density, on insect visitation (and thus reproductive success) can be scale-dependent, as suggested by several studies (Jakobsson et al. 2009, Ikemoto et al. 2011, Hegland 2014, Schmid et al. 2016). For example, Johnson et al. (2003) studied *Anacamptis morio* and found that its pollination success was correlated to rewarding species density, not at the individual scale (1 m²) but at a larger scale (100 m²).

In summary, our results reveal that variation in fruit production in *O. militaris* depends on individual (floral display size), population (conspecific density), and community (RSp density and richness and vegetation height) factors. In order to better understand the pollination mechanism of *O. militaris*, further field studies and experiments are needed to test the effect of these factors on pollinator foraging behaviour and species composition. Seed production and viability should be taken into account to describe more precisely the interaction effect between floral display size and local conspecific density. A higher seed production per fruit could for example compensate for reduced fruit set. Since sequential facilitation may occur, future studies should explore the role of the insect-pollinated species whose flowering period precedes that of *O. militaris*.

SUPPLEMENTARY DATA

Supplementary data are available in pdf at *Plant Ecology and Evolution*, Supplementary Data Site (http://www.ingentaconnect.com/content/botbel/plecevo/supp-data) and consist of: (1) occurrence and density (mean ± SE, in FUs, except when there were fewer than three populations or plots; then detailed data are shown) of rewarding species in the individual-scale study; and (2) occurrence and density (mean ± SE, in FUs, except when there were fewer than three populations; then detailed data are shown) of rewarding species in the population-scale study.

ACKNOWLEDGMENTS

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