Accepted Manuscript

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Please cite this article as: {http://dx.doi.org/

Effects of pollen species composition on the foraging behaviour and offspring performance of the mason bee *Osmia bicornis* (L.)

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Abstract

The effects of floral species composition on offspring performance of solitary bees are rarely studied under conditions where foraging behaviour of mothers is allowed to play a role. In a semifield experiment, we restricted foraging choices of the polylectic mason bee *Osmia bicornis* L. to flower species belonging to plant families presumably used to different extent*: Borago officinalis* L. (Boraginaceae), *Centaurea cyanus* L. (Asteraceae) and *Brassica napus* L. (*Brassicaceae*). We quantified the foraging behaviour and brood cell production by mother bees, and compared the quality of offspring in pure and mixed flower species stands. Offspring survival

in pure stands was expected to reflect the mothers' foraging preferences in the mixed stand. Pure stands of *B. napus* supported highest offspring survival, body mass and fraction of females produced. Offspring survival on *C. cyanus* and *B. officinalis* was very low. Larval mortality occurred earlier in brood cells provided with *B. officinalis* than in brood cells provided with *C. cyanus* suggesting different effects of pollen quality on early larval and later development. The time spent on different foraging activities correlated with lifetime reproductive output. However, in mixed stands, the proportion of time the bees were foraging on the different flower species did not differ significantly. Foraging behaviour may therefore not generally be a good proxy for the quality of floral resources for offspring production. Our results suggest that resources collected from one plant species may influence the usefulness of resources from another plant species. Bees may therefore overcome potentially deleterious effects of the suboptimal resources by mixing low- and high – quality resources. This may help generalist bees, such as *O. bicornis*, to cope with an unpredictable environment.

Zusammenfassung

Die Effekte der Artenzusammensetzung von blühenden Pflanzen auf das Gedeihen der Nachkommen von solitären Bienen werden selten unter Bedingungen untersucht, die zulassen, dass das Sammelverhalten der Mütter eine Rolle spielt. In einem Semi-Freilandexperiment, beschränkten wir die Sammeloptionen der polylektischen Mauerbiene *Osmia bicornis* L. auf Blütenarten aus vermutlich unterschiedlich stark genutzten Familien: *Borago officinalis* L. (Boraginaceae), *Centaurea cyanus* L. (Asteraceae) und *Brassica napus* L. (*Brassicaceae*). Wir bestimmten das Sammelverhalten und die Brutzellenproduktion von Bienenweibchen und verglichen die Qualität der Nachkommen bei reinem und gemischtem Blütenangebot. Die Überlebensrate der Nachkommen bei Angebot von nur einer Blütenart sollte die Präferenz der Mütter in gemischten Blütenbeständen wiederspiegeln. Reine Bestände von *B. napus* ergaben die höchste Überlebensrate, Körpermasse und den höchsten Weibchenanteil bei den Nachkommen. Bei *C. cyanus* und *B. officinalis* war die Überlebensrate sehr gering. Die Larvensterblichkeit setzte in mit *B. officinalis* verproviantierten Brutzellen früher ein als in Brutzellen mit *C. cyanus* als Proviant. Somit sollte es unterschiedliche Effekte der Pollenqualität auf die frühe larvale und die spätere Entwicklung geben. Die für die verschiedenen Sammelaktivitäten aufgewendete Zeit korrelierte mit der Gesamtproduktion an Nachkommen einer Mutterbiene. Indessen unterschieden sich die Zeitanteile, die bei gemischtem Angebot mit dem Sammeln an unterschiedlichen Blütenarten verbracht wurden, nicht signifikant voneinander. Das Sammelverhalten könnte deshalb kein guter Indikator für die Qualität von Blütenressourcen für die Produktion von Nachkommen sein. Unsere Ergebnisse legen nahe, dass Ressourcen, die von einer Pflanzenart gesammelt wurden, den Nutzen der Ressourcen von einer anderen Art beeinflussen könnten. Die Bienen könnten somit mögliche abträgliche Effekte einer suboptimalen Ressource durch das Mischen von qualitativ hoch- und minderwertigen Ressourcen

kompensieren. Dies könnte Generalisten wie der Mauerbiene *O. bicornis* helfen, mit einer nicht vorhersagbaren Umwelt zurechtzukommen.

Introduction

Herbivore growth, survival and reproduction are not only determined by the abundance of available resources, but also by their quality. Plants generally have a low nutrient content and often contain toxic allelochemicals and thus may represent suboptimal food sources for herbivores (Jermy 1984; Schoonhoven, van Loon, & Dicke, 2005). Most of our understanding of the role of plant quality in plant-invertebrate herbivore interactions originates from studies on folivorous insects, with much focus on how specialist herbivores deal with constraints imposed by plant defence chemistry (e.g., Zangerl & Berenbaum 2003). Substantially less attention is paid to the effects of food quality on generalist insect herbivores (Bernays & Graham 1988), and especially in pollen feeding species. Recent studies have begun uncovering the role of variation in pollen nutritional quality in wild bee development (Williams, 2003; Praz, Müller, & Dorn, 2008a; Eckhardt, Haider, Dorn, & Müller, 2014), and suggest that variation in pollen quality may have driven both dietary specialisation and generalism in plant – pollinator systems (Müller & Kuhlmann 2008; Roulston & Goodell, 2011; Sedivy, Dorn, Widmer, & Müller, 2013).

Pollen has been viewed as a nutrient-rich food source, where diet choices of bees were thought to be predominantly shaped by flower morphology and resource accessibility, and nutritional chemistry has been considered to play a secondary role (Roulston & Cane 2000). However, across different plant species, pollen shows large variation in nutritional properties like concentrations of essential amino-acids and fatty acids, and vitamin content (Roulston & Cane 2000). Besides nutrients, the amount and composition of pollenkitt may influence the pollen

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digestibility and thus nutrient uptake in the insect alimentary tract (Dobson & Peng 1997).

Members of the family Asteraceae, for example, possess pollen that is difficult to digest and may

contain low levels of essential amino-acids (Nicolson & Human 2013). Pollen may also contain a

range of allelochemicals such as alkaloids and glucosides, which may negatively affect pollinator

survival (Detzel & Wink 1993). For example, the pollen and nectar of some members of

Asteraceae, Boraginaceae, Ranunculaceae and Fabaceae contain allelochemicals with a

purportedly defensive function (Dodson & Stermitz 1986; Reinhard et al. 2009; Sedivy, Müller, &

Dorn 2011; Haider, Dorn, & Müller, 2014). While several studies report deleterious effects of

pollen due to suboptimal nutrient content or allelochemistry on managed honeybees, studies began

only recently to quantify the effects of pollen quality on life-history traits of solitary bees.

While specialist herbivores often detoxify or sequester allelochemicals, or physiologically adapt to

assimilate food of low nutrient content, generalist herbivores have to deal with suboptimal food in

other ways. Polylectic bees may exhibit large variation in their ability to develop on different

pollen species (Sedivy, Müller, & Dorn, 2011; Eckhardt, Haider, Dorn, & Müller, 2014). As a

result, mixing unsuitable and suitable pollen to balance nutrients or dilute toxins may increase

both offspring survival and reproductive output (Singer, Bernays, & Carriere, 2002; Eckhardt,

Haider, Dorn, & Müller, 2014). On the other hand, specialising in terms of flower handling (i.e.

flower constancy), may be beneficial over diet mixing because it allows more efficient resource

harvesting. To date, existing studies have tested the effects of pollen quality on bee development

and survival by artificially controlling the mixture and composition of pollen provided to bee

larvae.

The effects of food quality on survival and development ultimately depend on the foraging

decisions that mother bees make when allocating provisions into brood chambers. As provisioning

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rates impact offspring survival and quality (Müller et al. 2006; Roulston & Goodell 2011), natural selection is expected to affect foraging decisions. Consequently, mother bees may adjust foraging decisions to mix pollen of different quality and optimize offspring provisions. When bees can discriminate between pollen of different quality (Cook, Awmack, Murray, & Williams, 2003; Williams 2003; Leonhardt & Blüthgen 2012; but see Praz, Müller, & Dorn, 2008b), they should preferentially forage on flowers that provide high quality pollen for their offspring.

The aim of this study was to examine the impact of differences in flower species available to the polylectic solitary bee *Osmia bicornis* on foraging behaviour and reproductive output. We examined this under conditions when mother bees were provided with different unifloral resources and a multifloral mixture for offspring provisioning. In flight cages, we offered flower species, expected to have pollen of different quality, in pure and mixed stands to mated *O. bicornis* females. We subsequently compared the resulting variation in foraging behaviour and brood cell production, and in the survival rates, size and sex ratio of offspring. In addition, in the mixed stands containing all three flower species, we studied the preference ranking of foraging bees on the different flower species. *Borago officinalis* L. (Boraginaceae), *Centaurea cyanus* L. (Asteraceae) and *Brassica napus* L. (Brassicaceae) were chosen for our bioassays because they belong to plant families with putative differences in pollen quality, and because these plants are often sown in flower mixtures to attract pollinators. In addition, *B. napus* is a mass flowering crop that purportedly represents a predominant pollen source for early active wild bees in agroecosystems poor in flower resources. Presence of oilseed rape fields has been found to correlate positively with *O. bicornis* abundance in adjacent trap nests (Holzschuh, Dormann, & Tscharntke 2013), and pollen loads of field-collected *O. bicornis* often contain pollen of *Brassicaceae* (Haider, Dorn, Sedivy, & Müller 2014). However, the effects of *B. napus* floral resources on *O. bicornis* behaviour and development have not yet been investigated. The pollen of *C. cyanus* is not known to be toxic, but members of the Asteraceae often have a thick exine, with

protein and essential amino acid content at the lower end of the requirements of honeybees (Roulston & Cane 2000; Nicolson & Human 2013). The pollen of some members of *Boraginacea* contain toxic pyrrolozidine alkaloids (PA), but *B. officinalis* flowers are only known to contain low concentrations of thesinine, a PA compound not known for its toxic effects (Herrmann, Joppe, & Schmaus, 2002). We expected that *O. bicornis* will produce offspring of higher survival and mass on *B. napus* than on *C. cyanus* and *B. officinalis*. Higher offspring survival and mass, and more females were expected to be generated when foraging bees were provided with a mixed flower species stands than when their diet choices were restricted to pure stands of *C. cyanus* and *B. officinalis*. As a result of differences in floral resource quality, females provisioning their offspring in mixed stands were expected to preferentially forage on the flower species that provided the highest quality pollen for offspring development.

Materials and methods

Plants & Insects

Seeds of *Borago officinalis* L. and *Centaurea cyanus* L. were obtained from Cruydt-Hoeck (Nijeberkoop) and *Brassica napus* L. cv Helga seeds were obtained from Vreeken's Zaden (Dordrecht). Experimental plants were sown in a greenhouse compartment in February 2014. Four- to five-week-old seedlings were individually transplanted into 3 L pots filled with potting soil (Lentse potgrond©) and transferred into a heated tunnel where they stayed until April when they were subsequently transferred to an outdoor screen house and were supplied with equal amounts of slow-release fertilizer (Osmocote©) (10 g/pot).

Overwintering cocoons of the red mason bee (*Osmia bicornis* L.) originated from a population in the Nijmegen area. In the first week of May, a week before the onset of the experiment, cocoons were transferred to outdoor cages where bees emerged and were allowed to mate while simultaneously being exposed to all three plant species and a supply of honey, but not

to a nesting substrate. Three days later, two mated female bees of comparable size were randomly assigned to each experimental cage.

Experimental set-up

Twenty walk-in experimental cages (2 m x 2 m x 1.75 m, L x W x H) were made of insect nets (1 mm mesh size; Mononet 1000, Rovero Systems B.V., NL). Cages were laid out in two rows of ten on an area (50 m x 20 m) of bare soil covered by geo-textile weed membrane, with 2.5 m isolation distance between cages. Five replicated tents were installed for each of the flower treatments and were systematically randomized across the set-up (Appendix A: Supple. Fig. S1). In each cage, a group of 30 potted plants were placed that belonged to either one of the three monotypic stands or the mixture (10 pots per species). Within each cage, potted plants were placed into a 25 cm deep pit that was kept wet to prevent rapid desiccation of the potting soil. Three batches of plants were sown from each plant species in a succession so that flower resources in experimental cages could be refreshed and therefore flowers were present in the cages in abundance throughout the experiment (Appendix A: Supple. Fig. S2). In each cage, a trap nest was placed providing nesting substrate for bees. A trap nest comprised of two PVC tubes (20 cm x 10 cm, L x ø) affixed to a stake (Appendix A: Supple. Fig. S1). Each PVC tube contained 12 mason bee nest tubes (20 cm x 0.8 cm, L x ø) ([©]CJ Wildlife, Shrewsbury, UK). To construct brood cells, mason bees had access to soil either from the cage floor or from a dish of moist soil that was placed next to each trap nest.

Observation of reproduction and foraging behaviour

In addition to determining the total number of brood cells produced per cage during the experiment, we were also interested in the egg laying activity of individual bees over time. We therefore counted the number of newly made brood cells in each tent on days 12, 21, 34, 39, 40 and 46 days after starting the experiment. To estimate the brood cell production per bee per day

for each time point of observation, and for each tent, we divided the number of newly made brood cells by the number of bees still alive and by the number of days that elapsed since the previous census. In the second week of July, 47 days into the experiment, most of the surviving bees were killed by two days of heavy rainfall and the experiment was terminated.

During the experiment, the foraging behaviour of a bee within each cage was recorded on clear and sunny days, when bees were actively foraging and building nests (between 8:40 and 16:30). All cages in an observational round were visited within 2-3 days, and we strived for recording the same number of observation for all cages, where the sequence of recordings across the different treatments was randomized. Four observations were made for most cages yielding a total of 77 observations. To record behaviour, the observer entered the cage through a zip-fastened entrance and stood next to the cage wall as far from the trap nest and flowers as possible. Bees were allowed to habituate to the presence of the observer before selecting one of the two actively foraging bees and commencing to record its behaviour for a duration of 15 minutes using a handheld computer (Workabout Pro3, ©Psion Teklogix) and The Observer software 11.0 (Noldus Information Technology© 2009). The variables of interest were the following behavioural activities: rapid flight, hovering, foraging, standing still, walking, preening, and the place where behaviour was performed, such as on flowers or the trap nest. The duration of behavioural elements was extracted from the event log-files and the average time spent on each behavioural element was calculated for each tent.

Offspring survival, sex ratio and quality
At the end of the experiment, all nest tubes were collected and stored in a shed with temperature and humidity conditions closely approximating that of outdoors. In the second half of November, nesting tubes were carefully opened using a scalpel and the fate of the brood cells was determined.

The number of eclosed (i.e. fully developed) and living bees inside the cocoons was scored. Additionally, the number of dead offspring was recorded, and categorized according to their stage of development. Individuals could belong to viable eclosed adults. We recorded offspring mortality occurring during early larval development or possibly still in the egg stage, where brood cells contained largely intact provisions. Alternatively, offspring mortality occurred in the late larval, (pre)pupal and adult stages, where provisions were largely or entirely consumed. The sex of each pupa and adult was determined using morphological characteristics, and their dry weight was measured.

Data analysis

The effects of the four flower treatments on offspring performance (i.e. survival to adulthood, mortality of developmental stages) were compared using Generalized Linear Models with fractions modelled as logits: logit(p) = log(p/(1-p)), assuming a binomial distribution of errors, followed by linear contrasts for group comparisons. Correction for overdispersion was carried out by allowing the variance functions of the binomial distribution to have a multiplicative overdispersion factor (McCullagh & Nelder 1989). A similar logistic model was used to compare the proportion of female offspring between treatment groups. The dry weight (mg) of the successfully eclosed adults was compared by using General Linear Mixed Models with cage as a random factor, and flower treatment and sex of offspring as fixed effects.

To compare the number of brood cells between treatment groups, non-parametric KruskalWallis (K-W) tests were used. K-W tests were also used for the comparison of behavioural elements between treatments. To compare the foraging preferences across the three flower species within mixed stands, the proportion of time spent on each of the three flower species was calculated. A mixed model was used with flower treatment and the time of day as independent variables. Cage number was included as a random factor to account for possible correlation

between observations on the same bee individuals (PROC MIXED). To quantify if there was a possible effect of foraging behaviour (i.e., time spent foraging on flowers or engaged in rapid flight away from flowers) on the reproductive output (i.e., number of brood cells), ordinary regression analyses were performed with behavioural variables as explanatory variables and the number of brood cells as a response variable. Analyses assuming normality were followed by the inspection of the residuals that confirmed that normality assumptions were met acceptably. Analyses were carried out using SAS 8.2 (SAS Institute, Cary, NC, U.S.A.) and SPSS (IMB Statistics v19).

Results

Reproduction, offspring survival and quality

The experiment yielded a total of 460 brood cells. Most brood cells were produced in tents with *Borago* (158) followed by *Centaurea* (136) and the mixed stand (93), whereas the fewest were produced in the *Brassica* stands (73) (Fig. 1A). The average number of brood cells produced per cage showed large variation among tents, and no significant differences between treatment groups were found (K-W test, χ^2_3= 3.58, P=0.311). Examining the age-dependent reproductive output of bees showed that all bees began and ended their reproductive stage during the experiment and the observations therefore reflected life-time reproductive success (Appendix A: Supple. Fig. S3). The number of newly constructed broodcells per day depended on the age of the animals ($F_{1,18}$ = 10.55, P=0.005; Appendix A: Supple. Fig. S3). Females began constructing broodcells 12 days after emergence, and continued laying eggs and provisioning their offsprings until 46 days after eclosion, with the peak of reproduction observed at 21 days after emergence (Appendix A: Supple. Fig. S3). Also, age-dependent fecundity was not different between the flower treatments ($F_{3,18}$ = 0.6, P=0.622).

Offspring survival was different between flower treatments. The percentage of viable adults differed between flower treatments (χ^2_3= 59.36, P<0.001), with the highest percentages observed in the *Brassica* stands, and significantly lower percentages of eclosed adults in the *Centaurea* and *Borago* stands (Fig. 1B). The percentage of eclosed adults in the mixed stands was lower than that observed in the *Brassica* stands, but higher than those observed in the *Centaurea* and *Borago* stands (Fig. 1B). Larval diet also determined at which stage of development death occurred. Mortality in the egg and early larval stages was different between flower stands (χ^2_3= 76.26, P<0.001; Fig. 2A) as well as mortality rates in the later larval, (pre)pupas and adult stages (χ^2_3= 73.1, P<0.001; Fig. 2B). Interestingly, the mortality in these developmental stages showed contrasting patterns between the *Borago* and *Centaurea* stands, with egg and early larval mortality occurring predominantly in *Borago* and (pre)pupal and adult mortality in the *Centaurea* stands (Fig. 2AB). Egg and early larval mortality in the mixed treatment was lower than in the *Borago* stands, and was similar to the mortality observed in the *Centaurea* treatment, but higher than the mortality that was observed in the *Brassica* treatment (Fig. 2A). The mean mortality of the later developmental stages was significantly higher in the *Centaurea* stands than in the other three stands which had comparable low mortality rates (Fig. 2B).

The sex of 203 offspring could be determined. The fraction of female offspring ranged between 6.7% and 29.0% between treatment groups (Fig. 3A), where the overall effect of flower treatment was close to significant (χ^2_3= 7.31, P=0.063). The highest proportion of females was observed in the *Brassica* stands (29%), whereas the proportion of females was lowest in the *Centaurea* (6.7%) stand (*Brassica* vs *Centaurea* linear contrast z=2.35, P = 0.019, Fig. 3A). Pollen diet from the different flowers also affected offspring size (dry weight, $F_{3, 25.9}$ = 9.52, P<0.001, Fig. 3B). Posthoc tests revealed that individuals in the *Brassica* stands were heavier than those in the other flower stands (Fig. 3B). The majority of individuals on which the analysis was based were males,

and there were strong differences in the dry weight of the sexes ($F_{3, 25.7} = 96.33$, P<0.001). The lack of significant interaction between sex and flower treatment ($F_{3, 16} = 0.22$, P=0.884) indicated that the effects of floral resources on sizes of males and females were similar.

Foraging behaviour

Individuals spent most of the observation time foraging on flowers, hovering, staying inside their brood nest, or performing rapid flight behaviour across the cage, and considerably less time was spent on activities such as walking, preening or standing still (Appendix A: Supple. Table S1). The proportion of observation time spent on foraging on flowers positively correlated with the number of brood cells produced during the experiment (Fig. 4A, $F_{1,18}$=4.92, P=0.04, Pearson r = 0.46). Hovering flight that typically preceded landing on flowers or on trap nests, also correlated positively with the number of brood cells produced during the experiment ($F_{1,18}$=5.88, P=0.026, Pearson r = 0.496), whereas time spent on rapid flight behaviour away from flowers and trap nests negatively correlated with the number of brood cells produced per cage ($F_{1,18}$=6.47, P=0.02, Pearson r = -0.51). The flower treatments did not significantly influence the time spent on any of the behavioural activities. (Appendix A: Supple. Table S1). Furthermore, in the mixed stands, bees foraged on average 44%, 27% and 29% of their time on *Borago*, *Brassica* and *Centaurea* respectively but there was substantial variation between observations and we found no statistically significant differences in the foraging preferences of *O. bicornis* ($F_{2, 37.2}$ =1.09, P=0.35). Possibly for the same reason we found no indication that foraging on a particular species significantly increased or decreased during the course of the day (Fig 4B, Interaction flower treatment*time of day: $F_{2, 33.3}$ =1.14, P=0.33).

Discussion

The species of pollen that mother bees used for brood cell provisioning strongly impacted offspring development into adulthood and body size but in mixtures bees didn't select strongly against host plants supporting poor reproductive success. Previous studies showed that when providing a pure diet, *O. bicornis* was unable to develop on pollen of some members of the Asteraceae and Boraginaceae families, such as common tansy (*Tanacetum vulgare*) and viper's bugloss (*Echium vulgare,* Boraginaceae), respectively. In contrast, pollen of buttercup (*Ranunculus acris,* Ranunculaceae) and *Sinapis spp* (Brassicaeae) supported successful development (Sedivy, Müller, & Dorn, 2011; Eckhardt, Haider, Dorn, & Müller, 2014). We observed high larval mortality of *O. bicornis* when developing in pure stands of *C. cyanus* and *B. officinalis* and the high survival on *B. napus* floral resources observed here are in line with such phylogenetic differences in flower quality. However, the negative effects of *B. officinalis* on offspring survival are somewhat surprising, because its pollen is not known for its toxic effects (Herrmann, Joppe, & Schmaus, 2002). The observation that offspring mortality occurred in different developmental stages of *O. bicornis* on pollen of *C. cyanus* and *B. officinalis* also suggests different types of nutritional effects on bee larvae. Early larval mortality of *O. bicornis* was high on *B. officinalis*, indicating an acute negative (possibly toxic) effect of *B. officinalis* pollen on bee development. In contrast, the inability of larvae to reach later developmental stages or successfully eclose into viable adults on *C. cyanus* pollen may indicate more chronic effects of nutrient deficiency. The mechanisms of nutrient deficiency and toxicity are not mutually exclusive and may act in combination affecting bee larval development. In addition, differences in other pollen characteristics such as morphology can also affect pollen digestibility and access to nutrients (Suárez-Cervera, Marquez, Bosch, & Seoane-Camba, 2009).

In the mixed stands, the possibility to combine low-quality resources from *Centaurea* and *Borago* with high-quality resources from *Brassica*, increased the probability of *O. bicornis* offspring to develop into adults. Our findings comparing the mixed and the pure flower stands have implications for foraging decisions under floral resource heterogeneity. When food quantity is not limiting, foragers should choose the most profitable resource, in this case *Brassica* pollen. The foraging behaviour in the mixed stands and the lower offspring survival here compared to the pure *Brassica* stands suggest that this was not the case and *O. bicornis* likely mixed resources from different flower species even in the presence of better quality resource. One possible explanation for this may be related to information handling in heterogeneous environments. Bees may exhibit both innate and learned abilities to associate visual-olfactory cues with floral food characteristics such as pollen protein content (Dobson 1987; Praz, Müller, & Dorn, 2008a; Burger, Dötterl, & Ayasse, 2010; Leonhardt & Blüthgen 2012). However, learning to use cues associated with floral resources, or handling multiple flowers (i.e. operant conditioning) may entail constraints on foraging efficiency (Vet, Lewis, Papaj, & van Lenteren, 1990; Dukas & Real 1993). These constraints may be especially important in spatially and temporally highly heterogeneous environments (Menzel, Greggers, & Hammer, 1993), and foraging decisions may be selected against precise control over nutritional composition in offspring provisions in order to maximize brood cell production. However, these arguments only hold when resource quantity is not limiting. When the preferred resource is limited, females may choose not to discriminate and mix resources in order to maximize floral resource acquisition, even when they would otherwise be capable of discriminating. When low quality pollen is abundant, *O. bicornis* females may alleviate the negative effects of food quality on offspring by mixing pollen of different quality. An alternative explanation for the observed mixing of resources could be that if the high-quality floral resource may become more limiting later in the day, causing bees to shift from their more preferred flower species to the less preferred depending on the abundance of available pollen species (Vaudo et al.

2014). However, bees foraged consistently and at high frequency on *Borago*, a low-quality resource throughout the day, suggesting that this could not have been the main reason for mixing floral resources. Our data do not provide information on the type of resources provided by the different plant species in the mixed stands. A flower species may contribute much nectar but little pollen to a provision and such combination may affect physical and nutritional differences between provisions (e.g. density) with relevance for nutritional quality. Thus while our results demonstrate that uni- and multifloral stands have different effects on offspring quality and survival, in order to unravel the mechanism to which mixing can be credited to, studies such as the one described here should be complemented by analysis of floral provisions. Correlating foraging preferences under unlimited pollen resources with offspring performance on carefully controlled pollen diet mixtures should shed more light on the underlying mechanisms that govern diet mixing under pollen resource heterogeneity. Such experiments could also address the relative importance of floral resource quality and quantity in explaining offspring survival and performance under resource heterogeneity.

Sex ratios of *O. bicornis* observed in the pure *B. napus* stands were similar to sex ratios observed in controlled studies (Seidelmann, Ulbrich, & Mielenz, 2010) and in trap nests placed next to sown flower strips in Dutch agricultural landscapes (Suyker 2014; Radmacher & Strohm 2009). However, the proportion of females produced in the *Centaurea* stands was lower than in pure *Brassica* stands. A possible explanation for the male-biased sex ratios on *Centaurea* pollen could be a differential effect of pollen food on the survival of male and female progeny. Food quality and quantity both play important roles in influencing sex ratios in insects, but their effects on solitary wild bees is poorly studied. Due to the lack of information on the quantity and quality of provisions that the brood cells received in our experiment, we can only speculate on how food quality and quantity may affect offspring sex ratios. For example, when food quantity is limiting,

sex ratios may be influenced through sexual dimorphism. As the development of smaller males require less provision than that of females, limited provisions may result in more male biased sex ratios (Frohlich & Tepedino 1986; Helms 1994; Bosch & Vicens 2002). Besides floral resource quantity, quality of provisions may also affect sex ratios in insects in different ways, such as through differential survival of male and female offspring in insects (Awmack & Leather 2002; Ode 2006). In addition, as foraging bees may assess floral resource quality (e.g. Cook et al. 2003), the possibility of females controlling offspring sex in response to food quality cannot be excluded.

Provisioning brood cells with *B. napus* resulted in the highest survival and quality of *O. bicornis* progeny, suggesting that *B. napus* as a mass flowering crop could potentially contribute to the population growth of *O. bicornis*. Such positive effects of floral provisions on *O. bicornis* may therefore occur not only because of the high abundance of *B. napus* pollen in some landscapes, but also because of its high suitability in supporting *O. bicornis* development. However, oilseed rape is often treated with systemic insecticides such as neonicotinoids. As these insecticides have a strong negative impact on the reproductive success of *Osmia spp.* (Sandrock et al. 2014; Rundlöf et al. 2015), positive effects of oilseed rape on resource availability may be counteracted by toxicity effects (Woodcock, Isaac, Bullock, Roy, Garthwaite, et al. 2016). Our results show that foraging behaviour is not generally a good proxy for the quality of floral resources for offspring production. For lack of better data, foraging behaviour is often used as an indication of resource quality for bee species (Kleijn & Raemakers 2008). However, foraging preferences may be influenced by many factors such as presence and relative abundance of high- and low-quality resources and the number and identity of competitors. This study shows that the quality of individual plant species as a resource for generalist bees such as *O. bicornis* may additionally be influenced by resources that are collected from other plant species. By mixing low-quality

resources with high-quality resources bees may overcome potentially deleterious effects of the suboptimal resources. This may ultimately help them cope with an unpredictable environment.

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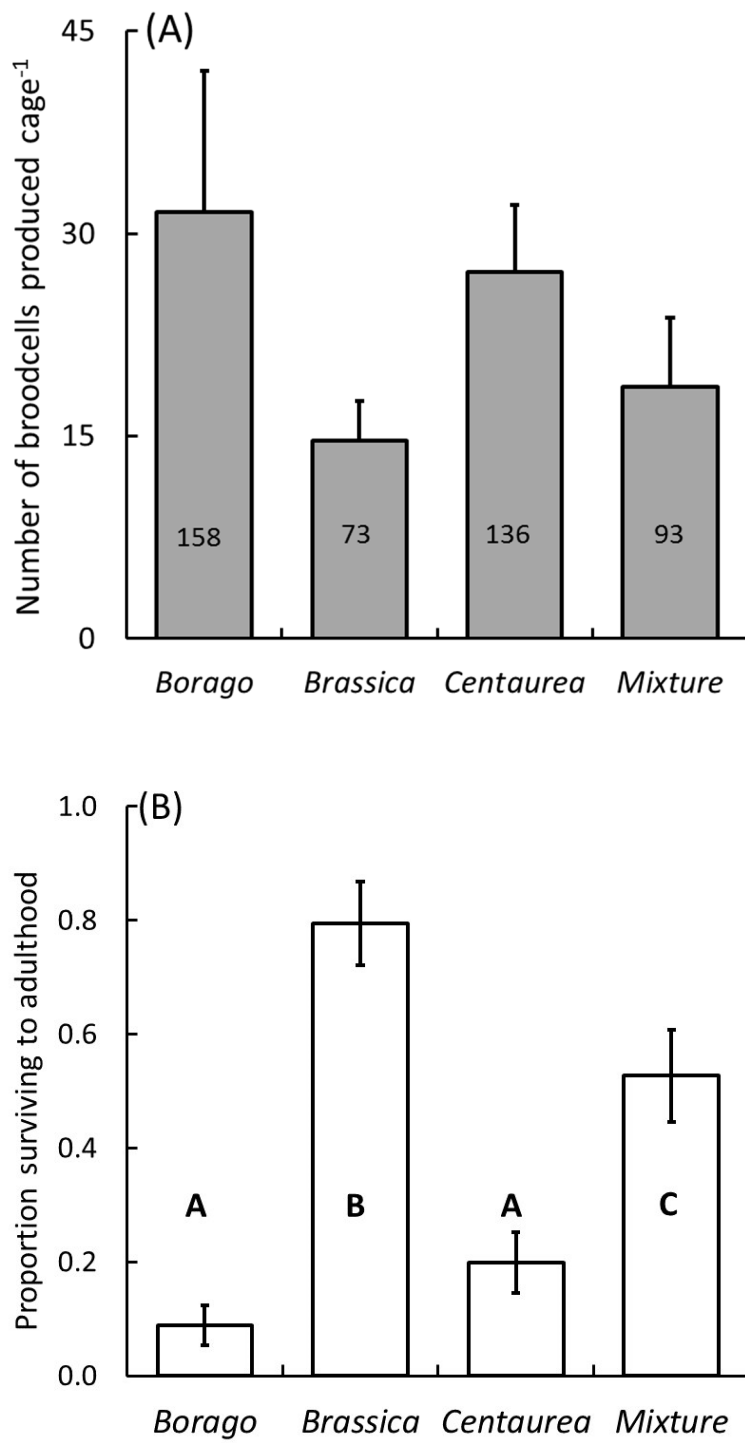webworms: causes and consequences. Evolution, 57, 806–815.

Figure legends

Fig. 1. (A) Average number (±SE) of brood cells produced per cage by two *Osmia bicornis* females. Numbers in bars are the total brood cell counts in that treatment. **(B)** Proportion (± SEM) of offspring surviving to adulthood when provisioned in flower stands of different species composition. Letters indicate significant differences between treatment groups.

Fig. 2. Proportions (± SE) of *Osmia bicornis* offspring suffering mortality in **(A)** early developmental stage (egg or early larva) or **(B)** during later development (late larva, (pre)pupa, adult). Letters indicate significant differences between treatment groups.

Fig. 3. The **(A)** proportion (± SE) of female progeny of *Osmia bicornis* when developing on floral food of different species composition. **(B)** Dry mass (±SE) of male (white bars) and female (grey bars) *O. bicornis* in the floral food treatments. Numbers in bars show sample sizes, letters indicate significant differences between treatment groups.

Fig. 4. The **(A)** total number of brood cells produced by *Osmia bicornis* in a cage in relation to the average proportion of time spent on foraging for flower resources. Solid line indicate fitted relationship. **(B)** Foraging preferences of bees during the day expressed as the proportion of time spent foraging on flowers of each of the three plant species in the mixed stands. Dashed lines indicate non-significant relationships that are added to aid visual interpretation of the data.
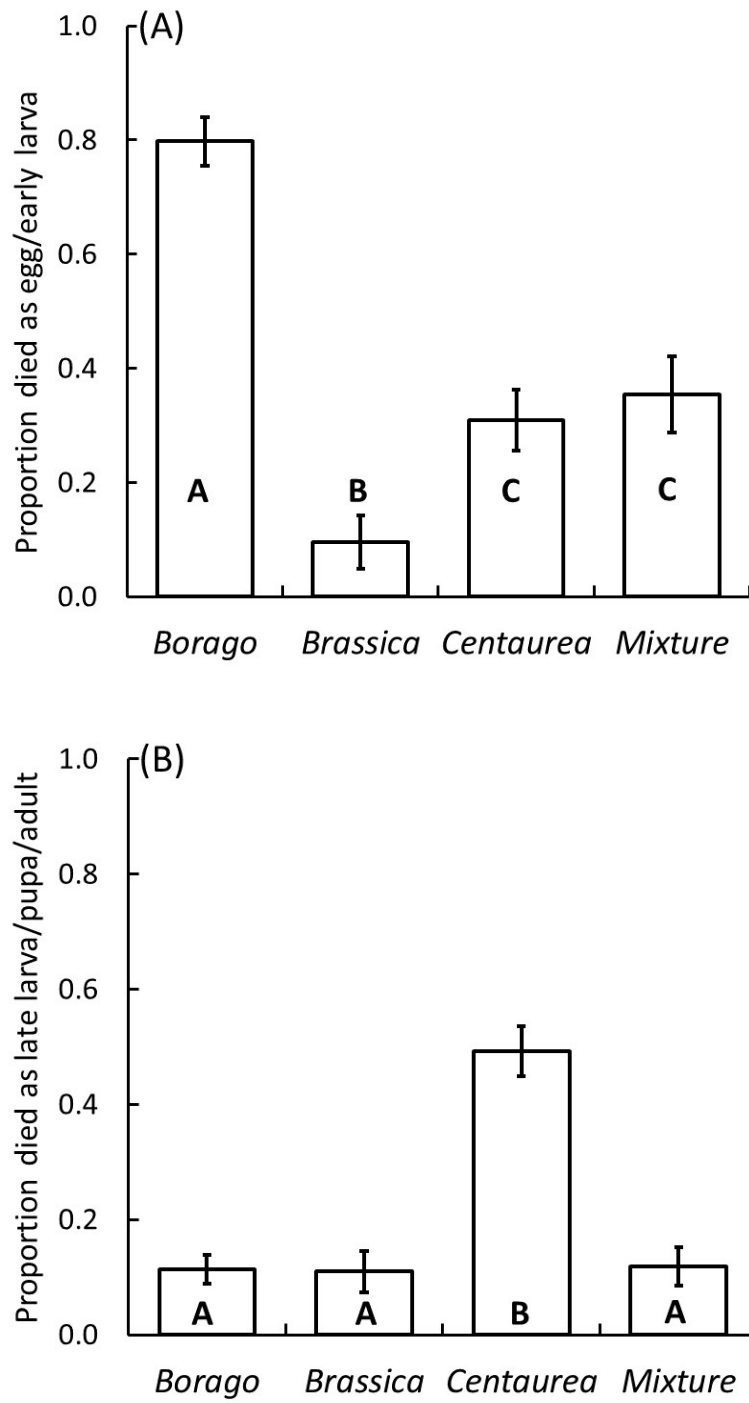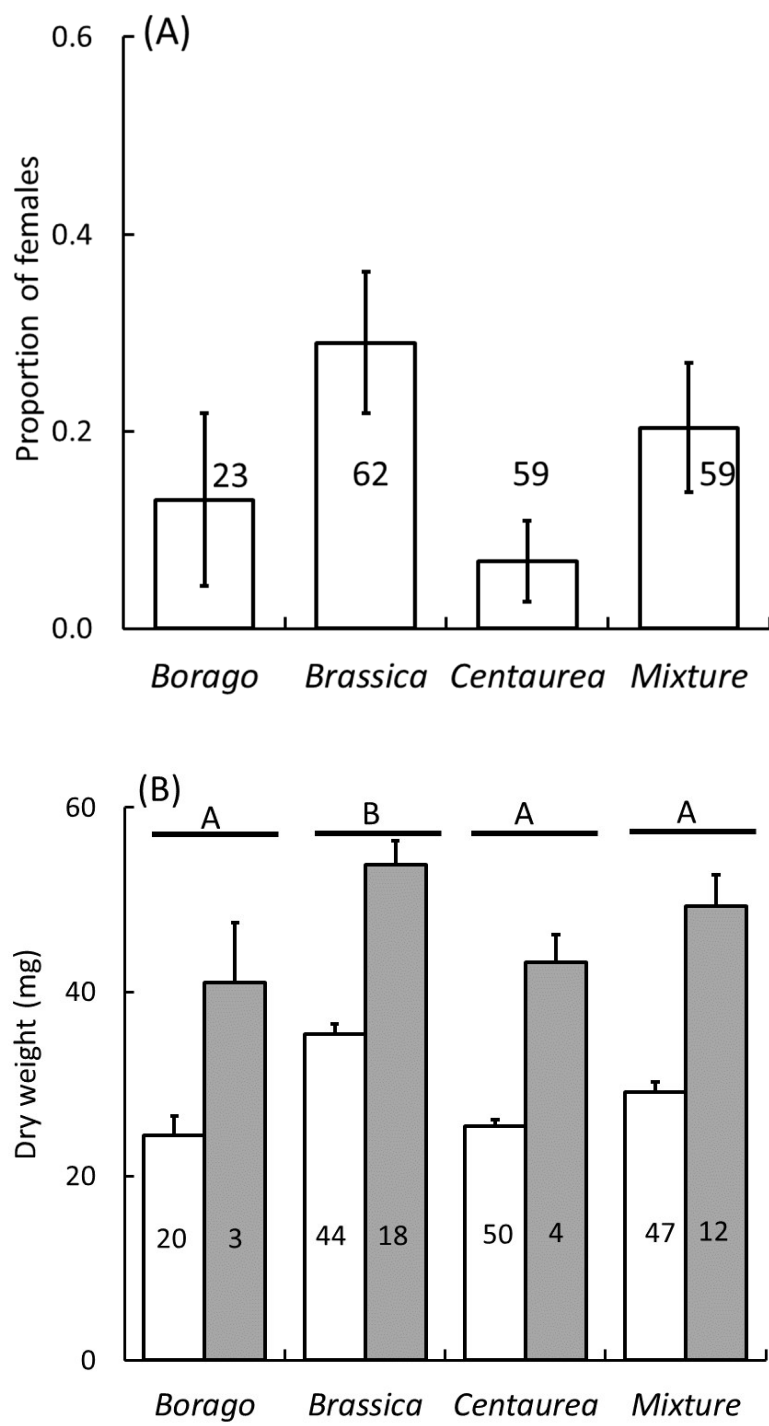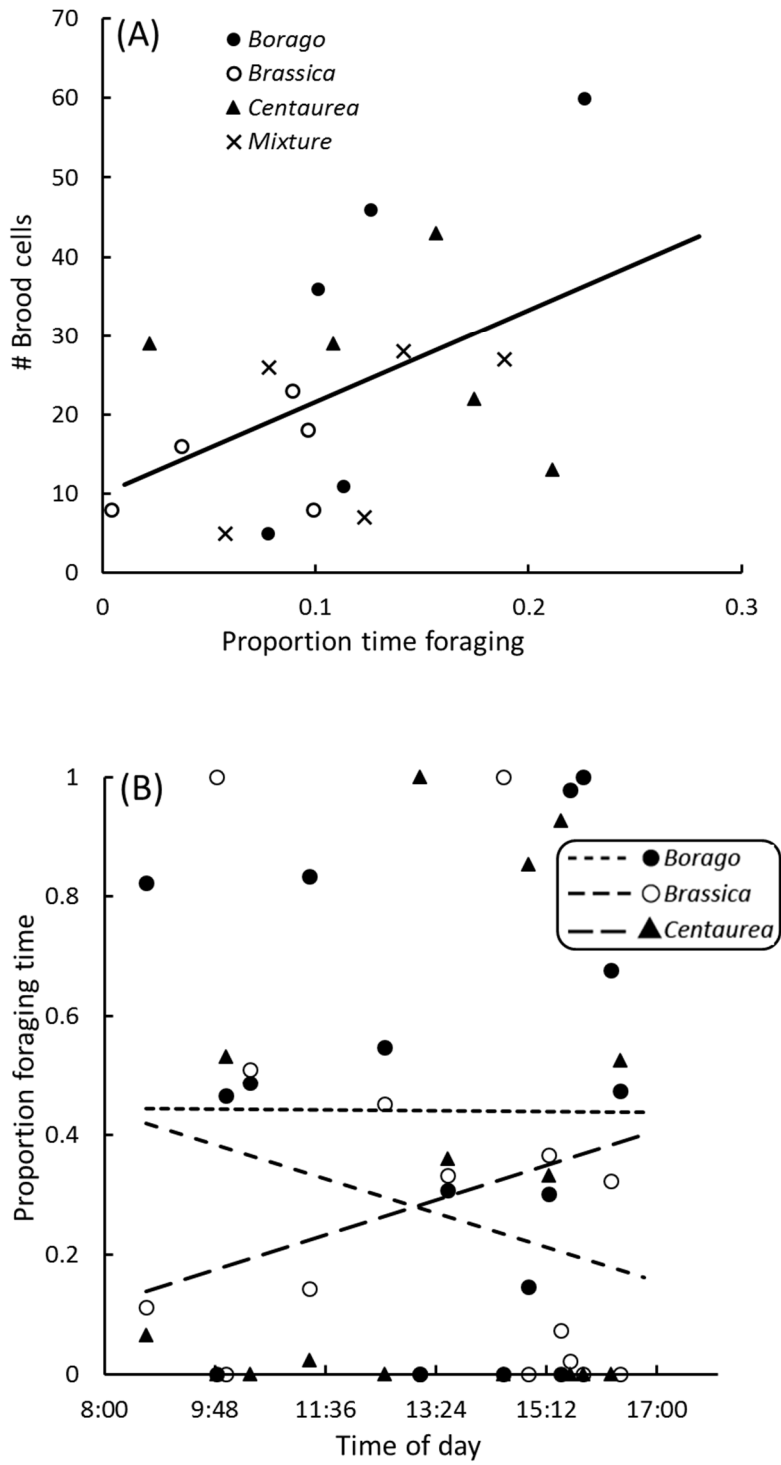
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