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Intraspecific size shifts in generalist bumblebees and flowers lead to low functional consequences

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Abstract

Body size is a trait that can affect plant–pollinator interaction efficiency and plant reproductive success. We explored the impact of intraspecific size shifts on the interactions between pollinators and flowering plants under controlled conditions. We considered two development conditions leading to the production of large and small individual flowers of *Borago officinalis* and *Echium plantagineum*. We also used the natural variability of worker size within bumblebee colonies to isolate small and large workers. We performed a fully crossed experiment with the two flower sizes of each plant species and the two sizes of bumblebee workers. Our results show that the size of both partners did not affect bee foraging behavior in most of the evaluated parameters and both bee sizes were equally efficient in depositing pollen. Significant differences were found only in pollen deposition across the life of a flower in small flowers of *B. officinalis*, with the greatest quantity of pollen deposited by small bees. We did not find a relationship between pollinator size and plant fitness. Our results suggest that generalist plant–pollinator interactions may be resilient to future potential mismatches in the size of the partners but remain to be tested if they are still resilient under the new environmental conditions resulting from global changes.

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KEYWORDS
bee behavior, bee size, *Bombus terrestris*, *Borago officinalis*, *Echium plantagineum*, flower size, plant fitness, pollen deposition

INTRODUCTION

Phenotypic plasticity enables species to display different phenotypes depending on the conditions where the individuals develop (Klingenberg, 2019), and most morphological traits display remarkable variability (Fusco & Minelli, 2010). This is especially relevant for traits that influence interactions between species. Body size is a trait that shapes plant–pollinator interactions (Corbet et al., 2001; Kendall et al., 2019; Klumpers et al., 2019; Morse, 1978; Stout, 2000) and plant reproductive success (e.g., Esposito et al., 2018). The size of pollinator bodies and the size of individual flowers influence the contact between the reproductive organs of plants (i.e., stigma and anthers) and the pollen-carrying areas on the pollinator's body, affecting pollen deposition (Mesquita-Neto et al., 2021; Solís-Montero & Vallejo-Marín, 2017).

Under the current changes of environmental conditions driven by global changes, phenotypes of organisms are expected to be affected, not always in the same direction (see Gérard, Vanderplanck, et al., 2020; Miller-Struttmann et al., 2015). Theoretical and experimental studies suggest that plants grown under higher temperatures tend to have fewer and smaller flowers (Carroll et al., 2001; Descamps et al., 2018, 2020), but there are species-specific differences in the effects (Kuppler et al., 2020; Scaven & Rafferty, 2013). Regarding bees, there is an overall trend of species having a smaller body size in warmer areas (Gérard, Martinet, et al., 2020; Nooten & Rehan, 2020), even if there are also some notable exceptions (Gérard et al., 2018; Ramírez-Delgado et al., 2016). Other local threats such as habitat fragmentation can also affect pollinator body size, bigger females being positively selected in fragmented areas because of the higher distances between resource patches (Gérard et al., 2021; Gérard, Martinet, et al., 2020). Overall, the resulting size probably depends on the mixture of the climatic and local (i.e., habitat quality) conditions faced by the individuals.

The alteration in the size of bees and flowers can have strong effects on the success of the interaction. Bee foraging efficiency could decrease if they cannot properly access nectaries and anthers due to the shift in the relative position of their body structures (e.g., tongue and legs) (Dohzono et al., 2011; Klumpers et al., 2019). Flowers could also be affected if the structures of bees and flowers do not come into contact, reducing pollen deposition or removal (Benitez-Vieyra et al., 2006; Johnson & Steiner, 1997).

In this work, we wanted to study the effect of intraspecific modification of individual flower and bee sizes on their interaction and plant fitness, in a global change scenario. For this purpose, we experimentally manipulated the sizes of flowers to obtain a significant difference between large and small ones (plants grown in optimal conditions versus heat and drought treatment, respectively) and selected bees with different sizes and performed a fully crossed experiment. We selected two widespread and generalist plant species with accessible reproductive structures (i.e., *Borago officinalis* and *Echium plantagineum*) and a common bumblebee species (i.e., *Bombus terrestris*). We wanted to study if modifications in the size of bees and flowers (1) have an effect on bees' flower-visiting behavior, (2) have an effect on the pollen deposition on the stigmas, and (3) have consequences on plant reproductive success. We expected that the modification of bee size would affect their foraging efficiency and their foraging patterns on the different flower sizes; that disruptive patterns (large bees on small flowers or vice versa) would result in lower foraging efficiency than symmetrical patterns (large bees on large flowers or small bees on small flowers); that when the traits of both partners resemble the traits occurring in natural conditions, there is maximum pollen deposition; and that for plant fitness, patterns would mirror those for pollen deposition.

MATERIALS AND METHODS

Study system

The two selected plant species were *B. officinalis* and *E. plantagineum* (Boraginaceae). *B. officinalis* flowers are actinomorphic, with five petals forming an open corolla. *E. plantagineum* flowers have five fused petals and show a campanulate-tubular shape (Figure 1). In both species, flowers are hermaphroditic, proterandrous, and self-compatible and contain four ovules (Castroviejo Bolibar et al., 2012). These species show floral trait plasticity under different stress conditions (Descamps et al., 2018, 2020). The optimal temperature for flower production in both species was 21°C (Descamps et al., 2018, 2020). The selected bee species was *B. terrestris* (Apidae), a primitively

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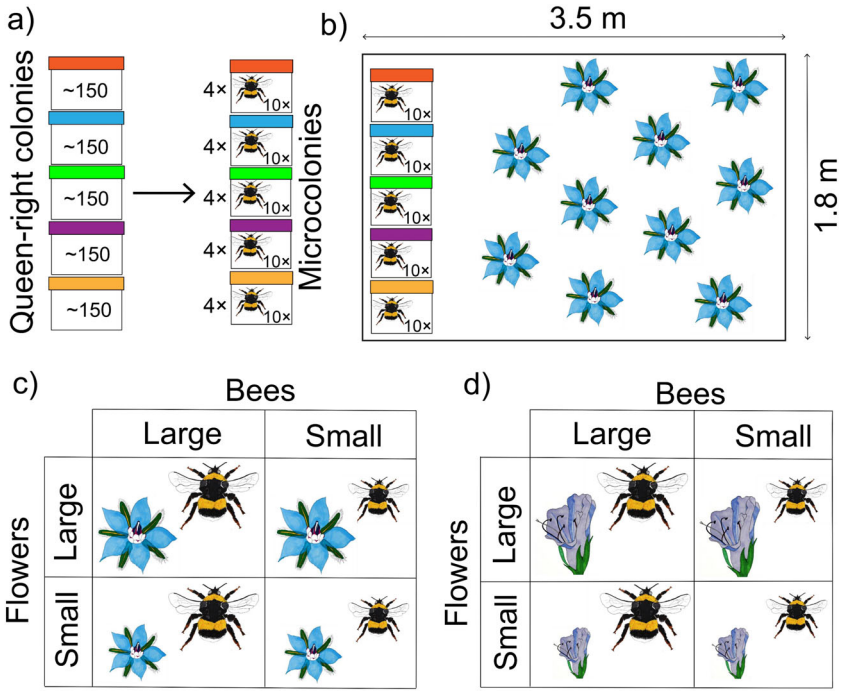

FIGURE 1 Experimental design. (a) From each queen-right colony (colonies where the queen is present), four derived microcolonies were set up. Two of these four microcolonies contained 10 large workers, while the other two contained 10 small workers. The same procedure was applied for 10 queen-right colonies, 5 in the first part and 5 in the second part of the experiment. (b) For each of the eight experimental treatments that were performed, one microcolony from each of five different queen-right colonies was selected. The five microcolonies contained bumblebees of the same size. The spatial disposition of each treatment consisted of the five bumblebee microcolonies located in one extreme of the space (left of the figure) and the pots of the corresponding plants were spread across the remaining surface. For each of the two plants species, *Borago officinalis* (c) and *Echium plantagineum* (d), four treatments were set corresponding to the four combinations of small and large flowers versus small and large bees.

eusocial and generalist species displaying a high polymorphism between workers, with differences in size between workers up to 10 times (Rasmont et al., 2008). *B. terrestris* is a managed species breed by commercial suppliers. Colonies were obtained from Biobest Group (Westerlo, Belgium).

Selection of different phenotypes

Seeds were provided by Plant World Seeds (Newton Abbott, UK) for *E. plantagineum* and by Cycle-en-Terre (Havelange, Belgium) for *B. officinalis*. Seeds were sown in a greenhouse and kept at 22°C. After four weeks, half of the plants were kept at 21°C with daily watering, while the rest were kept at 27°C and watered twice a week (further details in Appendix S1: Section S1). Thermic and drought stress negatively affect flower size of both species (Descamps et al., 2018, 2020). Plants grew at 27°C were treated as "small flowers" and plants grew at 21°C as "large flowers." For the bumblebees, 10 commercial queen-right *B. terrestris* colonies (i.e., colonies where the queen was present) of around 150 workers were purchased. The colonies were kept for two weeks in dark rearing chambers in optimal conditions at a constant temperature of 27 ± 1°C and 60% humidity (Vanderplanck et al., 2019). Colonies were fed ad libitum with sugar syrup (Biosweet, Biobest) and willow pollen. From each queen-right colony, 40 workers were extracted, 20 small and 20 large workers, and put into plastic microcolonies. Two microcolonies were composed of 10 large workers each, and two of 10 small workers each (Figure 1a) (see Appendix S1: Section S2 for further details).

Experimental setup

We performed a fully crossed experiment with the two plant species, the two sizes of flowers and the two sizes of bumblebees, resulting in eight treatments overall, that is, four treatments per plant species (Figure 1c,d). The pots of each flower size and species were divided into two groups, corresponding to the two treatments with the different bumblebee sizes (for small flowers, two groups of 80 plants for *E. plantagineum* and two groups of 90 plants

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for *B. officinalis*, and for large flowers, two groups of 35 plants for *B. officinalis* and of 34 plants for *E. plantagineum* per bee size treatment). The number of pots differed because many individuals in the large flower treatments never produced flowers. In each treatment, there were five microcolonies, all workers being of the same size category. The plastic microcolonies with the bumblebees were put inside wooden boxes to maintain the dark conditions inside. Holes were drilled as an exit for the bees. Plant pots and microcolonies were put together into long tables of a greenhouse, each table covered with insect-proof mesh leaving a flying cage of 12.6 m^3 (dimensions: $3.5 \times 1.8 \times 2$ m; Figure 1b). The average room temperature was 21°C. The experiment lasted five days, the period where bumblebees were let to forage on the plants.

PARAMETER MEASUREMENT

Phenotype of bumblebees and flowers

The length of the marginal cell on the left wing was used as proxy to evaluate the body size of bumblebee workers, which has been shown to be highly correlated with overall body size and tongue length in bumblebees (Nooten & Rehan, 2020). This measurement is referred throughout the text as "size." The marginal cell was measured in 50 bees of each of the two sizes (i.e., small and large), 25 workers of each size exposed to large and small flowers.

The traits measured on flowers were flower diameter, flower depth, and floral surface. As the floral traits were significantly correlated, we selected only flower diameter as a proxy for floral size (for further details, see Appendix S1: Section S3). It was measured as the distance between the tip of one petal and the tip of the second petal in a clockwise direction for *B. officinalis*, considering the two most distant points of the corolla when seen from above for *E. plantagineum*. It was measured on 15 randomly selected individuals, two flowers per individual ($n = 30$) per floral size and species. We used digital calipers (precision 0.01 mm, Facom 1500 mm digital calipers [Morangis, France]).

Bumblebees–flowers interactions

We followed foraging activity of the bumblebees in all treatments during the 5 days. Activity usually started at 9:30 a.m. and lasted until 3 p.m. During bumblebee activity, one forager was visually followed at a time for as long as possible until it was indistinguishable from other foragers, or it returned to the nest. We measured the total time during which foragers were followed, along with the number of flowers they visited during this time and the time spent visiting single flowers. We registered 24 (range = 19–35) foraging trips per treatment for *B. officinalis*, and 21 (range = 19–25) foraging trips per treatment for *E. plantagineum*.

On plants, we measured the deposition of pollen on the stigmas (1) during a single bumblebee visit and (2) pollen deposition across the life of a flower (2–3 days). For the single-visit measurements, 12 plants were bagged with mesh prior to opening the bumblebee nests. Once the bumblebees were let to forage, plants were progressively unbagged and observed until one of their flowers received one visit by a bumblebee. After the visit, the stigma of the visited flower was collected. For the pollen deposition across the life of a flower, freshly opened flowers were marked on the first two days after releasing the bees. The stigmas of the marked flowers were collected at the end of the last day of the experiment. For each measurement, 18–20 stigmas per treatment from 9 to 10 different individuals were collected. All stigmas were mounted on microscope glass slides, and the number of pollen grains was counted under the microscope (G = 400×).

Fitness

Around 25 fresh flowers per treatment were marked during the first two days after releasing the bees, from ±11 individuals (range = 9–14). After the end of the experiment, plants with marked flowers were maintained for three weeks in the greenhouse. Fruit set was considered as the proportion of marked flowers per individual producing at least one seed; seed set was the average number of seeds produced per fruit. Fruit and seed production were only measured in *E. plantagineum* because in *B. officinalis*, most plants died before producing fruits.

Statistical analyses

The data analysis was performed in R version 4.1.0 (R Core Team, 2021). We ran separate models for each plant species as we were interested in species-specific patterns. For the traits, we used generalized linear mixed model (GLMM) to compare small versus large flowers of each species and bumblebees. To compare flower sizes, we used a Gamma distribution including the individual plant as a random effect. For the bees, we used a Gamma distribution and used the flower size treatment from which they were collected as a random effect. For the

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interaction and fitness parameters, we only compared the effect of the two bee sizes inside each floral size for each plant species because of a different number of plants between the two sizes (many individuals with large flowers did not bloom). In all cases, the models consisted of using the treatment as the sole explanatory variable followed by a Tukey post hoc comparison to extract only the pairwise comparisons between the treatments with different sizes of bumblebees within the treatments with the same size of flowers. For bumblebee behavior, we performed GLM tests for the response variable time spent per flower and flowers visited per minute, using an inverse Gaussian distribution of the data and the log link function. For the pollen deposition per single visit and pollen deposition across the life of a flower, we performed GLMM using a negative binomial distribution of the data and individual plant as a random effect. For fruit set, we performed a GLMM with a binomial distribution of the data and individual plant as a random effect, and finally for the seed set, we performed a GLMM with a Poisson distribution of the data and individual plant as a random effect. The packages used for the analysis were "lme4" (Bates et al., 2015), "lmerTest" (Kuznetsova et al., 2017), and "AER" (Kleiber & Zeileis, 2008).

RESULTS

Phenotypes

Large bees were significantly larger than small bees ($p < 10^{-16}$; Figure 2a, Table 1; 3.37 ± 0.14 mm vs. 2.71 ± 0.16 mm, mean ± SD; Appendix S1: Tables S1 and S2). Large flowers were significantly larger than small flowers in *E. plantagineum* ($p < 10^{-9}$; Figure 2b, Table 1; 2.04 ± 0.27 cm vs. 1.37 ± 0.21 cm) and *B. officinalis* ($p < 10^{-9}$; Figure 2b, Table 1; 2.91 ± 0.16 cm vs. 2.42 ± 0.40 cm; Appendix S1: Tables S1 and S2).

Interactions

Small bumblebees spent significantly more time visiting large flowers of *B. officinalis* than large bumblebees did ($p = 0.01$; Table 1; Appendix S1: Table S2), while no significant differences were found on small flowers of *B. officinalis* ($p = 0.10$; Table 1; Appendix S1: Table S2). In *E. plantagineum*, small bees spent more time than large bees on small flowers ($p = 0.01$; Table 1; Appendix S1: Table S2), but there were no significant differences for large flowers ($p > 0.50$; Figure 2c). Considering the number of flowers visited per minute, there were no significant differences between large and small bumblebees in *B. officinalis* in either small or large flowers (all $p > 0.40$; Table 1; Appendix S1: Table S2) nor between the two sizes of bumblebees visiting the large flowers of *E. plantagineum* ($p > 0.80$; Table 1; Appendix S1: Table S2). A significant difference in the number of flowers visited per minute was found between both bumblebee sizes visiting the small flowers of *E. plantagineum*, with large flowers visiting more flowers per minute ($p < 0.001$; Table 1, Figure 2d; Appendix S1: Table S2).

For pollen deposition, the quantity of pollen deposited during a single visit was not affected by the size of the bumblebees in neither the small or the large flowers in *B. officinalis* nor *E. plantagineum* (all $p > 0.40$; Figure 2e, Table 1). For pollen deposition across the life of a flower, the size of the bumblebees had a significant effect on small flowers of *B. officinalis*, where the greatest quantity of pollen was deposited in the treatment with small bumblebees ($p = 0.004$; Figure 2f, Table 1). No significant effect of the size of the bumblebees on the pollen deposited on stigmas was found in the large flowers of *B. officinalis* ($p > 0.10$; Table 1), nor in either the small or large flowers of *E. plantagineum* (all $p > 0.70$; Table 1, Figure 2f).

Plant fitness

There was no effect of the size of the bumblebees on the fruit set of either small or large flowers of *E. plantagineum* ($p > 0.05$; Table 1, Figure 2g) nor in the seed set of either the small or large flowers of *E. plantagineum* ($p > 0.05$; Table 1, Figure 2h).

DISCUSSION

We showed that the size of bumblebees and flowers did not have a strong effect on the foraging behavior of bees and that small and large bees were equally efficient in depositing pollen during a single visit and in most cases and across the life of a flower. Differences were found only for the quantity of pollen deposited across the whole flowering period for small flowers of *B. officinalis*, with the greatest quantity of pollen deposited by small bees. Finally, bumblebee size had no effect on plant reproductive success in *E. plantagineum*.

Impact of size shift on bee–plant interactions

We found differences in foraging behavior between large and small workers in less than half of the comparisons,

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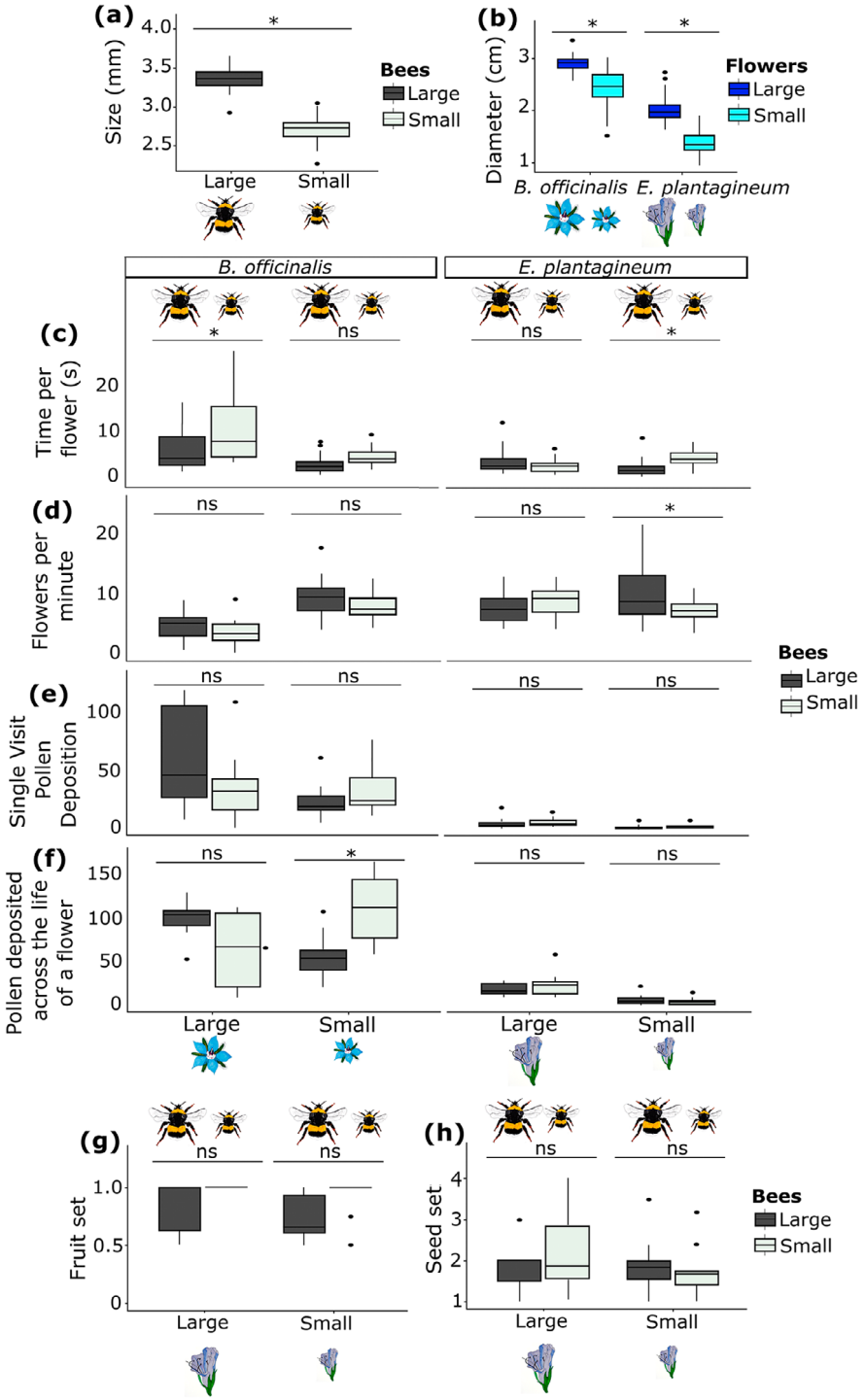

FIGURE 2 Comparison of (a) size of the two groups of bumblebees (measured as the length of the distal cell of the right wing), (b) diameter of the flowers of both sizes of the two plant species, (c) time in seconds spent per flower by the two groups of bumblebees on the two groups of flowers of the two plant species, (d) number of flowers visited per minute by the two groups of bumblebees on the two groups of flowers of the two species, (e) single-visit pollen deposition by the two groups of bumblebees on the two groups of flowers of the two species, (f) pollen deposited across the life of a flower by the two groups of bumblebees on the two groups of flowers of the two species, (g) fruit set by the two groups of bumblebees on the two groups of flowers of *E. plantagineum*, and (h) seed set by the two groups of bumblebees on the two groups of flowers of *E. plantagineum*. In all cases, asterisks above bars comparing the two groups of bumblebees on each flower size and species represent significant differences, and "ns" represents nonsignificant differences. In each boxplot, the central line represents the median, the upper and lower limits of the box represent the 75th and 25th percentiles, respectively, whiskers indicate variability outside the 75th and 25th percentiles (they are calculated as 75th percentile/25th percentile $\pm$ 1.5 $\times$ (75th percentile $-$ 25th percentile)), and points represent extreme values.

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TABLE 1 Comparison between group treatments: phenotypes of bees and flowers, interactions from the bee and from the plant point of view, and the fitness of the plants.

Variable	Species	Treatments	Data distribution	*N*	Parameter estimate	*p*
Phenotypes						
Bee size (mm)	*B. terrestris*	Large vs. small bees	Gamma	100	0.07	**10^{-16}**
Flower diameter (cm)	*B. officinalis*	Large vs. small flowers	Gamma	55	0.06	**10^{-9}**
	E. plantagineum	Large vs. small flowers	Gamma	61	−0.40	**10^{-16}**
Interactions—bee behavior						
Time per flower (s)	*B. officinalis*	LL-LS	Inverse Gaussian	39	0.90	**0.01**
		SL-SS	Inverse Gaussian	49	0.36	0.10
	E. plantagineum	LL-LS	Inverse Gaussian	39	−0.22	0.65
		SL-SS	Inverse Gaussian	38	0.59	**0.01**
No. flowers per minute	*B. officinalis*	LL-LS	Inverse Gaussian	39	−0.20	0.24
		SL-SS	Inverse Gaussian	58	−0.10	0.84
	E. plantagineum	LL-LS	Inverse Gaussian	39	0.08	0.85
		SL-SS	Inverse Gaussian	44	−0.42	**$<10^{-3}$**
Interactions—pollen deposition						
Single-visit pollen deposition (no. pollen grains)	*B. officinalis*	LL-LS	Negative binomial	37	−0.48	0.43
		SL-SS	Negative binomial	38	0.40	0.55
	E. plantagineum	LL-LS	Negative binomial	40	0.22	0.97
		SL-SS	Negative binomial	36	0.32	0.93
Pollen deposition across the life of a flower (no. pollen grains)	*B. officinalis*	LL-LS	Negative binomial	39	−0.46	0.18
		SL-SS	Negative binomial	40	0.76	**0.004**
	E. plantagineum	LL-LS	Negative binomial	40	0.30	0.81
		SL-SS	Negative binomial	41	−0.34	0.77
Plant fitness						
Fruit set	*E. plantagineum*	LL-LS	Binomial	64	4.84	1.00
		SL-SS	Binomial	78	1.92	0.07
Seed set	*E. plantagineum*	LL-LS	Poisson	59	0.23	0.58
		SL-SS	Poisson	65	−0.04	0.99

Note: The difference corresponds to the difference between the average of the treatments compared in the measured units. *p*-values lower than 0.05 are marked in bold.

Abbreviations: LL, large flowers with large bees; LS, large flowers with small bees; SL, small flowers with large bees; SS, small flowers with small bees.

and small bees always displayed longer times per flower. We assume that this pattern was related to intrinsic foraging differences between larger and smaller workers (Goulson et al., 2002; Spaethe & Weidenmüller, 2002). Nevertheless, there were no differences in foraging time and number of flowers visited per minute in the other comparisons. Previous studies showed that as long as bees can reach the nectar, there is no effect of their traits on the handling time of flowers (Morse, 1978; Naghiloo et al., 2021).

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The size of the bees visiting the plants in our study proved not to have a high importance for the adequate pollination service in the studied species. The anthers and stigmas of the two species are readily accessible to visitors, so both small and large bees can reach the pollen and nectar and touch the stigmas of both small and large flowers. Our results demonstrate that potential alterations in bee and flower size do not necessarily have strong consequences for the pollination of those plant species. Pollen deposition involves traits related to mechanical fit of the interaction (such as throat length, the distance between the rewards, and the fertile parts of the flowers), but not always the size (Cosacov et al., 2014). Only on small flowers of *B. officinalis* were there differences in pollen deposition between bee sizes, showing that different combinations of local environmental conditions can affect ecosystem functioning while others might go unnoticed. Nevertheless, other systems could be more impacted by size modifications, especially where the matching of sizes between flowers and pollinators has been shown to have consequences in the reproductive success of the species (Hattori et al., 2021).

Impact of size shift on plant fitness

We did not find differences in the production of fruits and seeds in *E. plantagineum* derived from the interaction with differently-sized bumblebees. The fruit of this plant is a tetrakene, a structure that typically does not produce four seeds even under hand pollination tests (Descamps, 2021). As the average of pollen grains deposited on the stigmas is similar to or higher than the number of ovules per flower in all treatments (Appendix S1: Table S2), there is probably not a quantitative limitation of pollen (Aizen & Harder, 2007). Even though we could not measure the fitness in *B. officinalis*, we do not expect an effect of bee size change since stigma pollen loads were always way higher than the number of ovules per flower. Finally, the relationship between the size of interacting partners and plant fitness may influence pollen removal and transport rather than pollen deposition (Benitez-Vieyra et al., 2006; Stanton & Preston, 1988).

Perspectives of research

We worked with two widespread and generalist plant species, and we may expect many wild common plants with accessible reproductive structures to show similar patterns. However, some plant species depend more on the traits of the pollinators visiting than others, according to the position and size of their reproductive structures (Avalos et al., 2021), and they will probably be more negatively affected by the change in the size of the bees visiting them. Moreover, the drivers of global change not only affect the size of flowers but also their multiple traits. Floral traits show covariation (Kuppler et al., 2021), and multiple traits seem to be affected in parallel with size due to stress, such as the scent, life span, phenology, plant size, and number of flowers produced per plant (Höfer et al., 2021; Kuppler & Kotowska, 2021). Increased temperature and drought affect the quantity and quality of floral resources in the studied species (Descamps et al., 2018, 2020; Descamps, Jambrek, et al., 2021) and in other species (Carroll et al., 2001; Descamps, Boubnan, et al., 2021; Rering et al., 2020), which could affect attraction and recognition of the plants in natural conditions and consequently their pollination and reproductive success (Descamps et al., 2018; Sato et al., 2006).

On the other hand, it was out of our scope to test the effect of thermal and drought stress on the bees themselves, even if it is proven that they are affected at different levels (Maebe et al., 2021), nor to test the effect of size modification on bee fitness. Future studies should assess the impact of stressed bees on plant–bee interactions, and how this could affect in turn as well as evaluate pollinator fitness in longer experiments. Most species of the genus *Bombus* are generalist and highly resistant species (Ghisbain et al., 2021; Rasmont et al., 2008), which can explain the low impact of the size shift on their behavior. The impact of size modification will be stronger for more specialist solitary bees, such as the species of the tribe Osmiini that are oligolectic on *Echium* (Sedivy et al., 2013).

CONCLUSIONS

We did not find a strong impact of the modification of the sizes of both bees and flowers on the bee–plant interactions nor on plant fitness. It seems that generalist pollinators are flexible and can adapt to modifications in the traits of their partners, while the effect on pollen deposition on plants depends on the combination of traits. This finding suggests that generalist plant–pollinator interactions may be resilient to shift in the size of the partners when impacted by global changes. Additional experiments are needed to define if the pollinators and the plants are impacted when they are also exposed to the expected environmental global changes during the interaction itself.

AUTHOR CONTRIBUTIONS

Denis Michez, Maxence Gérard, and Sara Reverté conceived the ideas and designed the methodology. Sara

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Reverté, Denis Michez, Julien Louvieaux, and Maxime Bodson prepared the experiment. Sara Reverté, Denis Michez, and Maxime Bodson collected the data. Sara Reverté, Maxence Gérard, and Maxime Bodson analyzed the data. Sara Reverté and Denis Michez led the writing of the manuscript. All the authors contributed substantially to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Reverté et al., 2023) are available from Dryad: https://doi.org/10.5061/dryad.gb5mkkwvn.

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