

RESEARCH ARTICLE

Flower-Visiting Butterflies Avoid Predatory Stimuli and Larger Resident Butterflies: Testing in a Butterfly Pavilion

Yuya Fukano^{1*}, Yosuke Tanaka², Sayed Ibrahim Farkhary³, Takuma Kurachi³

1 Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan, **2** Ueno Zoological Gardens, Tokyo Zoological Park Society, Tokyo, Japan, **3** Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan

* yuya.fukano@gmail.com



CrossMark
click for updates

OPEN ACCESS

Citation: Fukano Y, Tanaka Y, Farkhary SI, Kurachi T (2016) Flower-Visiting Butterflies Avoid Predatory Stimuli and Larger Resident Butterflies: Testing in a Butterfly Pavilion. PLoS ONE 11(11): e0166365. doi:10.1371/journal.pone.0166365

Editor: Jan M Hemmi, University of Western Australia, AUSTRALIA

Received: May 20, 2016

Accepted: October 27, 2016

Published: November 15, 2016

Copyright: © 2016 Fukano et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported in part by Grant-in-Aid for JSPS (Japanese society for promotion of science) Fellows (no. 26-3225). <https://www.jsps.go.jp/english/> The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

The flower-visiting behaviors of pollinator species are affected not only by flower traits but also by cues of predators and resident pollinators. There is extensive research into the effects of predator cues and resident pollinators on the flower-visiting behaviors of bee pollinators. However, there is relatively little research into their effects on butterfly pollinators probably because of the difficulty in observing a large number of butterfly pollination events. We conducted a dual choice experiment using artificial flowers under semi-natural conditions in the butterfly pavilion at Tama Zoological Park to examine the effects of the presence of a dead mantis and resident butterflies have on the flower-visiting behavior of several butterfly species. From 173 hours of recorded video, we observed 3235 visitations by 16 butterfly species. Statistical analysis showed that (1) butterflies avoided visiting flowers occupied by a dead mantis, (2) butterflies avoided resident butterflies that were larger than the visitor, and (3) butterflies showed greater avoidance of a predator when the predator was present together with the resident butterfly than when the predator was located on the opposite flower of the resident. Finally, we discuss the similarities and differences in behavioral responses of butterfly pollinators and bees.

Introduction

The relationship between flowering plants and insect pollinators is one of the most important mutualisms in terrestrial ecosystems [1–4]. The flower-visiting behaviors of pollinators have been shaped by mutualistic interactions with flowering plants, and the flower-visiting behaviors and preference of pollinators are influenced by flower traits such as color, shape, and odor [4–7]. However, mutualism with flowers is not the only biotic factor affecting the behavior of pollinators. Other biotic interactions such as antagonistic and competitive interactions are also important for pollinators in the community network [8,9]. Thus, several types of cues from antagonists (e.g. predators) and competitors can also affect the flower-visiting behaviors of pollinators.

First, flower-visiting insects may face predation risks from ambush predators on and near the flowers [10–13]. Because predation events have a considerable impact on the fitness of pollinators, pollinators have evolved the ability to avoid several predator-related cues. Pollinators change their flower-visiting behavior in response to the presence of live, dead and model predators [11,14–17]. These predator avoidance behaviors might be achieved by avoiding any foreign objects on the flower [18]. Moreover, pollinators can avoid flowers containing cues of past predation events [15,19]. Abbot (2006) found that flowers containing a freshly killed bumblebee or scent of the killed bumblebee received fewer bumblebee visitations than control flowers. The avoidance of predation risks by pollinators can reduce plant reproductive success and result in disruption of the mutualistic interaction between flowering plants and pollinators [16,20,21].

Second, if most of the flower nectar and pollen is consumed by pollinators, avoiding flowers occupied by live resident pollinators might be adaptive behavior for flower-visiting pollinators. On the other hand, if the nectar and pollen are not limited resources, following and preference for resident pollinators can be advantageous by reducing the energy cost of flower-searching behavior [22]. While many studies have found that flower-visiting animals do not show spatial aggregation, suggesting avoidance of resident pollinators [23–25], others studies have reported that flower-visiting animals are attracted to resident pollinators when searching for novel flowers [26–28]. Kawaguchi et al. (2007) found that pollinators adjust their response to the presence of conspecific pollinators depending on their familiarity with the flowers they are visiting [29]. Their study showed that bumblebees avoided visiting familiar flowers when conspecific bumblebees were present, but that they preferred visiting unfamiliar flowers when conspecific bumblebees were present. Furthermore, the presence of conspecific pollinators can be used as an indicator of safety for pollinators that experienced predation. Dawson & Chittka (2014) demonstrated that bumblebees prefer to feed with conspecific bumblebees when they are presented with a previously predator-infested flower. That study suggests that bumblebees can integrate information about predators and resident conspecifics for optimal flower-visiting behavior [30].

The effects of predators, conspecific or heterospecific pollinators on the flower-visiting behavior of pollinators have been extensively explored in recent years. However, the majority of studies have been done on Hymenoptera [18]. The effects of these factors on another important pollinator taxa, Lepidoptera, are relatively unknown. Compared with bees, butterfly species have different types of innate and learned preferences for floral traits, including color, shape, size, odor, and amino acids in nectar [4,31]. Also, butterfly and bee pollinators have different pollination niches [32]. Some studies have reported that butterflies avoid flowers with predator cues such as the presence of artificial spiders and models of spiders' forelimbs [16,18,21]. However, whether and how resident butterflies affect butterflies' visitations to flowers with and without the presence of predator cues is unknown. Here, we tested the effects of predators, conspecific or heterospecific residents on the flower-visiting behavior of butterfly species by observing the behavioral response of butterflies reared in captivity under semi-natural conditions in the butterfly pavilion of Tama Zoological Park.

Thousands of butterflies are released throughout the year in the butterfly pavilion of the insectarium at Tama Zoological Park (Tokyo, Japan). All adult butterflies fly freely in the pavilion. The pavilion provides several benefits for experimental studies of the flower-visiting behavior of butterflies. First, the pavilion has stable environmental conditions (e.g. temperature) with minimal impact from natural disturbances such as wind and rain. Second, a number of flower-visiting behaviors of many butterfly species can be easily observed in one location. Therefore, we can compare variations in visiting behavior between species and examine how wing size variation of butterfly species affect their behavioral responses to predators and

resident butterflies. Third, most individuals in the butterfly pavilion were reared in captivity and had no (or little) experience with predators. Although a few predators (mantises and spiders) manage to enter the pavilion, they are evicted as soon as they are discovered. Therefore, the butterflies in the pavilion can be regarded as displaying an almost exclusively innate response to predators on flowers.

In this study, we set up a dual choice experiment in the butterfly pavilion to explore the effects that the presence of predators and resident pollinators have on the flower-visiting behavior of butterflies. Specifically, we addressed following questions: (1) Do predator cues near an artificial flower and wing size of the visiting butterfly affect the flower-visiting behavior of butterflies? (2) Do the live resident butterfly and wing size differences between the resident and the visiting butterfly affect the flower-visiting behavior of butterflies? (3) How does the spatial relationship of predator cues and resident butterflies change the flower-visiting behavior of butterflies?

Materials and Methods

Experimental location and settings

All experiments were performed in the butterfly pavilion at Tama Zoological Park, Hino city, Tokyo, Japan by permission of Tama Zoological Park. The pavilion has a total floor space of 1140 m² and a maximum ceiling height of 16 m and is covered by a glass roof (Fig 1A). In the 5-month experimental period (Mar–July, 2015) and the 3-month pre-experimental period (Dec–Feb, 2014/15), adult butterflies of 23 species were released into the pavilion. The number of butterflies released each month, larval feeding plants, and the estimated number of generation in captive breeding for each species are included in S1 Table. Many plants, including *Ficus microcarpa*, *Ficus virgata*, *Cassia surattensis*, *Lantana camara*, *Pentas lanceolata*, and *Stachytarpheta dichotoma*, are grown in the pavilion as nectar sources and/or roosts for butterflies. The temperature is controlled by a hot water heating system and by opening or closing windows. All adult butterflies are kept under a natural day/night regime.

Two experimental sites were established 2 m away from the pavilion walkways to minimize the disturbance by visitors. At each site, we set two artificial flowers at opposite ends of a wooden board (38 cm × 11 cm × 1 cm). The height of the board from the ground was approximately 100 cm. Artificial flowers were included in the study rather than real flowers to be able to control the flower characters (e.g. shape, color, nectar and odor). The artificial flowers were created by arranging a flower-shaped yellow nylon on sponge (9 cm in diameter) in a white plastic dish (10 cm in diameter and 2 cm deep). The sponge was soaked in 10% sugar-water. The two artificial flowers were separated from one another by a center-to-center distance of 20 cm (Fig 1B). The artificial flowers and sponges were washed and soaked every day.

Experimental procedures

To examine the effects of predators and resident butterflies on the flower-visiting behaviors of butterflies, we performed two types of choice experiments: a predator experiment and a no-predator experiment. In the predator experiment, a dead mantis, *Tenodera aridifolia*, was set to the side of one of the two artificial flowers. The mantis was collected in the pavilion and dried at room temperature. We changed the position of the mantis every day to randomize the effect of the position. The mantis was positioned so that the head was located over the center of the artificial flower and the rear of the abdomen was facing away from the other artificial flower (Fig 1B). In the no-predator experiment, we did not put any object on the artificial flowers. The predator and the no-predator experiments were performed for 14 days (from March 6

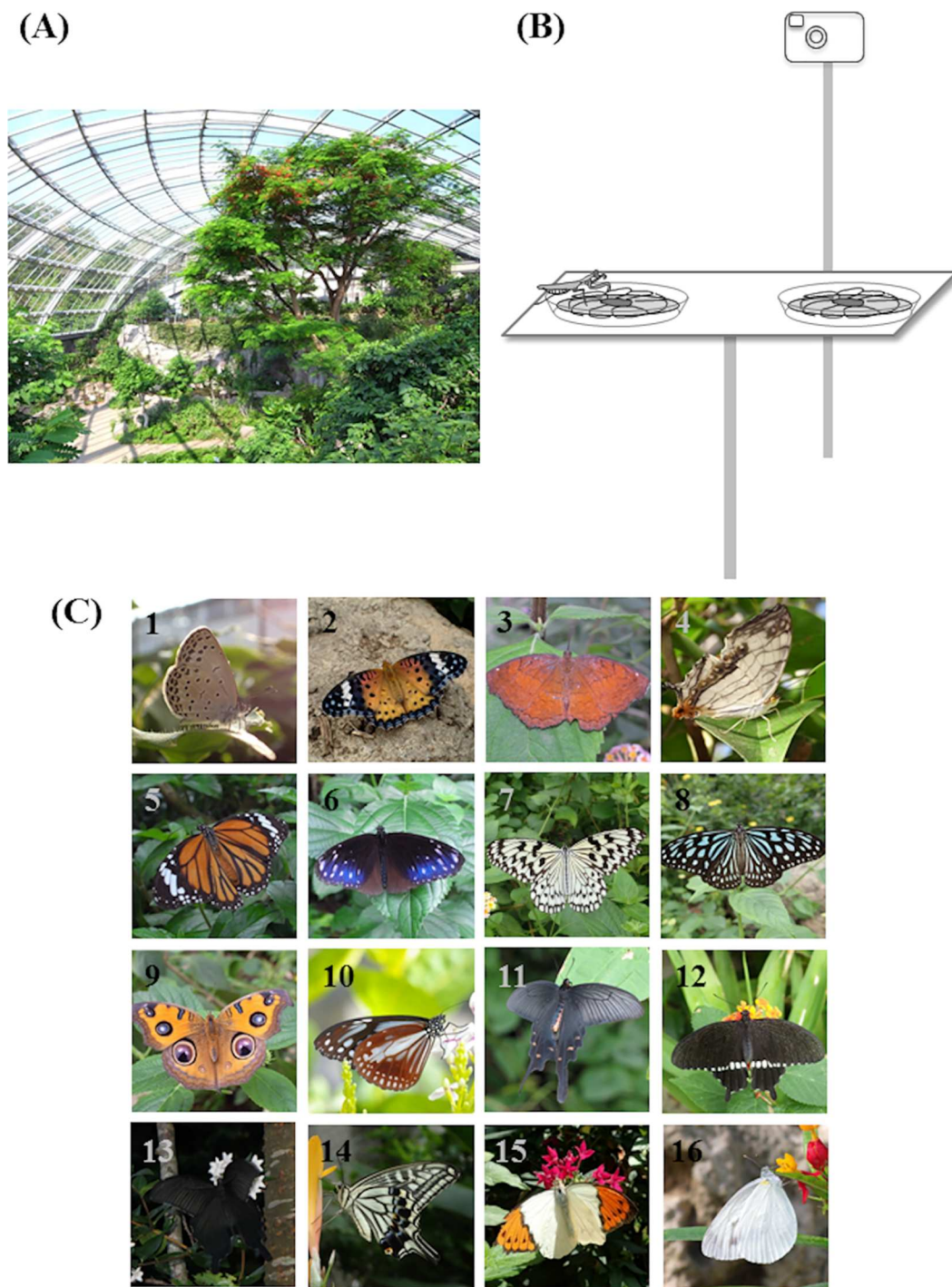


Fig 1. (A) The butterfly pavilion at Tama Zoological Park, Hino city, Tokyo, Japan. (B) Schematic diagram of experimental setup. (C) Butterfly species observed in the experiment. 1, *Pseudozizeeria maha*; 2, *Argyreus hyperbius*; 3, *Ariadne ariadne*; 4, *Cyrestis thyodamas*; 5, *Danaus genutia*; 6, *Euploea mulciber*; 7, *Idea leuconoe*; 8, *Ideopsis similis*; 9, *Junonia almana*; 10, *Parantica sita*; 11, *Byasa alcinous*; 12, *Papilio polytes*; 13, *Papilio protenor*; 14, *Papilio xuthus*; 15, *Hebomoia glaucippe*; 16, *Pieris melete*.

doi:10.1371/journal.pone.0166365.g001

to May 13) and 17 days (from May 15 to July 7), respectively. Video recording was done only during sunny conditions.

We recorded the flower-visiting behavior of butterflies between 0800 and 1300 with a digital video camera (Ricoh, WG-20). One recording period was approximately 84 minutes due to the camera's memory capacity, and recording was carried out twice a day at each experimental site. Video was recorded at 15 frames per second at a spatial resolution of 320 × 240 pixels. The camera was located approximately 120 cm away at an upward angle from the artificial flowers. After excluding data that contained any artificial disturbance by the pavilion's keepers, 4853 minutes for the predator experiment and 5530 minutes for the no-predator experiment were used for the analysis. It was not possible to score the data blind because our study involved focal animals in the field.

Data classification

For the statistical analyses, we identified the species of the visiting butterflies and classified all visiting behaviors observed in the predator experiments into three categories depending on the number of resident butterflies (Fig 2): "predator and no resident", i.e., visiting in a situation

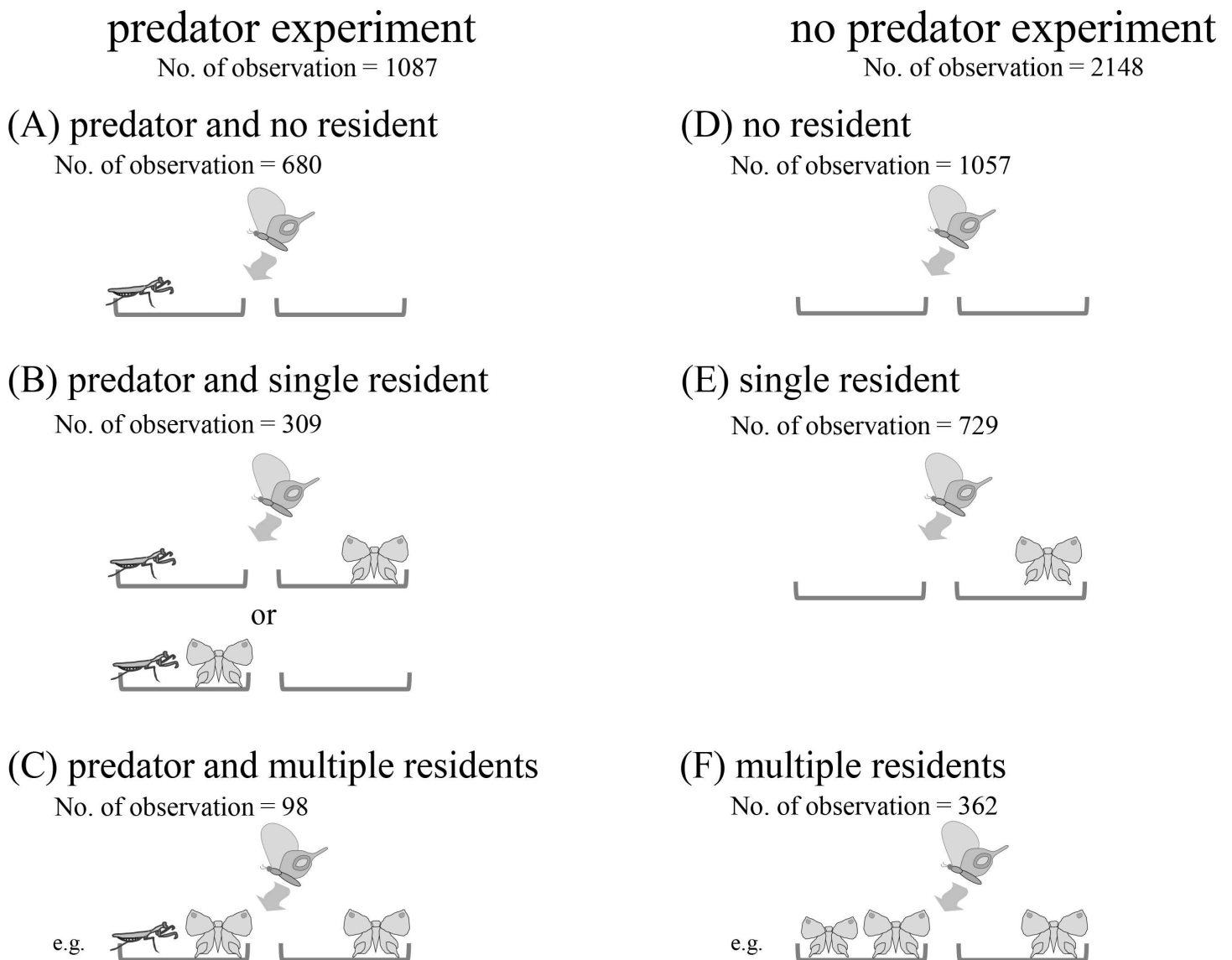


Fig 2. Schematic figures of data classification in the predator (A-C-) and the no-predator experiments (D-F).

doi:10.1371/journal.pone.0166365.g002

where the predator is located on one side with no resident butterfly; “predator and single resident”, i.e., visiting in a situation where the predator is located on one side and a resident is located on either the same or opposite side to the predator; and “predator and multiple residents”, i.e., visiting in a situation where the predator is located on one side and multiple residents are located on either side or both sides. In the same way, we classified the visiting behaviors of the no-predator experiment into three categories: “no resident”, i.e., visiting in a situation without any resident butterfly; “single resident”, i.e., visiting in a situation where a resident butterfly is located on either side; “multiple residents”, i.e., visiting in a situation where multiple resident butterflies are located on either side or both sides or either side.

To examine whether the species variation in wing size affect a visiting butterfly’s behavioral response to the presence of predators or resident butterflies, we measured the forewing length of three to five individuals of each butterfly species. For the statistical analysis, we used averaged lengths of each butterfly species as a representative value (S2 Table). The majority of butterfly species did not show sexual dimorphism, so we were unable to identify the sex of individuals from the video recordings.

Statistical analyses

To investigate the factors affecting the flower-visiting behavior of butterfly species, we adopted a generalized linear mixed model (GLMM) with the visiting choice (left or right as seen from video) and time spent on the artificial flower as response variables and species ID and setup (experimental site) as the random effect. We applied a binomial distribution for the analysis of visiting choice and a Gaussian distribution for the analysis of time spent on the flower. The models were fitted by using the `lmer` function in the “`lme4`” package in R. For all statistical analyses, we used the likelihood ratio test to evaluate the significance of the explanatory variables. We excluded the visiting data of butterflies that had not flown away from the artificial flower at the time recording was terminated.

To investigate how the presence of predators and the wing size of the visiting butterfly species affected the flower-visiting behavior, we analyzed the data of “predator and no resident” category (Fig 2A). In the analysis, we used the location of the predator, the wing size of the visiting butterfly, and their interaction as explanatory variables. To investigate how the presence and characters of a resident butterfly affected the flower-visiting behavior, we analyzed the data of “single resident” category (Fig 2E) and used the presence of the resident, type of resident butterfly (conspecific or heterospecific with visiting butterfly), wing size difference between visitor and resident species and their interactions as explanatory variables. To investigate the combined effects of a predator and a resident butterfly on flower-visiting behavior, we analyzed the data of “predator and single resident” category (Fig 2B). We used presence of predator, presence of resident, type of resident (conspecific or heterospecific), wing size of visiting species and wing size difference between visitor and resident species and their two- and three-way interactions as explanatory variables. If no significant effect of interactions were detected, the interaction terms were removed from the model to create the final model. Then we evaluated the significance of the explanatory variables in the final model. The data of “predator and multiple residents” category (Fig 2C), “no resident” category (Fig 2D) and “multiple residents” category (Fig 2F) were not used for analysis.

Results

In total, 3235 visitations by 16 butterfly species (1087 for the predator experiment and 2148 for the no-predator experiments) were observed (Fig 1C, S2 Table). For the predator experiment, we classified 680 visitations as “predator and no resident”, 309 visitations as “predator and

Table 1. Effects of presence of dead predator, wing size of the visiting butterfly species and the interaction on the visit choice and the time spent on the flower.

Response variables	Explanatory variables	d.f	LR-stat (Deviance)	P-value
Visit choice	Final model			
	Predator presence	1	25.942	<0.01
	Wing size of visitor	1	0.0145	0.90
	Removed interaction term			
	Predator presence × Wing size	1	0.2499	0.62
Time spent on the flower	Final model			
	Predator presence	1	3.0468	0.08
	Wing size of visitor	1	0.5216	0.47
	Removed interaction term			
	Predator presence × Wing size	1	1.8679	0.17

doi:10.1371/journal.pone.0166365.t001

single resident”, and 98 visitations as “predator and multiple resident”. For the no-predator experiment, we classified 1057 visitations as “no resident”, 729 visitations as “single resident”, and 362 visitations as multiple residents (Fig 2).

The effects of dead predator and the wing size

The presence of the dead mantis affected the visiting behavior of butterflies (Table 1). Butterflies significantly avoided visiting the predator side (Fig 3A) and spent moderately less time on that side (Fig 3B). Wing size of the visiting butterfly did not affect the visitor’s response to the predator (Table 1).

The effects of resident butterfly and the wing size

The type of resident species (i.e., conspecific or heterospecific with visitor) and interaction between the location of and type of resident butterfly did not affect the visiting choice and time spent on the flower (Table 2). On the other hand, the difference in wing size between the visitor and the resident species had a significant effect on the visiting avoidance (interaction between resident presence × wing size difference as shown in Table 2). The visiting butterfly avoided visiting the artificial flower occupied by the resident species when the wing size of the resident was larger than that of the visitor (Fig 4). The presence of resident butterfly, type of resident species and wing size difference between the visitor and the resident species did not affect the time spent on the flower.

The effects of coexistence of dead predator and resident butterfly

The interaction between the presence of the predator and the presence of the resident had a significant effect on the visiting choice (the interaction between predator presence and resident location in Table 3). When the resident was located on the side opposite the predator, butterflies avoided visiting the resident side rather than avoid visiting the predator side (Fig 5A). In contrast, when the resident was located on the same side as the predator, the visiting butterfly strongly avoided visiting the side occupied by both the resident and predator and preferred to visit the empty side (Fig 5B). The type of resident butterfly, wing size of visiting species, wing size difference between the visitor and the resident species and the other interaction terms had

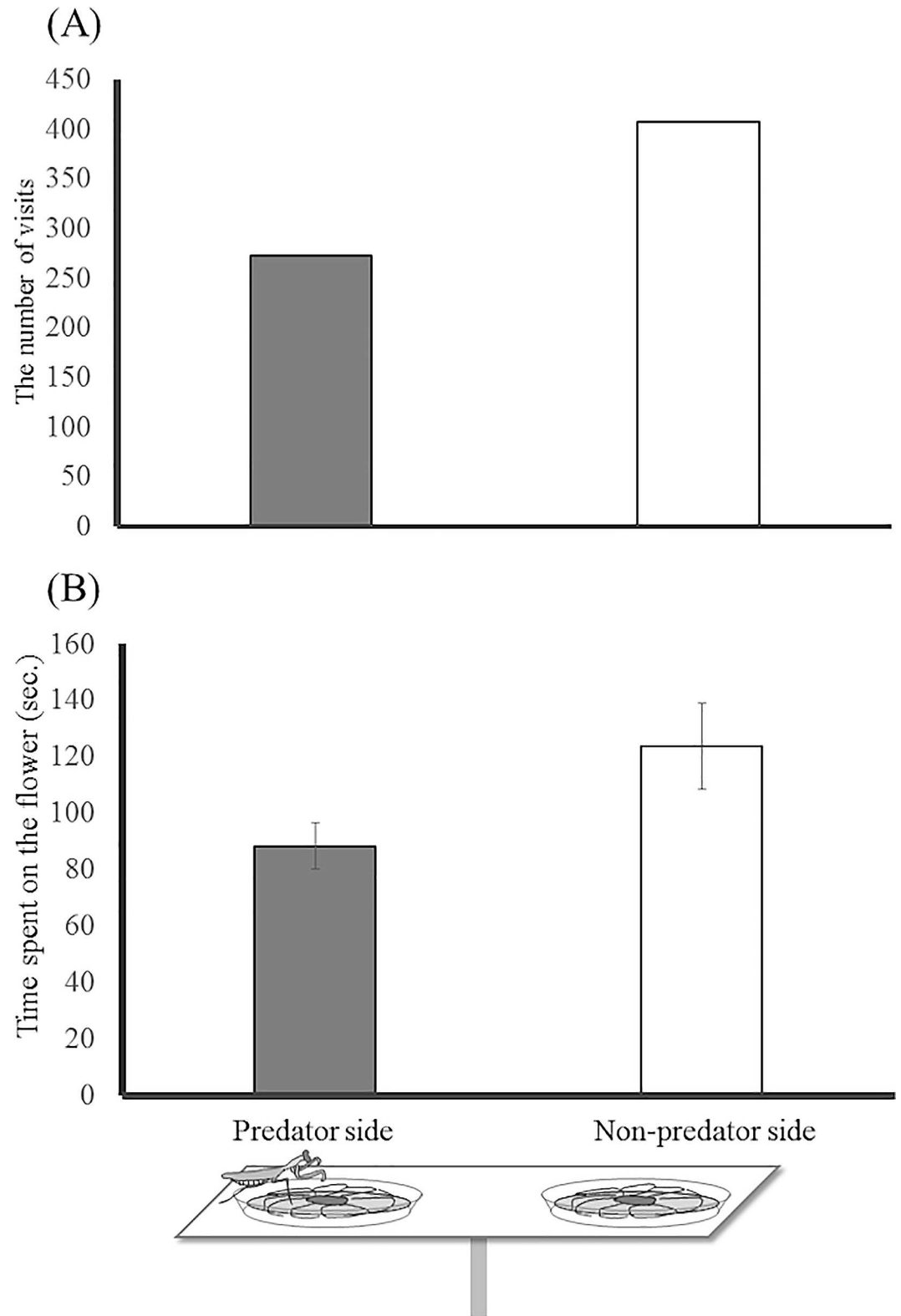


Fig 3. (A) The number of visits and (B) time spent on the artificial flower on the side occupied or not occupied by a dried specimen of *Tenodera aridifolia* (mean \pm SE).

doi:10.1371/journal.pone.0166365.g003

Table 2. Effects of presence of resident butterfly, type of resident butterfly (conspecific or heterospecifics with visitor), wing size difference between visitor and resident butterfly species and the interactions on the visit choice and the time spent on the flower.

Response variables	Explanatory variables	d.f	LR-stat (Deviance)	P-value
visit choice	Final model			
	Resident presence	1	5.924	
	Wing size difference	1	0.133	
	Type of resident	1	0.409	0.522
	Resident presence × Wing size difference	1	5.255	0.022
	Removed interaction terms			
	Resident presence × Types of resident	1	2.277	0.131
Time spent on the flower	Final model			
	Resident presence	1	1.889	0.169
	Type of resident	1	0.088	0.767
	Wing size difference	1	0.101	0.751
	Removed interaction terms			
	Resident presence × Types of resident	1	0.397	0.529
	Resident presence × Wing size difference	1	1.011	0.315

doi:10.1371/journal.pone.0166365.t002

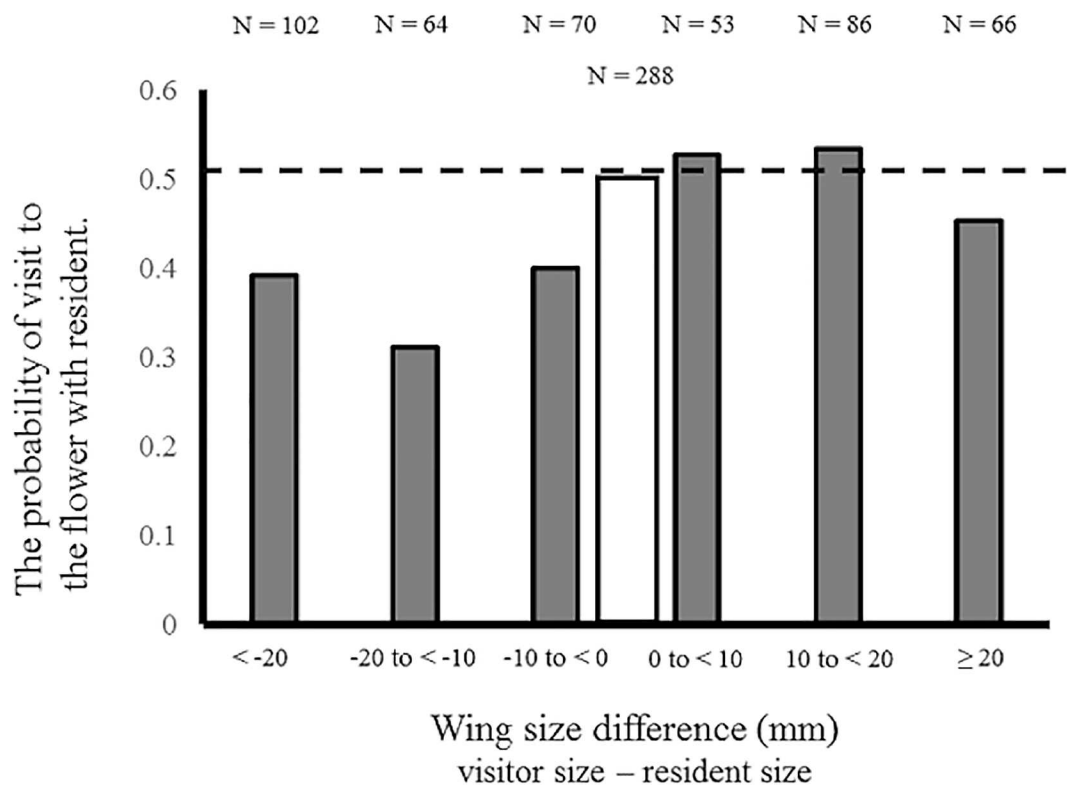


Fig 4. The probability of visit to an artificial flower occupied by a resident. Filled bar represents the average probability in each class of wing-size differences between the visitor and the resident butterfly species. Open bar represents the probability of visit to the flower occupied by a conspecific resident (wing size difference is zero).

doi:10.1371/journal.pone.0166365.g004

Table 3. Effects of presence of dead predator, presence of resident butterfly, type of resident butterfly (conspecific or heterospecifics), wing size of the visiting butterfly species, wing size difference between visitor and resident butterfly species and their interactions on the visit choice and the time spent on the flower.

Response variables	Explanatory variables	d.f	LR-stat (Deviance)	P-value	
Visit choice	Final model				
	Predator presence	1	7.935		
	Resident presence	1	14.742		
	Type of resident butterfly	1	0.16	0.689	
	Wing size of visitor	1	0.499	0.480	
	Wing size difference	1	0.269	0.604	
	Predator presence × Resident presence	1	61.301	< 0.01	
	Removed interaction terms				
	Predator presence × Type of resident butterfly	1	0.534	0.465	
	Resident presence × Type of resident butterfly	1	0.657	0.418	
	Predator presence × Resident presence × Type of resident butterfly	1	2.549	0.110	
	Predator presence × Wing size of visitor	1	0.019	0.890	
	Resident presence × Wing size difference	1	3.33	0.068	
	Time spent on the flower	Final model			
		Predator presence	1	0.15109	0.698
Resident presence		1	0.024	0.877	
Type of resident butterfly		1	1.27467	0.259	
Wing size of visitor		1	0.15921	0.690	
Wing size difference		1	0.19173	0.662	
Removed interaction terms					
Predator presence × Resident presence		1	0.00654	0.936	
Predator presence × Type of resident butterfly		1	0.01202	0.913	
Resident presence × Type of resident butterfly		1	1.13322	0.287	
Predator presence × Resident presence × Type of resident butterfly		1	0.09181	0.762	
Predator presence × Wing size of visitor		1	1.1605	0.281	
Resident presence × Wing size difference		1	0.0333	0.855	

doi:10.1371/journal.pone.0166365.t003

no effect on the visiting choice (Table 3). In this analysis, none of the variables affected the time spent on the flower (Table 3).

Discussion

In this study, we examined the effects of the presence of a dead predator and resident butterflies on the flower-visiting behavior of 16 butterfly species using dual choice experiment under semi-natural conditions in a butterfly pavilion. Firstly, we found that visiting butterflies avoided the flower with the dead predator. Secondly, visiting butterflies avoided the resident butterflies larger than the visitor. These results are consistent with the behaviors of bee pollinators [11,18,28,33,34]. Finally, butterflies showed greater avoidance of the predator when a resident was present together with the predator.

We observed that visiting butterflies avoided the flower with the dead predator (mantis). However, our experimental setting could not determine whether the butterflies truly perceived the dead mantis as a predatory cue or whether they were merely avoiding any foreign object on the flower. Previous studies have suggested that Hymenoptera pollinators portray

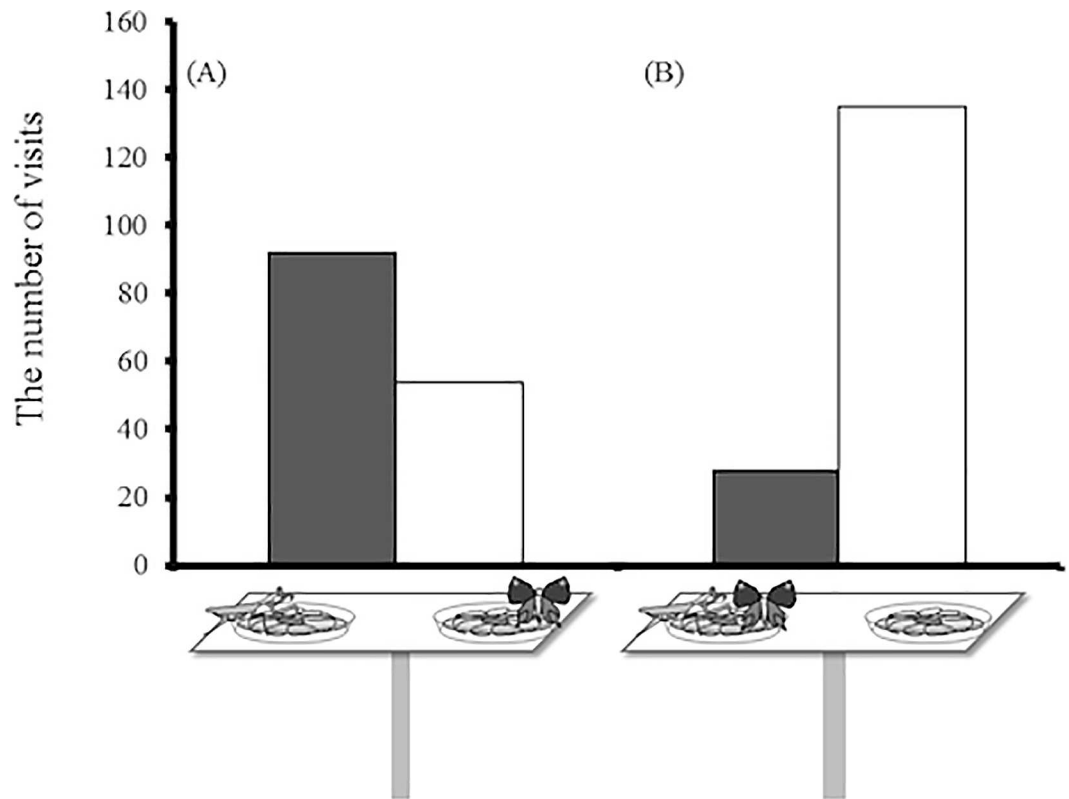


Fig 5. The number of visits to each artificial flower (A) when the resident butterfly is located on the side opposite the dried specimen of *Tenodera aridifolia* and (B) when the resident butterfly is located on the same side as the dried specimen of *T. aridifolia*.

doi:10.1371/journal.pone.0166365.g005

avoidance response to any object on flowers rather than avoidance of the predator per se [18]. In addition, Gonçalves-Souza (2008) demonstrated that flowers of *Rubus rosifolius* containing a spider replica or a sphere simulating a spider abdomen were visited less frequently by Lepidoptera pollinators than control flowers were under natural conditions. Considering the morphological variation of predators and high risk of missing the predator, avoidance response to any foreign object on flowers might be adaptive for insect pollinators. Therefore, the avoidance response to the dead mantis by the butterflies might be induced by the avoidance to any foreign object on flowers. Further experiments are required to determine what types of visual signals induce butterfly avoidance. The majority of butterflies in the pavilion did not have any experience of predation because there are few flower-dwelling predators in this environment. Thus, avoidance behavior might be attributed to an innate response rather than experience with predator attacks. The degree of avoidance shown by the butterflies in this study seemed to be weaker than that shown by wild butterflies in previous studies [16,18,21]. For example, a meta-analysis of the wild pollinator population revealed that signals of predation significantly decreased flower visitation rates by 36% and time spent on flowers by 51% [18]. Because the risk of being attacked by predators would be greater for wild butterflies than for the butterflies in the pavilion, the strong avoidance response reported in the wild might reflect a learned response to prior predation experiences.

Gonçalves-Souza et al. (2008) suggested that unpalatable or toxic butterflies might have no or little avoidance response to the predator cues. In this study, we could not test the effect of toxicity on the avoidance behavior because of the high collinearity between the wing size and

the potential toxicity (large butterflies tended to be toxic, [S2 Table](#)). However, the wing size of visiting butterflies did not affect the visiting behavior in response to the dead predator. This result suggests that the toxicity of the visiting butterfly also had little effect on the visiting choice and time spent on flower of the butterflies.

We found that the size difference between visiting and resident butterflies affected the flower-visiting behavior of butterflies: smaller butterflies avoided visiting the flower occupied by larger residents. Size-based dominance relationships among flower-visiting insects have been reported for bee and hoverfly species [28,33,34]. Our results indicate that a size-based dominance relationship also affects the visiting behavior of butterfly pollinators. Contact with other butterflies, especially for small butterflies, might damage their wings, and result in reduction of flight ability and sexual attractiveness. In the butterfly pavilion, the butterflies live in high densities and nectar sources are sometimes crowded with several butterflies. In this situation, the butterflies might learn the risks associated with approaching resident butterflies on flowers that are larger than they are on flowers. Future experiments will be needed to examine the size based dominance among wild butterfly pollinators.

Many species of adult butterflies have been observed to engage in mud-puddling behavior, aggregating at moist ground to feed on essential nutrients such as sodium. Otis et al. (2006) reported that two *Papilio* butterflies were highly attracted to artificial puddles with dead decoys of conspecific and heterospecific butterflies [35]. In contrast, our results showed that flower-visiting butterflies were not attracted to conspecific or heterospecific residents. Compared with the nutrients in the ground, flower nectar may be a limited resource for butterfly species.

When the resident butterfly was located on the opposite side to the predator, the visiting butterfly avoided the resident side and preferred the predator side ([Fig 5A](#)). This response is somewhat strange because the predator must be more “dangerous” than the resident butterfly. One possible explanation is that the resident butterfly might be more conspicuous than the dead predator to the visiting butterfly because resident butterflies often opened and closed their wings on the artificial flower. Another possibility is that while butterflies in the pavilion have learned the risk of approaching resident butterflies on flowers, the butterflies have not learned the risk of approaching predators. In either case, this result implies that the visiting butterflies did not perceive the dead mantis as a dangerous predator, but rather as a foreign object on the flower. On the other hand, butterflies showed greater avoidance of a predator when the predator was present together with the resident butterfly than when the predator was located on the side opposite the resident butterfly ([Fig 5B](#)). This result suggests that the combination of the dead predator and the resident living butterfly can be strong signals to avoid visiting the flower. Although the mechanism behind the strong avoidance of the combined cues of predator and resident butterfly is unclear, the presence of resident butterflies might emphasize the signal of the predator (or vice versa) for flower-visiting butterflies.

Our dual choice experiments focused on the local-scale response of flower-visiting butterflies to predators and resident butterflies. However, the broad-scale response of pollinators is also important for the understanding of the effect of predators and residents on the behavior of pollinators in the context of community ecology. Although the present study could not directly compare the data between the predator and no-predator experiments because the periods of the two experiments differed, the visitation rate (total number of visitations / total recorded time) and the species compositions of visiting butterflies seemed to differ between the no-predator and predator experiments ([S2 Table](#)). These differences might be accounted for by the broad-scale response of butterflies to the existence of the predator. Future studies are required to determine the effect of predators on the broad-scale response of insect pollinators. It is difficult to observe a number of butterfly pollination events under field conditions.

The present study indicates that experimental tests in a butterfly pavilion might be a useful approach for studies of butterfly behavior.

Supporting Information

S1 Table. Species list, description, and the number of released adults of each butterfly species in the butterfly pavilion at Tama Zoological Park.
(XLSX)

S2 Table. Abundance, composition, wing size and toxicity of butterflies observed in the predator and the non-predator experiments.
(XLSX)

Acknowledgments

We thank Nami Katada for butterfly photograph, Kei Eguchi and Kotone Arai for data collection, and members of the ethology group at TUAT and staff at Tama Zoological Park for their helpful discussions.

Author Contributions

Conceptualization: YF YT.

Data curation: SIF TK.

Formal analysis: SIF TK YF.

Funding acquisition: YF.

Investigation: YT.

Methodology: YF YT.

Project administration: YF YT.

Resources: YT YF.

Software: YF.

Supervision: YF YT.

Validation: SIF TK YF.

Visualization: YF.

Writing – original draft: YF.

Writing – review & editing: YF YT SIF TK.

References

1. Waser NM, Chittka L, Price M V, Williams NM, Ollerton J. Generalization in pollination systems, and why it matters. *Ecology*. 1996; 77: 1043–1060. doi: [10.2307/2265575](https://doi.org/10.2307/2265575)
2. Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. Pollination syndromes and floral specialization. *Annu Rev Ecol Evol Syst*. 2004; 35: 375–403. doi: [10.1146/annurev.ecolsys.34.011802.132347](https://doi.org/10.1146/annurev.ecolsys.34.011802.132347)
3. Steffan-Dewenter I, Potts SG, Packer L. Pollinator diversity and crop pollination services are at risk. *Trends Ecol Evol*. 2005; 20: 651–652. doi: [10.1016/j.tree.2005.09.004](https://doi.org/10.1016/j.tree.2005.09.004) PMID: [16701452](https://pubmed.ncbi.nlm.nih.gov/16701452/)
4. Willmer P. *Pollination and Floral Ecology*. Princeton, NJ.: Princeton University Press; 2011.

5. Hirota SK, Nitta K, Suyama Y, Kawakubo N, Yasumoto AA, Yahara T. Pollinator-mediated selection on flower color, flower scent and flower morphology of *Hemerocallis*: Evidence from genotyping individual pollen grains on the stigma. *PLoS One*. 2013; 8: e85601. doi: [10.1371/journal.pone.0085601](https://doi.org/10.1371/journal.pone.0085601) PMID: [24376890](https://pubmed.ncbi.nlm.nih.gov/24376890/)
6. Schemske DW, Bradshaw HD. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proc Natl Acad Sci U S A*. 1999; 96: 11910–11915. doi: [10.1073/pnas.96.21.11910](https://doi.org/10.1073/pnas.96.21.11910) PMID: [10518550](https://pubmed.ncbi.nlm.nih.gov/10518550/)
7. Waser NM, Price M V. Pollinator behaviour and natural selection for flower colour in *Delphinium nelsonii*. *Nature*. 1983; 302: 422–424. doi: [10.1038/302422a0](https://doi.org/10.1038/302422a0)
8. Zimmerman M, Pleasants J. Competition among pollinators: quantification of available resources. *Oikos*. 1982; 38: 381–383.
9. Suttle KB. Pollinators as mediators of top-down effects on plants. *Ecol Lett*. 2003; 6: 688–694. doi: [10.1046/j.1461-0248.2003.00490.x](https://doi.org/10.1046/j.1461-0248.2003.00490.x)
10. Craig CL, Wolf SG, Davis JL, Hauber ME, Maas JL. Signal polymorphism in the web-decorating spider *Argiope argentata* is correlated with reduced survivorship and the presence of stingless bees, its primary prey. *Evolution*. 2001; 55: 986–993. doi: [10.1554/0014-3820\(2001\)055\[0986:spitwd\]2.0.co;2](https://doi.org/10.1554/0014-3820(2001)055[0986:spitwd]2.0.co;2) PMID: [11430658](https://pubmed.ncbi.nlm.nih.gov/11430658/)
11. Dukas R, Morse DH. Crab spiders affect flower visitation by bees. *Oikos*. 2003; 101: 157–163. doi: [10.1034/j.1600-0706.2003.12143.x](https://doi.org/10.1034/j.1600-0706.2003.12143.x)
12. Morse DH. Predatory risk to insects foraging at flowers. *Oikos*. Blackwell; 1986; 46: 223–228.
13. Robertson IC, Maguire DK. Crab spiders deter insect visitations to slickspot peppergrass flowers. *Oikos*. 2005; 109: 577–582. doi: [10.1111/j.0030-1299.2005.13903.x](https://doi.org/10.1111/j.0030-1299.2005.13903.x)
14. Cembrowski AR, Tan MG, Thomson JD, Frederickson ME. Ants and ant scent reduce bumblebee pollination of artificial flowers. *Am Nat*. 2014; 183: 133–9. doi: [10.1086/674101](https://doi.org/10.1086/674101) PMID: [24334742](https://pubmed.ncbi.nlm.nih.gov/24334742/)
15. Dukas R. Effects of perceived danger on flower choice by bees. *Ecol Lett*. 2001; 4: 327–333. doi: [10.1046/j.1461-0248.2001.00228.x](https://doi.org/10.1046/j.1461-0248.2001.00228.x)
16. Gonçalves-Souza T, Omena PM, Souza JC, Romero GQ. Trait-mediated effects on flowers: Artificial spiders deceive pollinators and decrease plant fitness. *Ecology*. 2008; 89: 2407–2413. doi: [10.1890/07-1881.1](https://doi.org/10.1890/07-1881.1) PMID: [18831161](https://pubmed.ncbi.nlm.nih.gov/18831161/)
17. Tan K, Hu Z, Chen W, Wang Z, Wang Y, Nieh JC. Fearful foragers: honey bees tune colony and individual foraging to multi-predator presence and food quality. *PLoS One*. 2013; 8: e75841. doi: [10.1371/journal.pone.0075841](https://doi.org/10.1371/journal.pone.0075841) PMID: [24098734](https://pubmed.ncbi.nlm.nih.gov/24098734/)
18. Romero GQ, Antiquera P a P, Koricheva J. A meta-analysis of predation risk effects on pollinator behaviour. *PLoS One*. 2011; 6: e20689. doi: [10.1371/journal.pone.0020689](https://doi.org/10.1371/journal.pone.0020689) PMID: [21695187](https://pubmed.ncbi.nlm.nih.gov/21695187/)
19. Abbott KR. Bumblebees avoid flowers containing evidence of past predation events. *Can J Zool*. 2006; 84: 1240–1247. doi: [10.1139/z06-117](https://doi.org/10.1139/z06-117)
20. Dukas R. Bumble bee predators reduce pollinator density and plant fitness. *Ecology*. 2005; 86: 1401–1406. doi: [10.1890/04-1663](https://doi.org/10.1890/04-1663)
21. Munoz AA, Arroyo MTK. Negative impacts of a vertebrate predator on insect pollinator visitation and seed output in *Chuquiraga oppositifolia*, a high Andean shrub. *Oecologia*. 2004; 138: 66–73. doi: [10.1007/s00442-003-1405-2](https://doi.org/10.1007/s00442-003-1405-2) PMID: [14551828](https://pubmed.ncbi.nlm.nih.gov/14551828/)
22. Baude M, Danchin É, Mugabo M, Dajoz I. Conspecifics as informers and competitors: an experimental study in foraging bumble-bees. *Proc Biol Sci*. 2011; 278: 2806–13. doi: [10.1098/rspb.2010.2659](https://doi.org/10.1098/rspb.2010.2659) PMID: [21288951](https://pubmed.ncbi.nlm.nih.gov/21288951/)
23. Makino TT, Sakai S. Findings on spatial foraging patterns of bumblebees (*Bombus ignitus*) from a bee-tracking experiment in a net cage. *Behav Ecol Sociobiol*. 2004; 56: 155–163. doi: [10.1007/s00265-004-0773-x](https://doi.org/10.1007/s00265-004-0773-x)
24. Makino TT, Sakai S. Does interaction between bumblebees (*Bombus ignitus*) reduce their foraging area?: bee-removal experiments in a net cage. *Behav Ecol Sociobiol*. 2005; 57: 617–622. doi: [10.1007/s00265-004-0877-3](https://doi.org/10.1007/s00265-004-0877-3)
25. Palmer TM, Stanton ML, Young TP. Competition and coexistence: Exploring mechanisms that restrict and maintain diversity within mutualist guilds. *Am Nat*. 2003; 162: S63–S79. doi: [10.1086/378682](https://doi.org/10.1086/378682) PMID: [14583858](https://pubmed.ncbi.nlm.nih.gov/14583858/)
26. Kawaguchi L, Ohashi K, Toquenaga Y. Do bumble bees save time when choosing novel flowers by following conspecifics? *Funct Ecol*. 2006; 20: 239–244. doi: [10.1111/j.1365-2435.2006.01086.x](https://doi.org/10.1111/j.1365-2435.2006.01086.x)
27. Leadbeater E, Chittka L. A new mode of information transfer in foraging bumblebees? *Curr Biol*. Elsevier; 2005; 15: R447–8. doi: [10.1016/j.cub.2005.06.011](https://doi.org/10.1016/j.cub.2005.06.011) PMID: [15964260](https://pubmed.ncbi.nlm.nih.gov/15964260/)

28. Slaa EJ, Wassenberg J, Biesmeijer JC. The use of field-based social information in eusocial foragers: local enhancement among nestmates and heterospecifics in stingless bees. *Ecol Entomol.* 2003; 28: 369–379. doi: [10.1046/j.1365-2311.2003.00512.x](https://doi.org/10.1046/j.1365-2311.2003.00512.x)
29. Kawaguchi LG, Ohashi K, Toquenaga Y. Contrasting responses of bumble bees to feeding conspecifics on their familiar and unfamiliar flowers. *Proc Biol Sci.* 2007; 274: 2661–2667. doi: [10.1098/rspb.2007.0860](https://doi.org/10.1098/rspb.2007.0860) PMID: [17698483](https://pubmed.ncbi.nlm.nih.gov/17698483/)
30. Dawson EH, Chittka L. Bumblebees (*Bombus terrestris*) use social information as an indicator of safety in dangerous environments. *Proc R Soc B Biol Sci.* 2014; 281: 20133174–20133174. doi: [10.1098/rspb.2013.3174](https://doi.org/10.1098/rspb.2013.3174) PMID: [24789891](https://pubmed.ncbi.nlm.nih.gov/24789891/)
31. Alm J, Ohnmeiss TE, Lanza J, Vriesenga L. Preference of cabbage white butterflies and honey bees for nectar that contains amino acids. *Oecologia.* 1990; 84: 53–57. doi: [10.1007/BF00665594](https://doi.org/10.1007/BF00665594)
32. Rader R, Batomeus I, Garibaldi L, Garratt MPD, Howlett B, Cunningham SA, et al. Non-bee insects are important contributors to global crop pollination. *Proc Natl Acad Sci.* 2015; doi: [10.1073/pnas.1517092112](https://doi.org/10.1073/pnas.1517092112) PMID: [26621730](https://pubmed.ncbi.nlm.nih.gov/26621730/)
33. Kikuchi T. Role of interspecific dominance-subordination relationship on the appearance of flower-visiting insects. *Sci Rep Tohoku Univ Ser IV.* 1965; 31: 275–296.
34. Morse DH. Foraging relationships within a guild of bumble bees. *Insectes Soc.* 1982; 29: 445–454. doi: [10.1007/BF02226159](https://doi.org/10.1007/BF02226159)
35. Otis GW, Locke B, McKenzie NG, Cheung D, MacLeod E, Careless P, et al. Local enhancement in mud-puddling swallowtail butterflies (*Battus philenor* and *Papilio glaucus*). *J Insect Behav.* 2006; 19: 685–698. doi: [10.1007/s10905-006-9049-9](https://doi.org/10.1007/s10905-006-9049-9)