

Title: Nectar quality changes the ecological costs of chemically defended pollen

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Plants often compete in a marketplace that involves the exchange of floral rewards for pollination service [1]. This marketplace is frequently viewed as revolving around a single currency, typically nectar. While this focus has established pollinators such as bees as classic models in foraging ecology, in reality many plants provide both pollen and nectar, which vary in composition within and across species [2]. How this complexity impacts interactions between plants, pollinators, and co-flowering competitors is unknown. We explored how variation in two axes of reward chemistry – nectar sugar and pollen alkaloid content – impacted competition for bumblebee visits.

The effect of variation in one reward depended on the presence and quality of the other — bees discriminated against flowers with more defended pollen when all flowers offered the same quality nectar. However, bees preferred flowers with highly defended pollen when they offered higher quality nectar, suggesting that attractive nectar can overcome the ecological costs of defended pollen. Recognizing the interdependence of these floral currencies may help identify traits that drive indirect interactions between plants and clarify broader evolutionary patterns of floral reward phenotypes.

Despite growing interest in bees' nutritional requirements [3], we know little about how these needs impact ecological interactions with plants. Bees collect two major resources from flowers: nectar (primarily carbohydrates) and pollen (primarily proteins and lipids). Although bees' nectar preferences are well-established, the functional implications of pollen chemistry are largely unexplored. Pollen alkaloids, for example, are highly concentrated and diverse [2], and may play a role in managing pollen's dual role as reward and gamete [4]. Namely, they reduce bees' collection of pollen for consumption [5], which is potentially a loss of male plant fitness [6]. However, these pollen 'defenses' might also come with a cost (as in nectar [7]) if they cause plants to lose visits to less-defended competitors. The combined effects of nectar and pollen chemistry on bee preferences are unknown, but it is possible that nectar traits might allow plants to maximize pollinator attraction while minimizing pollen consumption.

To explore these possibilities, we measured bumblebees' (*Bombus impatiens*) preferences for two 'species' of artificial flowers (4 blue and 4 yellow). We presented individuals (N = 147, 6 colonies) with one of four competitive scenarios, counterbalancing color–reward pairings

(Figure 1A). Flowers had tubular corollas that required bees to crawl inside to collect rewards (Supplemental Experimental Procedures). Access at the flower's back allowed us to refill nectar (4 μ l) and pollen (2.5 ± 0.5 mg) after a bee left (Figure 1A). We manipulated the presence and quality of nectar (15% vs. 50% w/w sucrose), alongside each flower's level of pollen defense (pollen adulterated 5% w/w with neutral cellulose or the alkaloid quinine, within the natural range of pollen alkaloid concentrations [2]).

We found that the presence of nectar changed bumblebees' response to pollen alkaloids. When no nectar was present, bees left the patch quickly (Figure 1C), but their relative preference for flowers with more- vs. less-defended pollen did not change (pollen-only treatment; logistic GLMM: $\chi^2 = 2.27$, $p = 0.13$; Figure 1B). However, the addition of low-quality nectar to all flowers changed this — with each subsequent landing, bees were less likely to visit a flower with highly-defended pollen (nectar-presence treatment; logistic GLMM: $\chi^2 = 7.93$, $p = 0.0048$; Figure 1B).

Together these results suggest that the addition of nectar to flowers increased bumblebees' likelihood of detecting pollen alkaloids. If, as in honeybees, taste sensilla on the proboscis respond to quinine [8], the act of drinking nectar may increase its detection in pollen; alternatively, extended bout lengths associated with nectar presence may give bees greater opportunity to identify the source of defended pollen.

Beyond mere presence, the relative quality of nectar also shaped bees' responses to defended pollen. Bees preferred flowers with high-quality nectar and highly defended pollen over flowers with low-quality nectar and low pollen defenses (nectar presence vs. non-aligned treatments; logistic GLMM: $\chi^2 = 16.13$, $p = 0.000059$; Figure 1B). Moreover, bees always preferred flowers with high-quality nectar regardless of pollen presence or defense level, while pollen defenses did not impact bees' response to nectar differences (nectar only vs. non-aligned; logistic GLMM: landing \times treatment, $\chi^2 = 0.29$, $p = 0.59$; Figure 1B).

We also uncovered a patch-level effect of pollen defenses (Figure 1C; Supplemental Experimental Procedures): the presence of a single defended floral type reduced visitation to all flowers in the array (Poisson GLMM: $\chi^2 = 360.08$, $p = 2.2 \times 10^{-16}$). The magnet species concept posits that attractive flowers increase per capita visitation for all species in a patch [9]; our results raise the possibility of a reverse-magnet effect, whereby neighbors share the cost of defended rewards. Bumblebees visiting pollen-only flowers do not always assess pollen chemistry immediately [5] and this delayed assessment might make bees more likely to generalize pollen traits from an individual flower to the patch. Given that bees were more responsive to pollen chemistry while also collecting nectar, how this might change with floral architecture (e.g., whether or not rewards are offered in close proximity) is an open question. A reverse-magnet effect might also be driven by bees integrating the nectar and pollen quality of both flowers to assess patch quality. This could generate the ordered scaling of bout lengths we observed across groups whose arrays varied in overall quality (Figure 1C).

Our findings generate two predictions for plants pollinated by generalist bees in natural systems. First, because pollen defenses had rapid aversive effects, we expect that plants offering only

pollen should have low levels of pollen alkaloids when compared with close nectiferous relatives, relying upon mechanical dosing tactics [10] to regulate pollen collection (e.g., poricidal anthers in *Solanum*). Second, we expect that within a community, plants should show a positive correlation between pollen alkaloid and nectar sugar concentrations, given the compensatory role of high-quality nectar discovered here. Datasets characterizing both rewards in plant communities would speak to this latter hypothesis, and allow us to explore how competitive dynamics play out across a wider range of quality differences. Our finding that floral rewards interact in complex ways to guide bee foraging decisions lays the groundwork for taking a holistic view of floral reward phenotypes and ultimately extending these ideas to a community context.

Supplemental Information

Supplemental information includes experimental procedures and references, and can be found at <https://doi.org/10.1016/j.cub.2019.05.046>.

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Author Contributions

All authors contributed to the conceptualization and design of the experiment. C.R.A. and J.S.F. collected the data, and J.S.F. analyzed the data. All authors contributed to the writing of the manuscript.

Declaration of Interests

The authors declare no competing interests.

Caption Figure 1: Pollen defenses and nectar quality impact floral preference and bout lengths.

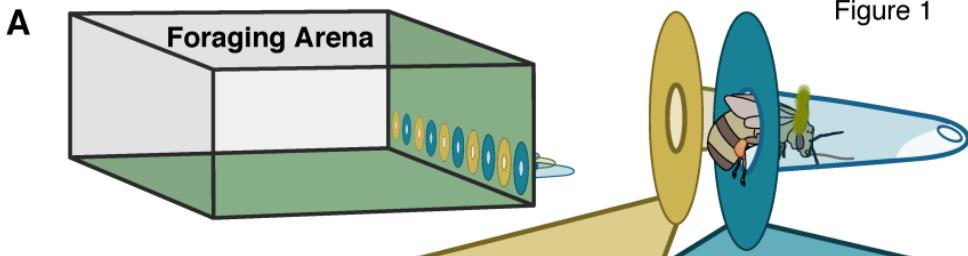
(A) Array and artificial flower design. A colony was connected to the foraging arena by a gated passageway. Flowers extended through the foraging arena wall to allow a researcher to refill nectar and chenille anthers loaded with floral-collected cherry (*Prunus avium* var. *Tieton*) pollen adulterated with powdered quinine or cellulose (pollen alk. above). Table shows the four floral-reward treatments. (B) Change in floral preference (modeled percentage of visits to Flower 1 and 95% confidence bands) over the course of a foraging bout (censored after there were fewer than 10 bees in a given treatment; details in supplement). Bees in all treatments showed significant shifts in preference except for the pollen-only treatment. (C) The number of bees remaining

foraging in each treatment across foraging bouts: average bout length differed significantly in each treatment.

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Figure 1



Treatment	Flower 1's Reward			Flower 2's Reward		
	Nectar + Pollen Alk.			Nectar + Pollen Alk.		
Pollen Only	None	+	Low	None	+	High
Nectar Only	15%	+	None	50%	+	None
Nectar Presence	15%	+	Low	15%	+	High
Non-Aligned	15%	+	Low	50%	+	High

