Learning about larceny: experience can bias bumble bees to secondary-rob nectarJessica L. Barker^{a,b,*}Anna Dornhaus^aJudith L. Bronstein^aFelicity Muth^c^aDepartment of Ecology and Evolutionary Biology, University of Arizona, Tucson, USA^bCurrent address: Aarhus Institute of Advanced Studies, Aarhus University, Aarhus, Denmark^cDepartment of Biology, University of Nevada, Reno, USA

*Corresponding author: jlbarker@aias.au.dk, +45 87 15 36 81

Abstract

How do nectar-feeding animals choose among alternative handling tactics? Such decisions have consequences not only for animal fitness (via food intake) but for plant fitness as well: many animals can choose to ‘rob’ nectar through holes chewed in the base of a flower instead of ‘legitimately’ collecting it through the flower’s opening, thus failing to contact pollen. Although variation among individuals in these nectar-foraging tactics is well documented, it is largely unknown why some individuals specialize (at least in the short term) on robbing, others on legitimate visitation, and others switch behaviors. We investigated whether the frequent tendency to rob nectar through previously constructed holes (secondary robbing) is influenced by prior foraging experience. In a laboratory experiment, we trained groups of bumble bees (*Bombus impatiens*) either to visit artificial flowers legitimately or to secondary-rob; a third group

received no training. On subsequent visits to flowers, all bees had the opportunity to use either foraging tactic. We found that experience did affect bees' tendency to secondary-rob: trained bees were more likely to adopt the tactic they had previously experienced. Untrained bees initially sampled both tactics, but over time preferred to secondary-rob. Experience also increased bees' success at gaining nectar from flowers, but only for legitimate visitation: that is, experience had a greater effect on the success of the less-preferred tactic. Overall, these findings highlight the importance of experience in animals' choices of alternative handling tactics while foraging, and help explain long-standing observations of variation in nectar-robbing behavior among individuals of the same population.

Significance statement

Animals that can adopt alternative behaviors frequently use only one of them. A widespread but little understood example of this is nectar-feeding, in which foragers can choose between 'legitimately' collecting nectar through a flower's opening or 'robbing' nectar via holes chewed through a flower's base. We showed in a laboratory experiment that prior foraging experience can bias bumble bees' choices to rob nectar from pre-existing holes (i.e., to 'secondary' rob). Initial exposure to flowers that could only be robbed or only be legitimately visited increased bees' tendency to subsequently adopt that behavior at the expense of sampling the alternative behavior, even when other bees seemed to prefer the alternative. These results help explain field observations that bees often specialize on a single nectar-collecting behavior and that there is individual variation in which behavior is adopted, contributing to our understanding of nectar-robbing from the animals' perspective.

Keywords

Nectar robbing, foraging, experience, decision-making, alternative tactics, bumble bee

Introduction

Foraging for floral nectar is widespread, and has important fitness consequences for both animals and plants. The majority of nectar-feeding animals are insects, commonly bees (Hymenoptera), butterflies and moths (Lepidoptera), and flies (Diptera), although other arthropods and many birds, notably hummingbirds, also feed on nectar (Irwin et al. 2010). Since foraging success is known to influence fitness (Schmid-Hempel and Schmid-Hempel 1998; Pelletier and McNeil 2003), how an individual manipulates a flower to gain nectar (its nectar-foraging tactic) will likely also impact fitness, because the costs and benefits resulting from different tactics may vary (Pyke 1980; Stephens and Krebs 1987; Gegear and Thomson 2004; Dedej and Delaplane 2005; Stephens et al. 2007). Since nectar-foraging is often associated with pollination, a forager's tactic can also have an impact on plant reproduction (Heinrich and Raven 1972; Goulson 1999; Irwin et al. 2010).

Nectar-foraging animals often have choices of alternative tactics for handling flowers. Nectar-foragers typically visit flowers through a flower's opening. This handling tactic is termed 'legitimate' because it can result in pollination, yielding reproductive benefits for the plant as well as energetic benefits for the animal (Irwin et al. 2010). However, many animals can also 'rob' nectar: they either chew a hole through the flower's corolla ('primary' nectar-robbing), or forage through holes already cut by primary robbers ('secondary' nectar-robbing) (Inouye 1980).

Primary and secondary nectar-robbers usually do not come into contact with a flower's anthers and stigma, and in some species, robbed flowers are subsequently avoided by legitimate visitors. Both robbing behaviors have been extensively documented in a wide variety of plant species (Irwin et al. 2001). Alone or in concert, they usually lead to reduced seed or fruit production (Irwin et al. 2010, Richman et al. 2016); but see Maloof and Inouye 2000; Richardson 2004).

While some nectar-foraging species feed on flowers using a single tactic, in others, substantial variation can be observed among individuals: some visit flowers purely legitimately, others exclusively rob nectar, while others adopt a mixed strategy, switching between legitimate visitation and one or both types of robbing (Bronstein et al. 2017). Although the natural history of nectar robbing is well-studied from the plant perspective (including its consequences for plant fitness), the factors affecting animals' decisions to visit legitimately versus rob nectar remain minimally understood (Irwin et al. 2010; Bronstein et al. 2017). The energetic costs and benefits of each tactic (Dedaj and Delaplane 2005) and morphological 'fit' between the nectar-forager and flower likely help explain variation in nectar robbing across species: for example, *Bombus* species with shorter tongues exhibit more nectar robbing (Newman and Thomson 2005). However, even within species, individuals within a population often vary in nectar robbing (e.g. *Apis mellifera*: J.L. Barker and J.L. Bronstein, unpubl. data).

Since learning plays a major role in determining other aspects of foraging for bumble bees and honey bees, including floral handling strategies (Woodward and Lavery 1992; Lavery 1994a; Chittka and Thomson 1997), it seems plausible that it may also play a role in determining whether or not individuals choose to rob. Previous studies suggest that social learning may be

important. In *Bombus terrestris*, exposure to the social cue of primary-robbled flowers resulted in a greater likelihood of primary robbing (Leadbeater and Chittka 2008). *Bombus lucorum* and *B. wurflenii* appeared to copy other individuals' use of one side of the flower ('handedness') when primary-robbing (Goulson et al. 2013). While these studies show that social experience influences nectar-robbing behavior within species, it is still not clear what role individual foraging experience plays.

Secondary robbing is ubiquitous in plants subject to primary robbing (Irwin et al. 2010; Richman et al. 2016), yet it is comparatively poorly understood. It is particularly of interest in a behavioral context because flower visitors commonly switch between legitimate foraging and secondary robbing, far more than they do between other foraging tactics (e.g., Richardson and Bronstein 2012; Richman 2017). Shifts away from potentially mutualistic, legitimate foraging behaviors towards a nonmutualistic "cheating" tactic has the potential to impact plant success, and indeed to destabilize pollination mutualisms (Bronstein et al. 2017).

In this study, we tested whether prior experience with either secondary robbing or legitimately visiting flowers biases individual bumble bees' decisions to rob nectar. If a bee's tendency to secondary-rob is not learned (rather, each individual has an *a priori* tendency to rob or not rob), then a bee's foraging tactic will not be affected by whether she has had any previous experience with robbing or with legitimate visitation. Alternatively, secondary robbing could arise from previous individual foraging experience: if a bee first encounters opportunities to gain nectar from secondary robbing but not from legitimate visitation, she may continue secondary-robbing flowers over time even if legitimate visitation is subsequently an alternative option that may

yield nectar rewards with equal or even greater success (Ravary et al. 2007). Finally, it is also possible that bees will initially have a preference for a particular foraging tactic (be it innate or learned from prior experience), but that this will change with subsequent experience of sampling both foraging tactics until the individual arrives upon the choice that more successfully yields higher net payoffs (that is, initial floral encounters do not bias longer-term tactic choices).

We carried out a laboratory-based foraging experiment using the bumble bee *Bombus impatiens*. We focused only on secondary robbing because this species has never been observed to chew robbing holes itself (i.e., to primary rob); many bees lack the chewing mandibles required to adopt this behavior. This species is, however, well-documented to secondary-rob previously robbed flowers in the field (Rust 1979; Irwin et al. 2010). We trained naïve bumble bees to collect nectar from artificial flowers which they could either only visit legitimately or only secondary-rob; a third group of bees received no training (Fig. 1). This initial training period was intended to capture how, in the field, individuals may gain experience via stochastic initial encounters with flowers in a given environment (for example, due to high variability in the rates of robbed flowers: Irwin and Maloof 2002), as well as via information learned in that environment over time. We then recorded the behavior of these three experimental treatment groups on ‘test flowers’ that bees could either visit legitimately or secondary-rob over a series of subsequent foraging bouts. This experiment provides insight into how experience via both initial encounters and learning affect tactic choice, but also how experience interacts with tactic type: that is, whether one tactic requires more experience to gain the same rewards.

Methods

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140 *Subjects and rearing*

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142 We used four commercially-reared *Bombus impatiens* colonies (Koppert Biological Systems,
143 Howell, Michigan). We kept two colonies in the laboratory at a given time (colonies A and B:
144 October-November 2013; colonies C and D: March-May 2014), and marked workers every few
145 days with honey bee queen tags (Bee Works, Orillia, Ontario) affixed with superglue, enamel
146 paint (Testor's, Vernon Hills, Illinois), or paint pens (Craft Smart, Irving, Texas).

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148 We housed each colony in a nestbox (39 x 10.5 x 23 cm), connected by a transparent plastic tube
149 (2 cm diameter) to a foraging arena (90 x 40 x 60 cm) (Fig. 2). By inserting plastic discs into the
150 tube connecting the nestbox and arena, we controlled individual bees' access to the foraging
151 arena. The nestboxes and foraging arenas were made of wood, with transparent Perspex roofs for
152 observation. We placed a sucrose feeder (containing 25% sucrose in water by volume (v/v)) in
153 each colony's foraging arena for approximately 1 week, to familiarize the bees with foraging in
154 the arena. We subsequently fed each colony by pipetting 25% v/v sucrose solution into all empty
155 honeypots twice daily, and kept replenished an *ad lib.* supply of pollen (honey bee-collected,
156 purchased freshly-frozen from Koppert Biological Systems, defrosted and ground before feeding
157 to bumble bees) in a dish next to the nest in the nestbox.

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159 *Artificial flowers*

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We constructed artificial flowers out of 0.25 cm thick yellow foam glued to make a cone (3.5 cm high x 3.5 cm diameter), with a 0.5 cm cut Eppendorf tube tip glued to the bottom to hold 20 μ l of ‘nectar’ (50% v/v sucrose solution). We used three types of flowers (Figs. 1 and 2):

a. Legitimate-visitation training flowers: the top of the cone was open, allowing a bee access to nectar by entering the flower (*B. impatiens* is not capable of nectar-robbing in the absence of a pre-existing hole, which was not provided in this treatment). We termed this tactic ‘legitimate visitation’.

b. Secondary-robbing training flowers: the top of the cone was closed with an additional circle of yellow foam, but there was a 0.5 cm hole cut into the flower, 0.5 cm above the Eppendorf tube. This allowed a bee to access nectar from outside the flower, at its base, by sticking her proboscis and/or head inside the opening. We termed this feeding tactic ‘secondary robbing’, the holes cut in these flowers representing those that a primary robber would make.

c. Test flowers: the flowers were open at the top and had holes cut into the base, allowing a bee access to nectar via both legitimate visitation and secondary robbing. We note that both ‘legitimate visitation’ and ‘secondary robbing’ are terms defined from the plant’s perspective (see Introduction). Recognizing that this terminology is not meaningful for artificial flowers, we use it here for consistency with field studies of nectar robbing. We also note that, in the field, the handling difficulty and nectar yield of each tactic will depend on the plant species and other factors: see Discussion.

General protocol

We assigned bees to one of three experimental treatments (Fig. 1):

Treatment 1: legitimate training. Bees ($N=14$) foraged from legitimate training flowers (training phase) before gaining access to test flowers (test phase).

Treatment 2: secondary-robbing training. Bees ($N=16$) foraged from secondary-robbing training flowers (training phase) before gaining access to test flowers (test phase).

Treatment 3: no training. Bees ($N=17$) had access to test flowers without any prior encounters with either type of training flowers (test phase only; no training phase).

In all training and test phases, we presented bees with an array of 10 artificial flowers (Fig. 2).

Each flower was mounted with Blue-Tack on a clear plastic tube (9.5 cm high x 2.5 cm diameter) that was glued to a piece of plywood (approximately 45 x 30 cm). The foraging arena was illuminated by a lamp with an incandescent 60W bulb, in a lab with fluorescent lighting. We mounted a video camera on a tripod next to the arena so that we could record bees' behavior in the arena (viewed from above, through the Perspex roof).

Training phase

For Treatments 1 and 2, we allowed 10-20 bees at a time access to an array of training flowers (either legitimate-training or robbing-training flowers: Fig. 2) in the foraging arena. These bees had foraged from a sucrose feeder in the arena, but were naïve to the artificial flowers. Whenever a bee made a rewarded visit (i.e., obtained nectar) on a flower, we recorded the bee's color/number tag, and refilled the flower (so that, as far as possible, all training flowers were always full).

We let bees forage from the training flowers until one bee made 10 rewarded visits. These 10 visits could be over multiple bouts, with a bout defined as a sequence of visits to flowers made without returning to the colony (i.e., a single foraging trip). When one bee had gained these 10 rewards, we did not remove her immediately but instead allowed her to finish her foraging bout and return to the colony by herself. Consequently, some bees that we tested made more than 10 rewarded visits. We then removed all other bees from the foraging arena, cleaned the mount for the flowers with isopropyl alcohol to remove any scent marks, and soaked the training flowers in warm water to remove any traces of nectar. Both were dried before re-use.

Test phase

Immediately after training, we tested each bee in Treatments 1 and 2 over a series of five foraging bouts. We carried out the tests on individual bees without others present, to ensure there were no social effects on which tactics bees used. We allowed the test bee access to an array of test flowers in the foraging arena; when she finished foraging (usually after around four rewarded flower visits), we allowed her back into the nestbox via the connecting tube, and counted this series of visits as a single test bout. We then waited for the bee to return to the arena for the next foraging bout. Between test bouts, we replaced the test flowers with clean filled ones, keeping the same mount. When the test bee was ready to resume foraging, we allowed her to return to the arena for another bout, with each test bee completing a total of five bouts (and approximately <10 minutes between each bout). The five bouts by each bee were always completed on a single day, and on the same day as that bee's training phase.

For bees in Treatment 3 (no training), we carried out the test phase in exactly the same way as in Treatments 1 and 2, with the exception that in the first bout, we allowed 10-20 bees into the foraging arena and when one bee gained a reward we removed all other bees in the arena, avoiding disturbance to the foraging bee. We did this by catching the other bees with forceps or a small plastic container, and either putting them back into the nestbox directly or letting them back through the connecting tube. Thus, the first bout was similar to the training phase in Treatments 1 and 2, in that every bee first entered the foraging arena with several other bees, but subsequent testing was on individual bees.

To determine whether foraging bees fully drained the flowers they visited, we measured nectar volumes in a subset of arrays after bees completed their trial, using 2 μ l microcapillary tubes (Fisher Scientific). All flowers on which bees gained the nectar reward ('successful visits') were completely depleted of nectar each time (i.e., bees consumed 20 μ l of nectar), regardless of whether they had been 'legitimately visited' or 'secondary-robbed'.

Analyses

For each visit in the test phase, we recorded:

- a. the foraging tactic used by the test bee (legitimate visitation or secondary robbing, as defined above: see *Artificial flowers*);
- b. the location of the flower in the foraging array (we did not refill flowers during testing, so a re-visit would be unrewarded unless the first visit was not successful);

- c. the times at which the test bee landed on the flower (defined as first contacting the flower with two legs) and left the flower;
- d. the times at which the test bee started and stopped attempting to obtain nectar (defined as the bee's head in the legitimate opening or the robbing-hole); and
- e. whether or not the test bee was successful at gaining the nectar reward (defined as the bee's head positioned over the nectar for more than 2 seconds, with abdomen pumping).

We used data from real-time observations for (a) and (b), and from video recordings for (c), (d) and (e). The videos were scored by observers who were blind to the experimental treatment. In some videos, the test bee's behavior was partially obscured by the flowers, and thus the sample sizes for (c), (d) and (e) are smaller.

We used R version 3.4 (R Core Team 2017) for statistical analyses. After checking that the residuals were normally distributed, we first ran repeated *G*-tests of goodness-of-fit (using the R packages RVAideMemoire (Hervé 2017) and dplyr (Wickham et al. 2017)) for each treatment, to determine whether bees' choice of tactic (legitimate visitation versus secondary robbing, measured as the proportion of legitimate visits pooled across all five bouts) differed from chance (50% probability of using each tactic). We also addressed whether treatments differed from each other and whether this changed across foraging bouts, by fitting linear mixed models (LMMs) using the R package nlme (Jose et al. 2017), all of which included the random factors 'bee' (individual) and 'colony'.

- a. Choice of foraging tactic (from real-time data): we used the proportion of legitimate visits in each bout as the response variable, with experimental treatment (legitimate training,

secondary-robbing training, or no training), bout (1-5), and treatment $\times$ bout interaction as predictor variables.

- b. Success at obtaining the nectar reward (from video data): we used the proportion of successful visits pooled across all bouts as the response variable, with experimental treatment, tactic type (legitimate visitation or secondary robbing) and treatment $\times$ tactic as predictor variables.
- c. Latency to access nectar the first time (from video data): we considered the first time a bee obtained nectar from a test flower, and defined ‘latency’ as the time between landing on the flower and beginning to feed. We wanted to know how long it took bees to use each handling tactic, so we used latency to access nectar rather than a measure of foraging efficiency derived from the total time spent on the flower. (We note that the total time on the flower did not differ for the different foraging tactics. Legitimate visits took 22.0 ± 0.9 seconds (mean $\pm$ SE), and secondary robbing visits took 23.5 ± 0.8 sec.) In our analyses, we used latency as the response variable, with experimental treatment, tactic type, treatment $\times$ tactic as predictors. We analyzed latency on the first visit only because we would expect treatment to have the strongest effect on a bee’s first visit compared to later visits.

When we found a significant effect of treatment, we ran Tukey post-hoc tests (using the R package lsmeans) to determine where the differences lay. When there was a significant interaction between treatment and another factor, we ran separate models on subsets of the data to examine the interaction in more detail.

Results

Prior use of one foraging tactic caused subsequent bias, persisting after opportunities to use both tactics

Bees showed a bias towards the foraging tactic on which they had been trained (Fig. 3). All trained bees used the tactic on which they had been trained on their first visit to test flowers. Across all bouts, most of the visits made by legitimate-trained bees were legitimate (proportion of legitimate visits: mean $\pm$ standard error (SE): 0.88 ± 0.03 , $N=66$ bouts by 14 bees; repeated G -test of goodness-of-fit against expected 0.5: $G_{14}=264$, $p<0.001$), while most of the visits made by secondary-robbing-trained bees were secondary robs (mean $\pm$ SE: 0.16 ± 0.03 , $N=77$ bouts by 15 bees; $G_{15}=245$, $p<0.001$). Untrained bees showed no bias towards either tactic on their first visit, but over the five bouts less than 50% of their visits were legitimate visits (mean $\pm$ SE: 0.32 ± 0.04 , $N=76$ bouts by 17 bees; $G_{17}=126$, $p<0.001$).

In addition to these departures from random choice, the behavior of bees in the three treatment groups both differed from each other and changed across the foraging bouts (Fig. 3). Legitimate-trained bees made a significantly higher proportion of legitimate visits across the five foraging bouts of the test phase than did secondary-robbing-trained and untrained bees, which did not differ from each other (linear mixed model (LMM) effect of treatment: $F_{2,41}=36.4$, $p<0.001$; differences confirmed with Tukey post-hoc tests: legitimate-trained versus secondary-robbing-trained bees; $t_{41}=10.8$, $p<0.001$; legitimate-trained versus untrained: $t_{41}=7.37$, $p<0.001$; secondary-robbing-trained versus untrained: $t_{41}=1.38$, $p=0.362$).

Untrained bees increased their frequency of robbing over the five bouts, but this was not the case for bees in the two training groups (significant treatment $\times$ bout interaction (LMM: $F_{8,160}=2.14$, $p=0.035$; Fig. 3). Rather, the trained bees persisted with using the tactic on which they had been trained, even after multiple foraging bouts in which they could sample both tactics. To determine how choices changed across bouts for each treatment, we examined the effect of bout in each treatment separately. Bout had a significant effect on untrained bees (LMM: $F_{4,55}=2.68$, $p=0.041$), but not on bees with training in either legitimate visitation ($F_{4,48}=1.49$, $p=0.220$) or secondary robbing ($F_{4,57}=0.858$, $p=0.494$).

Prior use of either tactic increased success obtaining nectar

In order to determine whether in the context of this experiment one tactic might have been more profitable than the other, we analyzed how tactic choices were related to bees' success obtaining nectar. Even without training, bees were highly successful at gaining nectar from flowers: bees in the no-training experimental treatment obtained the nectar reward in $80\pm 11\%$ (mean $\pm$ SE) of legitimate visits ($N=97$ visits by 12 bees) and in $90\pm 8\%$ of secondary-robbing visits ($N=177$ visits by 13 bees).

However, training increased bees' success at gaining nectar from flowers still further (Fig. 4). Bees that were trained in either legitimate visitation or secondary robbing were more successful gaining nectar on legitimate visits than were untrained bees (LMM effect of treatment: $F_{2,31}=17.0$, $p<0.001$). Untrained bees were more successful gaining nectar when secondary-robbing than when legitimately visiting flowers (LMM treatment $\times$ tactic interaction: $F_{2,27}=9.36$,

$p < 0.001$; differences confirmed with Tukey post-hoc tests: for legitimate visits, legitimate-trained versus secondary-robbing-trained bees: $t_{27} = 0.720$, $p = 0.754$; legitimate-trained versus untrained: $t_{27} = 3.88$, $p = 0.002$; secondary-robbing-trained versus untrained: $t_{27} = 3.70$, $p = 0.003$).

We further investigated the treatment $\times$ tactic interaction by running separate LMMs for each tactic type. We found that bees' training had a stronger effect on their success when visiting legitimately than when secondary robbing. For legitimate visits, treatment significantly affected bees' success ($F_{2,28} = 10.6$, $p < 0.001$): bees with no training were less successful at obtaining nectar legitimately than were legitimate-trained and secondary-robbing-trained bees. However, bees' success at secondary robbing was not affected by treatment ($F_{2,27} = 2.43$, $p = 0.107$).

Latency to obtain nectar on the first rewarded visit was not affected either by training (LMM: $F_{2,30} = 0.668$, $p = 0.520$) or by the foraging tactic used on the first visit ($F_{1,27} = 1.13$, $p = 0.296$) (Fig. 5).

Discussion

Initial floral encounters can bias bees to secondary-rob or not rob nectar

When do bees and other nectar-foraging animals secondary-rob floral nectar, rather than visiting flowers legitimately, when they have the option of gaining nectar from both tactics? Two long-standing observations from the field remain unexplained. First, bees are often observed using a single nectar-foraging tactic during a single foraging bout, even though they are capable of

employing alternatives (Richardson and Bronstein 2012; Bronstein et al. 2017; J.L. Barker and J.L. Bronstein, unpubl. data). Second, the extent to which individuals rob is observed to vary within a species and even within a population (Irwin et al. 2010; Richardson and Bronstein 2012). Our laboratory experiment offers an explanation for both of these field observations: we showed that bumble bees (*Bombus impatiens*) specialized on secondary-robbing or legitimate visitation depending on which of these tactics they had initially used (Fig. 3). Bees that had not had prior exposure to either tactic did not show an initial preference. Thus, secondary nectar robbing is affected by individual foraging experience.

Bees that had prior experience collecting nectar from our artificial flowers did not change their tactic choices over the subsequent five foraging bouts of the experiment, instead continuing to predominantly adopt the tactic they had already used (Fig. 3). In contrast, bees that had not previously encountered the artificial flowers did change their tactic choice over the course of the experiment. These untrained bees began by using both tactics, but were less likely to visit flowers legitimately over time, such that in later bouts they secondary-robbed at the same frequency as bees trained to do so (Fig. 3), implying that secondary robbing may be the optimal tactic on these artificial flowers. Together, the results indicate that although bees can sample alternative tactics and may eventually learn to use the more profitable one, repeated initial opportunities to employ one tactic can persistently bias bees' later choices. A possible explanation is that using a single handling tactic over the course of several bouts means that alternative tactics do not need to be stored in a bee's short-term memory (reviewed by Bronstein et al. 2017). Although insects are known to employ a broad range of cognitive abilities when visiting flowers (Chittka and Thomson 2005), this is, to our knowledge, the first evidence that

individual experience plays a role in determining whether individuals secondary-rob flowers or visit them legitimately.

Why might a bias based on initial encounters be beneficial?

Our finding that secondary nectar robbing was affected by the foraging environment to which bumble bees were first exposed is consistent with evidence that prior encounters can result in biased tactic choices in other aspects of nectar foraging, for example ‘handedness’ in flower-handling (Kells and Goulson 2001) and constancy to certain flower morphologies (Lavery 1994b; Ishii and Kadoya 2016). Floral constancy – the tendency to choose the same flower color or morphology repeatedly despite opportunities to forage from alternative types (Grant 1950; Heinrich 1976; Waser 1986) – is analogous to the decision to adopt a single handling tactic (nectar robbing versus legitimate visitation) on a given flower type (Bronstein et al. 2017). Many hypotheses for floral constancy invoke learning and cognitive mechanisms (Chittka et al. 1999; Chittka and Raine 2006; Chittka and Muller 2009), and can provide insight into the benefits of biases arising from prior encounters. Given that a tendency to bypass rewarding alternatives can be costly (Costa et al. 2016), when might such biases be beneficial?

First, since acquiring information about alternatives may be costly, it frequently pays to use a tactic that was previously successful. Bumble bees are less likely to switch flower morphotype when the environment is less variable, as the available tactics and their net benefits are also less variable (Chittka et al. 1997; Gegear and Thomson 2004; Keasar et al. 2013). Thus, during the short lifespan of a bumble bee worker, adopting a single foraging tactic that provides acceptable

nectar rewards may yield a higher expected net benefit than sampling many other tactics that are costly to acquire even if potentially more rewarding. Second, there may be gains from efficiency (Lavery 1980, 1994b; Lewis 1986; Keasar et al. 1996). Bumble bees switching between foraging tactics that require different motor patterns incur costs of increased memory load, handling time, and time flying between flowers (Chittka et al. 1999).

The effect of bee biases on plant pollination traits

On the artificial flowers we used, secondary nectar robbing was apparently preferred over legitimate visitation because it was the ‘easier’ tactic. Three results lead to this conclusion. First, bees that were not trained for a particular tactic preferred secondary-robbing as they gained experience with both tactics (Fig. 3): bumble bees prefer flowers that require simpler handling and less learning unless morphologically complex flowers are much more rewarding (Muth et al. 2015). Second, untrained bees in our experiment were more successful obtaining nectar via secondary robbing than legitimate visitation (Fig. 4). Third, training via prior use of either tactic increased bees’ success at legitimate visits, but not at secondary robbing: learning has been shown to disproportionately improve performance at more ‘difficult’ handling tactics (Heinrich 1979; Lavery 1980).

While bees have been shown to prefer secondary robbing over legitimate visitation on certain flower morphotypes in nature (Free 1968; Dedej and Delaplane 2005), it is unknown whether this is more generally the case. Bees are likely to profit from a secondary-robbing tactic only at times and places when primary-robbing rates are high; this is a tactic that can only be used in the

presence of primary-robbing species, and is likely only profitable once robbing-holes are abundant (Richman et al. 2017; J.L. Barker and J.L. Bronstein, unpubl. data) and when nectar has not been greatly depleted by primary robbing. The extent to which bees switch from legitimate visitation to secondary nectar robbing could also be influenced by specific features of different flowers and bees' interactions with them. For example, bees' choices of different flower morphotypes depended on whether their previous experience was successful (Chittka et al. 1997) and whether they encountered a given morphotype consecutively or mixed in with other flowers (Chittka and Thomson 1997). As different morphotypes often require different handling tactics, it is probable that these factors also affect bees' choices of handling tactics with regard to nectar robbing. An additional consideration to account for when bees may switch nectar-foraging tactics is whether those bees are also foraging for pollen on the same species. Because secondary robbing bypasses a flower's anthers, bees generally pick up little to no pollen per visit. Thus, pollen-collecting bees may be less likely to switch to secondary robbing than those exclusively foraging for nectar.

Plants are likely to gain greater fitness benefits when their floral visitors specialize on legitimate visits, as legitimate visitation generally transfers pollen and nectar robbing does not (Irwin et al. 2001). How, then, might plants be able to increase the likelihood of visitor specialization on legitimate visits? While one mechanism might be for the payoff from legitimate visitation to be greater than that from nectar robbing (e.g., plants may be able to reduce the payoff of primary robbing via thickened calyces (Irwin et al. 2010)), our study also suggests that plants may disproportionately decrease secondary robbing if bees' initial encounters, and success at extracting nectar, can be biased towards legitimate visits. Thus we would might expect selection

for floral traits that particularly affect naïve bees, such as traits like nectar guides that exploit sensory biases (Leonard et al. 2013).

Implications

Our experiment shows that bumble bees' initial opportunities to use a given nectar-foraging tactic affected their subsequent tactic choice and success. We suggest that this result can help explain why, in nature, some individuals rob nectar while others visit flowers legitimately or adopt mixed strategies (reviewed by Irwin et al. 2010; Bronstein et al. 2017). In the field, the frequency of flowers that can be secondary-robbed varies both spatially and temporally (Irwin and Maloof 2002). Individual bumble bees will likely encounter different floral environments depending on where and when they first leave the nest to begin foraging (Heinrich 1976). Consequently, if a naïve bumble bee started foraging from flowers before any of those flowers had been primary-robbed by other floral visitors (thus precluding secondary robbing), then she might be 'primed' by that environment to continue visiting flowers legitimately. However, our experiment addressed a relatively short time scale (five foraging bouts in a single day) and a fixed environment; field observations of honey bees suggest that they learn to secondary-rob as the frequency of primary-robbed flowers increases (Dedek and Delaplane 2004).

The artificial flowers we used likely required more similar handling techniques for both legitimate visitation and secondary robbing than is likely for real flowers, since in both cases bees merely had to find an opening. Similar handling techniques can enable transfer of motor skills (Dukas 1995; Chittka and Muller 2009), explaining why prior encounters with either type

of flower in our experiment (providing rewards only via legitimate visitation or only via secondary robbing), increased bees' success at legitimate visits compared to bees that had no prior encounters with any flowers (Fig. 4). The artificial flowers' morphology may also explain why bees' prior encounters did not affect their latency to find nectar on the first visit where they did find so (Fig. 5): the nectar reward was relatively straightforward to access via both handling tactics. In addition, each handling tactic on our flowers yielded the same volume and concentration of nectar. Whether this is the case in nature, and how differences in the nectar reward affect bees' behavioral flexibility, is an open question. Robbed flowers commonly contain less nectar, on average, than unrobbed flowers (Irwin et al. 2010), so bees that previously fed legitimately from unrobbed flowers may be more likely to switch to secondary-robbing on robbed flowers if continued legitimate visits result in decreased nectar returns. Secondary robbing may also allow bees to extract the last dregs of nectar more effectively (Dedek and Delaplane 2005).

In sum, we found that biases in bees' choice of nectar-foraging tactics arose from initial encounters with opportunities to use each tactic, and, within the time-scale and relative nectar rewards afforded by our experiment, were not overcome by subsequent exposure to additional tactic options of different 'preferability'. These results should apply broadly to the many situations where animals choose between alternative handling tactics for the same food sources (Custance et al. 1999; Aplin et al. 2013; Mondal et al. 2014). Thus, our study not only helps explain behavioral flexibility in nectar robbing, but also sheds light on the range of mechanisms by which biased tactic choices can arise.

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Ethical approval

All applicable international, national and institutional guidelines for the care and use of animals were followed.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Data availability

Upon acceptance, data will be archived at Dryad or *Behavioral Ecology and Sociobiology*'s preferred data depository. We have included our data in this submission as a supplementary Excel file.

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Figure captions

Fig. 1 Treatments 1 and 2 consisted of a training phase and a test phase. In the training phase, bees foraged from artificial flowers that they could either only legitimately visit (flower was open at the top; Treatment 1) or only secondary-rob (flower was closed at the top and had a pre-cut hole at the base; Treatment 2). In the test phase, bees were presented with artificial flowers on which they could use both foraging tactics (open at the top plus hole at the base). Treatment 3 had no training phase; bees foraged from the test flowers only

Fig. 2 Each colony was housed in a nestbox connected to a foraging arena; a series of discs inserted in the connecting tube allowed us to control individual bees' access to the arena. Bees foraged on an array of 10 artificial flowers. An array contained one of three types of flowers (legitimate training, secondary-robbing training, or test flowers), depending on the experimental treatment and phase of the experiment

Fig. 3 Bees that had been trained on one tactic (legitimate visitation or secondary robbing) predominantly used the trained tactic throughout the experiment, while bees with no training decreased their proportion of legitimate visits over the five foraging bouts. Boxes indicate the quartiles, and whiskers extend to values within 1.5 times the interquartile range. Numbers above the boxes are sample sizes (numbers of bees)

Fig. 4 Bees' training did not affect their success obtaining nectar via secondary robbing. For legitimate visits, bees that received no training were less successful than bees that had received either legitimate or secondary-robbing training. Bees with no training were less successful at obtaining nectar legitimately than via secondary robbing. Boxes indicate the quartiles, and whiskers extend to values within 1.5 times the interquartile range. Letters indicate groups that are significantly different, and sample sizes are given below the boxes

Fig. 5 Training on either foraging tactic (legitimate visitation or secondary robbing) did not affect bees' latency to obtain nectar on the first rewarded visit. Boxes indicate the quartiles, and whiskers extend to values within 1.5 times the interquartile range. Letters indicate groups that are significantly different, and sample sizes (number of bees) are given above the boxes