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ORIGINAL ARTICLE

Hummingbirds use taste and touch to discriminate against nectar resources that contain Argentine ants

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Abstract

Hummingbirds compete with other floral visitors for access to floral resources (nectar). Several hummingbird species, including Anna's (*Calypte anna*), Black-chinned (*Archilochus alexandri*), Allen's (*Selasphorus sasin*), and Costa's (*Calypte costae*) hummingbirds, make extensive use of non-native plants of urban areas of Southern California. Exploitation of urban ornamentals may expose hummingbirds to increased interactions with invasive Argentine ants (*Linepithema humile*), which are also frequently found foraging on flowers in such habitats. Here, we investigated the mechanisms by which hummingbirds interact with invasive ants at nectar resources in a series of aviary and wild experiments. When given a choice, hummingbirds avoided flowers and feeders with ants in or feeding at a sucrose solution. We identified specific ant-derived cues (visual, tactile, and gustatory) which are sufficient to elicit changes in bird foraging. Tactile and gustatory cues appeared to play the strongest role in mediating interactions with Argentine ants, with visual cues alone not enough to deter hummingbirds from feeding at sugar resources with ants. Our experiments provide support for interference competition at floral resources, where ants limit the birds' access to flowers and feeders.

Significance statement

Hummingbirds and invasive Argentine ants both visit and exploit floral resources. However, hummingbirds avoid nectar sources that are occupied by ants. Here, we detail a series of mechanistic experiments to determine the proximate cause of this avoidance behavior. We found that the touch and taste of ants is strongly aversive to both wild, free-foraging and aviary hummingbirds. In urban environments and under low water situations, Argentine ants and hummingbirds come into frequent competition for the same few flowers. This competition leads to changes in foraging behavior and may have negative effects on hummingbirds.

Keywords Invasive species · Pollination · Nectar · Resource competition · Hummingbird foraging

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Introduction

Hummingbirds compete intensely for access to flower nectar, both intraspecifically and interspecifically (Stiles and Wolf 1970; Slauson 2000). Competition for floral resources can be both interference and exploitative (Gill et al. 1982) and influences territoriality (Thomas et al. 1986), foraging strategy (Carpenter 1979; Gill 1988), and fitness (Temeles and Kress 2010). While the effects of intra-guild competition among hummingbirds has been relatively well-studied (Stiles and Wolf 1970; Ewald and Carpenter 1978; Feinsinger and Colwell 1978), how the foraging strategies and their pollination services of hummingbird shift in response to non-pollinator floral visitors,

such as Argentine ants, remains largely untested. One such species of interest, the Argentine ant (*Linepithema humile*), is an invasive species in California that utilizes floral nectar and displaces other floral visiting arthropods (Lach 2008), lowers pollinator visitation, and reduces plant reproductive success (LeVan et al. 2014). Despite overlapping in distribution with several hummingbird species, how these ants interact and compete with native hummingbirds has not yet been addressed.

In California, Argentine ants can be found in both urbanized areas as well as natural areas such as coastal sage scrub (Suarez et al. 1998) and riparian habitats (Holway 1995). Anna's (*Calypte anna*), Black-chinned (*Archilochus alexandri*), Allen's (*Selasphorus sasin sedentarius*), and Costa's (*Calypte costae*) hummingbirds all make extensive use of these habitats, potentially bringing them into contact with Argentine ants. *L. humile* is well-documented to deter resource collection of other ants (Lach 2005), herbivores (Ludka et al. 2015), and insect pollinators (Buys 1987; LeVan et al. 2014; Hanna et al. 2015; Sidhu and Wilson Rankin 2016). If Argentine ants engage in exploitative or interference competition with hummingbirds, this could potentially reduce the resource base for hummingbirds in ant-invaded areas and have implications for the pollination services offered by hummingbirds.

Hummingbirds have acute vision and taste perception, at least for sweetness (Martinez Del Rio 1990; Harris-Haller and Harris 1991; Fleming et al. 2004; Baldwin et al. 2014). Here, after anecdotally observing that hummingbirds seemed to avoid ants at commercial backyard feeders, we performed a series of experiments to examine the sensory mechanism by which they rejected flowers or feeders invaded by *L. humile*. We hypothesized that ants may be detected by hummingbirds at floral resources via a variety of cues. Hummingbirds may either see ants on or inside flowers (visual cues), touch ants as they probe flowers for nectar (tactile cues), or taste ants or ant byproducts when they consume nectar from flower containing ants or from which ants have previously fed (gustatory cues). Here, we describe a series of experiments to test whether the presence of ants affected subsequent hummingbird visitation to feeders or flowers and identify the proximate causes of hummingbird avoidance of ants.

Methods

Field observations: hummingbird foraging on flowers with and without ants

We conducted field observations of wild Allen's, Anna's, Costa's, and Black-chinned hummingbirds on the campus of UC Riverside, Riverside, CA, USA in October 2014. These species are generalist in their feeding preferences, and all species utilized the blooms of cultivated *Tecoma capensis* (Cape honeysuckle) for food, and its large, tubular flowers often attract ants. Thus, our first goal was to determine how hummingbirds interact with Argentine ants in a semi-natural situation. To control as many factors as possible, we first attracted wild hummingbirds to a location by hanging feeders roughly 1.5 m off the ground at two locations on the UC Riverside campus. Each feeder tended to be monopolized by a single male hummingbird that, when not feeding, perched in view, such that we could assign each visit to the feeder to an individual bird. To conduct the experiment, the focal feeder was then temporarily removed and replaced with two sets of 10 freshly harvested *T. capensis* flowers in vials of water: one set contained field-realistic levels of 50 Argentine ants, whereas the other, a control, contained no ants. As the nectar volume and concentrations of wild flowers vary (DTR, unpubl. data), we supplemented the nectar in the flowers by partially filling each flower with 500 μl of a 25% (*w*/*w*) sucrose solution to ensure that nectar did not deplete for the duration of the trial. Tanglefoot (Contech Enterprises, Inc.) was applied to the base of each vial to prevent any wild ants from accessing the flowers and to prevent any spread of ants between treatments. Each trial consisted of a single 60-min observation period. We noted the species, age, sex, and bird ID of each bird that visited the flowers and time and duration of each visit, as well as any behavioral responses of the birds to the feeder or each other. It was not possible to record data blind because our study involved focal animals in the field and aviary. After three trials with a given individual, that bird was either trapped and held temporarily in captivity to allow a new bird to visit the flowers, or the entire setup was moved into another bird's territory.

Aviary experiments

The field experiment allowed us to assess bird behavior in a natural context, but did not reveal which ant cues (visual, tactile, or gustatory) elicited the bird's responses. Ants are often visible on the flowers on which hummingbirds feed and may physically block the corolla of the flower. If they are inside the flower when a hummingbird inserts it tongue into the nectary, they may not be visually observable to the birds, but the birds may instead be able to feel or taste the ants. To further investigate how hummingbirds respond to these various cues provided by Argentine ants at food resources, we conducted a series of experiments in an aviary, using ten captive hummingbirds for each experiment.

All birds were banded and released at the end of the study. Because the birds were caught on campus, they all likely had prior experience with Argentine ants. All trials took place in one of two outdoor aviaries that contained between two and four Anna's and Black-chinned hummingbirds at a time. One aviary measured 3.6 m × 6 m ×

1.8 m tall and the second 2.7 m × 4.3 m × 1.8 m tall, which was enough space to house several hummingbirds at a time, each with their own perch. The birds were marked and thus individually identifiable. Each hummingbird was trained to use a 10-ml syringe as a feeder prior to being placed in the aviary. Similar to the field experiments, multiple birds were present in the same aviary and could visit both feeders during the experiments. The two feeders were positioned close enough to each other that the birds treated them as a single resource (i.e., like multiple flowers on a single inflorescence). While birds may have learned where the feeders were from observing each other, every bird probed each feeder itself and then made decisions as to which feeder to continue to visit, thus we considered each bird to be an independent sample. In the rare cases (2 of 101 trials) when one bird became territorial and began to prevent others from feeding at the feeders, the trial was discarded and the aggressive bird was removed from the experiment prior to beginning another trial.

For the experiments described below, we placed control and experimental feeders 10 cm apart, thus ensuring that the birds treated them as a single resource patch. Both feeders were suspended from the ceiling of the center of the aviary with an ant guard treated with pyrethrin (Perky Pet #245L), which kept wild ants from crawling onto the feeders and prevented experimental ants from escaping. The feeders were tilted down at a 35–45° angle such that gravity ensured the sucrose solution was always accessible.

The experimental feeder presented ant cue(s), while the control feeder lacked any ant cues. Each feeder consisted of a 10-cm^3 syringe filled with fresh 15% (*w*/*w*) sucrose solution suspended at a height of ~ 1.5 m. In some ways, this setup mimicked flowers that hummingbirds would feed on in the wild, in that the tip of the syringe did not contain sugar water (like a corolla of a flower), so the bill had to be physically inserted into the syringe to feed. Trials were conducted between 0930 and 1230 hours. Each trial consisted of two 30-min consecutive observation periods; the location of the ant cue feeder was randomly selected for the first period and switched for the second period, in order to control for any location bias. We noted the time and duration of each visit to a feeder and the identity of the visitor. We considered a visit to occur when a bird approached the feeder and inserted its bill into the syringe tip to feed, for > 1 s. Each bird participated in at least three trials on different days.

All ant cues

We used the setup described above to test hummingbirds' reactions to all the possible cues that ants provide about their presence ("ants feeding" in Table 1). For the first treatment, the experimental feeder was loaded with 20–25 ants, with an ant barrier (fluon) to prevent ants from leaving the feeder but which allowed ants to freely feed on the sugar solution. This provided tactile, gustatory, and visual cues to the hummingbird that ants were present on one of the feeders. In 60-min trials as described above, we noted the time, number and duration of each visit to the feeder as well as foraging behavior from each bird in the aviary.

Visual cues only

Ants crawling on the corolla of a flower will often be the first visual cue a hummingbird could use as an indication that ants are present in a flower and could cause a bird to reject that flower without attempting to feed. To determine how hummingbirds responded to visual cues only, without directly competing with ants for access to the nectar solution, we used the same setup as described above except that each feeder was wrapped in gauze soaked in a 15% sucrose solution and the feeder containing ants was treated with fluon to prevent ants from either leaving the feeder or accessing the tip of the syringe. This setup allowed ants to roam freely around on the feeder, and feed from the sucrose-soaked gauze, but not access the sucrose solution or come into physical contact with hummingbirds utilizing the feeder. Hummingbirds could see the ants, but would receive no tactile or gustatory cues when they went to feed at the feeder. Observations and data were collected as described above for aviary experiments.

Gustatory cues only I: dead ants

In many cases, ants in a flower will be visually hidden by the corolla, thus providing only gustatory and tactile cues as to their presence at the resource. Ants also sometimes drown in nectar sources. To assess whether encountering the taste of Argentine ants at a feeder affects hummingbird acceptance of a resource, we observed hummingbird response to whole dead ants placed in the nectar solution immediately prior to the experiment. One feeder consisted of 10 ml of a 15% (*w*/*w*) sucrose solution with 100 whole Argentine ants and one feeder with 10 ml of a 15% sucrose solution. Both feeders were wrapped in white tape to hide the sucrose solution from view, so that the birds could not view which solution had dead ants in it. Because the dead ants floated at the top of the nectar reservoir, furthest from the feeding tip, there was no opportunity for the birds to touch or lick the ant bodies. In this way, only fresh gustatory cues were available to the hummingbirds to use to detect ants in the feeder. Observations and data were collected in 60-min trials as described previously for aviary experiments.

Table 1 Types of ant-derived cues available in each experiment and whether behavioral avoidance was observed

Experiment	Cue type				Behavioral avoidance?
	Visual	Olfactory	Tactile	Gustatory	
Field	X	X	X	X	Yes
Ants feeding	X	X	X	X	Yes
No ant access	X	X			No
Dead ants in nectar				X	Yes
Prior ant feeding		X		Possible	No

Gustatory cues only II: ant contamination

Ants may not necessarily need to be physically present on a flower to influence hummingbird behavior. In the course of feeding on nectar, they may contaminate the nectar with microbiota or hydrocarbons from their cuticle, feces, or by depositing pheromones. While hummingbirds are not known to have a well-developed sense of smell, they do have well-developed taste receptors. Therefore, hummingbirds might be able to taste if ants contaminated nectar with such chemicals or waste. To investigate whether Argentine ant feeding on the feeder in some way affected nectar quality or the birds' preference, we tested hummingbird preference for nectar that had never been fed upon compared to that which had been previously fed upon by ants. Using the same setup as in prior experiments, two feeders were presented to 2–4 hummingbirds in an aviary at a time: a control feeder and an ant cue feeder on which wild Argentine ants had fed at for 1 h immediately prior to the experiment. Both feeders were rinsed with hexane, then cleaned with Liquinox to remove any ant cues from previous uses. The ant feeder was placed in a small container with 100 Argentine ants, which were allowed to feed on the sucrose solution in the feeder for 60 min, after which all ants were removed. No ants were present on either feeder at the time they were presented to the birds. The only cues available to hummingbirds would be any chemical cues the ants left behind after feeding. Data were collected as described previously.

Statistical analyses

All data were analyzed using generalized linear mixed models (GLMMs) in R v. 3.3.0 (R Core Team 2016). All means are reported ± standard errors (SE).

Field experiments We analyzed forager visitation by including number of flowers visited, number of foraging visits, and total time spent feeding as y responses, while ant treatment and treatment position (left or right) were fixed effects, and date and individual forager identity (including species and sex) were random effects.

Aviary experiments Using GLMMs, we analyzed forager visitation using Poisson error structure with the number of visits per individual was the dependent variable, ant treatment crossed with forager identity and trial number were fixed effects, and date and time were random effects. Significant fixed effects were subsequently analyzed by comparing least square means (Kuznetsova et al. 2013). To assess the effect of ants on visit duration, we looked both at (1) average visit duration per trial per bird and (2) total duration of all visits per trial per bird. For both cases, the duration measure was the dependent variable, ant treatment crossed with forager identity and trial number were fixed effects, and date and time served as random effects. To assess ant effects on resource consumption, volume consumed per feeder per trial was the dependent variable, with ant treatment and trial number as fixed effects, and date and time served as random effects.

Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Results

Field experiments

Wild hummingbirds feeding on flower cuttings showed a clear preference for flowers with no ants (Fig. 1). Hummingbirds approached and hovered in front of both sets of flowers, cocked their head to the side, and appeared to inspect individual flowers in each clump, but usually jerked backed upon contact with ants, or would briefly probe at flowers (for < 1 s) before moving on to ant-free flowers. Individual wild hummingbirds made over ten times as many visits (Fig. 1a: $F_{1,33} = 31.159$, $p < 0.0001$) and spent eightfold more time overall per observation period (Fig. 1b: $F_{1,33} = 38.641$, $p < 0.0001$) visiting the ant-free flowers as compared to the flowers experimentally inoculated with ants. There was no effect of treatment position (e.g., flowers on the left or flowers on the right) for any of the variables examined ($p > 0.20$).

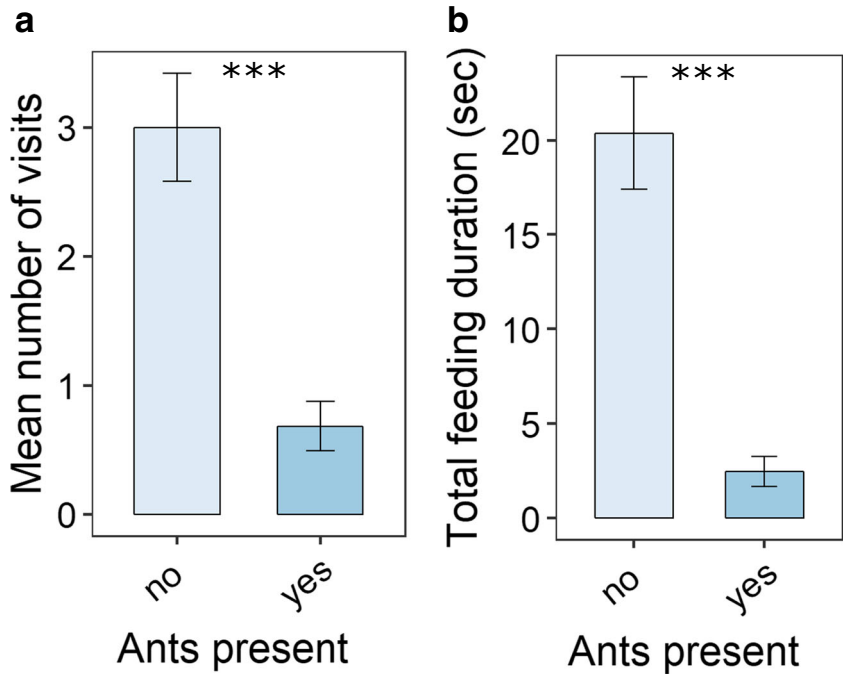

Fig. 1 Field experiments. Wild hummingbirds preferred flowers that lacked live ants ($N = 10$ individuals). **a** Mean number of visits to flowers with or without Argentine ants. **b** Total amount of time per trial spent feeding on flowers. *** indicates $p < 0.0001$. Error bars indicate SE

This effect was not due to exploitation competition: neither feeders nor flowers were ever fully depleted during foraging trials and any consumption by ants was too small to detect (i.e., < 0.1 ml).

All ant cues

In the aviary, birds showed a preference for feeders with no ants, relative to feeders with live ants (all three ant cues

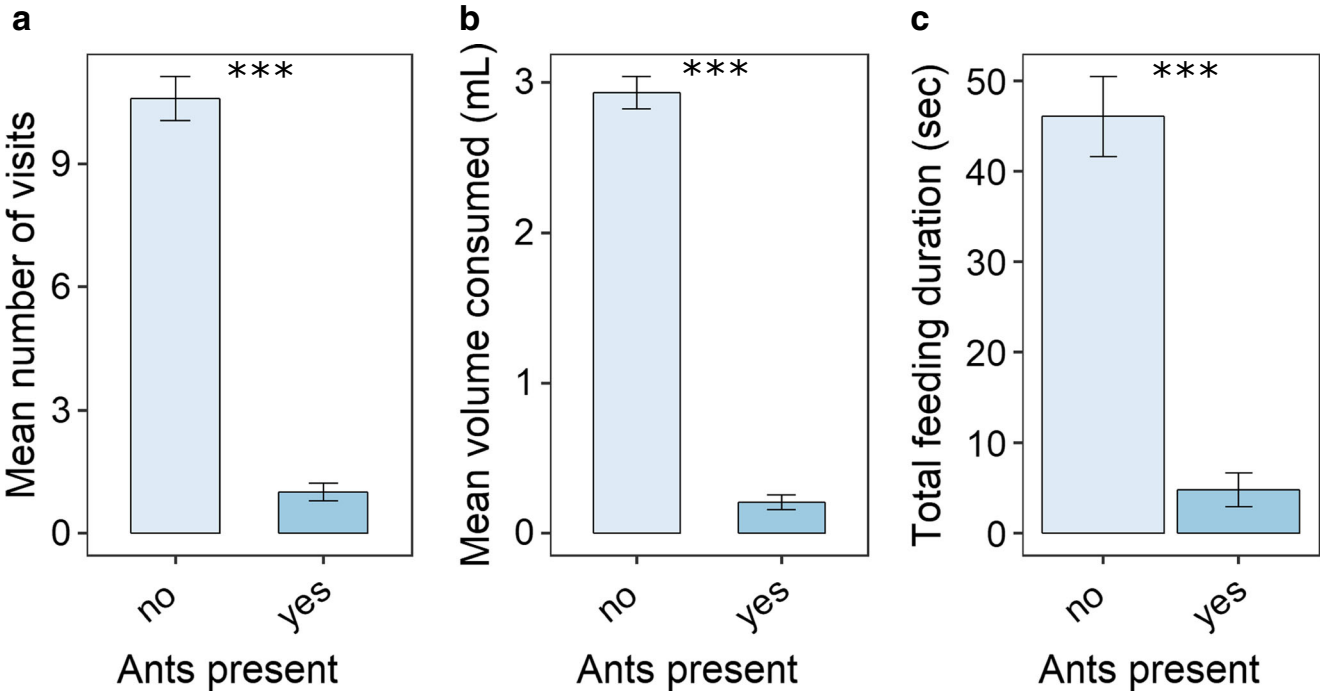

Fig. 2 All ant cues. In aviaries, hummingbirds preferred feeders that lacked live ants feeding on sucrose solution. **a** Mean number of visits to feeders with or without live ants for hummingbirds foraging in an aviary ($N = 12$ individuals, at least three observations/individual). **b** Volume of sucrose solution consumed per trial by ant treatment. $N = 12$ individuals, with at least three trials per individual. $F_{1,114} = 678.73$, $p < 0.0001$. **c** Hummingbirds preferentially spent time feeding upon feeders that lacked ants ($F_{1,95} = 464.84$, $p < 0.0001$). *** indicates $p < 0.0001$. Error bars indicate SE

present). Hummingbirds made more than tenfold more visits to control feeders than feeders with live ants (Fig. 2a; $F_{1,109} = 232.44$, $p < 0.0001$). There was an effect of individual (Fig. S1; $F_{11,109} = 4.86$, $p < 0.0001$) and individual × treatment interaction ($F_{11,95} = 6.98$, $p < 0.0001$), but no effect of trial ($F_{1,109} = 0.452$, $p = 0.50$), meaning birds preferentially visited the feeder without ants no matter what position it was in. Furthermore, hummingbirds consumed more than 14-fold more nectar (Fig. 2b) from control feeders compared to those with live ants present, with no effect of trial ($F_{1,114} = 0.059$, $p = 0.44$). Foragers spent nearly tenfold more time feeding at control feeders that lacked live ants as compared to feeders with ants (Fig. 2c). There was an effect of individual ($F_{11,91} = 3.63$, $p = 0.00028$), but all birds spent more time on control vs. treatment feeders (range 1.6- to 46-fold more time). There was an interaction of individual and treatment (Fig. S2; $F_{11,95} = 6.98$, $p < 0.0001$), though this interaction was driven predominantly by one bird, ANHU6, whose preference for control feeders was less strong than those of all other birds (Fig. S2). When ANHU6 was excluded from analysis, the individual by treatment interaction became non-significant ($p > 0.3$) while no other statistical patterns were affected. As with the experiment above, the amount of nectar consumed by ants was negligible.

Visual cues only

Birds showed no preference for either feeder when only the visual cue of ants on the syringe was present, without gustatory or tactile cues, or able to access the sucrose solution. There was no effect of ant treatment on number of visits to a feeder (ants 3.51 ± 0.27 visits vs. no ants 3.86 ± 0.26 visits, $F_{1,91} = 0.844$, $p = 0.36$); volume consumed (ants 2.0 ± 0.09 ml vs. no ants 2.15 ± 0.08 ml, $F_{1,105} = 2.46$, $p = 0.12$); or total duration spent feeding (ants 15.53 ± 1.14 s vs. no ants 18.83 ± 1.37 s, $F_{1,88} = 3.42$, $p = 0.07$). The birds were not deterred by the visual cues of ants on the physical feeder when ant cue was not present at the actual access point, where the bird's bill contacts nectar.

Gustatory cues I: dead ants

Hummingbirds avoided feeding on sucrose solution containing fresh dead ants but that lacked any visual cues of ants. Birds visited the non-ant feeder twice as often as the ant feeder (Fig. 3a). There was a significant effect of individual (Fig. S3; $F_{11,47} = 3.69$, $p = 0.0008$) but no effect of trial ($F_{1,47} = 0.226$, $p = 0.64$) on the number of foraging visits. Moreover, these birds consumed more than 3.5-fold of the sucrose solution (Fig. 3b; 2.80 ± 0.18 ml vs. 0.78 ± 0.11 ml). Birds also spent threefold more time feeding on sucrose solution that lacked dead ants (Fig. 3c; 20.09 ± 1.29 s vs. 7.15 ± 0.91 s) as compared to the feeder with ants in the sucrose solution, although there was an effect of individual ($F_{11,20} = 2.77$, $p = 0.022$). While *all* individuals spent *more* time feeding on control feeders than feeders with dead ants in the sugar solution, the degree to which they did so varied (Fig. S4).

Gustatory cues II: ant contamination

While hummingbirds avoided feeders actively being fed upon by ants and those where ants were in the nectar in the previous two experiments, birds did not discriminate between feeders from which ants had previously fed and feeders where ants have never visited. There was no effect of ant treatment on number of visits to a feeder (ants 5.33 ± 0.57 visits vs. no ants 5.49 ± 0.57 visits, $F_{1,60} = 0.10$, $p = 0.76$); volume consumed (ants 2.07 ± 0.15 ml vs. no ants 1.91 ± 0.11 ml, $F_{1,72} = 1.13$, $p = 0.29$); or total duration spent feeding (ants 21.04 ± 1.96 s vs. no ants 19.83 ± 1.74 s, $F_{1,60} = 0.23$, $p = 0.64$).

Discussion

Invasive ants can have strong negative impacts on pollinators and ant pollination is relatively rare (Hölldobler and Wilson 1990; Rico-Gray and Oliveira 2007; Dutton and Frederickson 2012). By competing with native vertebrate pollinators for floral rewards, the presence of ants in flowers can lead to shifts in pollinator behavior (Junker et al. 2010), decreased visitation, and lower seed set (LeVan et al. 2014) for some plants. In some cases, large-bodied invasive ants decrease the pollination and seed dispersal services of native vertebrate pollinators (Hansen and Muller 2009). Argentine ants were present on many species of flowers across campus where these birds were captured, and occasionally, ants overtook the feeders at which we caught birds. Therefore, we suspect that all the birds used in the experiment had prior experience with Argentine ants prior to their participating in foraging experiments. Wild hummingbirds avoided manipulated flowers with ants crawling on and in them to a similar degree as our captive feeder experiments, confirming that the observed behavioral avoidance is exhibited by free-foraging birds. Most birds approached the manipulated flowers and investigated multiple blossoms before finally selecting one for feeding. When attempting a floral visit, birds would quickly retract their bill from the flowers or feeders containing ants, although it was impossible to assess the exact cause of this response. Future experiments explicitly testing naiveté to ants and the role of learning in hummingbird's response to ants would provide further insights into the mechanisms underlying behavioral avoidance and ultimately, displacement from floral resources.

Here, hummingbirds avoided Argentine ants at manipulated flowers in the field, feeders with ants able to access the sucrose solution, and feeders with drowned ants. The birds did not exhibit a preference between feeders with ants and without ants

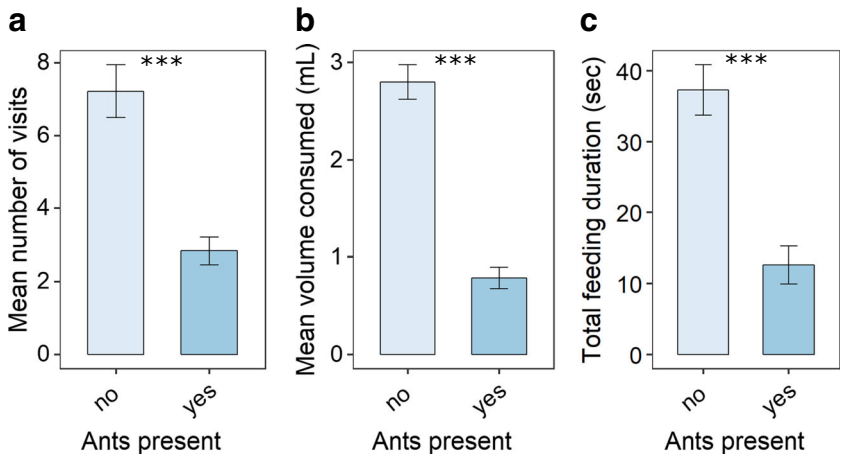

Fig. 3 Gustatory cues: dead ants. In aviaries, hummingbirds preferred feeders that lacked freshly killed ants in sucrose solution. **a** More visits were made to feeders with or without ants in the sucrose solution for hummingbirds foraging in an aviary ($N = 12$ individuals, at least three observations/individual) ($F_{1,47} = 39.43$, $p < 0.0001$). **b** Volume of sucrose solution consumed per trial by ant treatment. $N = 12$ individuals, with at least three trials per individual. More nectar was consumed from feeders with no ants ($F_{1,61} = 121.15$, $p < 0.0001$). **c** Hummingbirds preferentially fed upon feeders that lacked ants ($F_{1,42} = 119.84$, $p < 0.0001$). *** indicates $p < 0.0001$. Error bars indicate SE

if the ants could not access the sucrose solution nor did they distinguish feeders to which ants had had prior access ("ant contamination") but were not present during the trial. In total, our results suggest tactile and gustatory cues are most important to hummingbirds when encountering ants, with no single cue being the sole factor of discrimination between feeders. Visual cues alone did not deter hummingbirds from visiting feeders, while tactile and gustatory cues did (Table 1).

The importance of vision to foraging hummingbirds has received a lot of attention. Hummingbirds rely on visual cues to remain docked into feeders or flowers (Goller and Altshuler 2014), to find and select food sources (George 1980; Healy and Hurly 2003), and navigate a complex and shifting environment (Dakin et al. 2016). In a clear demonstration that visual cues of ants alone had no impact on foraging behavior, the birds in our study did not avoid feeders that had ants crawling on them—as long as ants were unable to access the sucrose solution. Given this reliance on vision, it may be somewhat surprising that visual cues alone were not sufficient to elicit feeder rejection. This is unlikely to be the result of the ants being invisible to hummingbirds, because hummingbirds routinely catch and consume insects of similar or smaller size to Argentine ants (e.g., Clark 1902; Beal and McAtee 1912; Legg and Pitelka 1956) either by fly catching or gleaning the insects off leaves (Clark and Russell 2012). Rather, this may be explained in part by the fact that hummingbirds must insert their bills into flowers in order to reach the nectar, such that ants visible on the outside of a flower may not equate with ants inside the flower which could interfere with a bird's ability to obtain nectar from that flower.

Argentine ants are avid nectar foragers, capable of drawing down nectar resources in flowers over time and with continual recruitment of workers (Buys 1987). Unlike in a wild setting, birds never came into contact with ants in the visual cues only experiment, and the feeders always contained sugar solution regardless of whether or not ants were present on the feeder. Hummingbirds thus received no negative feedback when they attempted to feed from the feeder with ants. Because we did not test the effects of exploitative competition on hummingbird foraging choice, it is possible that visual cues would be more important in natural settings where flowers with ants on them may differ in their nectar volume.

Hummingbirds will make repeated exploratory visits to non-rewarding flowers if they have sufficient energy (Hurly 1996). During testing, hummingbirds were often observed flying up to the feeders and hovering in front of it, cocking their head to the side and sometimes briefly (< 1 s) probing at parts of the feeder, which would be consistent with visual inspection of a feeder. However, they would not always choose the ant-free feeder after visually inspecting them. It is possible that the birds were either repeatedly testing the feeders to determine if they were suitable to feed on (as they have been shown to do with taste: (Bacon et al. 2011) or that they did not rely wholly on visual cues in order to avoid ants on feeders.

While we did not test the importance of tactile cues in isolation, we infer from our results that tactile cues play a strong role in determining hummingbird response to ants. Hummingbirds routinely come into contact with the stamens and pistil of flowers with their bills and foreheads when nectaring (e.g., Grant and Grant 1966, 1968); therefore, we would not expect them to instinctively respond negatively to any foreign objects touching their bill. Because tactile cues do not play a major role in maintaining contact with resources during hovering (Goller et al. 2017), it is unlikely that Argentine ants are interfering with a bird's ability to remain

docked to the feeders. This suggests that something about the ants' tactile feedback repulses hummingbirds, potential stimuli may be the ants crawling on the bird or even biting the bird. One bird, ANHU6 (Fig. S1), was the most tolerant of ants, spending almost 40% of its foraging time at the feeder containing ants. While further research may investigate the causing stimuli, the response patterns observed are consistent with interference competition, with Argentine ants limiting hummingbird access to the sucrose solution by their physical presence.

Hummingbirds can use odor to differentiate between rewarding and non-rewarding resources (Goldsmith and Goldsmith 1982; Ioale and Papi 1989), though the extent to which they rely on olfaction during foraging is not clear, as many hummingbird-pollinated plants exhibit little or no odors (Knudsen et al. 2004). In the ant contamination and gustatory cues only experiments, chemical cues were available to the birds in the form of (1) chemicals introduced by ants into the sucrose solution, (2) pheromone trails laid down by live ants, or (3) microbiota introduced by ants as they fed. Our data show that gustatory detection of chemical cues is the best explanation of the observed foraging responses in the gustatory cues only I and II experiments (Table 1). While hummingbirds responded strongly to the feeders with dead ants in nectar, this avoidance was only 25% as strong as their response to feeders with live ants actively feeding. Vannette et al. (2012) found that bacterial contamination of nectar reduced nectar consumption by hummingbirds. Since hummingbirds must sample a sugar solution before they can detect changes in taste, taste appears to play a secondary role to other cues in determining their reaction to ant-visited flowers. Past research has investigated hummingbirds ability to taste different sugar concentrations and types (Stiles 1976), but to our knowledge, this is the first documented instance of hummingbirds discriminating between sucrose solutions based on the taste of contaminants by ants present in the solution.

The hummingbirds used in our study did not discriminate between feeders upon which ants had fed and those they did not. The presence of ants in and at the edge of the nectar could introduce cuticular hydrocarbons, yeasts, or bacteria into the sucrose solution, which hummingbird could potentially taste and dislike. While such contaminants may be distasteful, we may not have detected aversion if the relatively small number of ants accessing the sucrose solution, compared to the large volume of liquid (10 ml) diluted any effect of the ants' presence. Moreover, the 1-h time period in which the sucrose solution was exposed to ants may have been insufficient for any contamination to build to levels detectable by a hummingbird. While ants are known to recruit to resources using pheromone trails (Deneubourg et al. 1990) and contaminate floral nectar with their microbiota (de Vega and Herrera 2012), this did not seem to affect subsequent hummingbird foraging—at least in the short term. Therefore, it is most likely that the birds in this study were unable to detect such recent resource occupation by ants.

Conclusions

Hummingbirds and invasive Argentine ants compete for the same floral resources. Given a choice, both wild-caught and wild, free-foraging hummingbirds avoided flowers and feeders with live ants or ants drowned in the sucrose solution. Our mechanistic experiments provide strong support for the potential of interference competition at floral resources, with ants limiting access to flowers and feeders at which they were present. Tactile and gustatory cues appear to be the most important mechanisms by which ants are detected and avoided by hummingbirds. The degree to which Argentine ants and hummingbirds naturally compete at flowers is currently unknown and has received little attention. However Argentine ants are insidious invaders capable of invading both urban and natural areas used by hummingbirds, and thus have the potential to cause shifts in hummingbird foraging behavior across their range.

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Compliance with ethical standards

Conflict of interest There are no conflicts of interest to report.

Ethical approval This study followed all applicable national and institutional guidelines for the care and use of animals and was approved by the University of California, Riverside Institutional Animal Care and Use Committee (IACUC protocol #20130018). Hummingbirds were captured from the wild on the UCR campus under USFWS Bird Banding permit #23516 and CA Fish and Wildlife Permit #SC-006598.

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