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ORIGINAL ARTICLE

Olfaction foraging in visually oriented tropical arboreal ants *Oecophylla smaragdina*: Implications for insect predation studies using artificial sentinel prey

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Abstract

Because of the brief and cryptic nature of predatory behavior, sentinel prey has been widely adopted as an indirect way to identify predators and understand trophic interactions. However, sentinel prey presents only static visual cues, potentially biasing toward visually oriented predators while ignoring those that utilize other sensory cues for foraging. Despite this, the effectiveness of sentinel prey has rarely been tested. In this study, we focused on the weaver ant *Oecophylla smaragdina* Fabricius (Hymenoptera: Formicidae), a keystone predator widespread in the Asian and Australian tropics. As this species has large eyes and is known to visually navigate in its arboreal habitats, we hypothesized that it relies on visual cues to forage and that its predatory behavior will be captured by caterpillar-shaped sentinel prey. Ants were collected as colonies, and preference trials on baits were conducted using combinations of olfactory and static visual cues, including the caterpillar shape. Surprisingly, *O. smaragdina* showed little or no preference for baits in the absence of olfactory cues and did not differentiate the shapes of baits regardless of olfactory cues. Our results indicate that *O. smaragdina* is likely to make predatory decisions based primarily on olfactory cues, whereas visual cues might be used for other behaviors. Furthermore, *O. smaragdina* is likely to be left out by the predation studies using sentinel prey models, which is particularly alarming considering the dominant role of this species in the trophic interactions of tropical rainforests. Our study demonstrates that morphological characteristics, arboreal habitats, and visually oriented behavior do not necessarily suggest the use of static visual cues for predatory decisions. We suggest that sentinel prey models should not be used alone when the dominant predators are unlikely to use visual cues to make predatory decisions.

KEYWORDS
arboreality, dummy caterpillar, foraging behavior, Formicidae, Hymenoptera, olfaction, preference trial, sensory cues, vision, weaver ant

INTRODUCTION

Predators are a key ecosystem component that regulates population dynamics and ecosystem functions through an evolutionary arms race between predators and their prey (Peckarsky et al., 2008). Besides directly consuming prey, predators can indirectly shape prey development, behavior, dispersion, and aggregations (Lima, 1998; Peckarsky et al., 2008; Preisser et al., 2007; Schmitz et al., 2004). Predation on herbivores, one of the major sources of prey for many predators, indirectly affects plants and primary production through trophic cascading (Finke & Denno, 2004, 2005). Despite the ecological significance of predation, the cryptic and transient nature of predation poses a challenge to understanding its dynamics, especially at larger spatial and temporal scales (Crawley, 2009; Howe et al., 2009).

Live prey may be used as baits to directly measure predation events; however, it is difficult to record the identity of the predators without intensive observation or video recordings (Friend, 1995). Alternatively, the use of sentinel prey models, such as plasticine, molded to resemble prey, has been used to indirectly infer the coarse identity of the predators (e.g., birds, ants, wasps, and snails) using imprints left from attacks (Howe et al., 2009; Rößler et al., 2018). This method has been widely adopted in ecological studies (Liu et al., 2020; Roslin et al., 2017), and the flexibility of this method has given rise to experimental manipulations of numerous visual cues related to finding prey (e.g., texture, color, and shape) to understand the sensory ecology of the animals in question (Pan et al., 2021; Sam et al., 2015; Zvereva et al., 2019).

It is important to note that although sentinel prey models visually resemble natural prey (from a human perspective), they only provide static visual cues and lack motion, chemical, and tactile cues of natural prey. The sensory cues utilized by predators vary depending on the predator identities and habitats where the interactions occur (Kielty et al., 1996; Short, 2020; Webster et al., 2007). Thus, the accuracy of predation rate inferred from artificial sentinel prey may be highly variable among habitats where predators may or may not utilize static visual cues to distinguish the shapes and colors of their prey. For example, invertebrate predators vary widely in their sensory cues used for foraging compared with vertebrate predators that rely on visual cues (Jackson & Pollard, 1996; Klärner & Barth, 1982; Wen et al., 2017). However, few studies have examined the foraging ecology of dominant invertebrates in a given ecosystem so that the potential bias introduced by artificial sentinel prey could be corrected. Testing their response to artificial sentinel prey will provide direct insights into the effectiveness of this method in understanding predation.

Due to their ubiquitous occurrence and abundance, ants are an exemplar of the major predator groups in tropical regions (Floren et al., 2002; Jeanne, 1979; Schmitz et al., 2000). As artificial sentinel prey are generally placed on the leaves and stems of plants (Howe et al., 2009), marks left by ants can be attributed to those with arboreal affinities. Although several studies concluded that ants were responsible for a large portion of attacks on plasticine caterpillars (e.g., Leles et al., 2017; Liu et al., 2020; Tiede et al., 2017; Tvardikova & Novotny, 2012), their conclusions were based on the bite marks on the plasticine models and they did not directly observe ants attacking them. A recent study by Zvereva and Kozlov (2023) found that arboreal ants did not attack plasticine caterpillars in boreal forests. This leaves a question as to whether arboreal ants of tropical regions, especially dominant species such as weaver ants, *Oecophylla smaragdina* Fabricius (Hymenoptera: Formicidae), attack plasticine models. *O. smaragdina* is one of the dominant species in the Indo-Australian tropics and is known as one of the most active predators in the agricultural and natural systems of this region (Forbes & Northfield, 2017; Tsuji et al., 2004). If plasticine models fail to capture predation by this arboreal ant species, it has profound implications not only for ecological studies but also for the management of pest control in production lands where plasticine models are used to monitor the effectiveness of predation by *O. smaragdina* (Denan et al., 2023).

Several pieces of evidence have indirectly suggested that, unlike many ant species that rely on olfactory cues, *O. smaragdina* relies on visual cues for foraging (Lokkers, 1990; Mishra & Bhadani, 2017), albeit not tested with behavioral trials. Studies have demonstrated that their ability to discriminate patterns, well-developed eye morphology, and diurnal foraging lifestyle were correlated with the necessity of vision during foraging (Lokkers, 1990; Mishra & Bhadani, 2017). The arboreal nature of this species also corroborates with their visual capacity: Canopy-dwelling organisms use visual cues due to better light conditions and rapid evaporation of chemical cues in the canopy (Loiselle & Farji-Brener, 2002; Short, 2020; van Oudenhove et al., 2011). However, no experimental studies have been conducted to directly examine the utilization of visual cues in the food preference of *O. smaragdina* (but for the role of visual cues in navigation during foraging, see Jander & Jander, 1998).

In this study, we aim to examine the effectiveness of the use of sentinel caterpillars in estimating predation rates using *O. smaragdina* as a model. We employed manipulative experiments in controlled laboratory conditions using both edible baits and plasticine sentinel models to test the relative importance of olfactory and static visual cues for their predatory decisions, connecting the sensory ecology of the weaver ant with the effectiveness of sentinel caterpillars in capturing predation by them. We hypothesized that *O. smaragdina* prioritizes visual cues over olfactory cues for predation. Furthermore, because sentinel caterpillars are visually effective in resembling prey, *O. smaragdina* preferentially attacks a caterpillar shape over other shapes. That is, artificial caterpillar models effectively capture their predatory behavior.

MATERIALS AND METHODS

Ant collections

Nests of *O. smaragdina* were collected in Mengla County, Xishuangbanna Dai Autonomous Prefecture, located in the monsoonal tropical region of southern Yunnan Province, China, in November 2020. We included ants from two distinct habitats to explore the connection between the difference in ecological factors among habitats and the potential adaptation by *O. smaragdina* in different populations. We visited two locations representative of the dominant habitats of *O. smaragdina*: a tropical rainforest in Bubeng (21°36′ N, 101°34′ E) and a rubber plantation located within Xishuangbanna Tropical Botanical Garden (XTBG; 21°55′ N, 101°15′ E) in Menglun. In total, three leaf nests were collected from the rainforest canopies using a canopy crane (80 m high, 60 m jib length) established in Bubeng that allowed us to access canopy trees at various heights. All three ant nests were collected from one of the dominant canopy tree species in this region, *Parashorea chinensis* Y.K. Yang & J.K. Wu (Dipterocarpaceae), at heights over 50 m above ground using a pruning knife. In the rubber plantation, we collected three ant nests on rubber trees (*Hevea brasiliensis* Müll.Arg., Euphorbiaceae) at approximately 5–10 m high by cutting the nests from tree branches using a pruning knife. All nests were kept separately in plastic boxes during transfer and provided with sugar water.

Ant nests from the rainforest were larger (130–300 mm in diameter) than those collected from the rubber plantation (110–240 mm in diameter). We, therefore, confirmed the species identity using DNA barcoding (Figure S1). Additionally, as relative eye size may be associated with ants' visual capacity, we measured and confirmed that relative eye size did not vary between ant nests and collecting localities (Figure S2).

Experimental design

All behavioral trials were conducted within 2 weeks of the ant nest collections. We designed four manipulative experiments to test (1) the importance of visual shape in the absence of olfactory cues (visual-only experiment, Figure 1A), (2) the importance of olfactory cues and natural prey (olfaction and natural shape experiment, Figure 1B), (3) the interaction between visual and olfactory cues (olfaction and caterpillar shape experiment, Figure 1C), and (4) the effects of olfactory cues without visual cues (darkroom experiment, Figure 1D). All experiments were conducted within a plastic box with each ant colony placed in the middle, and four bait treatments at each of the four corners of the box (Figure 1). Bait treatments were placed in transparent polypropylene plastic tubes (see below for more details).

As we had three large and three small nests collected from the two habitats, we used a large box (55 × 37 × 38 cm) for large nests and a small box (18 × 28 × 18 cm) for small nests. We left the baits in large tubes (120 mL; 44 mm

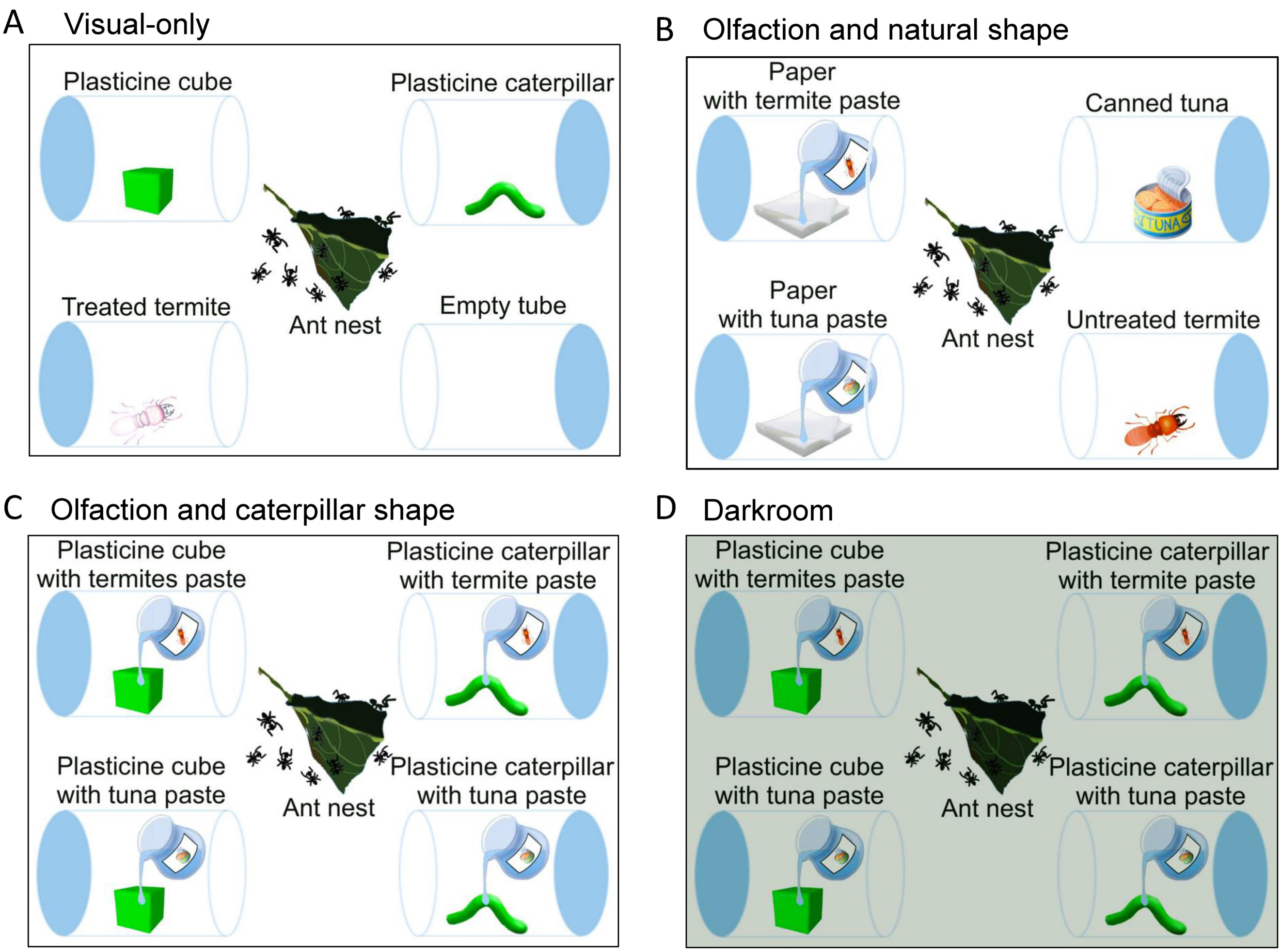

FIGURE 1 Conceptual illustrations depicting the design of the four experiments: (A) visual-only experiment, to assess the effect of visual cues; (B) olfaction and natural shape experiment, to test the effect of olfactory cues in the presence or absence of natural visual cues of the baits; (C) olfaction and caterpillar shape experiment, to test the interaction between olfactory cues and abstract visual cues of plasticine models; and (D) darkroom experiment, to test the effect of olfactory cues in the absence of visual cues.

diameter, 105 mm depth) for the large boxes and small tubes (5 mL; 15 mm diameter, 49 mm depth) for the small boxes. Non-drying sticky glue (tanglefoot) was applied to the rim of the box (approximately 2–4 cm width) to prevent ants from escaping. Before the experiments, ants were starved of food for at least 24 h before each experiment began but provided with water. To quantify the ants' preference for the various types of bait, we recorded the number of ants present inside each tube every 10 min for 1 h. In addition, we recorded the time when an ant first entered the tube.

Visual cues

The visual-only experiment (Figure 1A) tested the effect of prey shape in the absence of olfactory cues using the bait treatments consisting of (1) plasticine caterpillars, (2) plasticine cubes, (3) dead termites treated by critical point drying to minimize olfactory cues, and (4) an empty tube as a control. We used plasticine caterpillars, a classic model method widely used in ecological and behavioral studies (Rößler et al., 2018), and plasticine cubes, which differ from plasticine caterpillars by shape only. To construct plasticine baits, we used non-toxic green plasticine (Newplast; Newclay Products, Newton Abbot, UK). Plasticine caterpillars (3 mm diameter × 30 mm length) were molded using a metal syringe (a syringe used for pastry molding), and the same quantity of plasticine was molded into cubes (7 mm length) by hand. We selected the termite species *Odontotermes yunnanensis* Tsai & Chen (Isoptera: Termitidae) for use in our experiments. This termite species is a preferred prey species for *O. smaragdina* under natural conditions in the study area (X. Shen, pers. obs.). Termites were collected from their nests, and only worker termites were collected and killed in a freezer. To minimize olfactory cues from termites, we prepared specimens by using a Leica EM CPD300 critical point dryer (Leica Microsystems, Wetzlar, Germany). Termite specimens were preserved in absolute ethanol, and critical point drying was conducted using the following program: cooling at 15°C, slow CO_2 admittance with 120 s of delay, 16 exchange cycles with the speed set to "6," followed by a slow heating process at 40°C, and a slow gas discharge. This method removes polar and non-polar compounds while preserving organisms' natural shape. Although we did not quantify the amount of olfactory cues this process reduced, supercritical drying has been proven to be effective for chemical extraction as well as effective olfactory cue removal in several fields (Brunner, 2010; Maheshwari et al., 1995). Empty tubes were used as a control to test whether ants were attracted to the plastic tubes per se. The same control was not replicated in the following three experiments, as the same ant colonies were subjected to the four experiments. For large nests, we placed three sentinel models (caterpillars or cubes) or 30 termites in each tube. For small nests, one sentinel model (caterpillars or cubes) and 10 treated termites were used.

Olfactory and visual (natural shape) cues

The olfaction and natural shape experiment (Figure 1B) tested the importance of visual and olfactory cues using the bait treatments consisting of (1) termite olfactory cues only, (2) tuna olfactory cues only, (3) termite olfactory and visual cues, and (4) tuna olfactory and visual cues. We used canned tuna because it has been a preferred bait in previous studies (Andersen, 1992; Narendra et al., 2012). To prepare olfactory cue treatments, we prepared paste by crushing worker termites or canned tuna using a sterilized pestle and mortar. A small quantity of distilled water was added while crushing the bait until it became fine and smooth. The paste was applied to a filter paper (15 × 15 mm) until saturated. Three pieces and one piece of paper were placed in large and small tubes, respectively. Termite olfactory and visual cue treatment consisted of freshly killed, untreated termites (30 in large tubes, 10 in small tubes). Tuna olfactory and visual cue treatment consisted of canned tuna (1.2 g of tuna in large tubes, 0.4 g of tuna in small tubes).

Olfactory and visual (caterpillar shape) cues

The olfaction and caterpillar shape experiment (Figure 1C) tested the importance of olfactory and visual cues of plasticine models using the bait treatments consisting of (1) tuna paste applied to plasticine caterpillars, (2) tuna paste applied to plasticine cubes, (3) termite paste applied to plasticine caterpillars, and (4) termite paste applied to plasticine cubes. We applied the tuna or termite paste to the plasticine cubes and caterpillars until all surfaces were covered. The larger test tubes contained three sentinel models in each tube, whereas the smaller test tubes contained one sentinel model.

Absence of visual cues

The darkroom experiment (Figure 1D) tested the importance of olfactory cues in the absence of visual cues. To be consistent, the experimental setups were the same as the olfaction and caterpillar shape experiment (Figure 1C) but conducted in a dark room to eliminate visual cues. We used an infrared camera (Sony FDR-AX60) placed approximately 50 cm above the experimental setups to monitor ant behavior. As an important caveat to this experiment, the infrared cameras made it difficult to verify whether ants were inside (or on) the tube. Therefore, for this experiment, we counted the ants overlapped with the tubes in the infrared video clips. Given the imaging difficulties, we did not measure the time when an ant first entered the tube.

Statistical analysis

Statistical analyses were performed using R v.4.1.2 (R Core Team, 2021). We tested the differences in ant preference (i.e., the number of ants in the experimental tubes) to different baits in each of our four experiments. A generalized linear mixed model was used, initially with Poisson distribution, but overdispersion was found when fitting our data using the glmer() function in the lme4 package (Bates et al., 2007). As a result, we fitted our data with a generalized linear mixed model using Template Model Builder in the glmmTMB package (Brooks et al., 2017b) with a negative binomial distribution to account for count data and to control for zero inflation (Brooks et al., 2017a; Hardin et al., 2007). The candidate models in each experiment included the four experimental treatments (bait type), time intervals, habitats (rainforest and rubber plantation from which ant nests were collected), and the interaction between bait type and time as fixed effects. All models were constructed with the identity of ant nests as a random factor, a single zero-inflation parameter applied to all observations, and the size of the nests as an offset in all candidate models (Bolker, 2016). Nest size was estimated in volume (cm^3), and this was included as an offset to relax the assumption that more individuals from larger nests forage and attack baits (Bolker, 2016). The best model was selected based on the lowest AIC and its significant difference from the null model (Arnold, 2010). After the best model was selected for each experiment, type II Wald χ^2 tests were performed to determine the significance of fixed factors using the car package (Fox & Weisberg, 2018). We used the emmeans package to run Tukey's honestly significant difference tests as a post hoc test to identify the pairwise significance between estimated marginal means of treatments (Searle et al., 1980). Model predictions were generated using the best model and the original dataset, with the offset colony size set to 8000 cm^3. The predictions were used to plot the regression lines with the observed data in scatter points.

In addition to the number of ants in the experimental tubes, we also assessed the time when an ant first entered the experimental tubes. Because the nature of the data violated the assumptions of a linear model, we adopted a nonparametric test (Kruskal–Wallis rank sum) to compare the time when ants first arrived among the four types of baits in each experiment.

RESULTS

Visual cues

The estimated marginal mean (±SE) of the number of ants was the highest in the empty tubes (2.12 ± 0.98), followed by the tubes with treated termites (1.85 ± 0.84), plasticine cubes (1.68 ± 0.77), and plasticine caterpillars (1.23 ± 0.57). The best model included bait type, which was marginally significant ($\chi^2 = 8.054$, df = 3, $p = 0.045$). Post hoc analysis suggested significant differences between the empty tubes and the tubes with plasticine caterpillars, but not in other pairs, including the comparisons between the plasticine caterpillars and cubes and between treated termites and empty tubes (Table S1). The effect of time was excluded from the best model, as the number of ants visiting the tubes did not change over time (Figure 2A). No significant difference was found in the time when an ant first entered the experimental tube (Kruskal–Wallis rank sum test: $\chi^2 = 1.384$, df = 3, $p = 0.71$) (Figure 3A).

Olfactory and visual (natural shape) cues

The largest number of ants was observed on papers with tuna paste (5.72 ± 1.93), followed by tuna (3.09 ± 1.08). Termite papers were visited less frequently (1.50 ± 0.55), and untreated termites were hardly visited by ants (0.94 ± 0.36) (Figure 2B). The best model included bait type ($\chi^2 = 123.03$, df = 3, $p < 0.001$) and time ($\chi^2 = 33.96$, df = 1, $p < 0.001$), but no interaction was included. Post hoc pairwise analysis indicated significant differences between tuna and termite baits, regardless of visual cues (Table S1). No significant difference was found in the time when an ant first entered the experimental tube ($\chi^2 = 1.306$, df = 3, $p = 0.73$) (Figure 3B).

Olfactory and visual (caterpillar shape) cues

Aligning with the olfaction and natural shape experiment, plasticine cubes with tuna paste (4.19 ± 1.48) and plasticine caterpillars with tuna paste (3.58 ± 1.26) attracted more ants than plasticine caterpillars with termite paste (1.75 ± 0.64) and plasticine cubes with termite paste (1.63 ± 0.61) (Figure 2C). The best model included bait type ($\chi^2 = 39.34$, df = 3, $p < 0.001$) and time ($\chi^2 = 23.75$, df = 1, $p < 0.001$), but not their interaction. Post hoc pairwise comparisons showed a significant difference between tuna and termites regardless of the plasticine shape (Table S1). Time was also a significant factor in the model, showing the overall increase in the recruitment of ants, especially tuna baits. No significant difference was found in the time when an ant first entered the experimental tube ($\chi^2 = 0.83$, df = 3, $p = 0.84$) (Figure 3C).

Absence of visual cues

The darkroom experiment showed a consistent pattern of the preference for tuna baits, with the largest number of ants visiting tuna cubes (5.03 ± 3.23) followed by tuna caterpillars (3.50 ± 2.26), termite cubes (1.90 ± 1.25), and termite caterpillars (1.87 ± 1.23) (Figure 2D). Both bait type ($\chi^2 = 31.90$, df = 3, $p < 0.001$) and time ($\chi^2 = 38.52$, df = 1, $p < 0.001$) were included in the final model, but not their interaction.

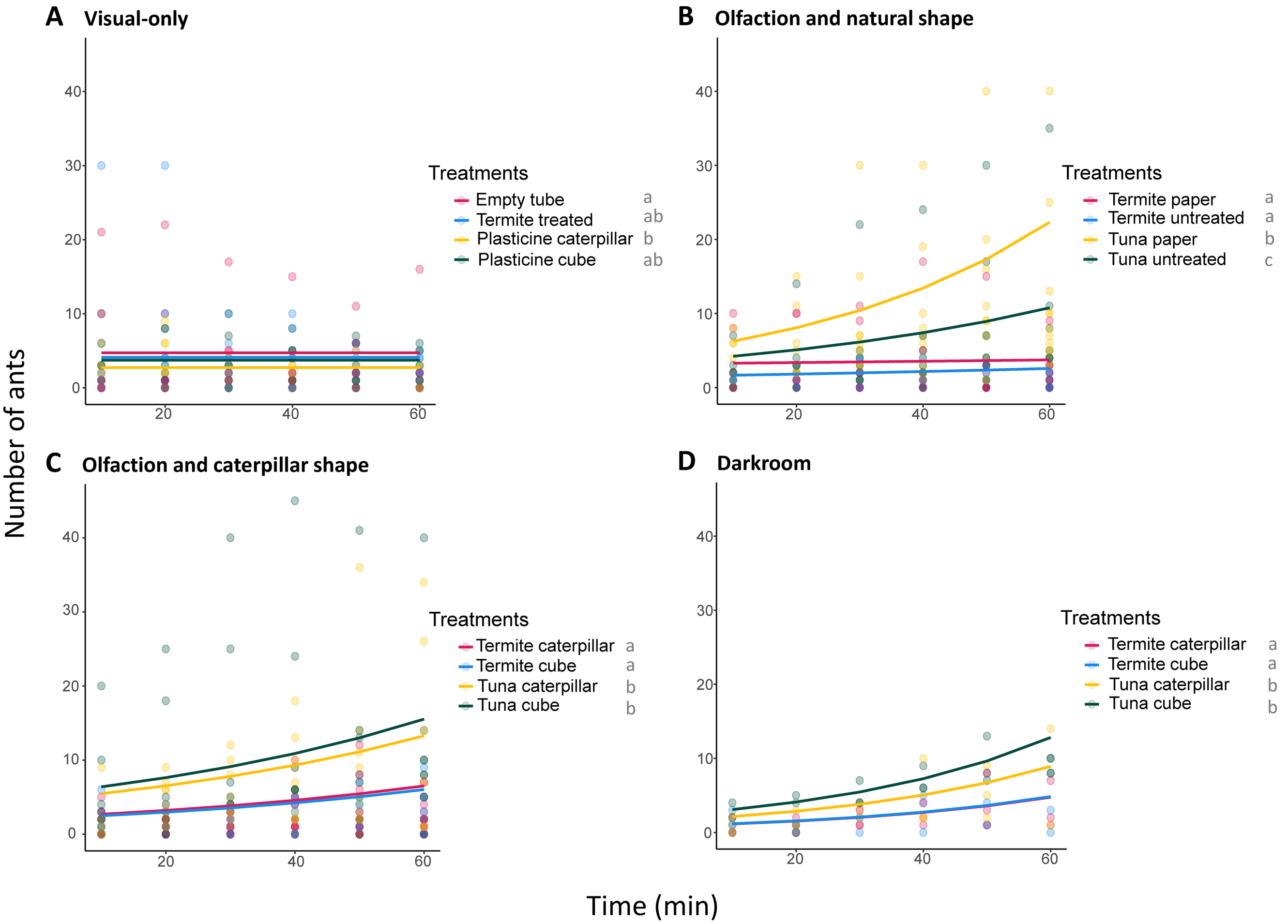

FIGURE 2 The number of ants found in each of the four experimental tubes over time (10-min intervals) in the experiments (A) visual-only, (B) olfaction and natural shape, (C) olfaction and caterpillar shape, and (D) darkroom. All regression lines are predictions based on the best model. Treatments within a panel followed by a different letter are significantly different (Tukey's honestly significant difference test of estimated marginal means: $p < 0.05$). Note that the two regression lines in (D) ('termite caterpillar' and 'termite cube') overlap almost entirely.

DISCUSSION

We used an experimental approach to test the efficacy of plasticine caterpillars to assess the predation rate and the importance of static visual and olfactory cues for foraging by *O. smaragdina*. To our surprise, the ants did not show a preference for plasticine caterpillars compared with plasticine cubes. Both plasticine caterpillars and cubes (those without olfactory cues) were hardly visited or attacked by the ants. Ants showed a strong preference for tuna compared with termites regardless of the visual cues, suggesting that static visual cues are not a decisive factor for prey selection. Previous studies suggested that arboreal ants have evolved to rely more on visual cues and less on olfaction compared with ground-dwelling ants (Jaffe & Perez, 1989; Short, 2020). However, their conclusions were drawn predominantly on neural anatomy, while behavioral tests were lacking. Our results suggest that *O. smaragdina*, one of the dominant arboreal ants in this region, relies on olfactory cues for prey selection. Studies with similar behavioral trials on other ant species in different habitats are needed to further elaborate on the relative importance of sensory cues across different habitats and behavioral contexts.

We found a consistent preference for tuna over termite baits. *O. smaragdina* has been used as an efficient biological control agent against termites (Musyafa et al., 2019), and during this study, some individuals were observed to carry the untreated termites out from the tube, demonstrating that ants do forage on termites. On the other hand, tuna has been commonly used as a bait for *O. smaragdina* and has been shown to be a preferred bait because of its high protein content (Lach & Hoffmann, 2011; Pimid et al., 2019). Additionally, the preference for tuna over termites may be attributable to the lack of motion cues in our termite baits. One study (Paluh et al., 2014) showed that sentinel prey with motion increased predation rate, which may also be the case with *O. smaragdina*.

Our study suggests that the sentinel plasticine prey is not attacked by dominant arboreal predators such as *O. smaragdina* that was thought to be visually oriented. Even though caterpillars are the prey of *O. smaragdina* in their

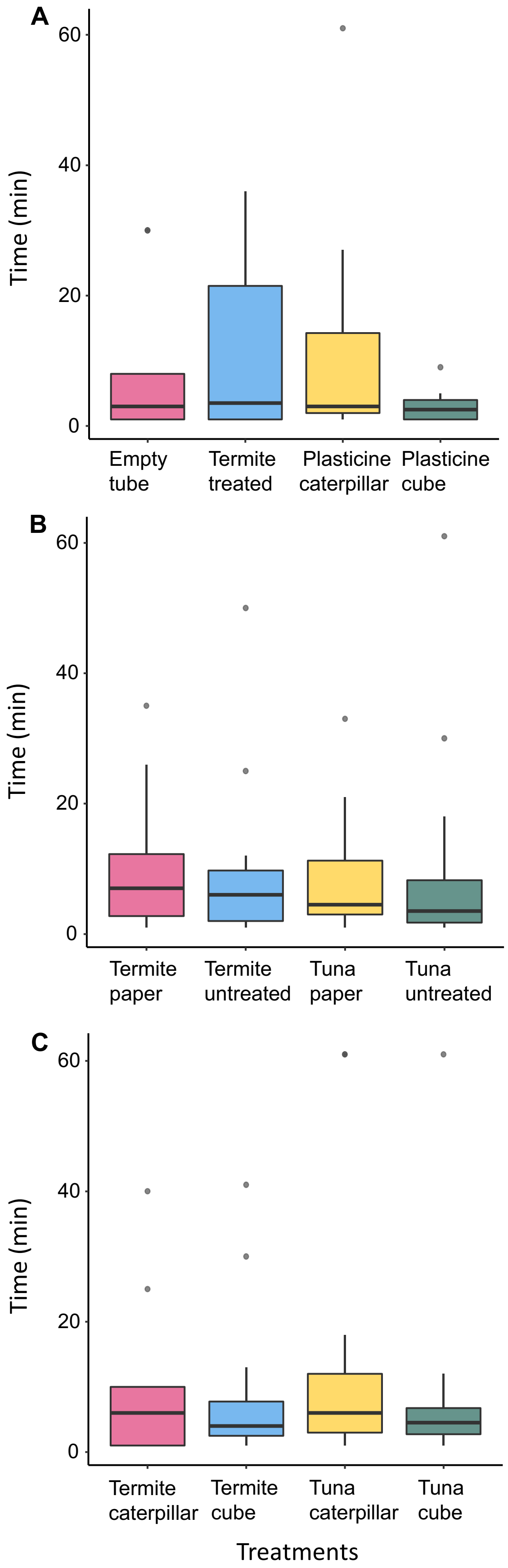

FIGURE 3 Box plots showing the time (min) of first entry by an ant to the experimental tube in the experiments (A) visual-only, (B) olfaction and natural shape, and (C) olfaction and caterpillar shape. As the infrared camera was not clear enough to confirm whether ants were inside or outside the tubes, we did not test this for the darkroom experiment.

natural habitat, ants may not detect caterpillars (and other prey) using static visual cues but rather, the movement of the caterpillar, odors emitted directly by the caterpillar, or indirectly, by the volatile compounds released from the damaged plants (Paluh et al., 2014; Vet & Dicke, 1992). Although a study based on the use of sentinel caterpillars showed an increase in predation rate in the canopy compared with the understory (Loiselle & Farji-Brener, 2002), such studies using sentinel caterpillars miss out on a non-negligible portion of predators that need more than immobile visual cues.

We infer that sentinel caterpillars might underestimate predation rates in habitats where dominant predators, such as *O. smaragdina*, do not attack sentinel prey models. Incorporation of olfactory cues to sentinel caterpillars may better approximate the actual predation rate. This can be achieved by applying specific nutritional compounds (e.g., protein, carbohydrates, and fat) or general odor cues on the exterior of the sentinel model, as was done in this experiment. Alternatively, one can knead edible particles (e.g., sugar and lard) in the plasticine or replace plasticine with edible material such as dough, as Sam et al. (2015) did. These methods combine visual and olfactory cues and may capture the predation of olfaction-oriented predators; however, sentinel models with olfactory cues may attract non-predatory species, such as scavengers, in the field (Nimalrathna et al., 2023).

The use of caterpillar-shaped plasticine sentinel models is a well-established experimental approach widely used to study predation pressures at various spatial (elevation, latitude) and temporal (seasons, time of the day) scales (Molleman et al., 2016; Pan et al., 2021; Richards & Coley, 2007; Roslin et al., 2017). Although plasticine lacks olfactory and other cues of natural prey, other studies have demonstrated that the attack rates on plasticine caterpillars were comparable to that of real caterpillars, as long as the relative differences in attack rate stay congruent between sentinel models and natural prey (Howe et al., 2009; Richards & Coley, 2007; Sam et al., 2015). Recent studies, however, challenged this notion, as they found the attack rate on plasticine caterpillars was substantially lower than that on real caterpillars (Nimalrathna et al., 2023), and variable levels of discrepancies between real prey and sentinel prey models across different habitats (Rodriguez-Campbell et al., 2024). The results of the latter study are particularly alarming, as the use of sentinel prey models may not necessarily reflect the relative differences in predation rate across habitats.

Our study and other recent studies suggest that using plasticine prey models is a rough proxy and does not necessarily equate to using natural prey. Sentinel prey may underestimate the predation rate compared with live prey, which provides multiple cues, including motion, chemical, and tactile cues that may attract predators. In contrast, sentinel prey may overestimate the predation rate because of the lack of predator evasion mechanisms (Rößler et al., 2018; Zvereva et al., 2019; Zvereva & Kozlov, 2023). The use of sentinel prey models and its extensions should always be treated with caution, especially when the sensory ecology of target predator communities is unknown (Rodriguez-Campbell et al., 2024).

AUTHOR CONTRIBUTIONS

Lin Yan: Conceptualization (supporting); data curation (equal); formal analysis (lead); investigation (equal); methodology (equal); validation (equal); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Samuel Paul Kagame:** Conceptualization (supporting); data curation (equal); formal analysis (supporting); investigation (equal); methodology (equal); validation (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Yang Liu:** Conceptualization (supporting); data curation (equal); investigation (equal); methodology (equal); validation (equal); writing – original draft (supporting); writing – review and editing (supporting). **Takafumi Mizuno:** Conceptualization (supporting); data curation (supporting); formal analysis (supporting); investigation (supporting); methodology (supporting); project administration (supporting); resources (supporting); software (supporting); validation (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Akihiro Nakamura:** Conceptualization (lead); data curation (supporting); formal analysis (supporting); funding acquisition (lead); investigation (supporting); methodology (equal); project administration (lead); resources (lead); software (supporting); supervision (lead); validation (equal); visualization (equal); writing – original draft (supporting); writing – review and editing (supporting).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in GitHub at https://github.com/linnyan/Oecophylla-.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. DNA sample preparation.

Figure S1. Molecular phylogenetic tree constructed by the maximum likelihood method.

Figure S2. Relative eye size of (A) ants collected from rainforest and rubber plantations, and (B) major and minor workers.

Table S1. The output of Tukey's honestly significant difference test that compared the treatment pairs.

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