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# Estimating predation pressure in ecological studies: controlling bias imposed by using sentinel plasticine prey

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## Abstract

Sentinel plasticine prey has been increasingly used to estimate predation pressure. The use of plasticine prey may, however, bias the results, as this method was originally designed to account for predation by organisms that can visually recognize the shapes and colors of their prey. To evaluate the limitations of using sentinel plasticine prey, we compared predator attack rates between real prey – dead and live mealworms, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) – and plasticine models in a monsoonal tropical rainforest of southeastern China. The attack rates by invertebrates were highest on dead prey followed by live prey and plasticine models, whereas the attack rates by vertebrates were lowest on dead prey, and did not differ between live prey and plasticine models. These results confirm that bias imposed by using the plasticine models is affected by the type of predators. In addition, we tested the validity and generality of the premise that predators can distinguish the shapes of plasticine model prey and preferentially attack a caterpillar-like shape over other shapes. To test this hypothesis, we conducted three independent experiments in China, Papua New Guinea, and Finland. In the two latter localities, predation rates on plasticine caterpillars were higher than on models of other shapes, whereas in China, these differences were not significant. Taken together, our study suggests that plasticine models may underestimate the predation by invertebrates to a greater extent than predation by vertebrates, and the preference of model shape by predators may be locality-specific, presumably due to differences in the composition of the predator community. We propose that predation be estimated on both live and plasticine prey in future studies to measure the potential bias imposed by using plasticine models and its variation among various habitats and predator groups.

KEYWORDS
ants, China, Coleoptera, dummy caterpillars, Finland, monsoonal tropical forest, Papua New Guinea, predator, *Tenebrio molitor*, Tenebrionidae, Xishuangbanna, Yunnan

## INTRODUCTION

Predation (including parasitism) is an ecological process through which biodiversity is shaped and maintained (Chesson, 2000; de Groot et al., 2002). It is the main cause of mortality of insect herbivores, especially in the early stages of their development (Sih et al., 1985; Berryman et al., 1987; Howe et al., 2009). Cascading predator–insect herbivore interactions are key processes to mediate herbivory and maintain sustainable populations of host plants (de Groot et al., 2002; Low et al., 2014). The importance of predation in ecosystem functioning and community assembly highlights the use of appropriate methods to assess predation, especially where predation rates and the identities of predators remain unclear (Furlong, 2015; Sam et al., 2015).

Identifying predators and quantifying predation pressure are challenging. The event of a predator attacking an invertebrate prey occurs relatively fast and, hence, is rarely encountered, particularly if the predators are small, or hide after attacking the prey (Howe et al., 2009; Ferrante et al., 2014; Low et al., 2014). Direct observation of predation events is possible (Baer & Marquis, 2020), but it is labor-intensive and affected by observer bias (Ferrante et al., 2014). Moreover, the disappearance of prey cannot be reliably attributed to predation if observers fail to see the predation events (Richards & Coley, 2008; Baer & Marquis, 2020). These issues have made indirect observations imperative to measure predation pressure and assess predator identity.

The use of artificial sentinel prey models is one of the indirect approaches commonly used in ecological studies (Rößler et al., 2018). Artificial sentinel prey models are made of various materials such as dough (lard or flour), paraffin wax, and plasticine to mimic the shape, color, and size of prey (Remmel & Tammaru, 2009; Rojas et al., 2014; Roslin et al., 2017). These materials are non-toxic and can be easily molded into reasonably accurate features of the prey (Low et al., 2014; Bateman et al. 2017). Also, they are soft enough to record bite and scar marks of predators (Low et al., 2014). Sentinel models have been used for ecological studies as early as the 1970s, but the materials were molded into relatively large prey items such as snakes (e.g., Madsen, 1987; Brodie III, 1993) and lizards (e.g., Castilla et al., 1999) to estimate predation by visually oriented vertebrates (e.g., avian predators) (Hinman et al., 1997). Subsequently, their use was extended to estimate predation by invertebrates among a variety of habitat types by molding the plasticine into the shape of a caterpillar (Loiselle & Farji-Brener, 2002).

Oil-based plasticine is often the material of choice to make caterpillar-shaped sentinel models (Howe et al., 2009). It can maintain its shape under various climatic conditions and it is cheap to produce models without sophisticated equipment or procedures, making it suitable for large-scale spatial studies (Howe et al., 2009; Low et al., 2014; Roslin et al., 2017; Zvereva et al., 2019). Experiments using plasticine caterpillars are aided by the establishment of comprehensive image databases showing the imprints of predator attacks, to identify the predators at coarse (e.g., arthropod, bird, mammal, or reptile) and more detailed taxonomic levels (e.g., ant, beetle, rat, possum, gecko, or snake) (Low et al., 2014). Manipulative studies using plasticine caterpillars are widely employed to improve our understanding of complex predator–prey relationships (Remmel & Tammaru, 2009; Tvardikova & Novotny, 2012; Volf et al., 2021) and to investigate predation pressure among habitats and across time (Howe et al., 2009; Mappes et al., 2014; Seifert et al., 2016; Ferrante et al., 2017; Roslin et al., 2017; Liu et al., 2020; Zvereva & Kozlov, 2021).

Plasticine caterpillars neither exhibit behavioral characteristics nor release chemical (olfactory) cues of natural prey. The lack of moving and other behaviors may underestimate the predation rate, as invertebrates such as spiders and wasps may rely on behavioral cues (Spangler, 1986; Nyffeler, 1999; Libra et al., 2019; Baer & Marquis, 2021). On the other hand, the lack of defensive behaviors, such as escaping, hiding, and limiting activities when disturbed, may overestimate the predation rate (Yack, 2022). The lack of chemical cues specific to natural prey and the presence of other chemical cues released from the plasticine model may also bias the estimation of predation pressure (Greeney et al., 2012). It is known that ants utilize chemical cues released from real caterpillars (Vet & Dicke, 1992; Sam et al., 2015), and some parasitoids use chemical signals of prey excrements (Agelopoulos et al., 1995) and herbivore-induced plant volatiles (Lövei & Ferrante, 2017; Volf et al., 2021). Studies utilizing plasticine caterpillars, nevertheless, assume that predation bias was equal for predators utilizing visual, tactile, behavioral, or olfactory cues among habitats and across time (Howe et al., 2009; Ferrante et al., 2014; Mappes et al., 2014; Zvereva & Kozlov, 2021), without systematically scrutinizing this assumption (but see Tvardikova & Novotny, 2012; Suzuki & Sakurai, 2015).

Studies using plasticine caterpillars make an implicit assumption: predators recognize the shape of plasticine as their prey, and preferentially attack caterpillar-shaped plasticine models over other shapes. A study conducted by Tvardikova & Novotny (2012) tested this assumption by comparing the attack rates on caterpillar- and ball-shaped plasticine models; they found higher attack rates on the caterpillar-shaped models in an equatorial tropical forest. In a habitat where visually oriented predators, such as insectivorous birds, are common, predation pressure is expected to be greater on caterpillar shapes than on other shapes of plasticine. This, however, may not hold true where non-visual predators, such as invertebrates, are dominant. The generality of this premise, however, has not been tested among localities.

Here, we evaluated the potential estimation bias caused by the use of plasticine models. Our study tested two hypotheses. First, we hypothesized that the rate of attack, especially by invertebrates, is lower for plasticine caterpillars than for real prey due to the lack of olfactory and behavioral cues. Second, we hypothesized that predators attack caterpillar-shaped plasticine models at a higher rate than models of other shapes, but this is not the case if invertebrate predators dominate. To test the first hypothesis, we compared attack rates between real prey – dead and live mealworms, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) – and plasticine models (caterpillar and cube shapes) in a monsoonal tropical rainforest of southwestern China. To test the second hypothesis, we compared our results, specifically in relation to the relative differences in attack rates on various shapes of plasticine models, with two independently conducted studies in Papua New Guinea (PNG) and Finland that also tested predation rates on various shapes of plasticine models. This was done to seek the validity and generality of the premise that predators distinguish the shapes of plasticine and preferentially attack caterpillar models over other shapes of plasticine. We then propose a way to measure and alleviate the potential bias in the use of sentinel plasticine caterpillars to estimate predation rates.

## MATERIALS AND METHODS

### Field sites

We tested the first hypothesis in a monsoonal tropical forest in Xishuangbanna Dai Autonomous Prefecture of Yunnan Province, southwestern China. Monsoon climate prevails in this region with distinct wet and dry seasons (Cao et al., 2006; Lan et al., 2012) (Table 1). The experiment was conducted in an old secondary rainforest in November 2017, at the end of the rainy season.

To test the second hypothesis, we included two additional studies conducted in PNG and Finland. In PNG, the field experiment was conducted in three lowland secondary and/or primary tropical forests and one montane primary rainforest between December 2010 and March 2011. In the lowland, the study sites are characterized by a humid climate with a mild dry season from July to September. The montane primary rainforest is characterized by a lower montane humid climate with a mild dry season from April to September (Tvardikova & Novotny, 2012) (Table 1). In Finland, the field experiment was conducted in a managed mixed coniferous forest located in Kustavi, southwestern Finland from May to September 2019, when both ant and bird activities were high (Table 1).

### Model preparation

For the experiments in China, oil-based, non-toxic green Newplast clay (Newclay Products, Newton Abbot, UK) was used to prepare artificial caterpillars and cubes, and live and dead prey were prepared using commercially available mealworms (*T. molitor*). Caterpillar-shaped plasticine models were molded by a metal syringe (a sugar press) to make a smooth round shape (0.3 cm diameter, 3 cm long), resembling the common size and shape of lepidopteran larvae (Sam et al., 2015; Roslin et al., 2017). Following the standard method used by previous studies, these caterpillars were bent in the middle to mimic the natural 'inchworm' posture of caterpillars (Roslin et al., 2017; Pan et al., 2021). Plasticine cubes (0.5 × 0.5 × 0.5 cm) were made using the same, green-colored plasticine that was pressed and cut using a metal syringe. All plasticine caterpillars and cubes were stored and transported to the plots using 2-ml plastic microcentrifuge tubes. To prepare dead and live prey, we chose 2- to 3-cm-long mealworms that were 8–10 weeks old and golden brown in color. Dead prey were prepared by killing the mealworms in situ by submerging them in a water tub for 10–15 min; they were set on the leaves immediately afterward.

In PNG, the plasticine models were made from similar oil-based, non-toxic green clay (Koh-i-noor Hardtmuth, České Budějovice, Czech Republic). Using a metal syringe, the caterpillar shape was molded (0.3 cm diameter, 1.5 cm long), and the ball shape was hand-rolled (0.5 cm diameter). In Finland, non-toxic, oil-based green clay (PC Chemical Plant 'Luch', Yaroslavl, Russia) was used to make clay models hand-molded into caterpillar-shape (0.4–0.5 cm diameter, 2.5–3.0 cm long), balls (1 cm diameter), and 'bricks' (1.4 × 0.65 × 0.65 cm). Unlike in China, caterpillar-shaped plasticine models used in PNG and Finland were not bent.

### Experimental setup

In China, we set up a total of five experimental plots (12 × 10 m) within the rainforest. These plots were at least 100 m away from each other and at least 100 m away from the habitat edge. Within each plot, we deployed plasticine caterpillars and cubes, dead and live mealworms (30 for each treatment, totaling 120) for 5 days resulting in a total of 3000 days of caterpillar deployment (120 baits × 5 plots × 5 days). These plasticine models and mealworms were randomly distributed within each plot area keeping a distance of about 1 m between the samples.

The plasticine models and mealworms were pinned on the understory leaves using entomological pins at a height of 30–100 cm above the ground. Live and dead mealworms were wrapped in the middle (approximately 0.5 cm width) by light-blue moldable synthetic adhesive (non-toxic, non-soluble materials sold as Blu Tack; Bostik, Middleton, MA, USA) before pinning through their bodies and leaves. This ensured that the live mealworms could not escape by wiggling whereas the adhesives covered the wound made by the pin. The adhesive also assisted in the identification of the predators, as the attacking predators left imprints in it (Ho et al., 2016). To successfully find and retrieve the samples later, we placed orange flagging tapes with information on experimental treatments approximately 20–30 cm from the samples on the same tree branches. This ensured to retrieve information in cases where samples were lost.

All sample locations were visited every 24 h for 5 days after the samples were deployed. At each visit, we checked for signs of attack on the plasticine and mealworm samples, and replaced damaged samples with new ones. When organisms were found to be feeding on the samples, we photographed them to aid in predator identification. In cases where entire samples (including entomological pins and/or adhesive) were lost from the leaves and were also not found on the nearby ground, we considered those samples taken away by a large vertebrate, as suggested

**TABLE 1** Information on the three independent studies, conducted in China, Papua New Guinea (PNG), and Finland, of predation of plasticine caterpillars vs. plasticine models of other shapes

| Country | Site | Date | Habitat | Latitude, longitude | Elevation (m above sea level) | Mean annual precipitation (mm) | Mean annual temperature (°C) | Research hypothesis tested[a] | Bait type | Predator group |
|---|---|---|---|---|---|---|---|---|---|---|
| China | Xishuangbanna | 11–15 Nov 2017 | Old secondary rainforest | 21°54'N, 101°16′E | 500–550 | 1532 | 21.0 | 1 and 2 | Mealworms (dead and alive), plasticine (caterpillar and cube) | Invertebrates, vertebrates |
| PNG | Wanang 1 | Dec 2010–Mar 2011 | Mosaic of primary and secondary lowland rainforest | 5°14'N, 145°11′E | 125 | 3600 | 26.5 | 2 | Plasticine (caterpillar and ball) | Invertebrates, vertebrates |
|  | Wanang 3 |  | Contiguous lowland primary forest | 5°14'N, 145°05′E | 120 |  |  |  |  |  |
|  | Ohu |  | Fragment of lowland primary forest | 5°16'N, 145°41′E | 170 |  |  |  |  |  |
|  | Kotet |  | Montane primary rainforest | 6°10'N, 146°50′E | 1700 | 4000 | 17.0 |  |  |  |
| Finland | Kustavi | 26 May–21 Sep 2019 | Managed mixed coniferous forest with regrowth of birches | 60°32'N, 21°18′E | 20 | 582 | 7.0 | 2 | Plasticine (caterpillar, ball, and 'brick') | Vertebrates[2] |

[a] Hypothesis 1: the rate of attack, especially by invertebrates, is lower for plasticine caterpillars than for real prey due to the lack of olfactory and behavioral cues. Hypothesis 2: predators attack caterpillar-shaped plasticine models at a greater rate than models of other shapes, but this is not the case if invertebrate predators dominate. [2] Only vertebrate predators were included in this study as very few imprints were left by invertebrates.

by Low et al. (2014), and deployed new samples (of respective types) in their place. Any damaged samples that had dropped from the leaves were collected for predator identification and were replaced with new ones. Dropped but intact plasticine caterpillars were placed back on the same leaf. We also replaced the live mealworms if they were no longer moving (though pinned live mealworms generally stayed alive for >24 h). Dead mealworms with signs of decay were also replaced with freshly killed ones. When live mealworms went missing with intact adhesives and entomological pins left on the leaves, we considered they had 'escaped', and the samples were removed from subsequent analyses.

All damaged plasticine and mealworm samples were considered predated and brought back to the laboratory for identification of predators. We examined imprints on the plasticine, adhesive, and mealworms with bare eyes and hand lenses, and compared them with pictorial references provided by Low et al. (2014) and with our photographed organisms. Predators were identified as ants, other invertebrates, or vertebrates (these included no more than five lost samples). However, we merged the data on attacks by ants and other invertebrates for analyses as we could not ascertain the predator identity based on the imprints on some of the plasticine caterpillars. Our predator identification was coarse as we took a conservative approach to avoid misidentification of predators.

In PNG, in total 30 trees with a distance ranging from 15 to 30 m were haphazardly selected along a single 2175-m-long transect at each of the four forest sites. On each tree, five caterpillar-shaped and five ball-shaped plasticine models were deployed at 3–5 m above the ground with at least 30 cm distance from one another (5 models ×2 shapes ×30 trees ×4 locations = 1200 models deployed). Plasticine models were pinned on the distal half of the leaves and pinheads were buried in the clay. The models were checked after 24 h of exposure. Imprints left on the models were carefully inspected and identified as vertebrate and invertebrate attacks. Models that were not recovered ('missing') were treated as attacks by vertebrates. Other details of experimental design are provided in Tvardikova & Novotny (2012).

In Finland, a total of 10 trees taller than 3 m were randomly selected with a distance of 10–20 m in between. On each tree, one caterpillar, one ball, and one 'brick' shaped model was attached at least 20 cm apart in the outer part of the crown at 1–1.5 m above the ground. To fix the models, we penetrated a wire (0.3–0.5 mm diameter) through the plasticine models, and attached the end of the wire to a tree branch. The models were left exposed for 5–20 days and the predator attack imprints were inspected to identify possible predator groups. Missing plasticine models were considered as predation by vertebrates (birds). The damaged models were repaired by hand or replaced (if the damage was too severe) and redeployed for another 5–20 days. This was repeated 14× on each tree, totaling 420 records of predation (3 shapes × 10 trees × 14 intervals). As very few imprints were left by invertebrates, we only analyzed the damages inflicted by birds in this study.

## Data analysis

We first calculated the mean daily attack rates for each experimental treatment. Daily attack rates were calculated in the three localities as follows: in China, by dividing the number of damaged models by the number of deployed (minus 'escaped') models over 24 h within each plot; in PNG, by dividing the number of damaged models by the number of deployed models over 24 h within each tree; and in Finland, by dividing the number of damaged models by the number of deployed samples and then further dividing it by the number of days for which the models were left on the tree branches. For Chinese and PNG data, we calculated attack rates for invertebrates and vertebrates separately as well as combined.

For Chinese data, we used generalized linear mixed models (GLMM) using 'template model builder' ('glmmTMB' package; Brooks et al., 2017) in which we incorporated attack rates as response variables with binomial error structure, and the four experimental treatments – live mealworms, dead mealworms, plasticine caterpillars, and cubes – as a fixed factor. As predators may be spatially aggregated and attack the prey in their vicinity, we included plots as a random factor to control for spatial autocorrelation. We carried out the analysis of deviance based on the type II Wald $\chi^2$ test to check the significance of the experimental treatments (using the ANOVA function available from the 'car' package; Fox et al., 2019). Then, we conducted pairwise post-hoc tests using the 'emmeans' package (Lenth et al., 2020), where we specified to use Tukey's tests to compare estimated marginal means (least-square means) of the experimental treatments.

We used 'glmmTMB' instead of conventional GLMM (e.g., the 'glmer' in the 'lme4' package) as our daily predation rates were measured over 5 days in each plot and temporal autocorrelation had to be accounted for. We first used the commonly used autocorrelation structure (AR1) which assumes exponential decline with increasing distance (days between sampling events), but the 'glmmTMB' function had convergence issues. We, therefore, adopted the more flexible Toeplitz covariance structure, which assumes no exponential decline between sampling events (Brooks et al., 2017). To account for potential zero-inflation of the data, we compared two models, which included either binomial or zero-inflated binomial distributions of the response data.

For PNG and Finnish data (used to test the second hypothesis), we analyzed the attack rates on plasticine molded into caterpillar-shapes and other shapes by fitting GLMM using the 'glmer' function available from the 'lme4' package (Bates et al., 2015). For PNG data, the response variables were attacks (binomial data) by vertebrates or invertebrates with binomial error structure, and the predictor variable was model shape (caterpillar and ball), with location as a random factor to control for spatial autocorrelation. For Finnish data, the response variable was the presence or absence of bird attacks and the predictor variable included plasticine shapes (caterpillar, balls, and 'bricks'), with trees as a random factor. Summary statistics were generated using the 'ANOVA' function in the 'car' package, and pairwise post-hoc tests using the 'emmeans' package. All statistical analyses were carried out in R v.4.0.2 (R Core Team, 2019).

## RESULTS

In China, we identified predators as invertebrates and vertebrates (birds and rodents) based on the bite and scar imprints left on the plasticine models (Figure 1, Table S1). Although observations of scar imprints suggested that over 50% of damage was inflicted by ants, we did not conduct a separate analysis for ants alone, as it was difficult to confirm predator identity. A total of 21 live mealworms were regarded as 'escaped'. Daily observations and photography revealed diverse predatory and non-predatory organisms that were attacking and feeding on live and dead mealworms and plasticine models, albeit much less frequently (Figure 2). This included a large number of scavengers, such as earwigs (Dermaptera) and snails (Gastropoda). A large number of predators, such as jumping spiders (Arachnida, Salticidae), harvestmen (Opiliones), and centipedes (Chilopoda) (Figure 2) were also recorded; they cannot usually be identified based on the imprints on plasticine models.

To test the first hypothesis, we compared the attack rates on live and dead mealworms with plasticine model prey in China. The daily attack rates by all invertebrates and vertebrates were highest on dead mealworms, followed by live mealworms, and then followed by both plasticine caterpillars and plasticine cubes ($\chi^2 = 458.6$, d.f. = 3, $P < 0.001$) (Figure 3B). When the total attack rate was subdivided into predator groups, the attack pattern for invertebrates was very similar to the total attack rate ($\chi^2 = 510.1$, d.f. = 3, $P < 0.001$) (Figure 4), suggesting that invertebrates were responsible for the differences in overall attack rates. For attacks by vertebrates, the direction was opposite to that for invertebrates: the attack rate was lower on dead mealworms than on plasticine models, and the predation rate on live mealworms was intermediate ($\chi^2 = 12.3$, d.f. = 3, $P < 0.001$) (Figure 4).

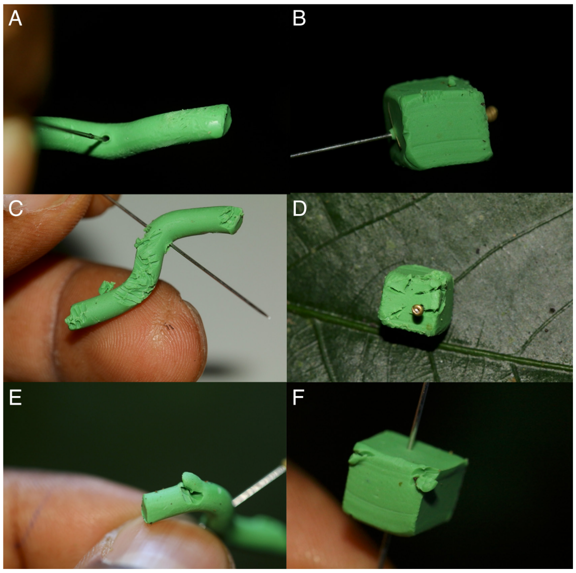

**FIGURE 1** Bite and scar imprint likely left by (A,B) ants, (C,D) non-ant invertebrates, and (E,F) vertebrates (rodents) on plasticine (A,C,E) caterpillars and (B,D,F) cubes deployed in a monsoonal tropical rainforest of southeastern China

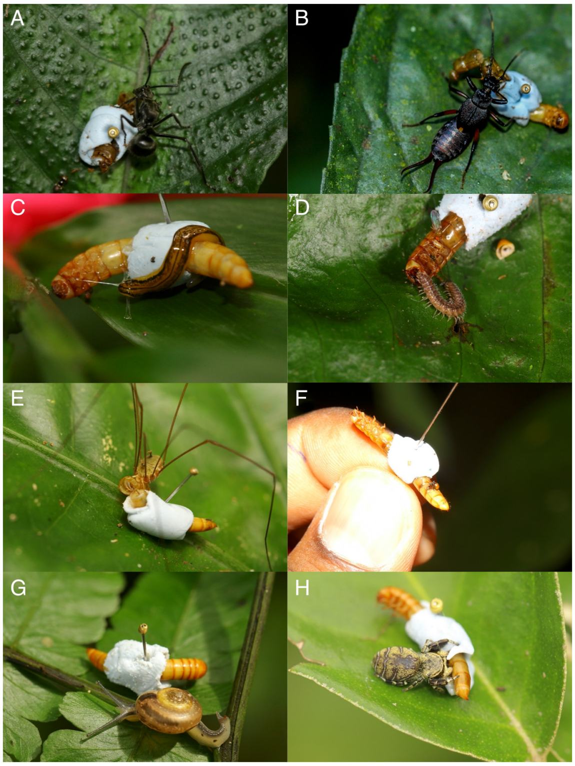

**FIGURE 2** Various invertebrates observed on live mealworms (*Tenebrio molitor*) deployed in a monsoonal tropical rainforest of southeastern China: (A) ant, (B) dermapteran, (C) platyhelminth, (D) centipede (also a fly), (E) opilionid, (F) beetle, (G) land snail, and (H) spider

To test the second hypothesis, we compared the results of three independent experiments conducted in China, PNG, and Finland. In China, there were no significant differences between attacks on plasticine models of the two shapes (Figure 3B). In contrast, the total attack rates (vertebrates and invertebrates combined) were significantly higher for plasticine caterpillars than for plasticine balls in PNG ($\chi^2 = 57.7$, d.f. = 1, $P < 0.001$) (Figure 3C, Figure S1). Similar results were found when the attack rates by vertebrates ($\chi^2 = 32.2$, d.f. = 1, $P < 0.001$) and invertebrates ($\chi^2 = 18.9$, d.f. = 1, $P < 0.001$) were considered separately (Figure S1). The data from Finland also showed a significant effect of model shape on predator attack rates; plasticine

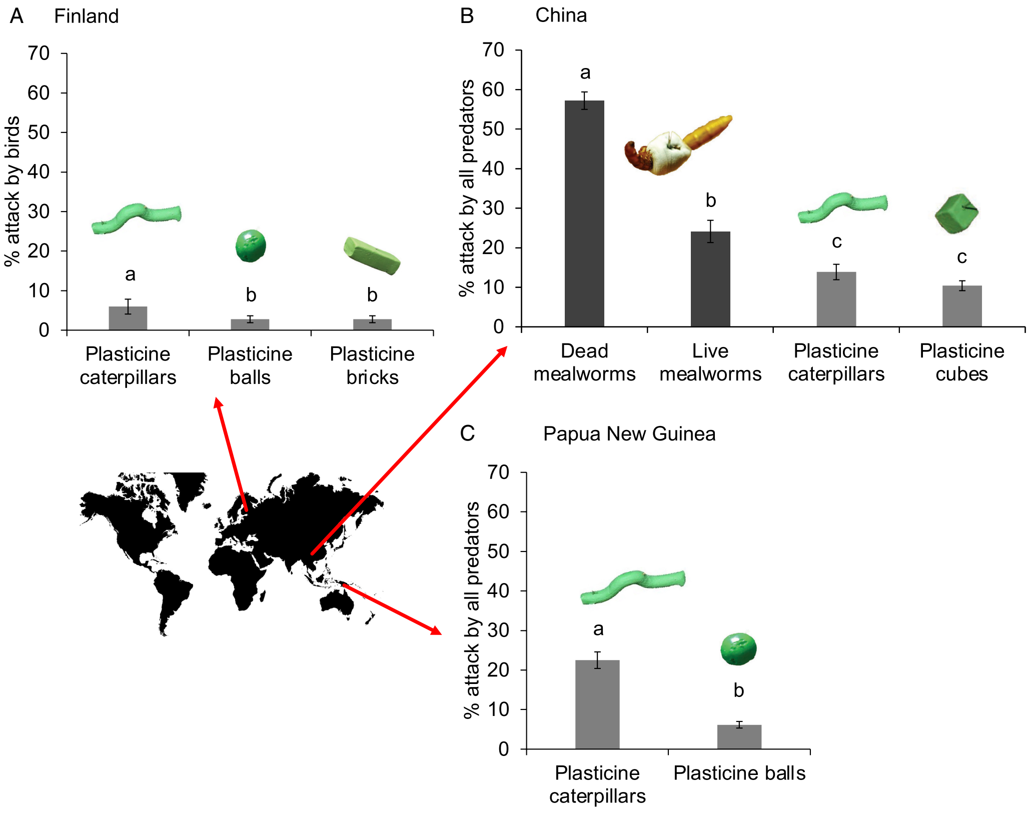

**FIGURE 3** Mean (± SE) daily attack rates (%) by predators (vertebrates only or vertebrates and invertebrates combined) on live or dead mealworms (*Tenebrio molitor*) and plasticine prey items at three study sites: (A) Finland, (B) China, and (C) Papua New Guinea. Means within a panel capped with different letters are significantly different (GLMM: $P < 0.05$). Note that plasticine caterpillar models in Finland and Papua New Guinea were not bent

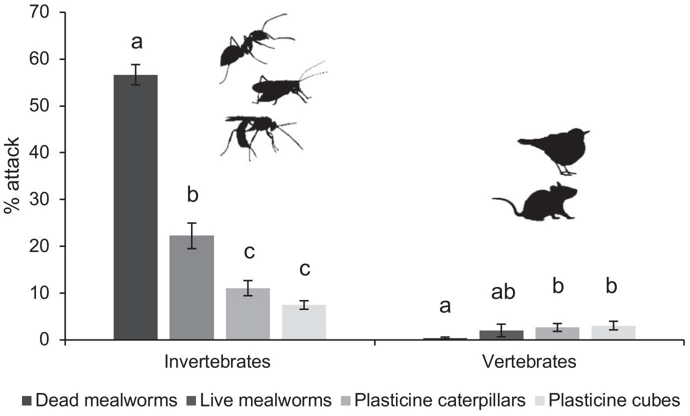

**FIGURE 4** Mean (± SE) daily attack rates (%) by invertebrate and vertebrate predators on dead and live mealworms (*Tenebrio molitor*) and plasticine caterpillars and cubes deployed in a monsoonal tropical rainforest of southeastern China. Means within a predator group capped with different letters are significantly different (GLMM: $P < 0.05$)

caterpillars received more attacks by birds than plasticine balls and 'bricks' ($\chi^2 = 10.0$, d.f. = 2, $P = 0.007$) (Figure 3A).

## DISCUSSION

The daily attack rates on plasticine caterpillars (6–20.6%) documented in China were within the same range as previously recorded (utilizing the same plasticine caterpillars) in the same locality and a similar habitat (Leles et al., 2017). The critical question is whether the predation rates found by us and in other studies using plasticine sentinel prey over- or underestimate the real predation intensity and whether such estimation bias differs among common predator groups that occur in a particular locality at a given time. Hence, a quantitative evaluation of the advantages and limitations of utilizing plasticine models is necessary, owing to the surging trend to use this method for predation studies (Lövei & Ferrante, 2017).

Our study supported the first hypothesis, as we found higher invertebrate predation on mealworms than on plasticine caterpillars. These results suggest the possibility of underestimated predation intensity by invertebrates using plasticine caterpillars. Unlike invertebrates, attack rates by vertebrates (mainly birds) were not significantly different between live mealworms and plasticine caterpillars, suggesting the suitability of plasticine caterpillars to estimate predation rates by birds and other vertebrates. However, our study was conducted at the end of the rainy season when predation by vertebrates, especially birds, is relatively low (Pan et al., 2021). Further scrutiny is required to confirm the usefulness of plasticine models in different seasons, especially during the breeding season of birds (spring) in this locality. Higher attack rates by invertebrates recorded for mealworms, relative to plasticine caterpillars, may be related to behavioral, olfactory, as well as visual cues of the live prey that may signal to numerous groups of foraging invertebrates (Saavedra & Amo, 2018). Consequently, we observed live mealworms being attacked by several predator groups that were not usually detected when using plasticine caterpillars. These predators include jumping spiders and centipedes that are likely to be attracted by the movement and olfactory cues of the prey (Nyffeler, 1999).

The highest attack rates were found on dead mealworms, followed by live mealworms and plasticine models. Between dead and live mealworms, the distinctions could be driven by various factors such as the inability of dead prey to defend itself against enemies (Gentry & Dyer, 2002), or the chemical substances emitted by the dead and decaying prey (Vet & Dicke, 1992; Weiss et al., 2004). Therefore, dead prey can be attacked by various non-predatory groups, such as scavengers (saprophages and coprophages), as was observed in this study. We believe that the highest contribution was likely to be caused by ants, which is in line with the study by Griffiths et al. (2018), who reported the highest proportion of organic material removal (dried biscuit, sunflower seeds, and fish) by ants in tropical rain forests. A similar study was conducted by Nagy et al. (2020), who compared the predation rates between plasticine and real (but only dead) caterpillars in grass and wheat fields. They observed much higher predation rates on dead than on plasticine caterpillars and the majority of damage was inflicted by ants and scavengers (Nagy et al., 2020). Hence, the high attack rates on dead mealworms found in the present study were perhaps not a true representation of predation rate; it is rather likely to reflect the removal of animal carcasses by invertebrates (Griffiths et al., 2018).

To test the second hypothesis (caterpillar-shaped plasticine models vs. other shapes), we compared relative differences in attack rates for three independent field studies, to assess the generality of the premise that predators distinguish the shapes and preferentially attack plasticine caterpillars over plasticine models in different shapes. In PNG and Finland, we found significantly higher attack rates on plasticine caterpillars than on models in other shapes. In China, however, there was no significant difference in attack rates between plasticine caterpillars and cubes, regardless of the predator groups examined. Inconsistent results among the localities may be partly explained by differences in study design and timing among the three studies. For example, longer duration of the experiments in PNG and Finland may have resulted in accumulation of more bird attacks. Differences in size, color, shape, and material of the plasticine and the presence or absence of flagging tape are all likely to have an effect on the predation rates and possibly the outcomes of the experiments (Mänd et al., 2007; Remmel & Tammaru, 2009). Despite such differences in experimental design among the three studies, we fully standardized the analyses within each study so that relative (and not absolute) differences in predation rates remain valid. Inconsistent results among the three studies, therefore, suggest that attack rates on models of different shapes depend on predator composition of a particular locality at a given time. In particular, when bird predation is intensive (PNG and Finland), the shape of model prey is more important for estimation of predation, whereas when invertebrate predation is dominating (China), the shape of model prey does not affect estimates of predation rates. It is also interesting to note that non-caterpillar-shaped plasticine models, even of such unnatural shapes as cubes or bricks, sustained some damage among the three localities, suggesting that some predators (and perhaps non-predators such as frugivores) attack plasticine regardless of its shape.

Leles et al. (2017) suggested that plasticine caterpillars could provide a reliable estimation of ant predation based on a behavioral experiment using the tropical trap-jaw ant, *Odontomachus rixosus* Smith. Although they found that *O. rixosus* prefer plasticine caterpillar-shaped over cube-shaped models, ants of this genus have one of the largest eye sizes in the tropics and other regions of Yunnan (Figure S2 shows the relative eye size of ants found in this region). The same result, therefore, may not be expected for other ant species which do not distinguish prey shape. This was supported by unpublished experiments showing that some of the dominant ant species in our study areas – *Oecophylla smaragdina* (Fabricius) in the Chinese, and *Formica* spp. in the Finnish sites – did not attack plasticine models (regardless of their shapes) (A Nakamura & EL Zvereva, unpubl.). *Oecophylla* is known to have well-developed eyesight (Mishra & Bhadani, 2017), but perhaps they rely more on olfactory and behavioral cues rather than stationary visual cues to hunt their prey. This has serious implications, as studies utilizing plasticine caterpillars may have failed to document predation by dominant ants that do not attack plasticine caterpillars. Roslin et al. (2017), for example, showed that the predation rate increases with decreasing latitude, primarily due to increased predation by arthropods in the tropical regions. Their argument may be valid, as the distribution of predatory arthropods is concentrated in areas at lower latitudes and elevations (Guénard et al., 2017; Camacho & Avilés, 2019). We argue, however, that the use of plasticine models may have disregarded a large proportion of predators, including ants, that may not attack plasticine caterpillars.

In light of the results of our study, we suggest that, whenever possible, plasticine experiments should be 'calibrated' by employing other methods (using real live prey, for example, like in our study) so that the discrepancies between predators that attack plasticine caterpillars and those that do not could be estimated. The 'calibration' of the plasticine experiments can be done by comparing attack rates between live prey and plasticine caterpillars among sites. Although neither plasticine models nor live prey can estimate the true predation pressure (Richards & Coley, 2008; Baer & Marquis, 2020), we can measure the relative differences in predation by organisms (especially invertebrates) utilizing different cues for hunting. If the discrepancies are minimal and attack rates between live prey and plasticine caterpillars are directly related (Figure 5), predation pressure is generally well-represented by the attack rates on plasticine caterpillars. In our study, this was found for vertebrate predation, where similar attack rates were observed between live prey and plasticine caterpillars. If the attack rates on plasticine caterpillars are higher (overestimation) or lower (underestimation) than on live prey, yet, the magnitude of over- or underestimation stays the same among sites or across time (Figure 5), bias can be assumed to be equal and relative differences can be compared. Problems arise when the magnitude of over- or underestimation varies among sites due to differences in predator composition (Figure 5). In that case, unequal bias should be incorporated into the analysis by estimating the amount of deviation in attack rates between live prey and plasticine caterpillars.

The choice of live prey items is crucial and challenging (and this is the very reason why sentinel plasticine preys are used to estimate predation rates in many studies). Our study used mealworms by wrapping them in light-blue adhesives and pinning them onto leaves through the body (so that the live mealworms cannot wiggle out of the adhesives to escape). In contrast to the green plasticine, resembling the color of real lepidopteran larvae and natural leaves – the reflectance spectra of the materials used in this study are found in Aslam et al. (2020) and Pan et al. (2021) –, mealworms are generally brown to light brown. Also, pinning the body of live mealworms changed their behavior and oozing body fluid might have attracted different sets of organisms (Deml & Dettner, 1995), though the wound was sealed with adhesive and most insects stayed alive for more than 24 h. Consequently, the use of live mealworms has its own bias in estimating predation pressure. For example, vertebrate predators that use visual cues might have seen the adhesive and consequently avoided these mealworms. In addition, we used coleopteran instead of lepidopteran larvae; hence, the results may not reflect true predation on lepidopteran larvae. It is best to use caterpillars that are reared or collected from the field as live models (Richards & Coley, 2008; Sam et al., 2015), but this is

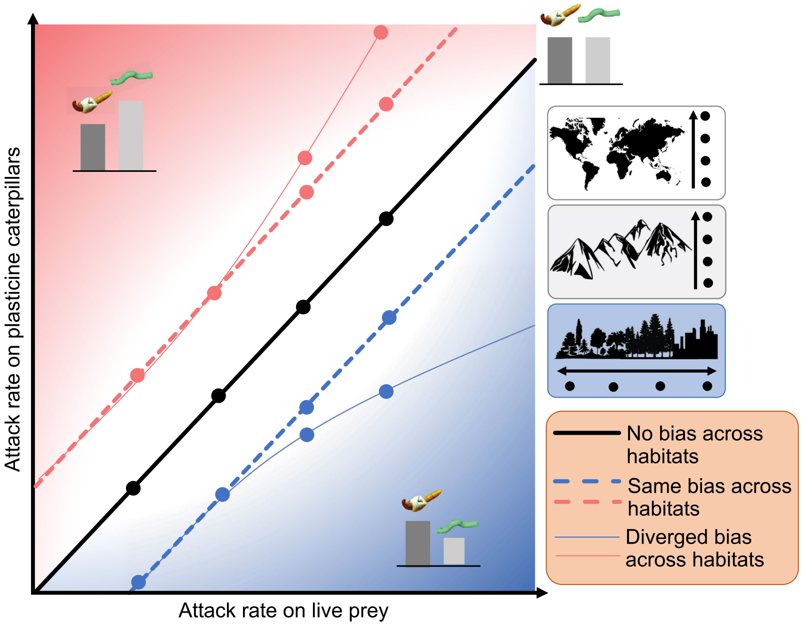

**FIGURE 5** A hypothetical diagram illustrating potential bias that could be detected by comparing plasticine models and real prey. If predation pressure is estimated using plasticine caterpillars across latitudes, elevations, or habitats (sampling sites denoted by dots), predator composition may vary, potentially resulting in biased estimation among habitats. The magnitude of such bias can be estimated by comparing attack rates on live prey (mealworms in our study, but other prey items can be selected to better reflect the locally available natural prey) and plasticine caterpillars. If no bias is present (solid black line), attack rates between live prey and plasticine caterpillars are directly proportional, and attack rates on plasticine caterpillars can be compared among sites. Attack rates may be biased resulting in overestimation (red area) or underestimation (blue area). However, if the magnitude of bias stays the same among sites (dashed lines), then direct comparisons can be made among sampling sites. The problem arises when the magnitude of the bias changes among sites (blue and red solid lines), and deviations from live prey should be incorporated in the analysis

often not a feasible option, especially when a large number of models is required for field-based observations. Other commercially available species may not reflect the colors of naturally occurring caterpillars. Despite such potential shortcomings, the use of live prey attracts predators that recognize non-visual cues and provides useful benchmark information, which should be incorporated when predation is estimated using plasticine caterpillars. It should also be noted that handling (bare hands vs. gloves), material, color, size, and posture (e.g., 'inchworm' posture vs. straight) of plasticine models all become potential sources of bias and these issues should be carefully considered before implementing the experiment (Howe et al., 2009; Sam et al., 2015). We suggest that: (1) the live prey should be able to move so that they can present behavioral cues; (2) if possible, naturally occurring prey should be used, but commercially available prey can be an alternative; and (3) reflectance spectra of live prey, plasticine models, and natural leaves should be checked (Aslam et al., 2020; Pan et al., 2021), so that potential bias between real and plasticine models can be estimated.

## CONCLUSION

Our study demonstrated that sentinel plasticine models may underestimate predation rate, especially by invertebrates that use mostly non-visual cues to detect their prey. In addition, plasticine models, irrespective of their shapes, may attract predators and perhaps non-predators, potentially undermining the assumption that predators distinguish the shapes of plasticine models and attack only caterpillar-shaped models.

The use of dead prey is likely to attract non-predatory scavengers, but the use of live prey is not an unbiased method to estimate predation pressure either. Nevertheless, live prey attract predators that otherwise cannot be detected using conventional plasticine models. We recommend to use both live prey and plasticine models, at least in pilot studies, to understand the potential inherent bias in the use of the plasticine method, so that more robust estimations and comparisons of predation pressure among habitats can be made. This is especially important for invertebrates, whose hunting strategies likely vary among habitat types, and consequently attack rates on plasticine caterpillars may not represent relative differences in predation pressure among habitats.

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## SUPPORTING INFORMATION

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

**Figure S1** Mean (± SE) daily attack rates by (A) all predators, (B) invertebrates only (mainly ants), and (C) vertebrates only (mainly birds) on plasticine caterpillars (black bars) and plasticine balls (grey bars) in Papua New Guinea. Means within a predator group capped with different letters are significantly different (GLMM: $P < 0.05$).

**Figure S2** Distribution of relative eye size of ant species collected across an elevation gradient in Yunnan, China.

Open dots denote Odontomachus spp., which was used to analyze aggressive behavior towards plasticine caterpillars compared with plasticine cubes (Leles et al., 2017). Data derived from Fontanilla et al. (2019).

**Table S1** Sample sizes and number of damaged prey items by three groups of predators among four types of prey (treatments) deployed in a monsoonal tropical rainforest of southeastern China

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