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ORIGINAL ARTICLE

Do prey shape, time of day, and plant trichomes affect the predation rate on plasticine prey in tropical rainforests?

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Abstract

Predation can effectively limit insect herbivores with cascading effects on plant community composition and diversity of tropical rainforests. Assessing variation in predation is therefore important to understand the mechanisms structuring complex rainforest ecosystems. Variation in predation with time of day may provide herbivores with temporal enemy-free space. Trichomes (plant hairs) may provide spatial enemy-free space by increasing climbing resistance for walking arthropod predators and by scattering bat echolocation calls. Artificial model prey is commonly used to measure predation pressure on insect herbivores. Whether model prey shape is sufficient to deceive predators and whether attacks represent actual predation however remain unresolved. We used artificial, plasticine prey to assess temporal and spatial variation in predation in two Panamanian rainforests and tested whether model prey shape is as important for prey recognition by predators as often assumed. We assessed the effect of prey shape and size, time of day, and trichomes on predation by comparing attacks on caterpillar- and humanoid-shaped figurines. We find higher nocturnal than diurnal predation in one but not the other forest, suggesting that herbivores may benefit from enemy-free space during the day in some forests. We find no evidence for an effect of trichomes on predation in the two plant species tested. Equal attack numbers on caterpillar- and humanoid-shaped objects challenge the idea that the visual resemblance of model prey alone is sufficient to deceive predators. We conclude that attacks on model prey represent a variety of responses to novel objects (e.g. exploration, aggression, possibly predation) and urge caution when interpreting their results.

Abstract in Spanish is available with online material.

KEYWORDS
enemy-free space, insects, multitrophic, Panama, predation, predator–prey interactions, prey shape, trichomes

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1 | INTRODUCTION

Insect herbivores cause tropical trees to lose around a quarter of their foliage (Arnold & Asquith, 2002) and can directly affect the plant community composition of rainforests (Szefer et al., 2020). Insect herbivores may further contribute to the high diversity of tropical rainforests by mediating the coexistence of plants with different defensive strategies (Coley & Kursar, 2014). Predation can effectively limit herbivory (Harrison & Banks-Leite, 2020; Kalka et al., 2008; Styrsky et al., 2006) with cascading effects on plant performance (Styrsky et al., 2006), community composition (Harrison & Banks-Leite, 2020), and diversity (Chesson & Kuang, 2008). Assessing temporal and spatial variation in plant–herbivore–predator interactions is crucial to advance our understanding of the mechanisms affecting the structure and stability of tropical rainforest communities.

Predation can affect the chemical traits, physiology, and behavior of insect herbivores (Greeney et al., 2012) and drives their behavioral search for enemy-free space, i.e., times or localities at which predation risk is reduced. Direct evidence of diurnal variation in predation within forests is scarce and the few existing studies report inconsistent results of either higher (Seifert et al., 2016) or lower (Ferrante et al., 2017) daytime versus nighttime predation. Nocturnal feeding by undefended caterpillars of some temperate and subtropical Lepidoptera species has been suggested to be a behavior that avoids predominately diurnal predators (Berger & Gotthard, 2008; Heinrich, 1979). In tropical forests, however, caterpillar activity seems not to vary between day and night (Novotny et al., 1999) and predator exclosures had a stronger positive effect on insect herbivore abundance at night than during the day (Kalka et al., 2008). We thus suggest that higher nocturnal predation in tropical forests may provide herbivores with temporal enemy-free space during the daytime.

Spatial enemy-free space could be provided by trichomes (plant hairs). Non-glandular trichomes are a common feature of tropical rainforest plants (Ichie et al., 2016) and present a structural defense against insect herbivores by reducing the insects' mobility and feeding efficiency (Gorb & Gorb, 2019; Hanley et al., 2007; Levin, 1973). It thus seems surprising that butterflies often prefer pubescent plants for oviposition on which their offspring suffer from reduced growth rates and survival (Jones & Agrawal, 2019; Kariyat et al., 2017). Such oviposition preference in swallowtails has been explained by reduced mobility of arthropod predators, which captured fewer caterpillars on pubescent plants (Fordyce & Agrawal, 2001). Predation is the greatest source of larval mortality for many insect herbivores (Cornell & Hawkins, 1995) and may drive the selection of pubescent food plants because the positive effect of reduced predation outweighs associated costs in larval growth.

While trichomes should not affect predation by birds or flying arthropods, they may compromise the ability of two main predator groups to detect and access their insect prey. Walking arthropods, and ants in particular, are important and abundant predators of insect herbivores in tropical rainforests (Leponce et al., 2021; Novotny et al., 1999). Ants have been shown to be impaired by trichomes (Davidson et al., 1989) and to prefer climbing glabrous stems over stems covered with stiff trichomes while foraging (Gorb & Gorb, 2019) possibly due to the increased friction imposed by downward pointing stem trichomes (Vermeij, 2015). Stem trichomes could provide herbivores with enemy-free space because walking arthropod predators avoid the higher energy expenditure of climbing pubescent stems during opportunistic foraging if other substrates are available.

Predation efficiency of bats, another major group of predators of insect herbivores in neotropical rainforests (Kalka et al., 2008; Kalka & Kalko, 2006), could be affected by foliar trichomes. The acoustic

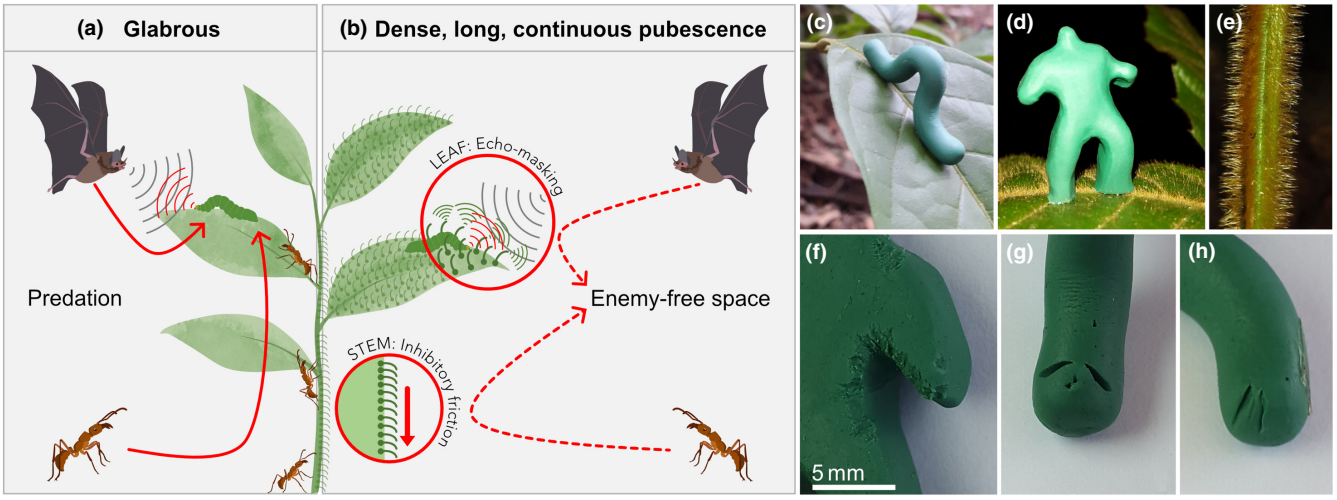

FIGURE 1 Illustration of hypothesis 2: (a) successful echolocation of a caterpillar by bats and ascent of walking arthropod predators on glabrous plants. (b) Foliar trichomes reduce the acoustic specular effect and mask prey echoes, and stem trichomes inhibit arthropod ascent, thereby creating enemy-free space for herbivores on pubescent plants. (c) Artificial caterpillar on a glabrous *Cupania cinerea* leaf, and (d) hulk on a pubescent *C. rufescens* leaf. (e) Stem pubescence of *C. rufescens*. Attack marks by (f–g) chewing arthropods, and (h) a bird. Scale bar applies to f–h only.

specular effect by which smooth surfaces, e.g., glabrous leaves, reflect echolocation calls like a mirror makes it easy for bats to locate prey resting on a smooth surface (Geipel et al., 2019). Rough surfaces however can scatter echolocation calls (Clare & Holderied, 2015) and reduce the detectability of prey. Surface protrusions of just 30-µm can alter reflected echoes sufficiently to impact bat behavior (Habersetzer & Vogler, 1983). We thus hypothesize that several millimeter-long foliar trichomes can scatter bat echoes and reduce the predation risk of insects. In conclusion, the primary plant defenses stem and foliar trichomes may reduce predation pressure and provide herbivores with enemy-free space on pubescent plants (Figure 1).

Measuring predation of insect herbivores in natural settings is complicated as predation often happens quickly with predators leaving no traces of their prey. Artificial, plasticine caterpillars in which predators leave distinctive attack marks without removing the objects (Howe et al., 2009; Lövei & Ferrante, 2017) are thus commonly used to estimate predation risk of insect herbivores. Such model prey offers many advantages, including its cheap and simple production, wide applicability (Howe et al., 2009), and comparability of results across studies and treatments. However, model prey lacks olfactory and chemical characteristics that predators use as cues for prey identification (summarized in Howe et al., 2009; Lövei & Ferrante, 2017). This is generally accepted as an explanation for the typically lower estimates of predation derived by using model prey compared to those derived using real prey (Lövei & Ferrante, 2017). However, we know little about the effect of model prey shape on predation estimates. It is widely assumed that attacks on model prey represent predation events because predators perceive objects mimicking prey color and shape as real prey. This assumption may hold true for birds and some arthropod predators, e.g. wasps and mantids (Greeney et al., 2012), which mainly use visual cues and start attacks from a distance. Most arthropods – that typically cause most attacks on plasticine prey in tropical forests (Liu et al., 2020; Seifert et al., 2016) – however, get much closer to their prey before attacking it and other cues may be of greater importance. Together with the repeated appearance of marks left by non-predators on plasticine objects (Pfennig et al., 2007) this fuels a debate on whether plasticine caterpillars record predation specifically, or otherwise a range of animal responses to a novel object, possibly including predation.

Here, we use plasticine prey to quantify variation in predation pressure on insect herbivores in tropical rainforests. We hypothesize that *(a) predation will be higher during the night than during the day*. Stem trichomes may increase climbing resistance for walking arthropod predators and foliar trichomes may disturb the acoustic specular effect for bats. We thus hypothesize that *(b) predation will be higher on glabrous than on pubescent plants*. Finally, we test the widespread assumption that prey shape is a decisive factor for prey recognition by comparing attacks on caterpillar-shaped versus humanoid-shaped objects resembling The Incredible Hulk superhero (hereafter "hulks"). We hypothesize that *(c) predation will be higher on caterpillars than on hulks* because predators recognize model prey by its shape.

2 | METHODS

We conducted two experiments in the understory of two lowland rainforest sites in Panama to assess temporal and spatial variation in predation and test the importance of model prey shape and size for predator recognition. Both experiments were performed in closed-canopy sites with flat terrain and at the end of the dry season to minimize the impacts of frequent rainfall which can dislodge model prey (Howe et al., 2009).

2.1 | Island experiment

We compared diurnal and nocturnal attacks on plasticine caterpillars and hulks placed on glabrous and pubescent plants in the understory of Barro Colorado Island (BCI) in Panama [9°09′37"N, 79°50′45"W; 2600 mm/yr rainfall, 3 month dry season (Leigh, 2019)] in February 2020. To standardize host plant height and distance among hosts, we cut similar-sized branches and arranged them in a 10 × 10 matrix with a distance of 1 m between individual branches and alternating between a pubescent and a glabrous species. Branches were cut from young trees with a maximum height of 3 m that grew individually (not clustered) within a 1 km radius of the experimental site. We cut between one and three 40 to 80 cm long branches per plant, which had a minimum of two undamaged leaves, a branch diameter of 0.8 to 1.2 cm, and vertical growth with leaf angles similar to those of small saplings. Directly after they were cut, branches were placed into sealed water containers that were buried in the soil to keep the branches hydrated for the duration of the experiment and mimic a naturally growing plant. We selected sites with minimal understory vegetation and our experimental branches did not touch any plants that were naturally growing in our sites. Small flags marked the experimental area and facilitated retrieval in the understory, while avoiding effects on predator movement and bias through direct tagging of plants.

The congeneric tree species *Cupania rufescens* Triana & Planch. and *C. cinerea* Poepp. (Sapindaceae) were selected as focal species as both coexist in the BCI forest and their structural similarity precludes any potential effects of leaf shape and general plant architecture on predation. *Cupania rufescens* is a medium-sized tree growing in tropical rainforests from Mexico to Brazil. Young plants have dense pubescence on stems, branches, and both surfaces of the compound leaves (Figure 1d–e) with obovate-oblong leaflets with serrated margins [7–22 cm long × 3.5–10 cm wide (Woodson et al., 1976)]. *Cupania cinerea* occurs in lowland tropical rainforests from Costa Rica to Bolivia as a medium-sized tree, has compound leaves, obovate leaflets and also a serrated margin (6–17 × 3.5-7 cm), but all plant parts are glabrous (Figure 1c) except for soft trichomes

covering the lower leaf surface (Woodson et al., 1976) that should not affect predation by bats or arthropods. None of the species is myrmecophytic. Many lepidopteran caterpillars feed on the upper leaf surfaces of *C. rufescens* and *C. cinerea*, with six species known to feed on both (Janzen & Hallwachs, 2019). Six out of seventeen and four out of twelve recorded herbivore species associated with *C. rufescens* and *C. cinerea* respectively, are inconspicuously brown or green, without obvious defenses, and 28–46 mm long (Janzen & Hallwachs, 2019). We used plasticine caterpillars to visually mimic this subset of the tree species' known natural herbivores.

Predation pressure was measured using model prey. Caterpillars (50×4 mm) and hulks (with a height of 30 mm that equaled the maximum height of the bent caterpillars) were molded from green, odorless (to humans), non-toxic Newplast (Newclay Products Ltd., Newton Abbot, UK). Caterpillars were shaped and bent to mimic the posture of common geometrid caterpillars. Hulks resembled small (but fearsome) humanoid figurines, modeled on the superhero of Marvel comics (Marvel Worldwide Inc.), and were shaped using custom made plastic molds. We used hulks as a control to the more naturally shaped caterpillar models to test whether objects resembling natural prey are more recognized as such by potential predators. All objects were modeled and handled using surgical gloves to avoid leaving unwanted cues (e.g., scent) to predators. One hundred hulks and one hundred caterpillars were placed on 50 plants per host species (i.e., one object of each type per plant). For each plant, we selected two undamaged leaves that grew in opposite directions and at a similar height, 30 to 80 cm above the ground. We attached the objects close to the midrib on the upper leaf surface with a small amount of fast-setting glue (Loctite Superglue, Henkel AG, Düsseldorf, Germany).

For four consecutive days (total of 96 h), plasticine objects were inspected at 12-hour intervals at dusk and dawn (18.15 to 19.15 h and 06.15 to 07.15 h) to differentiate diurnal from nocturnal predation. We removed attacked objects from the sites without replacement and identified predator attack marks using reference pictures (e.g., Low et al., 2014).

2.2 | Mainland experiment

To assess the impact of model size as an additional effect and test for a possibly confounding effect of host plant transplantation in the island experiment, we conducted a follow-up experiment using uncut saplings growing in a close-by mainland forest site in Gamboa, Parque Nacional Soberanía (9°9′42″N, 79°44′43″W), in early-March 2021. Following the same procedure as for the island experiment described above, we glued 27 caterpillars, 27 hulks, and 27 small caterpillars (30×2 mm) close to the midrib on the upper side of leaves of 27 tree saplings (one object of each type per sapling). In this follow-up experiment, however, we did not select specific plant species, but chose saplings of various species that were naturally growing in the field site. All plants were of similar size (40–100 cm tall), with simple, elliptic to ovate, smooth-edged leaves, and without any foliar or stem pubescence.

2.3 | Statistical analysis

2.3.1 | Predation risk

We analyzed predation risk as the total percentage of attacked objects at the end of each experiment using pairwise two-sided Fisher's exact tests and calculated the estimated odds ratio (OR) as a measure of effect size (package "rstatix"). Given our relatively small sample sizes and attack numbers, many values in the contingency table of expected results for Chi-square tests were ≤5, in which case the Fisher's test is the preferred analytical method as it does not rely on an approximation but is exact (McDonald, 2009). We performed pairwise tests with Holm-adjusted p-values to correct for multiple comparisons to identify variation in predation risk with time of day and pubescence for each possible object-experiment combination and for the predation risk averaged across objects per experiment. To test for interaction effects, we calculated pairwise tests for all combinations of pubescence, time of day, and object in the island experiment and all combinations of object and time of day in the mainland experiment. Finally, we compared overall, diurnal, and nocturnal predation risk across objects between the island and mainland experiment. Missing objects were excluded from the analysis.

To facilitate comparison with other studies we additionally provide mean daily predation risk, which is the predation risk divided by the number of exposure days (e.g., Molleman et al., 2016; Seifert et al., 2016).

2.3.2 | Predation rate

To incorporate the temporal component of predation, we calculated predation rates (PR) as the ratio between the number of attacked objects and the time at risk, i.e., the number of hours that each object was exposed, totaled over all objects per object type. For each object we added the hours of exposure until one of three possible events: attack, loss, or the end of the experiment. An object that remained without attack until the end of the experiment added 0 to the nominator and the full experimental time of 96 hours to the denominator. An attacked object added 1, and a lost object added 0 to the nominator. As we cannot know when exactly an attack or loss occurred within a 12-hour census interval, we assume that the event happened on average halfway through the respective census period (Cummings, 2019) and thus added 6 hours to the time at risk. Hence, each attacked or lost object contributed the hours of pre-event censuses plus the 6 hours of the census period during which the event occurred to the denominator. Predation rates can thus be understood as the predicted number of attacked objects in 1000 object-hours of exposure (Cummings, 2019).

We calculated predation rates for the full experimental period (96 hours) and separately for diurnal and nocturnal time periods (48 hours each) for each object and each object-pubescence combination (epi.conf in "epiR"). To test for statistic differences, we conducted pairwise comparisons of estimated point differences at

a confidence level of 0.95 (ratedifferences in "fmsb"). All analyses were performed in R (R Core Team, 2021).

3 | RESULTS

A total of 11.0% and 16.9% of objects were attacked in the island and mainland experiment, resulting in mean daily predation risks of 2.8% and 4.2%, respectively (Table 1). In both experiments, predation was almost exclusively due to arthropods (Figure S1). In the island experiment, one out of 22 attacks was due to a bird (Figure 1h), and on the mainland, one out of 13 attacks was caused by a small mammal (Figure S1). We lost one hulk during the island experiment, and one hulk and three small caterpillars during the mainland experiment, equaling 0.5% and 4.9% of exposed objects.

3.1 | Variation with time of day

In the island experiment, predation risk across objects was 5.5% both during the day and during the night (Table 1). Even within objects, diurnal and nocturnal predation risk was almost identical being 1% higher at daytime for caterpillars (OR = 1.2, $p = 1$) and 1% higher at nighttime for hulks (OR = 0.8, $p = 1$; Figure 2a & Table S1). Likewise, predation rates ($\pm$ *SE*) did not differ between day (1.2 ± 0.4) and night (1.2 ± 0.4) across objects ($p = .99$) but was slightly higher at night for hulks on pubescent plants ($p = .046$; Table S2).

In the mainland experiment, the predation rate was significantly higher at night than during the day ($p = .047$; Figure S2, Tables 1 and S2). A non-significant, yet consistent trend of a higher nocturnal predation rate was evident for all three object types but was strongest for small caterpillars that were five times more likely to be attacked during the night than during the day ($PR_{day} = 0.9 \pm 1.3$, $PR_{night} = 5.0 \pm 2.6$, $p = .09$; Table 1). Similarly, nocturnal predation risk was three times higher for caterpillars, and doubled for hulks (Table 1, Figures 2 and 3).

3.2 | Variation with host plant surface

Neither predation rate (Table S2, Figure 2a), nor predation risk differed between glabrous and pubescent host plants when averaged across objects and in the separate analyses for hulks and caterpillars, with a total of 11 objects attacked on each plant type (OR = 1, $p = 1$; Table S1).

3.3 | Variation with prey object

In the island experiment, hulks and caterpillars experienced an equal predation risk of 11% (OR = 1, $p = 1$; Table S1) and equal predation rates of 1.2 ± 0.4. In the mainland experiment, we found a strong, albeit statistically not significant (all $p > .2$, Tables S1 and S2), trend towards higher predation risk and predation rate in small caterpillars (risk = 25%; $PR = 3.1 \pm 1.4$) as compared to caterpillars ($risk_{mainland} = 14.8\%$, $risk_{island} = 11\%$; $PR_{mainland} = 1.7 \pm 1$, $PR_{island} = 1.2 \pm 0.4$) and hulks ($risk_{mainland} = 11.5\%$, $risk_{island} = 11\%$; $PR_{mainland} = 1.3 \pm 1$, $PR_{island} = 1.2 \pm 0.4$; Table 1, Figures 2 and 4). Predation risk and rate did not differ between the experiments for caterpillars, hulks or across the two object types (Tables S1 and S2).

4 | DISCUSSION

We used artificial prey to evaluate spatial and temporal variation in predation pressure on insect herbivores in two tropical rainforest sites and test the importance of model prey shape for predator recognition. Predation was higher at night than during the day in one of our sites, but did not vary with time of day in the other site. Whether insect herbivores benefit from temporal enemy-free space may thus be locality-specific. Predation did not differ between objects placed on pubescent or glabrous *Cupania* plants, suggesting that in this plant genus trichomes may not provide enemy-free space to insect herbivores. Object shape did not affect the number of attacks, suggesting that prey shape may not be as important for predator recognition as previously assumed. Our results thus highlight the need for caution when interpreting model-prey-derived estimates of predation.

Mean daily predation risk was 2.8% in the island experiment and 4.2% in the mainland experiment (Table 1). While these levels are low, they are comparable to the 2.4% of artificial caterpillars attacked per day in other lowland rainforests (Tvardikova & Novotny, 2012). Model-prey-derived estimates of predation typically underestimate actual predation pressure (Lövei & Ferrante, 2017) and a daily mortality rate of 5% has been projected to result in a mortality risk of 66% over a caterpillar's life span (Tvardikova & Novotny, 2012). Finally, parasitoid attacks are not captured well by this method, yet further increase the mortality risk of real caterpillars. Thus, our results support previous evidence that predation strongly affects the abundance of herbivorous caterpillars in tropical rainforests.

4.1 | Variation of predation with time of day

Nocturnal predation was significantly higher than diurnal predation in our mainland forest experiment, where objects were 3.5 times more likely to be attacked at night than during the day. However, this effect was not seen in the island experiment, where diurnal and nocturnal predation were notably similar (Figure 3 and Table 1). The result of our mainland experiment is in accordance with a higher nocturnal predation on stick insect nymphs in the BCI forest (Berger & Wirth, 2004) but contrasts with studies in other tropical rainforests that found that attacks peaked during the day (Novotny et al., 1999; Seifert et al., 2016).

In our study, 83% of attacks were caused by chewing arthropods and we suspect that beetles, orthopterans, and ants were

TABLE 1 Predation risk as the total percentage of attacked objects at the end of the island and mainland experiments

	Day			Night			Total		
	Risk (%)	Mean risk (% 24/h)	PR ± SE	Risk (%)	Mean risk (% 24/h)	PR ± SE	Risk (%)	Mean risk (% 24/h)	PR ± SE
Island experiment									
Caterpillar									
Glabrous	4.00	2.00	0.85 ± 0.75	4.00	2.00	0.86 ± 0.77	8.00	2.00	0.87 ± 0.51
Pubescent	8.00	4.00	1.71 ± 0.10	6.00	3.00	1.29 ± 0.90	14.00	3.50	1.55 ± 0.66
Total	6.00	3.00	1.28 ± 0.59	5.00	2.50	1.08 ± 0.55	11.00	2.75	1.21 ± 0.40
Hulk									
Glabrous	10.00	5.00	2.21 ± 1.13	4.00	2.00	0.85 ± 0.76	14.00	3.50	1.60 ± 0.68
Pubescent	0.00	0.00	0.00 ± 0.00	8.00	4.00	1.73 ± 1.01	8.00	2.00	0.86 ± 0.50
Total	5.00	2.50	1.07 ± 0.55	6.00	3.00	1.29 ± 0.60	11.00	2.75	1.22 ± 0.40
Combined									
Total	5.50	2.75	1.17 ± 0.39	5.50	2.75	1.18 ± 0.39	11.00	2.75	
Mainland experiment									
Small caterpillar									
Total	4.17	2.08	0.90 ± 1.27	20.83	10.42	4.99 ± 2.56	25	6.25	3.09 ± 1.43
Caterpillar									
Total	3.70	1.85	0.80 ± 1.13	11.11	5.56	2.42 ± 1.67	14.81	3.70	1.66 ± 0.97
Hulk									
Total	3.85	1.92	0.83 ± 1.17	7.69	3.85	1.67 ± 1.48	11.54	2.88	1.29 ± 0.90
Combined									
Total	3.90	1.95	0.84 ± 0.58	12.99	6.50	2.90 ± 1.00	16.88	4.22	

Note: Mean predation risk is predation risk averaged per 24 hours. Predation rate (PR) ± standard error (SE) is the number of attacked objects divided by the time at risk, i.e., the sum of hours each individual object was exposed to predation totaled over all objects of each type and can be interpreted as number of attacked objects per 1000 objects exposed for 1 hour.

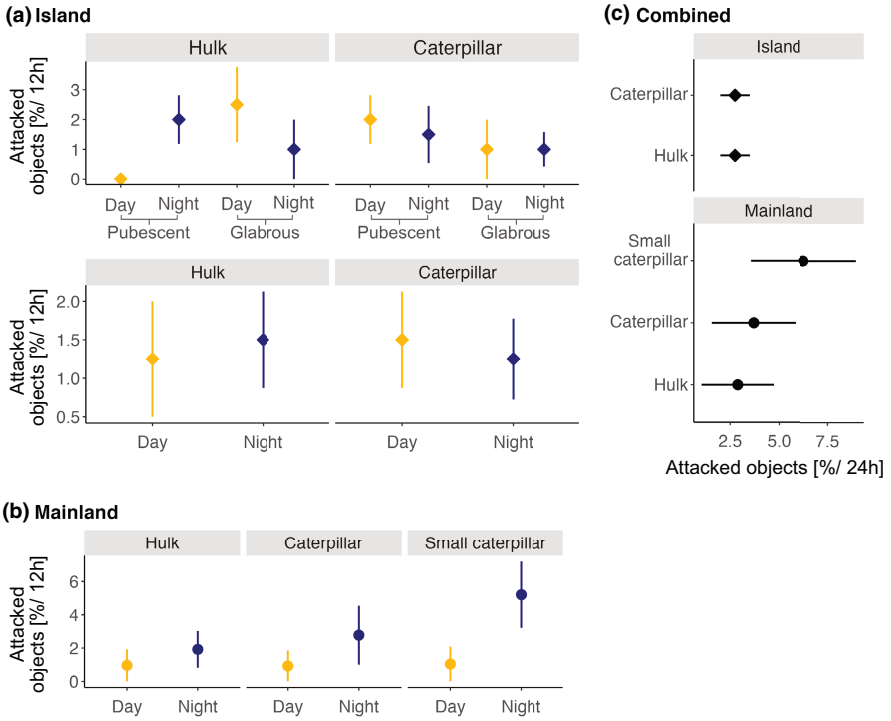

FIGURE 2 Proportion of attacked objects per 12-hour census interval ± standard error expressed as diurnal (yellow) versus nocturnal (blue) predation risk for (a) each combination of plant type × object type (upper row) and averaged over plant types (lower row) in the island experiment and (b) mainland experiment. (c) Comparison of daily predation risk per object type.

main predators (Figure S1). Carabid beetles (Ferrante et al., 2017) and orthopterans (Novotny et al., 1999) have been identified as important nocturnal predators of insects in forests. Ants also are dominant predators in tropical rainforests (Leponce et al., 2021), where they typically cause the overwhelming majority of attacks on artificial caterpillars (e.g., Molleman et al., 2016; Seifert et al., 2016). While ant attacks were reported overall not to vary between night and day (Novotny et al., 1999; Seifert et al., 2016), shifts in ant community composition were shown to explain a marked increase in attacks within the first hours of the night in a tropical rainforest in China (Liu et al., 2020). We suggest that spatial variation in predator community composition and temporal niche partitioning might explain contradicting results between our experiments and among studies on diurnal variation in predation. A few particularly active or aggressive species that peak in their activity at a specific day- or nighttime might be sufficient to skew trends in either direction. Ultimately, our results do not support the assumption that nocturnal feeding by caterpillars is an effective strategy to escape predation (Berger & Gotthard, 2008) and in our mainland forest site the opposite may be the case with herbivores potentially benefiting from reduced predation pressure during the day.

4.2 | Plant trichomes of *Cupania rufescens* do not provide enemy-free space

For our two host plant species we found no effect of pubescence on predation. An equal 11 out of the 100 objects placed on glabrous and pubescent plants respectively were attacked (Table 1 and Figure 2a), hence providing no support for the idea that trichomes of *Cupania* species provide insect herbivores with enemy-free space.

Due to the complete lack of detected attacks by bats, we cannot draw conclusions on the effect of trichomes on bat predation. The gleaning bat *Micronycteris microtis* – a common insectivore on BCI (I. Geipel, personal communication) – has been shown to use echolocation to discriminate between the surface structure of prey and attacked model prey made of crumbled aluminum similarly as real prey, while neglecting dummies made from paper and smooth aluminum (Geipel et al., 2013). It thus seems likely that some bats use the combination of object shape and surface structure to differentiate between motionless insect prey and the environment. Given the complexity of the forest understory and the similarity of prey and background (e.g., imagine stick insects versus sticks), these skills can be expected to be extremely precise. It remains to be tested whether the difference in reflective properties between plasticine and chitin could explain the lack of bat attacks on plasticine model prey in our study. We recommend using a different material or real sentinel prey in future studies to address the question of whether trichomes may reduce the acoustic specular effect.

Arthropods readily attacked our objects despite the lack of prey-specific behavioral or chemical cues (Figure 1f–g). Coming from the forest floor, walking arthropods can neither visually detect model prey, suggesting that foraging may be largely opportunistic. Earlier studies have shown that the identity of glabrous plants does not affect attacks on artificial (Molleman et al., 2016) or live prey in tropical rainforests (Novotny et al., 1999). Trichomes were reported to be overall harmful for predatory insects across 47 studies (Riddick & Simmons, 2014) and to present a physical barrier to ants (Gorb & Gorb, 2019). However, here we show that walking arthropod

predators are not deterred by dense, downward pointing stem trichomes of *C. rufescens* (Figure 1e) and attack model prey on both glabrous and pubescent plants. In conclusion, we did not find evidence that *C. rufescens* foliar or stem trichomes can provide enemy-free space.

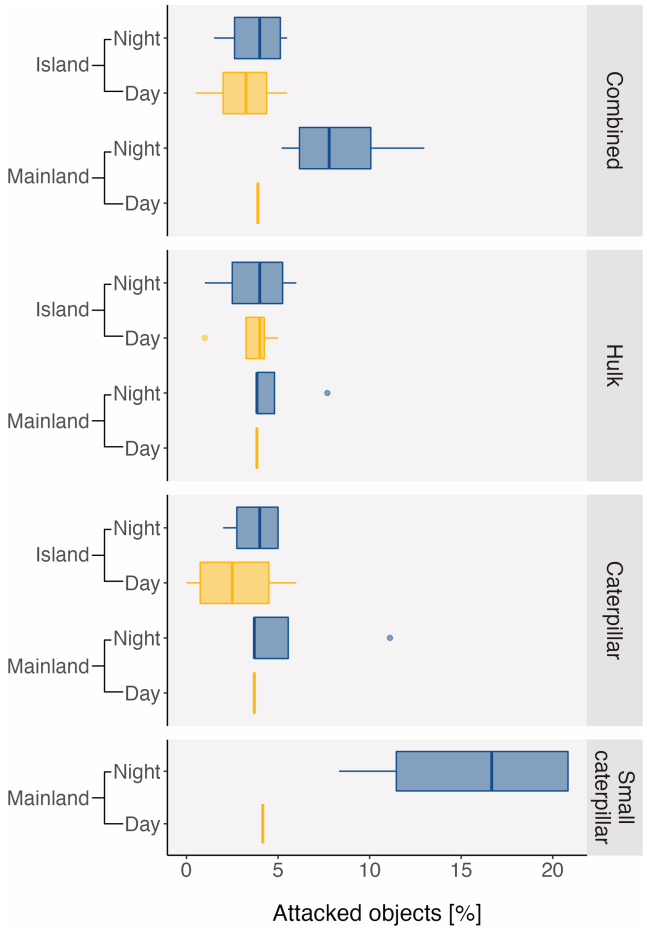

FIGURE 3 Diurnal (yellow) and nocturnal (blue) predation expressed as cumulative percentage of attacked objects over time for small caterpillars, caterpillars, and hulks in the mainland and island experiment.

4.3 | Not object shape, but size matters

Small caterpillars received twice as many attacks as caterpillars and hulks (Figure 4a). Our finding agrees with a reported negative correlation between model prey length and number of arthropod attacks across 45 studies (Lövei & Ferrante, 2017). Smaller predators may prefer smaller prey due to a reduced risk of injury as compared to attacking large prey (but see: Molleman et al., 2016), while vertebrate predators may prefer bigger prey (Remmel & Tammaru, 2009).

Object shape did not affect our predation estimates and the humanoid-shaped hulks were attacked as frequently as large caterpillars (Figure 3). Predators are argued to confuse plasticine caterpillars with palatable and undefended prey due to their resemblance in color and shape (Howe et al., 2009; Lövei & Ferrante, 2017); hence, this method has been used widely to test for predation of herbivores, as we did here. While predators were shown to not be attracted by the plasticine material itself (Tvardikova & Novotny, 2012), bite marks by non-predatory insects and rodents on plasticine snakes (Bateman et al., 2017; Pfennig et al., 2007), and video recordings of tree crickets and moths feeding on artificial caterpillars (Molleman et al., 2016) show that a variety of species can cause "attack marks" on model prey with only some of them being predators of true caterpillars. Some of the attacks on our hulks may present opportunistic predation triggered by object size and unsuspicious coloring. Given their humanoid shape, we however suggest that many attacks result from defensive, exploratory, or aggressive behavior and do not reflect predation attempts.

Artificial prey simulates visual prey cues but lacks any motion, chemical, and tactile cues. It is thus surprising that arthropods that rely on chemical and tactile cues accounted for 21 of 22 attacks in our island and 12 of 13 attacks in our mainland experiment. They were also responsible for more than 90% of attacks on artificial caterpillars

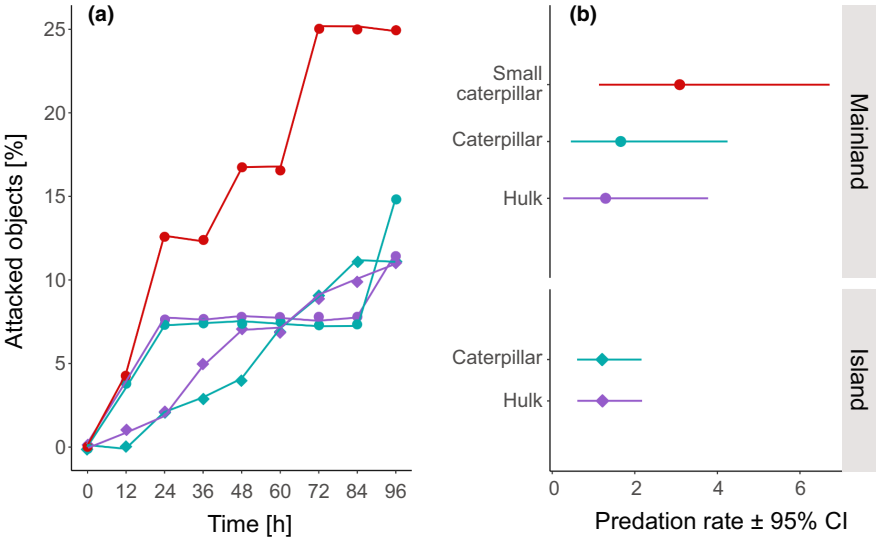

FIGURE 4 (a) Cumulative percentage of attacked objects over time and (b) mean predation rate with 95% confidence intervals for small caterpillars (red), caterpillars (turquoise), and hulks (purple) in the island (diamond) and mainland (circle) experiments.

in other tropical forests (Liu et al., 2020; Seifert et al., 2016). Birds, which are mainly visually oriented, however caused only one out of 35 attacks in our study and are typically responsible for less than 5% of attacks on model prey in the understory of tropical lowland forests (e.g., Liu et al., 2020; Seifert et al., 2016). Bird attacks on model prey might increase in relative importance with disturbance (Posa et al., 2007), habitat openness (Williams-Guillen et al., 2008), and elevation (Tvardikova & Novotny, 2012). Nonetheless, the coarse identification of taxa causing attack marks in studies like ours, the inability to distinguish the context of attacks, and a lack of knowledge of how predators perceive and process prey-derived information, raise the question of whether attacks on artificial prey actually present predation or whether they include other behaviors too. We show that model shape is less important than it theoretically should be and thus call for caution when interpreting attack marks as caterpillar specific predation.

5 | CONCLUSIONS

Our results suggest that variation in predation with time of day may be locality specific, as predation was higher at night than during the day in one but not the other forest site. We find no evidence of enemy-free space being provided by the trichomes of *Cupania rufescens* plants. Further studies including a larger set of forest sites and plant species are needed to determine the generality of our findings.

Caterpillar- and humanoid-shaped objects got attacked with equal frequency. The fact that the shape of plasticine prey had no effect on attack rates by invertebrate predators is novel and striking. Our results challenge the idea that the visual resemblance of model prey alone is sufficient to deceive predators, question the nature of attacks on artificial prey, and imply that further studies are needed to explain how predators use prey-derived cues to decide for or against an attack. While model prey studies have their value in our quest to understand complex multitrophic interactions, we urge greater caution when interpreting their results.

AUTHORS' CONTRIBUTIONS

AW, LM and IG developed the study idea and design. AW and IG conducted the field experiments. AW led the writing of the manuscript and conducted the statistical analyses. All authors contributed to the manuscript and gave final approval to the publication.

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CONFLICT OF INTEREST

The authors declare that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available from https://zenodo.org at http://doi.org./10.5281/zenodo.5674690.

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