Behavioral Ecology

The official journal of the
ISBE
International Society for Behavioral Ecology

Behavioral Ecology (2023), 34(6), 1055–1064. https://doi.org/10.1093/beheco/arad076

Original Article

Golden mimics use multiple defenses to counter generalist and specialist predators

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Received 24 November 2022; revised 9 June 2023; editorial decision 28 August 2023; accepted 7 September 2023

Many prey species employ multiple defenses during interactions with predators. Multiple defenses can provide a selective advantage against a single predator at different stages of the interaction or attack, as well as against multiple predator types. However, the efficacy of multiple defenses both during different sequences of an attack and against multiple predator types, remains poorly understood. We measured and classified defensive traits used by five mimics (Müllerian and Batesian) of the myrmecomorphic golden mimicry complex and one non-mimetic species. We then performed predatory trials using two different predators that differed markedly in their body size, trophic specialization, and how they handle prey—one being an ant specialist (spider) and the other a generalist which avoided ants (skink). We identified 12 defensive traits and classified them into four groups (primary, chemical, mechanical, and behavioral), which were strongly correlated. Skinks were much less likely to attack and capture mimics than the ant-eating spider predators. Our results show that multiple defenses (five or six) were used against each predator. The defensive behaviors and features that were most effective against skinks included appendage waving and large body size, whereas the golden "shine" warning signal, large body size, cuticle thickness, and defensive gland size were most effective against spiders. Most defenses appeared to be predator-specific. We conclude that potential prey in the golden mimicry complex have been selected for multiple defenses because of their vulnerability to different predator types and consequently, the efficacy of some of these defenses likely represents a trade-off.

Key words: **Araneae, Batesian mimicry, Formicidae, Heteroptera, multimodal signaling, Müllerian mimicry.**

INTRODUCTION

Most prey are vulnerable to more than one type of predator and have evolved multiple lines of defense as a consequence (Caro 2005; Ruxton et al. 2018; Winters et al. 2021). Defenses are typically classified as either primary, which prevents physical contact between predator and prey or secondary, which enhances the chances of survival following contact with a predator. Secondary defenses can be chemical, mechanical, or behavioral (Ruxton et al. 2018), and are detected by different sensory systems including vision, hearing, touch, olfaction, and gustation. During a predatory attack, different defenses can act simultaneously or sequentially at different stages of the predatory sequence (Caro 2005). Multiple defenses can increase the efficiency of information transfer by acting on several sensory modalities (Rowe and Guilford 1999) or by playing a different role: one defense can educate predators while others can elicit innate avoidance (Hauglund et al. 2006). Theoretically, suites of defenses should be concentrated at the early stages of the interaction because late-stage defense might be more costly (Wang et al. 2019). Investment in defensive structures and behavior is related to the type of the predator: early-stage defenses (e.g., crypsis) are favored against generalist predators while late-stage defenses (e.g., chemicals) are used against specialist predators (Broom et al. 2010).

Alternatively, multiple defenses should afford protection against an array of predators. Yet, evidence to support this hypothesis is limited. Predators vary in foraging behavior and how they attack and subjugate prey (Greeney et al. 2012). Potential prey thus experience selective pressures from multiple predators (Ratcliffe and Nydam 2008) and have evolved a suite of defenses (e.g., Van Buskirk 2001) composed of general and specific defenses (e.g., Kishida and Nishimura 2005). A key prediction is a positive association between the number of defenses and the number of different predators (Wang et al. 2019).

Interestingly, multiple defenses have been mainly studied in aposematic species in which conspicuous coloration is accompanied by pungent odors or disturbance sounds (Rowe and Guilford 1999). Aposematic species often form Müllerian mimetic complexes (or rings) composed of a variable number of species. Such complexes are often phylogenetically conserved, with mimics deploying similar defenses (Symula et al. 2001; Williams 2007; Alexandrou et al. 2011; Wilson et al. 2015). An exception to this is the myrmecomorphic golden mimetic complex consisting of over 100 species of

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phylogenetically unrelated arthropods, all characterized by a golden dorsal shine, including ants, heteropterans, hemipterans, wasps, and spiders (Pekár, Petráková, et al. 2017). Members of this complex possess a wide variety of defenses such as spines, defensive glands, stings, aggression, or escape. Pekár, Petráková, et al. (2017) quantified the defenses and estimated an index of noxiousness for the majority of species within the complex. Species noxiousness within the complex was highly variable, suggesting that the complex contains Müllerian, quasi-Batesian, and Batesian mimics.

Multiple defenses often act simultaneously and possibly synergistically not only in Müllerian, but also Batesian, mimics. For example, ant-mimicking species imitate not only coloration but also body shape, body size, and the behavior of their model (Ceccarelli 2008; Pekár and Jarab 2011; Nelson and Card 2016; Shamble et al. 2017; Pekár et al. 2020). Although these traits are mainly visual, they may provide information of different quality, or reinforce other signals, as in the case of aposematic species (Rowe and Halpin 2013). Beside primary defense traits, myrmecomorphic species may use secondary defenses, such as defensive behaviors (e.g., Pekár et al. 2011).

Here, we measured multiple defensive traits expressed by selected members (ants, bugs, and spiders) of the golden mimicry complex representing Müllerian, Batesian, and quasi-Batesian mimics. We then examined the efficacy of these defenses and when they were employed by staging encounters between mimics and two predators—one specialist (ant-eating spider), and one generalist (lizard). The two predators were visually oriented but differed markedly in their trophic specialization and how they handle prey. While skinks catch arthropod prey using their mouth, subdue them by crushing them in their jaws and then swallowing them, *Servaea* spiders capture prey by biting and perform extra-oral digestion, thus potentially avoiding the effect of some secondary (chemical and mechanical) defenses. Therefore, we expected that the different defensive traits would be effective against different predators and at different stages of the predatory sequence. The main aims of this study were to uncover the role of multiple defenses during encounters with single predators and to evaluate the extent to which multiple defenses protect the mimic against multiple predator types.

METHODS

Study animals

We used five species from the golden mimicry complex (Pekár, Petráková, et al. 2017) and one non-mimetic species (house cricket, *Acheta domesticus*; shortened to *A. domesticus* hereafter). The mimetic species included the following three ant species (Figure 1): *Polyrhachis ammon* (Fabricius) (shortened to *P. ammon* hereafter), *Polyrhachis vermiculosa* Mayr (shortened to *P. vermiculosa* hereafter), and *Camponotus aeneopilosus* Mayr) (shortened to *C. aeneopilosus* hereafter); one true bug species: *Daerlac nigricans* Distant (shortened to *D. nigricans* hereafter); and one spider species: *Myrmarachne luctuosa* (L. Koch) (shortened to *M. luctuosa* hereafter). Based on the assessment of a number of defensive traits, such as presence of spines, thick cuticle, and defensive chemicals (Pekár, Petráková, et al. 2017), the ants are Müllerian mimics, the bug is a quasi-Batesian, and the spider is a Batesian, mimic. These species were selected in order to represent a wide spectrum of mimics, because they co-occur with the predators used, and because of their availability. Crickets were used as a non-mimetic control because they represent a familiar prey to skinks and their refusal would indicate an absence of motivation to forage.

Defensive traits

We quantified the defensive traits (Table 1) for 10 individuals per species. First, we measured the occurrence of biting (in ants) following a simulated attack with a pair of pincers. The thorax of an ant was grabbed once by a pincer for a second and then the ant was released. Then, one of us (S.P.) gently restrained the animal between fingers and smelled them to detect whether they actively emitted noxious chemicals (i.e., formic acid). Noxious chemicals in arthropods are typically volatile and therefore readily detectable by the human olfactory system. Escape speed was then measured in the laboratory using a Canon Legria HF G10 video camera. Mimics were released into an arena (50 × 30 cm) with a very thin film of butter on the sides to prevent escape. We filmed each individual for a period of 30 s and prodded the animal with forceps every 10 s. Escape speed was estimated from the footage using Kinovea software (www.kinovea.org) as an average speed per 5 s following disturbance. From the footage (30 s), we also measured frequency of antennae (insects) or foreleg (spider) waving as the number of waves per s.

Following the behavioral trials, all specimens were killed through exposure to ethyl acetate in a sealed container and then mounted on transparent tape to imitate the natural body position. In order to quantify size and shape, we photographed each animal using a Canon Legria HF G10 camera. We illuminated specimens from the side (approximately at 45°) using two fluorescent bulbs (13-W

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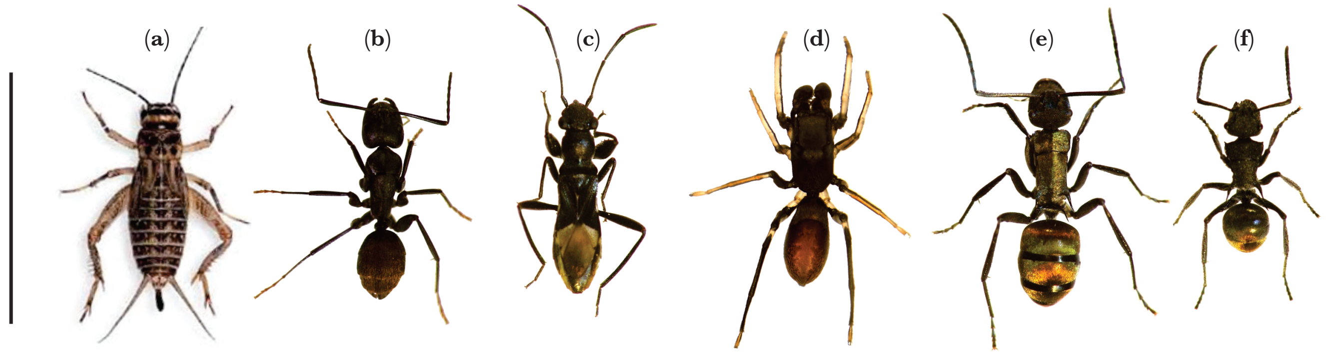

Figure 1

Habitus of study species. A. cricket *Acheta domesticus*, B. ant *Camponotus aeneopilosus*, C. true bug *Daerlac nigricans*, D. spider *Myrmarachne luctuosa*, E. ant *Polyrhachis ammon*, F. ant *Polyrhachis vermiculosa*. Scale = 1 cm.

Table 1

List of defensive traits and behaviors of five mimics and one non-mimetic species classified into four groups

Defenses	*Polyrhachis ammon*	*Polyrhachis vermiculosa*	*Camponotus aeneopilosus*	*Daerlac nigricans*	*Myrmarachne luctuosa*	*Acheta domesticus*
Primary						
Golden area proportion	0.65 (0.04)	0.38 (0.01)	0.46 (0.01)	0.36 (0.03)	0.37 (0.02)	0
Body shape	0.27 (0.01)	0.27 (0.01)	0.25 (0.01)	0.26 (0.01)	0.23 (0.01)	0.33 (0.01)
Total body size (mm)	9.04 (0.08)	5.98 (0.05)	8.04 (0.17)	7.40 (0.14)	7.04 (0.28)	5.93 (0.10)
Frequency of appendage waving (per s)	6.3 (0.97)	9.6 (0.98)	9.6 (1.08)	6.4 (0.6)	7.0 (1.12)	2.6 (0.9)
Chemical						
Spray chemicals	1	1	1	0	0	0
Gland size (mm^2)	2.84 (0.16)	1.62 (0.06)	2.43 (0.09)	0.57 (0.03)	0	0
Mechanical						
Number of spines	4	6	0	0	0	0
Total spines length (mm)	3.54 (0.08)	2.96 (0.08)	0	0	0	0
Jaw size (mm)	1.00 (0.02)	0.71 (0.03)	0.84 (0.02)	0	0	0
Cuticle thickness (mm)	0.04 (0.002)	0.03 (0.002)	0.02 (0.001)	0.02 (0.001)	0.02 (0.001)	0.15 (0.001)
Behavioral						
Frequency of biting	0.1	0	0.3	0	0	0
Escape speed (cm/s)	0.18 (0.03)	0.15 (0.01)	0.26 (0.01)	0.19 (0.02)	0.25 (0.02)	1.03 (0.35)

Values are means (± SE) estimated from 10 measurements in the case of traits showing variation.

daylight ReptiGlo 2.0 UVB) with a similar light spectrum to natural light. We then analyzed the images using custom-made image analysis software (Ježek 2015) to quantify body size and shape. The software extracts a binary mask of the whole body from the image. From the mask, the circularity index (Herrera-Navarro et al. 2013) was calculated as a measure of body shape (the lower the value, the more ant-like [articulated] shape). In addition, the total body size, the area with a golden shine on the head, thorax, abdomen or prosoma, and opisthosoma, were measured from photographs using ImageJ software (Schneider et al. 2012).

Last, the size and number of spines, and size of mandibles were measured using a stereomicroscope with an ocular micrometer for each specimen. The cuticle thickness was measured by slicing the thorax (mesosoma) or prosoma perpendicularly using a blade and then measuring it under the stereomicroscope with a precision nearest to 0.01 mm. To measure the size of any glands, the gland was dissected from the gaster (ants) or thorax (bugs) using fine forceps, placed on a glass slide in a drop of water, and enclosed by a cover slide. The gland was approximately oval; thus, two perpendicular diameters of the gland were measured using an ocular micrometer from which an area assuming a regular ellipsoid shape was calculated.

We grouped the measured traits into four groups based on the traditional classification (primary, chemical, mechanical, and behavioral) of defenses. As three of these groups (Table 1) included more than two highly correlated traits, we reduced the dimension of these groups using PCA to a single vector (scores along PCA1) following scaling (Supplementary Figure S1). To study relationship between the four groups of defenses, we used Spearman correlations as the scores did not approach a normal distribution and the relationships were not linear.

Response of predators

We used two visually oriented predators to test the efficacy of their defenses, one representing a generalist vertebrate predator, which is much larger than the mimics, the other was a specialized arthropod predator similar in size to the prey. Both predators have good color discrimination ability and both readily respond to visual movement (Peaslee and Wilson 1989; Barbour et al. 2002; Zurek et al. 2010, 2015; Fleishman et al. 2011; New et al. 2012). Importantly, they would have no difficulty detecting moving prey, or, discriminating golden coloration.

The generalist predator ($N = 26$) was the eastern water skink (*Eulamprus quoyii* (Quoy & Gaimard)). Only adult skinks, which came from a captive colony, were used. They were first generation captive-bred. The parental animals were collected locally, on campus at Macquarie University and in metropolitan Sydney. Skinks were housed individually in an opaque (white) plastic tub (70 × 50 × 40 cm) in which trials were performed. One individual of each prey species (*A. domesticus*, *C. aeneopilosus*, *D. nigricans*, *M. luctuosa*, *P. ammon*, and *P. vermiculosa*) was released into the tub in a constrained randomization. Ants were offered first (at random), because they are Müllerian mimics and the predators could experience a range of primary and secondary defenses. The other prey species were then offered at random. From recorded video footage we recorded the latency to spot (time from prey release to when skink turned to see the prey), latency to approach (time from spotting to when the skink started to move toward prey), latency to attack (time from approaching to actual capture), frequency of tongue-flicking (chemoreception) from predator approach to capture, attack and capture frequencies, and whether the captured prey was subsequently consumed or discarded (the whole prey body was either swallowed or spat out).

For the specialist predator ($N = 28$), we used adult females of the jumping spider *Servaea incana* (Keyserling). This species is myrmecophagous (McGinley et al. 2015). Spiders were collected by hand under the bark of gum trees on the Macquarie University campus, then placed individually in Eppendorf tubes, and kept at 23 °C for about 2 weeks before the experiment. During this time, they were fed small crickets. All trials were conducted in a Petri dish (8 cm diameter). All prey were individually released into the dish randomly and removed after the trial. In contrast to experiments with skinks, the randomization was not constrained as the spiders are myrmecophagous. We measured the size of spiders and mimics using calipers and the predator/prey total body size ratio was recorded prior to each trial. Because the predation behavior of spiders differs from that of skinks, not all behavioral events that occurred in skinks were measured in spiders. In each trial, we

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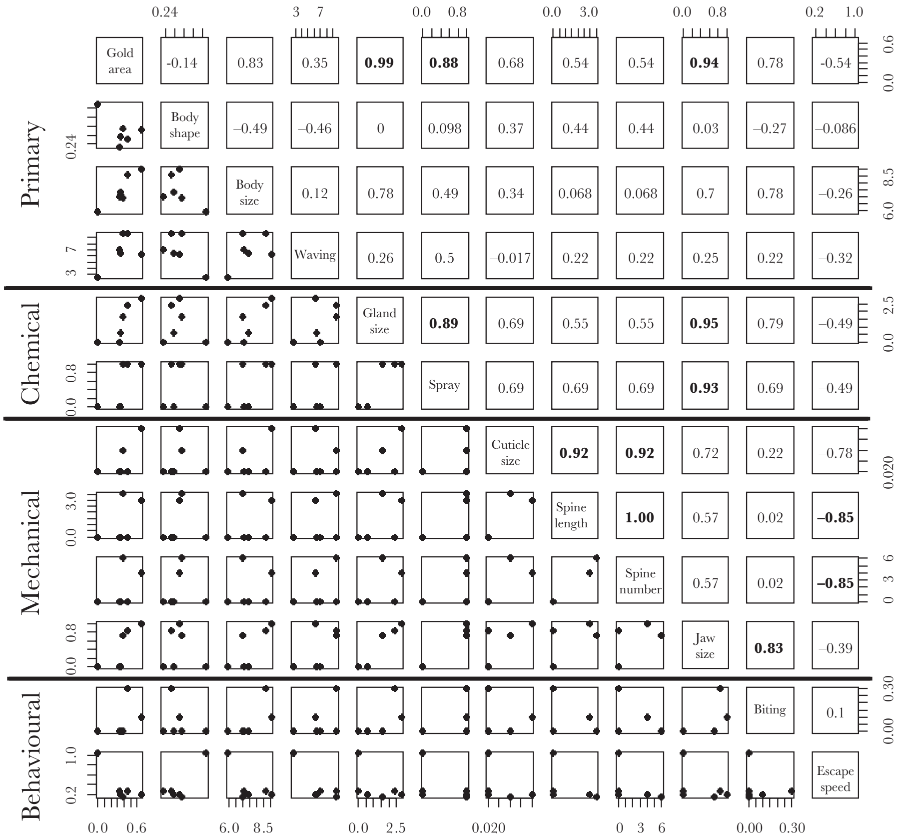

Figure 2
Relationship (Spearman correlation) between 12 measured traits of defense (arranged in four groups) in the five mimics and one non-mimetic species. Significant values ($P < 0.05$) are in bold.

recorded latency to attack (i.e., time from orienting to prey to when they first pounced on prey), latency to capture (i.e., time from the first attack to capture), number of attacks, and attack and capture frequencies. All captured prey were consumed.

We used Generalized Estimating Equations (GEE) from the geepack package (Yan and Fine 2004) because we had a blocked design (repeated use of the same individuals with different prey). GEE is an extension of the GLM for correlated data (Pekár and Brabec 2018). Data on latencies were subjected to GEE with Gamma errors (GEE-g); data on relative frequencies were subjected to GEE with binomial errors (GEE-b); and data on counts were subjected to GEE with Poisson errors (GEE-p). Exchangeable association structure was used in all cases. Treatment contrasts were used for post hoc comparisons. The linear predictors in models of skink response included only a prey species as an explanatory variable, while in models of spider response the predator/prey body ratio was also included. All analyses were performed in R (R Core Team 2021).

Effect of defenses on predator behavior

Primary defenses are used ahead of subjugation whereas secondary defenses are employed during subjugation and consumption (Ruxton et al. 2018). Some defense traits could be used both before and during subjugation. Thus, we used Spearman correlations to examine the relationships between primary defensive traits (Table 1) and observed behaviors of predators prior to subjugation (Supplementary Table S1), and secondary defense traits (Table 1) during subjugation and consumption (Supplementary Table S1).

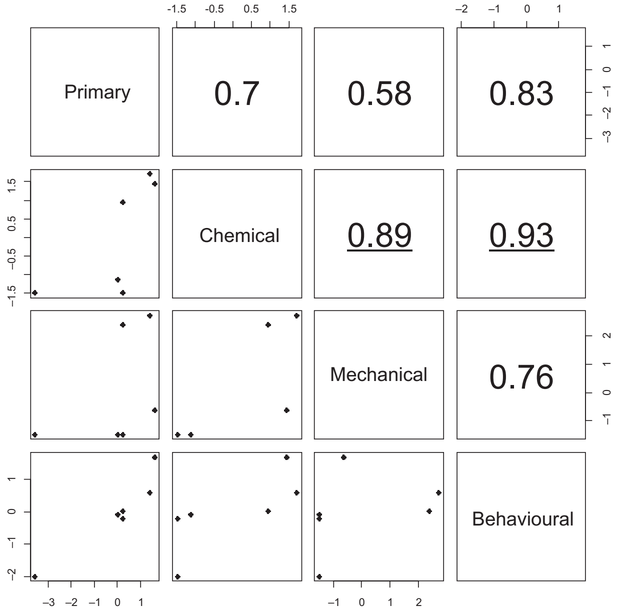

Figure 3
Relationship (Spearman correlation) between PCA scores of four groups of defenses in the five mimics and one non-mimetic species. Significant values ($P < 0.05$) are underlined.

Although some trait values are not independent among species due to common ancestry (e.g., among three ant species), we did not

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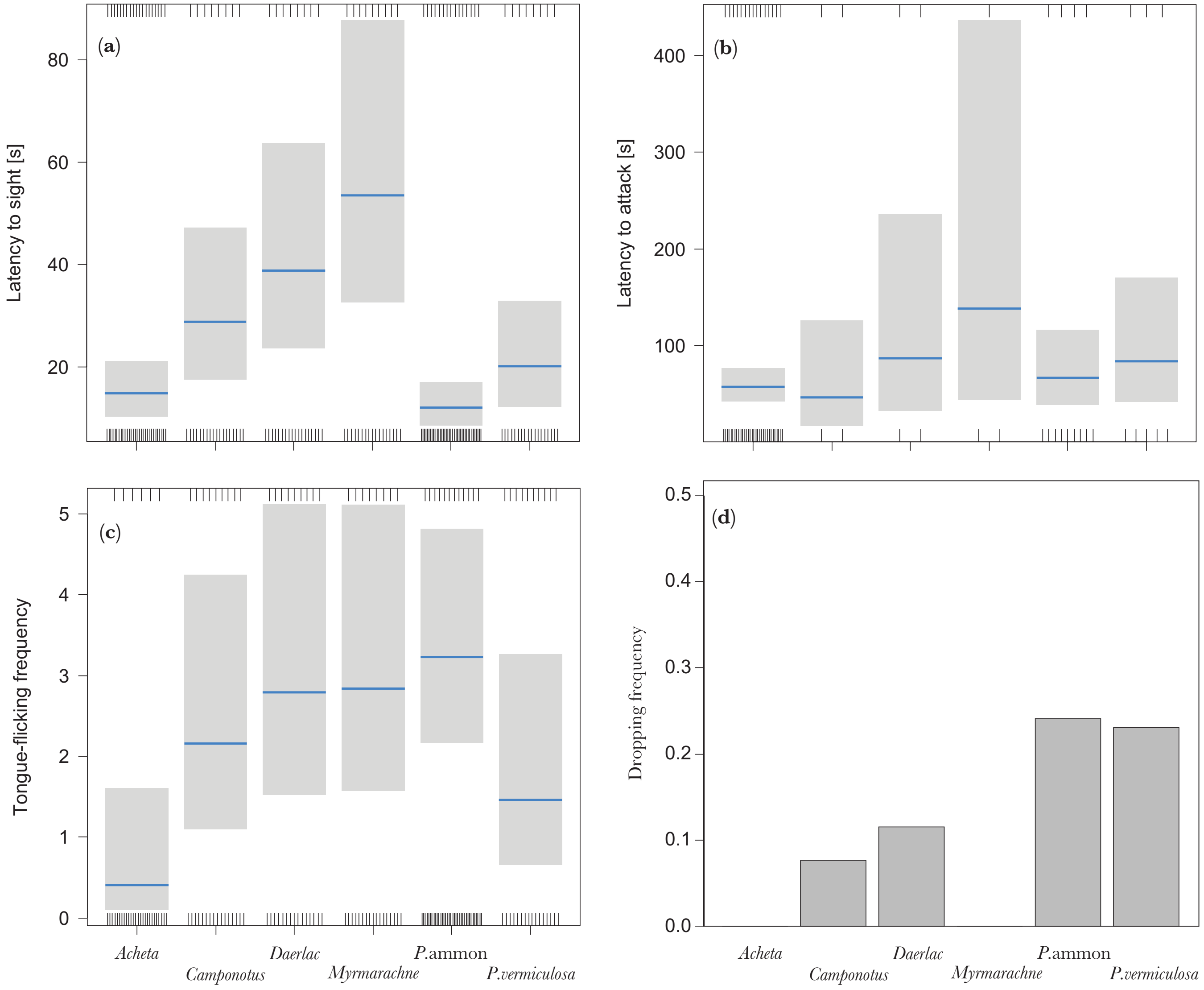

Figure 4

Comparison of the latency to sight (A), the latency to attack (B), the tongue-flicking frequency (C), and the prey-release frequency (D), by skinks, toward six prey species. Blue lines are estimated means and gray bars are estimated 95% confidence intervals (A–C). Bars (D) are means. *Acheta domesticus* is non-mimetic, *M. luctuosa* is Batesian mimic, *D. nigricans* is quasi-Batesian, and others are Müllerian mimics. Rugs on the horizontal axes represent measurements: those on the lower axis are below, while those on the upper axis are above, the estimated mean.

control for phylogenetic relationship because of small sample size and the fact that we were interested in their effect against tested predators not in inferences on a general evolutionary scale.

RESULTS

Defensive traits

Four defense traits (golden area, total body size, body shape, and frequency of appendage waving) were classified as primary defenses in all species. Two traits (expulsion of chemicals and gland size) were classified as chemical defenses. Four traits (number of spines, total spine length, cuticle thickness, jaw size) were classified as mechanical defenses. Finally, two traits (escape speed and frequency of biting) were classified as behavioral defenses. The pairwise (Spearman) correlation between 12 measured defenses was mostly positive (Figure 2) but negative correlations were found between escape speed and other defenses. When we combined the individual traits into one of four groups, the first axis of each PCA explained more than 70% of the variance. Three groups of defenses were highly positively correlated (Figure 3), including behavioral defenses.

All mimics possessed the primary defense traits: golden area, total body size, body shape, and appendage waving. In terms of secondary defenses, biting and chemicals were performed by ants and the true bugs. Two ant species possessed spines. Collectively, ants were the most heavily defended with six to eight traits, followed by *D. nigricans* with three and *M. luctuosa* with two.

Response of predators

All prey were approached by skinks at a similar latency of ~2 min (GEE-g, $\chi^2_5 = 3.7$, $P = 0.60$). Skinks sighted prey at significantly

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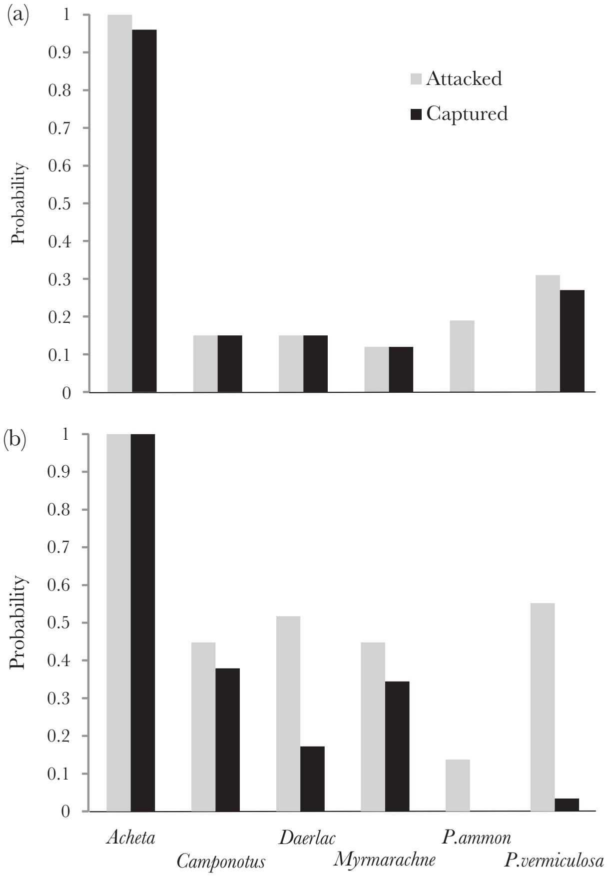

Figure 5
Comparison of the probability of attack and capture for six prey species by skinks (A) and spiders (B). *Acheta domesticus* is non-mimetic, *M. luctuosa* is Batesian mimic, *D. nigricans* is quasi-Batesian, and others are Müllerian mimics.

different latencies (GEE-b, $\chi^2_5 = 138$, $P < 0.0001$): ants (Müllerian mimics) were detected earlier than mimetic spiders (Batesian mimic) and *D. nigricans* bugs (quasi-Batesian mimic) (Figure 4A). The attack on prey occurred at significantly different latencies (GEE-g, $\chi^2_5 = 26.9$, $P < 0.0001$, Figure 4B): attack on *P. vermiculosa* was significantly earlier than on *A. domesticus* (contrasts, $P = 0.004$). Skinks tongue-flicked the different prey prior to attack at significantly different frequencies (GEE-p, $\chi^2_5 = 24.7$, $P = 0.0002$, Figure 4C): skinks tongue-flicked at a higher frequency in response to *P. ammon* ants than all other prey (contrasts, $P < 0.05$). The probability of attack was significantly different among prey species (GEE-b, $\chi^2_5 = 26.9$, $P < 0.0001$): while *A. domesticus* was always attacked, all mimics were infrequently attacked (Figure 5A). The probability of capture was significantly different among prey species (GEE-b, $\chi^2_5 = 125.9$, $P < 0.0001$): *P. ammon* was never captured (Figure 5A). The probability of releasing captured prey was significantly different among species (GEE-b, $\chi^2_5 = 28.1$, $P < 0.0001$, Figure 4D): *A. domesticus* and *M. luctuosa* were never released, unlike other prey species.

Spiders stalked prey following detection. The latency to attack differed significantly among prey (GEE-g, $\chi^2_4 = 19.4$, $P = 0.0007$, Figure 6A): *M. luctuosa* was attacked significantly later than *P. ammon*. The probability of attack was significantly different among prey species (GEE-b, $\chi^2_5 = 58.2$, $P < 0.0001$): while *A. domesticus* was always attacked, all mimics were infrequently attacked (Figure 5B). The attack probability changed significantly with prey/predator body size ratio (GEE-b, $\chi^2_1 = 26.5$, $P < 0.0001$): spiders attacked mainly prey smaller than themselves (Figure 6B). The latency to capture did not differ significantly among prey (GEE-g, $\chi^2_4 = 1.7$, $P = 0.79$, Figure 6C). Similarly, the number of attacks did not differ significantly among all mimics (GEE-p, $\chi^2_4 = 3.6$, $P = 0.47$). The probability of capture was significantly different among prey species (GEE-b, $\chi^2_5 = 107$, $P < 0.0001$): *P. ammon* and *P. vermiculosa* were rarely captured (Figure 5B). The probability of capture changed significantly with prey/predator body size ratio (GEE-b, $\chi^2_1 = 72.5$, $P < 0.0001$): spiders captured prey that were mostly half their size (Figure 6D). Successful capture was in all cases performed head-on: *Servaea* bit prey behind the head on the dorsal side of the pronotum/prosoma and held on firmly with their chelicerae so that the mandibles/chelicera of prey were facing away from the attacker.

Efficacy of defenses on predator behavior

In trials with skinks, five defense traits had a significant relationship with the attack sequence (Supplementary Table S1A). Two primary defenses were important before attack. Specifically, more intensive waving of an appendage by prey was associated with fewer tongue-flicks in lizards and a more ant-like shape was related to lower probability of attack (Figure 7A). One primary and two secondary defenses were important following initial attack: larger body size was related to lower probability of capture (Figure 7A) and species with thicker cuticles and larger glands had a higher probability of being released (Figure 7A).

In trials with spiders, six defense traits had a significant relationship with the attack sequence (Supplementary Table S1B). Three primary and one secondary defense were important before attack. Specifically, the larger the golden area, the larger the body size, and the larger the gland, the shorter the latency to attack (Figure 7B) or the lower the probability to attack (Figure 7B). Two secondary defenses were important after initial attack. Namely, the larger the body size, the higher the escape speed, the higher the capture probability (Figure 7B); and the thicker the cuticle, the lower the probability of capture (Figure 7B).

DISCUSSION

We found that members of the golden mimicry complex possess a range of primary and secondary defenses. This is typical for species using warning coloration (aposematic Müllerian mimics) as they advertise their unpalatability (achieved by chemical and/or mechanical traits). We found that Batesian mimics of this complex (spiders and bugs) possess few defensive traits and are thus better classified as quasi-Batesian mimics.

We found all four groups of defenses (primary, chemical, mechanical, and behavioral) to be positively correlated with each other. This is expected for aposematic species (e.g., Ruxton et al. 2018). Interestingly, behavioral defenses were also positively correlated with other defensive groups with the exception of escape speed, which was negatively correlated with behavioral defenses. A positive correlation is expected when behavioral and other defense types are used synergistically. For example, defensive behavior was significantly positively correlated with morphology in scorpions (Van der Meijden et al. 2013), in which the use of stinger is coupled with behavior. Escape, however, should act independently of other

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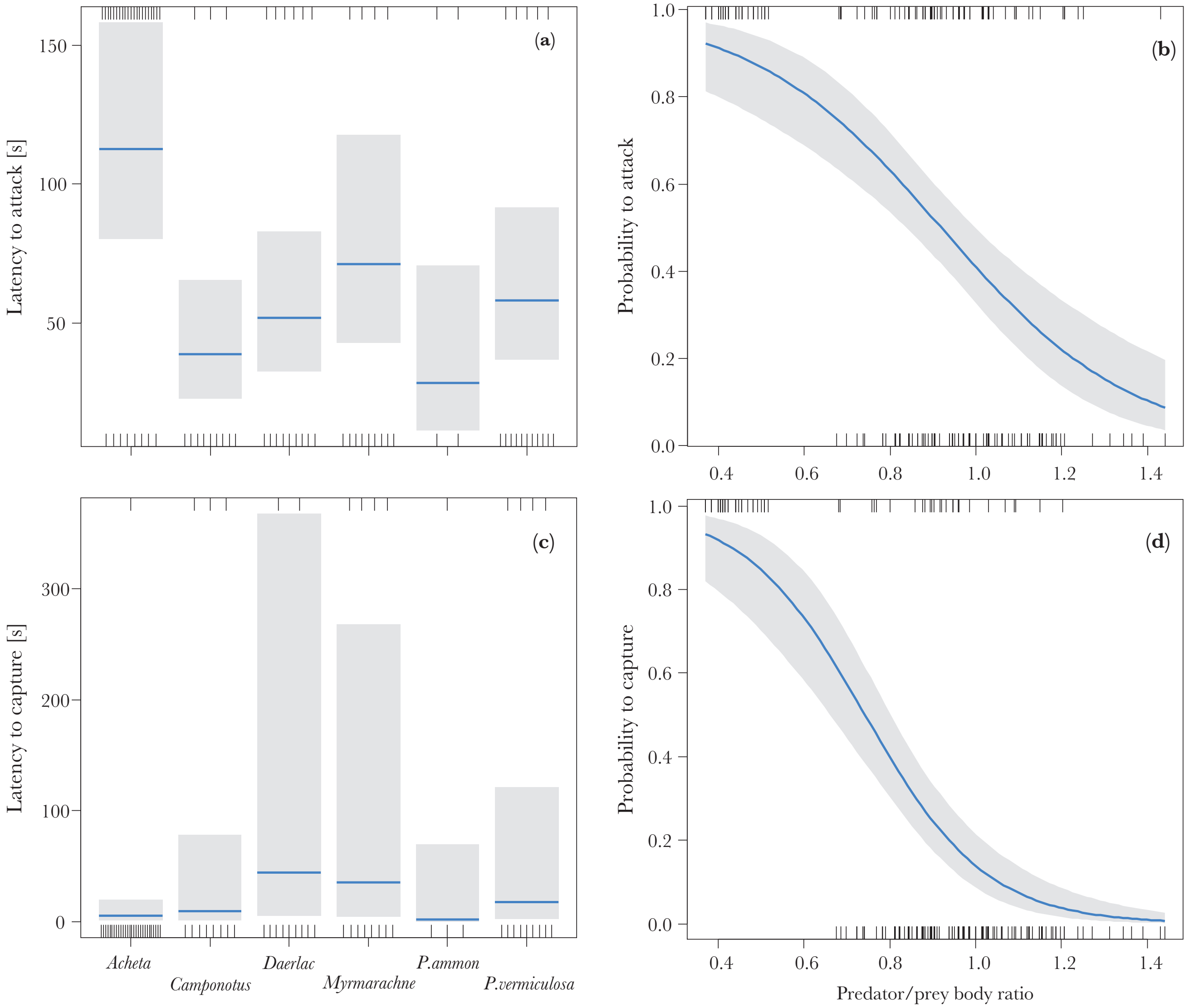

Figure 6

Comparison of the latency to attack (A), relationship between prey-to-predator body size ratio and probability to attack (B), the latency to capture (C), and relationship between prey-to-predator body size ratio and probability of capture (D) by *Servaea* spiders to six prey species. Blue lines are estimated means and gray bars (A, C) or bands (B, D) are estimated 95% confidence intervals. *Acheta domesticus* is non-mimetic, *M. luctuosa* is Batesian mimic, *D. nigricans* is quasi-Batesian, and others are Müllerian mimics.

defenses. Thus, the negative correlation between escape speed and the primary defenses could be a traded-off: more armature or mechanical defenses at the expense of sprint speed. For example, this relationship has previously been demonstrated in cordylid lizards, in which more heavily armored species could venture farther from their refuges but they had reduced sprint speed (Losos et al. 2002).

We predicted that different defensive traits would be effective against different predators and at different stages of a predatory sequence. Overall, out of 12 traits, seven (coloration, appendage waving, body shape, body size, gland size, escape speed, and cuticle thickness) were important at any stage of attack by skinks, spiders, or both, either encouraging or discouraging attack. Among primary defenses, coloration discouraged attack by spiders while appendage waving discouraged attacks by skinks. One primary defense trait, large body size, discouraged both predators from attack. Among secondary defenses, higher escape speed was more likely to prompt spiders to attempt prey capture. And two defenses, gland size and cuticle thickness, were important for both, however, in opposite directions. As expected, for ant-eating spiders, chemical defenses were ineffective because of how they handle prey. By using extra-oral digestion (sucking their prey without crushing it), spiders might be able to avoid contact with chemical defenses. Spiders also tended to attack behind the head which allowed them to avoid spines on the thorax and petiole.

Surprisingly, jaws and spines, which are expected to play a role during subjugation, were not important. Ants possess powerful mandibles which are used against enemies in combination with their aggressive behavior. Powerful mandibles allowed ants to escape from arthropod (Hénaut et al. 2014; Larabee and Suarez 2015), as well as from vertebrate, predators (e.g., Vogel 1983). Similarly, spines can serve as an effective defense not only against vertebrate (Dornhaus and Powell 2010; Ito et al. 2016), but even

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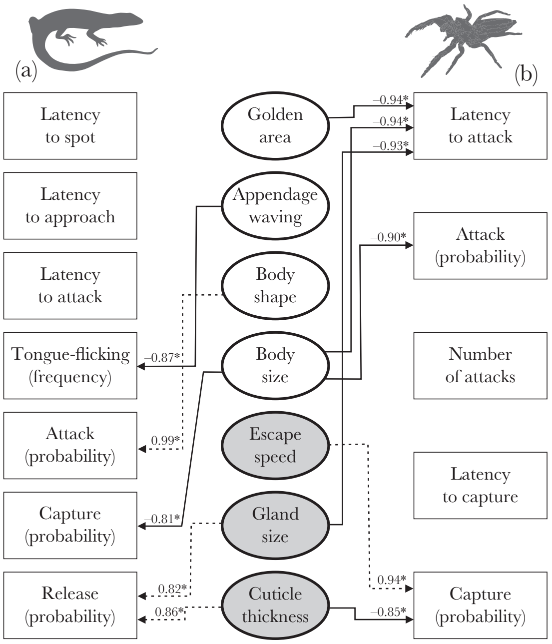

Figure 7

Relationship between selected traits (ellipses) and observed response (rectangles) of skinks and spiders. The effect of traits is displayed by arrows with a value of the Spearman correlation. Full lines are used for traits that discouraged and dashed lines are used for traits that encouraged attack. Asterisk marks significant values ($P < 0.05$). Displayed defensive traits are either primary (empty ellipses) or secondary (gray ellipses) defenses.

some invertebrate, predators (Mikolajewski et al. 2006). In the case of spiders, which grab prey by their legs or head during attack, spines are not very effective. For example, Blanchard et al. (2020) found only slightly higher survival of ant species with spines during encounters with another salticid spider. In our study, the salticid failed to catch most *Polyrhachis* ants. It appeared as if the spines of ants were physically used against the spider which made the spider quickly retreat. In *Polyrhachis* ants, spines are not conspicuous as they are a similar color to the body and thus easily overlooked by predators. Predators probably did not recognize their presence until only after capture. *Polyrhachis ammon* possess fewer but longer spines than *P. vermiculosa*. Yet, while *P. ammon* was not eaten by skinks, *P. vermiculosa* was eaten. However, we do not know whether it was the spines that caused skinks to drop *Polyrhachis* ants.

The importance of large body size to survival was particularly surprising for skinks—lower capture probability for larger ants. But prey size, particularly in the range of sizes of the ants and mimics in this experiment, should not have been a constraint for skinks, whose gape size easily accommodates arthropod prey. In spiders, the size of prey, which can be larger than the spider, is an important predictor of prey capture success (e.g., Nentwig and Wissel 1986). Here, we found that *Servaea* spiders captured relatively large prey, which is in agreement with abilities of other prey-specialized spiders (Pekár and Toft 2015; Pekár, García, et al. 2017).

We observed that chemical defenses were deployed against both predators. Both *Polyrhachis* and *C. aeneopilosus* ants possess formic acid which can be used both as an odor to signal ant identity, as well as a noxious substance during consumption. In our study it obviously acted as an odor prior to attack in spiders and as a defense after attack by skinks. Odors are important in prey identification both in spiders (Vickers and Taylor 2018) and birds (Winters et al. 2021). Even though skinks were observed to direct tongue-flicks toward prey during approach, they did not appear to use this information prior to attack as they frequently attacked both *Polyrhachis* and *C. aeneopilosus* ants.

Behavioral defenses were either aggressive (biting) or defensive (escape) and, therefore, not surprisingly, they were not correlated. This was most apparent in the behavior of the mimics. *Polyrhachis* ants and *D. nigricans* bugs, which deployed aggressive behavioral defenses, moved about in the arena seemingly unaware of the predator present. Conversely, *M. luctuosa*, which did not bite, either displayed intensively or tried to escape. The role of some behavioral traits, such as escape, was limited because the prey were contained in an arena. Furthermore, in the field, the prey could not only escape but also hide, for example, in leaf litter and debris, which was also not possible in the laboratory setup. Thus, the estimated attack and capture probabilities are likely to be the maximal possible as is typical for such artificial conditions.

We expected the golden body coloration of the mimics to be important for the predators, as has been found for birds (Marples et al. 1994; Hauglund et al. 2006; Winters et al. 2021). Surprisingly, this appeared to only be the case for spider predators. But in contrast to expectation the coloration appeared to be attractive for ant-eating spiders. This is likely because these spiders may recognize their preferred prey by the golden coloration. Given that lizards have good visual acuity and color discrimination (Barbour et al. 2002; Fleishman et al. 2011; New et al. 2012), this was unexpected. It might in part be because only a single prey item was available to the predator at a time, thus, it could not choose a more profitable one without defensive body coloration.

The predation pressure by the two predators on six prey species was similar only to a certain extent. Specifically, the most defended species, *P. ammon*, was not captured by either predator. There was also a much lower overall attack rate in skinks on all mimics compared to spiders. This is because skinks are generalist predators, preferring prey other than ants (Daniels 1987), whereas the *Servaea* spiders are ant specialists (McGinley et al. 2015). In addition, *Servaea* were equally likely to attack and capture any of the mimics offered, while skins were more selective. As *Servaea* are expected to target ants as prey, this may suggest that the prey we offered in this experiment all carried recognizable "ant" cues, even the non-ant mimics. However, during the attack, when confronted with secondary defences, some mimics had a higher survival probability than ants.

Simulations show that early-stage (primary) defenses are favored against generalist predators while late-stage (secondary) defenses are effective against specialist predators (Broom et al. 2010; see also Pekár et al. 2011). Results from this study support this prediction: generalist skinks attempted to attack all mimics at lower frequency than the specialized spider predator, indicating that the primary defenses were effective. However, spiders and skinks were similarly affected by both primary and secondary defenses. Alternatively, myrmecophagus specialized predators are more enticed than a generalist predator by prey with an ant-like appearance.

Several hypotheses have been proposed to explain the existence of multiple and multimodal antipredator displays, including increased detection, to startle predators, or enhancement of psychological effects (Rowe and Guilford 1999). It appears that the combination of primary defenses could improve detection at least by spiders. For example, movement improved detection of visual signals (Rowe 1999). While moving mimics could more effectively display the aposematic

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coloration which affected the decision of spider predators. We observed a startle response only in spiders following an attack on *Polyrhachis* ants. Unfortunately, our design was not suited to assess how, for example, odor could enhance learning of aposematic coloration.

We conclude that by using multiple defensive traits, mimics from the golden mimicry complex gain protection from multiple predator types. Defenses provided different levels of protection against generalist and specialist predators and multiple defenses provided protection across multiple stages of attack. Overall, prey were poorly protected against the specialist predator, which was generally able to circumvent both primary and secondary defenses, but better protected against the generalist predator across all attack stages. Any one suite of traits cannot be optimal against any one predator type and likely represents a classic trade-off whereby specialist predators such as ant-eating spiders are less deterred by these defenses compared to generalist predators such as lizards. Nonetheless, the level of protection the defenses exhibit appears to be adaptive.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

We thank Patricio Lagos for his help with the skink experiment and two anonymous reviewers for useful comments.

FUNDING

The study was supported by the Czech Science Foundation (grant no. 19-09323S awarded to S.P.).

ETHICAL APPROVAL

The experiments with skinks were performed according to Animal ethic guidelines AEC Reference No. 2013/033-3.

AUTHOR CONTRIBUTIONS

Stano Pekar (Conceptualization [Lead], Data curation [Lead], Formal analysis [Lead], Funding acquisition [Lead], Investigation [Lead], Methodology [Lead], Writing – original draft [Lead]), Martin J. Whiting (Conceptualization-Equal, Methodology-Equal, Resources-Equal, Writing – review & editing-Equal), Marie E. Herberstein (Conceptualization-Equal, Methodology-Equal, Resources-Equal, Writing – review & editing-Equal).

DATA AVAILABILITY

Analyses reported in this article can be reproduced using the data provided by Pekár et al. (2023).

Handling Editor: Ulrika Candolin

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