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Nature's disguise: Empirical demonstration of dead-leaf masquerade in *Kallima* butterflies

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ABSTRACT

Animals deploy diverse color-based defenses against predators, including crypsis, mimicry, aposematism, and masquerade. While crypsis, mimicry, aposematism have been extensively studied, the strategy of masquerade —where organisms imitate inedible or inanimate objects such as leaves, twigs, stones, and bird droppings —remains comparatively underexplored, particularly in adult butterflies. The Indian oakleaf butterfly (*Kallima inachus*) exemplifies this phenomenon, with its wings resembling dead leaves, providing a classic example of natural selection. Although it has long been postulated that these butterflies evade predation by being misidentified as dead leaves, direct experimental evidence is lacking. In the current study, using domestic chicks as predators, we manipulated their prior experience with dead leaves (model objects) while maintaining constant exposure to butterflies to test whether dead-leaf masquerade provides a protective advantage by preventing recognition. Results showed a marked delay in the initiation of attacks by chicks familiar with dead leaves compared to those with no prior exposure or those exposed to visually altered leaves. Chicks with prior dead-leaf experience required a similar amount of time to attack the butterflies as they did to attack dead leaves. These findings provide the first empirical demonstration of dead-leaf masquerade in *Kallima* butterflies, shedding light on its evolutionary significance. Our study highlights the effectiveness of masquerade in inducing the misclassification of butterflies as inanimate objects, showcasing the precise mimicry achieved by these organisms when viewed in isolation from the model objects. This study advances our understanding of the evolution of masquerade and its role as a potent antipredator strategy in nature.

Keywords: Antipredator defense; Camouflage; Dead-leaf butterfly; Masquerade; Natural selection; Visual recognition

INTRODUCTION

The animal kingdom is a battlefield of survival, where organisms have evolved diverse color-based antipredator strategies, ranging from crypsis to aposematism, each tailored to specific ecological demands (Cott, 1940; Cuthill et al., 2017; Ruxton et al., 2018). Among these strategies, crypsis and aposematism are prominent; the former aims to prevent detection, while the latter employs conspicuous warning coloration to deter predators (Cuthill et al., 2017; Ruxton et al., 2018). However, masquerade represents a particularly fascinating defense mechanism, wherein organisms imitate inedible or inanimate objects, such as leaves, twigs, stones, and bird droppings, to evade recognition by predators (Cott, 1940; Cuthill et al., 2017; Duarte et al., 2017; Edmunds, 1974; Endler, 1978; Quicke, 2017; Ruxton et al., 2018; Skelhorn, 2015). For example, the giant swallowtail caterpillar (*Papilio cresphontes*) closely resembles bird droppings, while the early thorn moth caterpillar (*Selenia dentaria*) mimics the appearance of twigs (Edmunds, 1974). Although masquerade is remarkably widespread, particularly among insects in both larval and adult stages (Cott, 1940; Edmunds, 1974; Poulton, 1890; Thayer, 1918), its protective function through misidentification has only recently been substantiated through empirical research (Skelhorn et al., 2010a).

Masquerade differs fundamentally from crypsis, which aims to prevent detection, instead relying on the misidentification of an organism as an inedible or uninteresting object (model) after it has been detected (Quicke, 2017; Ruxton et al., 2018; Skelhorn, 2015; Skelhorn et al., 2010a). The ground-breaking work by Skelhorn et al. (2010a) provided crucial evidence of

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this mechanism using the early thorn moth caterpillar (twig-masquerader) as prey and domestic chicks (*Gallus gallus domesticus*) as predators. By manipulating prior exposure of chicks to twigs while maintaining exposure to caterpillars, a marked shift in behavior was observed. Notably those familiar with twigs display a notable delay in attacks and handled the caterpillars with greater caution compared to those without twig experience or those exposed to manipulated, non-caterpillar-like twigs. This behavior strongly suggests that learned aversion to twigs was extended to twig-masquerading caterpillars, resulting in their misidentification as inanimate objects. This study provided the first empirical evidence for the protective function of masquerade through misidentification. Building on this, Skelhorn and colleagues, as well as other researchers, delved deeper into the underlying mechanisms of masquerade, investigating the influence of factors such as model object size, density, and microhabitat selection on the effectiveness of the strategy (Higginson et al., 2012; Mark et al., 2021; Skelhorn et al., 2010b, 2010c; Skelhorn & Ruxton, 2011a, 2011b, 2014; Yu et al., 2022). Despite these advancements, significant questions remain regarding the overall prevalence and ecological impact of masquerade in natural environments, warranting further investigation.

Within Lepidoptera, masquerade is a prominent strategy among caterpillars, especially twig masquerade (Higginson et al., 2012; Quicke, 2017; Ruxton et al., 2018). These larvae, constrained by limited mobility, are under constant predation pressure, necessitating the evolution of robust anti-predator defenses (Cott, 1940; Edmunds, 1974; Higginson et al., 2012). Cryptic coloration provides initial defense by allowing organisms to blend into their surroundings, thereby avoiding detection (Higginson et al., 2012; Skelhorn et al., 2010a; Thayer, 1918). However, twig masquerade enhances this strategy by enabling caterpillars to closely resemble twigs, confusing predators and reinforcing their already impressive invisibility (Poulton, 1890; Ruxton et al., 2018; Skelhorn et al., 2010a).

Investigating the effectiveness of masquerade in flying insects, such as butterflies and moths, is essential due to their unique lifestyles and potential constraints (Cuthill et al., 2017). Unlike caterpillars, butterflies and moths possess the advantage of flight, potentially reducing their reliance on masquerade as a primary defense mechanism (Cuthill et al., 2017; Quicke, 2017). However, their frequent movement during foraging and mating exposes them to predators, posing a challenge for maintaining effective masquerade (Higginson et al., 2012). Additionally, the vibrant colors often employed for attracting mates and defending territories can interfere with camouflage and compromise the effectiveness of masquerade (Wallace, 1889; Watson, 1936). Despite these challenges, some butterflies and moths demonstrate remarkable cryptic resting behavior, adopting postures that enhance their masquerade (Quicke, 2017). However, direct evidence supporting the protective function of masquerade in adult butterflies is currently lacking.

To address this research gap, we investigated the effectiveness of dead-leaf masquerade in adult butterflies, focusing on a life stage distinct from the well-studied caterpillars. Unlike previous studies, our research centered on the resemblance of adult butterflies to dead leaves. Various species exhibit a remarkable resemblance to dead leaves, with the genus *Kallima* Doubleday, 1849 (Lepidoptera: Nymphalidae) serving as a notable example. Indian oakleaf butterflies (*K. inachus*) feature distinct leaf-like patterns and markings on the underside of their wing, which enhance their masquerade ability. Exclusive to Asia, the *Kallima* genus comprises 11 distinct species (Frederic Moore, 1879; Nakamura, 2014; Shirôzu & Nakanishi, 1984). Since the late 19th century, the Indian oakleaf butterfly has served as a compelling case study for how natural selection can gradually shape animal morphology (Beddard, 1892; Morgan, 1903; Poulton, 1890; Suzuki, 2017; Wallace, 1889; Weissman, 1902). While some scientists have contested whether such complex masquerade could have evolved gradually (Goldschmidt, 1945; Punnett, 1915; Watson, 1936), the past decade has witnessed renewed interest in its origin and evolution, particularly from a genetic perspective (Suzuki et al., 2014, 2019; Wang et al., 2022; Yang et al., 2020). However, the extent to which this adaptation protects against predation remains a compelling yet unexplored question.

In the present study, we explored potential dead-leaf masquerade in *K. inachus* using an established experimental approach (Skelhorn et al., 2010a). By exposing naïve predators to both modified and unmodified dead leaf models, as well as an empty control arena, we aimed to observe their subsequent interactions with individual *K. inachus* butterflies. We hypothesized that if dead-leaf masquerade confers a protective advantage, predators with prior exposure to these models would display significantly greater hesitation in attacking butterflies compared to those without such exposure. Our findings not only contribute to a broader understanding of masquerade as an antipredator strategy but also advance the field by exploring a novel dimension—dead-leaf masquerade in adult butterflies.

MATERIALS AND METHODS

Domestic chick predators

Two-day-old domestic chicks (*Gallus gallus domesticus*) were obtained from a small free-range facility and reared under controlled laboratory conditions at 28±1ºC, following established protocols (Ma et al., 2020; Yu et al., 2022). The chicks were housed in five separate boxes (50 cm×40 cm×35 cm), each accommodating up to 16 chicks. Two of these boxes were designated for “buddy” chicks. The floor of each box was lined with newspapers to maintain cleanliness. The chicks were exposed to a 14 h light (L):10 h dark (D) cycle, using uncovered fluorescent lights that provided a full daylight spectrum, consistent with methods used in previous studies (Skelhorn et al., 2010a). Importantly, none of the chicks had prior exposure to real or modified dead leaves.

Starting from day 4, the chicks were provided with *ad libitum* access to water and food (Chick food, CP feed, CP group, Zhengzhou, China), except during training and experimental trials, during which they were temporarily deprived of food for 1 h—a common practice in rearing and experimental settings. The chicks were allowed to acclimatize to their housing conditions for the first two days. Regular monitoring was conducted to ensure adequate food and water consumption, maintain cleanliness, and assess overall health and well-being. Upon completion of the experiments, all chicks were donated to a farmer located near the city.

Prey species

Adult specimens of the dead-leaf butterfly (*Kallima inachus*) (Figure 1A) were used as the focal prey in our study.

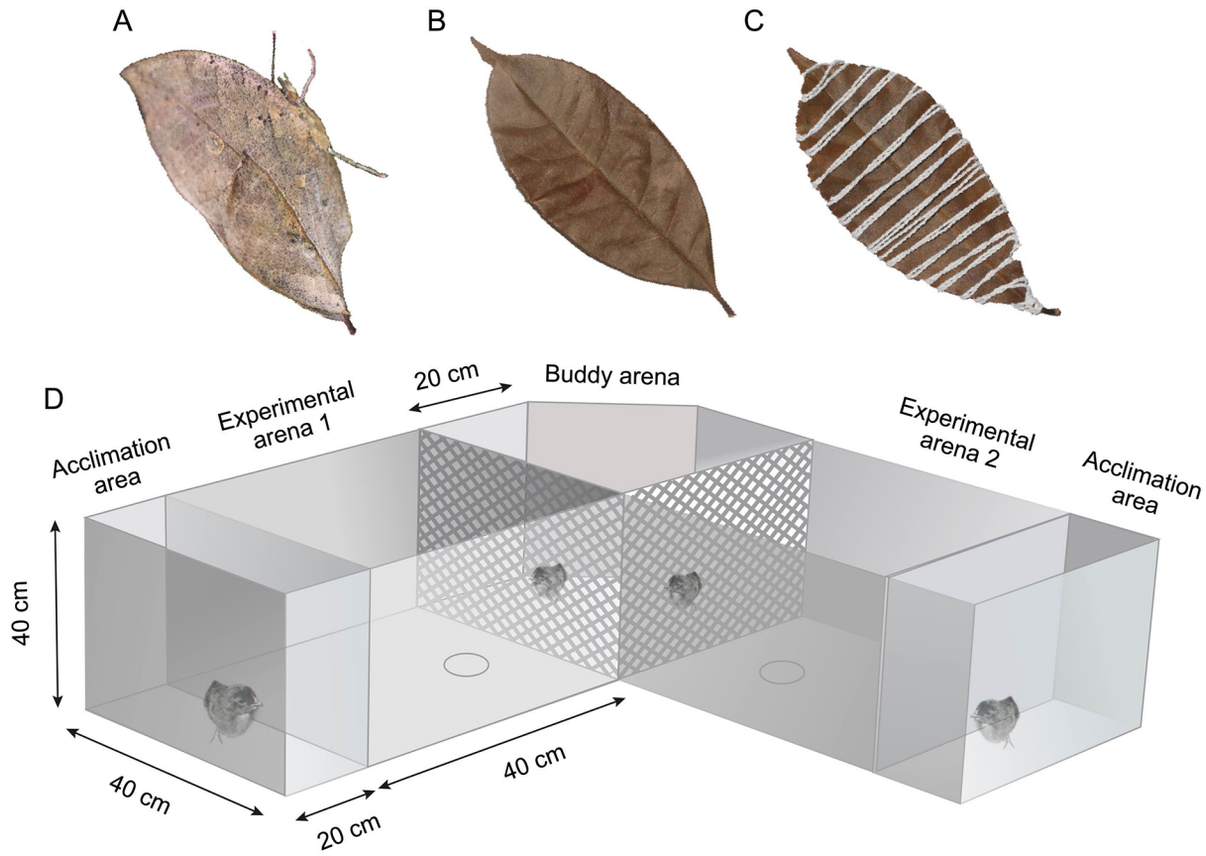

Figure 1 Prey, model objects, and chick predation experimental arenas with a partitioned "buddy" arena

A: Dead-leaf butterfly *Kallima inachus*. B: Dead leaf of the fragrant olive (*Osmanthus fragrans*). C: Modified dead leaf of *O. fragrans*. D: Two experimental arenas with a partitioned "buddy" arena, each equipped with an acclimation area.

Commonly found in tropical East and Southeast Asia, including southern China (Corbet & Pendlebury, 1992; Shirôzu & Nakanishi, 1984), this species is renowned for its remarkable resemblance to the leaves of Asian evergreen oaks, such as *Quercus lanata* (Alexander, 2019). The larvae of this species predominantly feed on *Strobilanthes* shrubs (Corbet & Pendlebury, 1992; Yang et al., 2005; Zhou et al., 2005).

Dry leaves from the fragrant olive tree, *Osmanthus fragrans,* collected in Wuhan, Hubei Province, China, were chosen as model objects. Although *K. inachus* shows a resemblance to the dead leaves of *Quercus lanata*, this species is known for its remarkable polymorphism (Wang et al., 2022), displaying variations that do not specifically mimic the dead leaves of any particular tree species. The selection of *O. fragrans* leaves was based on their availability and close similarity in size, color, and shape to dead-leaf butterflies (Figure 1B).

To ensure uniform developmental conditions, *K. inachus* pupae were sourced from a single lepidopteran breeder in Jiangsu Province, China, and reared under controlled conditions (25–27°C, 80% relative humidity (RH), 16L:8D photoperiod). A total of 50 adult females were housed in a designated cage (200 cm×150 cm×150 cm)(length×width×height), with access to spoiled fruits and a 10% sugar solution as their food source. For the experimental trials, 24 adult females were randomly selected from this population.

Ethics statement

This study was conducted in full compliance with the ASAB/ABS guidelines for the use of animals in research, as well as with all applicable laws of China where the experiments were performed and the legal requirements of Hubei University concerning research ethics and animal treatment. No ethical approval was required for this study.

Training

The training procedures employed in this study were adapted from a previously established protocol (Skelhorn et al., 2010a). In brief, on day 2, 80 chicks were transferred to the housing cages and allowed to acclimatize to the laboratory environment without access to food (although water was provided). On day 3, 48 chicks underwent a training regimen designed to familiarize them with consuming food (Chick food, CP feed, CP group, Zhengzhou, China) placed on the white laminated cardboard floor of the experimental setup (Figure 1D). This setup consisted of three arenas: two experimental arenas (each 60 cm×40 cm×40 cm)(length×width×height) and a "buddy" arena. Each experimental arena included an acclimation area (20 cm×40 cm×40 cm)(length×width×height) partitioned by a removable opaque barrier. The "buddy" arena, characterized by five partitions (40 cm×40 cm×20 cm×20 cm×40 cm)(length×width×height), was separated from the two experimental arenas by wire mesh. During both the training sessions and subsequent experimental trials, two chicks (out of 32), designated as "buddy" chicks, were placed in the "buddy" arena, where they were visible to the test chicks in the experimental arenas, thereby mitigating potential distress from solitary placement in the test arena. The "buddy" chicks were rotated every two trials, housed separately from the test chicks, and not exposed

to insect prey during the experiment, although water was provided.

On day 3, all test chicks underwent three training trials, conducted at regular intervals between 0700h and 1900h. During each trial, chick food was scattered across the floor of the test arena, and a test chick was placed in the acclimation area of each test arena, positioned approximately 45 cm from the "buddy" arena. Before each trial commenced, the test chick was allowed to acclimate in the designated area for 5 min. The training trial began upon the removal of the opaque barrier separating the acclimation and experimental areas and lasted for 3 min. In the first trial, chicks were introduced to the arena in groups of three; in the subsequent trial, group size was reduced to two, and in the final trial, each chick was placed individually in the arena. By the end of trial 3, all chicks were consistently consuming chick food within the arena.

Experience manipulation trials

On day 4, chicks were divided into six groups, each consisting of eight individuals (Figure 2). In two of these groups, each test chick was exposed to an unmodified dead leaf of similar size (approximately 8 cm×4 cm) during each trial, simulating the natural conditions under which avian predators may encounter dead leaves. In another two groups, the test chicks were presented with a dead leaf of comparable size but modified by binding it with a white cotton thread, altering its visual appearance while preserving its structural complexity and odor (Figure 1C). The remaining two groups served as controls, with the test chicks exposed to an empty arena. The dead leaf was positioned at the center of the arena, approximately 20 cm from each wall.

At the start of each trial, a test chick was randomly selected within each group and introduced to the acclimation area 25 cm from the training stimulus (either an unmodified leaf, a modified leaf, or nothing) and 45 cm from the "buddy" arena, oriented to face the stimulus. Following a 5 min acclimation period, the trial commenced upon the removal of the opaque barrier. Each test chick participated in three separate sequential 2 min trials at regular intervals between 0700h and 1900h, with 1 h of food deprivation prior to entering the arena. To ensure consistency and independence, each dead leaf was used only once. During the trials, the latency to first peck the dead leaf and the number of pecks were recorded, except in the control group. We confirmed that all chicks pecked the leaf during the first 2 min trial. If a chick failed to peck the leaf during the first trial, it was replaced with another until one did. Chicks were trained in random order, and all training sessions were conducted within the experimental arenas (Figure 1D).

Testing trials

On day 5, each test chick participated in a single testing trial. At the onset of the trial, a single test stimulus was placed on its side on the floor of the experimental arena, positioned 20 cm from the "buddy" arena and 20 cm from the walls of the experimental arena. After 1 h of food deprivation, the test chick was introduced into the acclimation area, positioned 25 cm from the test stimulus. The test stimulus consisted of either an adult *K. inachus* butterfly (8 cm×4 cm) or a similarly sized dead leaf (8 cm×4 cm), strategically positioned 45 cm from the "buddy" arena (Figure 2). To ensure consistency, live butterflies were euthanized by refrigeration at approximately 4°C before use, and dead leaves were similarly refrigerated. Following a 5 min acclimation period, the trial began with the removal of the opaque barrier. The test chick was allowed to enter the experimental arena and remained there until it pecked at the butterfly/dead leaf within a 5 min timeframe. The latency to the first peck of the stimulus was recorded.

Within each group of chicks, half were presented with a butterfly in experimental arena 1 and the other half were presented with a dead leaf in experimental arena 2. The assignment of chicks to one of these conditions was randomized. The test stimulus varied among groups according to prior training of the groups: one group had been trained with unmodified dead leaves, one trained with modified leaves, and one trained in the empty arena. All groups encountered a single adult butterfly, while one group from each training condition was exposed to a single unmodified dead leaf (Figure 2). Each freshly euthanized butterfly and each dead leaf was used only once.

Data analysis

All statistical analyses were performed using R v.4.3.2 (R

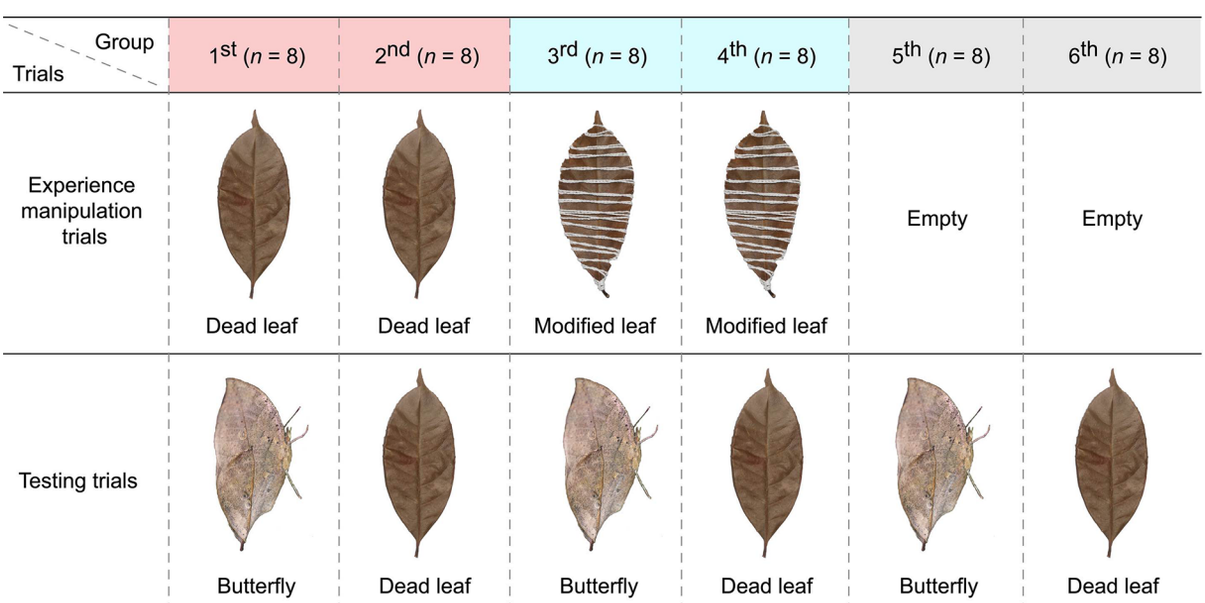

Figure 2 Testing chicks were divided into six groups of eight

Core Team, 2023). All statistical tests were two-tailed, with normality assessed using the Shapiro-Wilk test and homogeneity of variance evaluated using Levene's test.

For the experience manipulation trials, two separate Kruskal-Wallis tests were applied to assess the effects of dead leaf manipulation on the latency to first peck and the total number of pecks by chicks.

In the testing trials, five separate Kruskal-Wallis tests were performed, followed by multiple pairwise comparisons with Holm correction. The first Kruskal-Wallis test compared the latency to peck butterflies or leaves across all six experimental groups (Groups 1–6; Figure 3) to test the *a priori* prediction that chicks with experience with unmodified dead leaves (Groups 1 and 2) would exhibit longer latency to attack leaves/butterflies compared to chicks with experience with modified leaves (Groups 3 and 4) or naïve chicks (Groups 5 and 6). The second test validated the prediction that chicks with experience with unmodified leaves (Group 1) would exhibit longer latency to attack butterflies than chicks with experience with modified leaves (Group 3) or naïve chicks (Group 5). The third test compared the latency to peck butterflies or leaves among Groups 1, 2, 5, and 6. The fourth test verified the prediction that chicks with experience with unmodified leaves (Group 2) would exhibit longer latency to peck leaves than those with experience with modified leaves (Group 4) or naïve chicks (Group 6). Due to the presence of two outliers in Group 4, the fifth test was conducted excluding these outliers.

In addition, three pairwise comparisons were conducted using unpaired Wilcoxon rank-sum tests. The first comparison tested the prediction that chicks with experience with unmodified leaves (Groups 1+2) would exhibit longer latency to attack butterflies or leaves than naïve chicks (Groups 5+6). The second comparison tested the *a priori* prediction that chicks with experience with unmodified leaves would exhibit longer latency to attack leaves (Group 2) than butterflies (Group 1), which would suggest that the resemblance between masqueraders and their model objects is not perfect when viewed independently from the model objects. The third comparison tested whether chicks with experience of modified leaves would exhibit longer latency to attack leaves (Group 4) than butterflies (Group 3).

RESULTS

Experience manipulation trials

The latency to peck the first leaf did not significantly differ between chicks exposed to dead leaves and those exposed to modified leaves (Kruskal-Wallis test: χ^2=0.065, P=0.968, df=2; Figure 3A). Similarly, there was no significant difference in the total number of pecks across the three training trials (χ^2=2.129, P=0.345, df=2; Figure 3B). These findings suggest that the modification of dead leaves did not significantly affect the propensity of chicks to attack them. Chicks showed similar levels of reluctance to peck at both dead and modified dead leaves, and their tendency to stop pecking occurred at comparable rates.

Testing trials

The first Kruskal-Wallis test revealed a significant difference in the latency to attack butterflies or leaves among the six experimental groups during the final testing trials (χ^2=15.33, P=0.009, df=5; Figure 4). Specifically, when comparing groups 1, 3, and 5, where chicks were tested with butterflies, results showed that chicks with prior experience consuming unmodified dead leaves (Group 1) exhibited a significantly longer delay, indicating greater reluctance, in attacking *K. inachus* butterflies compared to chicks with prior experience eating modified dead leaves (Group 3) or naïve chicks (Group 5) (χ^2=8.17, P=0.017, df=2). Pairwise comparisons further confirmed significant differences in the latency to attack butterflies between Groups 1 and 3 (z=2.58, P=0.029), as well as between Groups 1 and 5 (z=–2.35, P=0.037), while no significant differences were detected between Groups 3 and 5 (z=0.23, P=0.818) (Figure 4). These results suggest that chicks misidentify masquerading butterflies as dead leaves.

When comparing Groups 1+2 (experienced with unmodified dead leaves) to Groups 5+6 (naïve), chicks with prior exposure to unmodified dead leaves exhibited a significantly longer delay in attacking butterflies or leaves compared to the naïve chicks (Groups 5+6) (Wilcoxon rank-sum test: W=43.5, P=0.002, n=16). Further analysis using the third Kruskal-Wallis test (χ^2=10.34, P=0.016, df=3) showed significant differences in attack latency between Groups 1 and 5, Groups 1 and 6, and Groups 2 and 5, but not between Groups 2 and 6

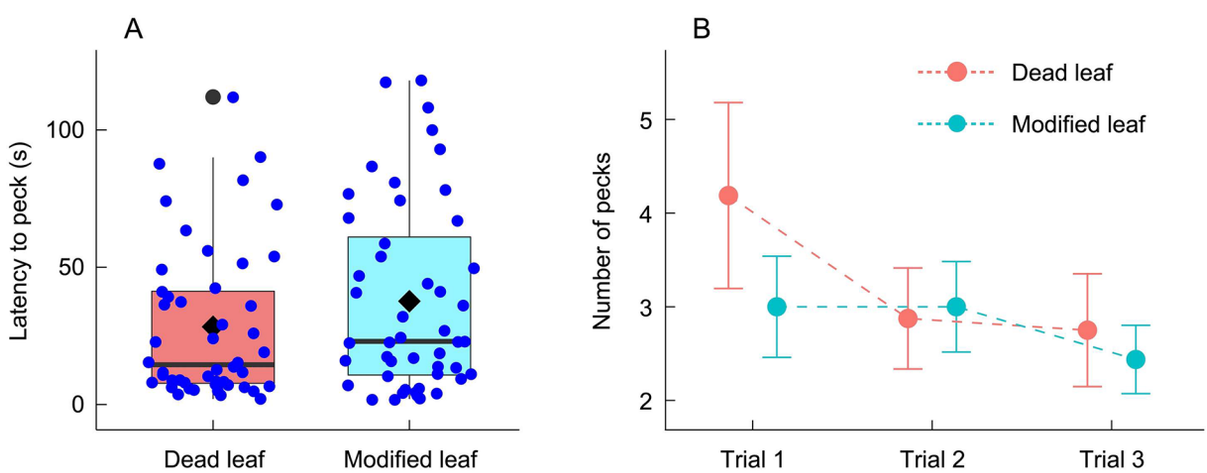

Figure 3 Results from training trials

A: Time (s) taken to first peck during training trials (n=48). B: Total number of times (mean±standard error of the mean (SEM)) that chicks pecked the training stimulus (unmodified dead leaf or modified dead leaf) across three training trials (n=48). Boxes show medians (lines), means (black rhombi), and interquartile ranges (IQRs); whiskers indicate lowest and highest values within 1.5×IQRs, and black solid circles represent outliers.

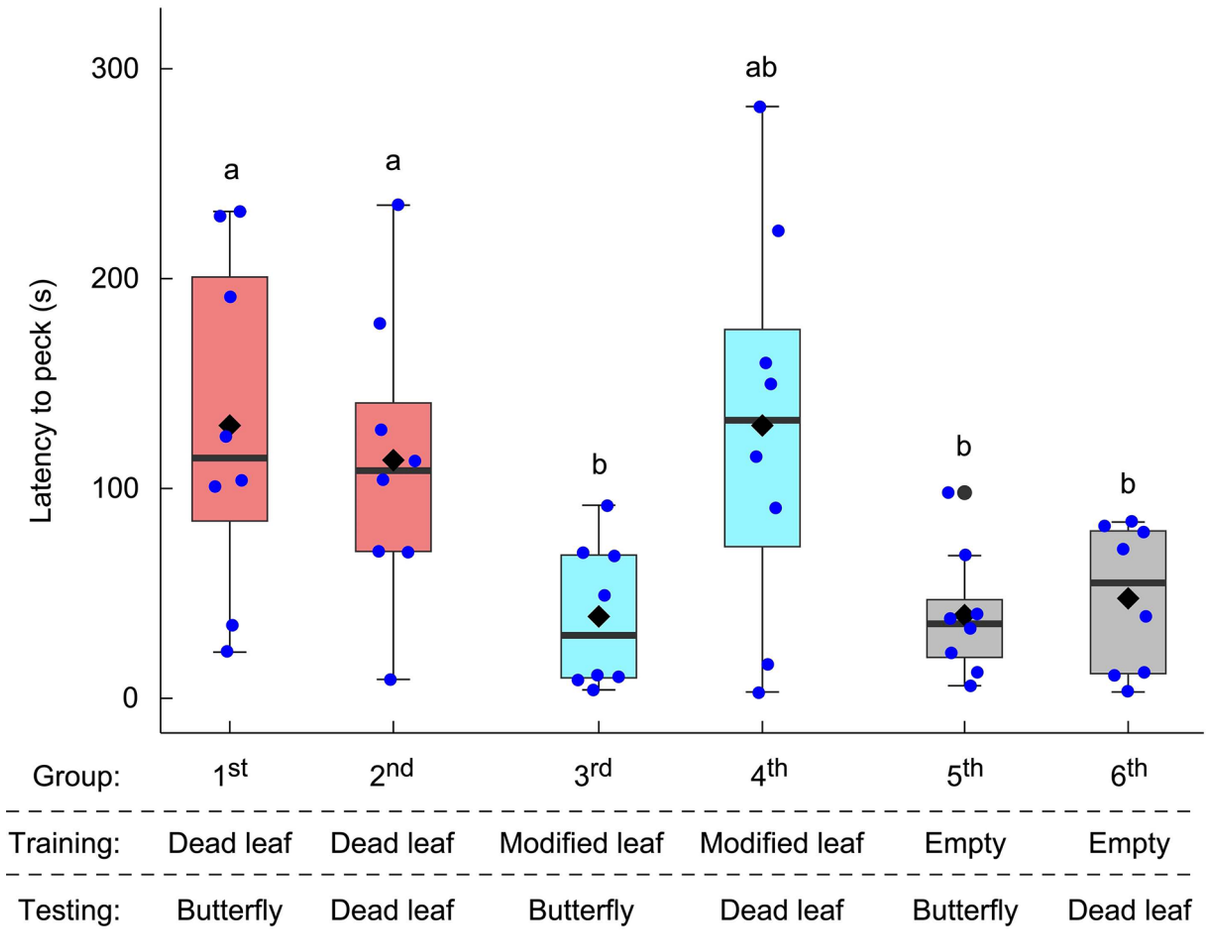

Figure 4 Results from testing trials

Time (s) taken to first peck test stimulus (unmodified dead leaf or modified dead leaf). Boxes show medians (lines), means (black rhombi), and interquartile ranges (IQRs); whiskers indicate lowest and highest values within 1.5×IQRs, and black solid circles represent outliers.

(Figure 4).

No significant difference was observed in the latency to peck dry leaves among Groups 2, 4, and 6, regardless of whether the outliers in Group 4 were included (χ^2=5.14, P=0.077, df=2) or excluded (χ^2=3.88, P=0.143, df=2). Pairwise comparisons, with or without the outliers, showed no significant differences in latency to peck leaves between chicks with experience consuming unmodified dry leaves (Group 2) and naïve chicks (Group 6), nor between chicks with experience consuming unmodified dry leaves (Group 2) and those with experience consuming modified dry leaves (Group 4) in the testing trials (Figure 4). These results indicate that chemical cues, such as scent, along with tactile cues like texture, may influence the effectiveness of masquerade in *K. inachus*.

Chicks with prior experience consuming unmodified dry leaves exhibited no significant differences in the time taken to attack butterflies (Group 1) compared to pecking dry leaves (Group 2) (W=34.5, P=0.833, n=8) (Figure 4). This suggests that the resemblance between masqueraders and their model objects is more convincing when each is viewed in isolation. Chicks exposed to modified dry leaves showed a slightly shorter latency in attacking butterflies (Group 3) compared to pecking dry leaves (Group 4), but the difference was not significant (W=13.0, P=0.052, n=1). These findings imply that non-visual cues, such as scent and texture of real leaves, may play a role in the effectiveness of masquerade in *K. inachus*.

DISCUSSION

Our results demonstrated the high efficacy of dead-leaf masquerade in *K. inachus* butterflies, extending beyond mere predator evasion. This strategy effectively induced predators to misidentify the butterflies as inedible or inanimate objects. Chicks exposed to real dead leaves developed a learned aversion, which extended to the butterflies, indicating that predators trained with dead leaves generalized their aversion to both leaves and butterflies. These findings support our hypothesis, offering empirical evidence for the role of masquerade in promoting misidentification.

This study advances our understanding of masquerade beyond the well-documented examples in twig-mimicking caterpillars, such as the Brimstone moth (*Opisthograptis luteolata*) (Skelhorn et al., 2010a), by exploring dead-leaf masquerade in adult butterflies. Results showed that chicks learned to associate real dead leaves with non-edible objects, and those exposed to unmodified leaves generalized this aversion to both leaves and butterflies. In contrast, naïve chicks, which had no prior experience with dead leaves, did not exhibit the same learned aversion. This suggests that misidentification is acquired through direct experience with real dead leaves. Similar to the protection observed in twig-mimicking caterpillars (Skelhorn et al., 2010a), dead-leaf butterflies gain significant protective benefits from their masquerade, particularly when viewed in isolation from their model objects. This protective advantage was most

pronounced in predators that had previously encountered real dead leaves, highlighting the remarkable adaptability and effectiveness of masquerade as an antipredator strategy in nature. However, the absence of a significant difference in latency to peck dead leaves between chicks exposed to unmodified leaves (Group 2) and those exposed to modified leaves (Group 4) suggests the potential involvement of chemical and tactile cues, in addition to visual information, in dead-leaf masquerade in *K. inachus* butterflies.

Masquerade in adult lepidopterans, much like in their larval stages, serves as a crucial protective function, although its manifestation undergoes fundamental shifts between these life stages. While caterpillars, limited in their mobility, depend heavily on stationary camouflage for protection (Cott, 1940; Edmunds, 1974; Higginson et al., 2012), as exemplified by twig-mimicking species (Skelhorn et al., 2010a), adult butterflies and moths leverage their flight capabilities. In these adults, masquerade involves not only visual mimicry but also behavioral adaptations, allowing them to adopt the appearance of inanimate objects, such as dead leaves, particularly when at rest with closed wings (Cuthill et al., 2017; Quicke, 2017), as observed in our study species. This type of masquerade in adult lepidopterans extends beyond visual resemblance to include behavioral adaptations, such as specific movements or wing positioning, to further enhance the illusion of inanimacy (Quicke, 2017; Stevens & Merilaita, 2009). While the fundamental principle of masquerade—deception through mimicry—is shared between lepidopteran larvae and adults, their strategies and adaptations differ significantly, reflecting the unique vulnerabilities faced at each life stage. Caterpillars rely on physical resemblance and immobility for protection (Skelhorn et al., 2010a), while adults incorporate flight-based camouflage tactics to complement their masquerade strategies. This distinction highlights the nuanced and adaptive nature of masquerade throughout the lepidopteran life cycle. However, further research is needed to fully elucidate how masquerade functions and evolves across different life stages of lepidopterans and other arthropods. Comparative studies involving other populations of *K. inachus* or closely related species could also reveal potential variations in the benefits and strategies associated with masquerade.

The dead-leaf masquerade strategy observed in *K. inachus* butterflies demonstrated a striking level of accuracy. Contrary to our initial predictions, predators with prior exposure to unmodified dead leaves exhibited a similarly prolonged hesitation when attacking both the butterflies (Group 1) and dead leaves (Group 2), suggesting a high degree of visual resemblance between the two. This contrasts with studies on twig masquerade in caterpillars, where the mimicry is less precise (Skelhorn & Ruxton, 2010). This disparity may indicate differing selection pressures for achieving perfect resemblance in various types of masquerade. The underlying reasons for this discrepancy remain unclear. One possibility is that selective pressure for perfect dead-leaf masquerade in more intense than for twig masquerade. Alternatively, while twig-masquerading caterpillars may evolve to resemble specific host plants more closely over time (Skelhorn & Ruxton, 2010), *K. inachus* may have evolved to achieve a more generalized masquerade that effectively resembles numerous objects simultaneously. This theory is supported by recent research demonstrating the remarkable polymorphism within this species (Wang et al., 2022), as well as observations in other masquerading species, such as those mimicking bird droppings (Quicke, 2017; Skelhorn, 2015). It is important to note that our experiment only considered the behavior of predators when a dead-leaf masquerading butterfly was presented in the absence of a model object. Exploring how the simultaneous presence of model objects or increased environmental complexity affects the likelihood of dead-leaf butterflies being misidentified as their model subjects will provide avenues for future study.

Interestingly, predators with prior exposure to modified dead leaves exhibited a slightly quicker attack response toward butterflies (Group 3) compared to dead leaves alone (Group 4), although this difference was not statistically significant. Several potential reasons could explain this outcome. One possibility is that predators trained with modified dead leaves may have perceived the butterfly as a novel stimulus due to its somewhat different appearance (e.g., visible head, legs, and antenna) when positioned on the arena floor compared to dead leaves lying on their side. This novelty effect might have triggered a quicker attack response. Another possibility may be that the modified leaves presented visual cues that intrigued the predators more than familiar, unaltered dead leaves, potentially capturing their attention and eliciting a faster attack. Alternatively, it is plausible that chemical or tactile cues other than visual information may influence dead-leaf masquerade in *K. inachus*. These findings highlight the need for further research to unveil the specific mechanisms and factors contributing to this unexpected result.

While our experiments indicate that adult *K. inachus* butterflies benefit from being misidentified as inedible or inanimate by avian predators within a laboratory environment, natural selection in a natural environment may differ. Thus, future studies are needed to test the effectiveness of dead-leaf masquerade in *Kallima* butterflies in the wild.

CONCLUSIONS

The remarkable resemblance of adult *K. inachus* butterflies to dead leaves while at rest provides compelling evidence that such mimicry can lead to misidentification by avian predators, thereby conferring a survival advantage. Our findings emphasize the need for further research to fully elucidate the complex and multifaceted nature of masquerade across various life stages in lepidopterans and other arthropods. Furthermore, our study highlights the importance of considering chemical and tactile cues in the effectiveness of dead-leaf masquerade in *Kallima* butterflies. It is crucial to recognize that while masquerade offers protection, it may come with associated costs, potentially manifesting as reduced effectiveness, behavioral constraints, increased susceptibility to generalist predators, and developmental trade-offs. A comprehensive understanding of these trade-offs, as well as the context-dependent effectiveness of different masquerade strategies, is essential for developing a robust theoretical framework. In conclusion, this study lays the groundwork for future research into the dynamics and ecological implications of masquerade. By encouraging a deeper exploration of its diverse nature, we can achieve a richer understanding of this fascinating and ecologically significant phenomenon.

DATA AVAILABILITY

Data, analyses, and codes in this article are available from the Dryad Digital Repository: https://datadryad.org/stash/share/2qXJEInir9d6HQWwMZN5Y0igbzGHW33_P4MNXqT1kV0.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

D.L., L.Y., and Z.T.Z. conceptualized, devised, and designed the study. L.Y., H.Z.C., and Z.T.Z. conducted field and lab work. D.L., L.Y., S.C.Z., and Z.T.Z. analysed the data. All authors contributed to the writing and editing of the manuscript. All authors read and approved the final version of the manuscript.

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