A systematic review and meta-analysis of anti-predator mechanisms of eyespots: conspicuous pattern vs eye mimicry

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We declare we have no competing interests.

Author contribution

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All gave final approval for publication and agreed to be held accountable for the work performed therein.

Data Accessibility

Raw data, analysis script and supplementary materials are available at https://ayumi-495.github.io/eyespot/ and https://zenodo.org/doi/10.5281/zenodo.13147018.

Abstract

Eyespot patterns have evolved in many prey species. These patterns were traditionally explained by the eye mimicry hypothesis, which proposes that eyespots resembling vertebrate eyes function as predator avoidance. However, it is possible that eyespots do not mimic eyes: according to the conspicuousness hypothesis, eyespots are just one form of vivid signals where only conspicuousness matters. They might work simply through neophobia or unfamiliarity, without necessarily implying aposematism or the unprofitability to potential predators. To test these hypotheses and explore factors influencing predators' responses, we conducted a meta-analysis with 33 empirical papers that focused on bird responses to both real lepidopterans and artificial targets with conspicuous patterns (i.e., eyespots and non-eyespots). Supporting the latter hypothesis, the results showed no clear difference in predator avoidance efficacy between eyespots and non-eyespots. When comparing geometric pattern characteristics, bigger pattern sizes and smaller numbers of patterns were more effective in preventing avian predation. This finding indicates that single concentric patterns have stronger deterring effects than paired ones. Taken together, our study supports the conspicuousness hypothesis more than the eye mimicry hypothesis. Due to the number and species coverage of published studies so far, the generalisability of our conclusion may be limited. The findings highlight that pattern conspicuousness is key to eliciting avian avoidance responses, shedding a different light on this classic example of signal evolution.

Keywords

Aves, butterfly, caterpillar, interspecific communication, predator-prey interaction, warning signal

Background

Naturalists have long pondered the evolution and function of the many signals and cues animals use to communicate [1–9]. Visual signals, such as vibrant colours and contrasting patterns, have attracted more interest from researchers than other signals, likely because our species is visually oriented [1, 10, 11]. Eyespot patterns, characterised by concentric rings of different colours with a light outer ring and a dark centre [12], are well-known patterns believed to reduce predation. Although eyespots have been researched for a long time [12–15], researchers continue to debate why eyespots might deter predation.

Three hypotheses have been proposed to explain why eyespot patterns can contribute to prey survival (reviewed in [12, 14, 15]; Fig. 1). First, the eye mimicry hypothesis suggests that eyespots play a role in deterring predators from attacking prey and reducing predation risks by mimicking the eyes of vertebrates [16–18]. This hypothesis predicts that if the pattern has specific characteristics (e.g., eye-like shape) and is presented as a pair, predation avoidance will increase, assuming eyespots imitate potential predators. Second, the conspicuousness hypothesis posits that eyespots are simply conspicuous patterns that prevent attacks due to negative predator responses caused by sensory bias, neophobia, or sensory overload [12, 14]. The hypothesis states that the eye-like shape and patterns arranged in pairs do not necessarily deter predators. Rather, it is their conspicuous appearance that makes them effective predator deterrents, and any resemblance to eyes is coincidental. Eyespots can act as an aposematic signal for potential predators. For example, if the size of the pattern (one of the measures of conspicuousness) increases, the avoidance effect will also increase. Third, the deflection hypothesis suggests that predator attacks should be directed towards eyespots to avoid damage to vital body parts [19–23]. The eye mimicry and conspicuousness hypotheses are usually applied to explain large eyespots, while the deflection hypothesis is used to interpret the function of small ones [12, 14, 15]. The first two of these hypotheses focus on how eyespots prevent predators from attacking, specifically whether it is because they resemble eyes or are conspicuous. The third hypothesis focuses on whether eyespots divert a predator's attack away from vital body parts by drawing the predator's attention to them. Thus, in this third hypothesis, whether the eyespots resemble eyes or are conspicuous is not the central issue [12, 14, 15]. Although there seems to be little disagreement in the deflection hypothesis ([24–26], but see also [27]), why large eyespots can intimidate avian predators has been controversial [12, 14]. This is because while the eye mimicry and conspicuousness hypotheses are not mutually exclusive, the key mechanism that explains why predators react negatively to eyespots is clearly different.

Lepidopterans, such as butterflies and moths, have been the leading models for testing the eye mimicry and conspicuousness hypotheses. A typical empirical study has adult individuals, caterpillars, or their models as prey, with birds as predators (reviewed in [12, 14, 15]). According to the eye mimicry hypothesis, avian predators perceive the eyespots as the eyes of a potential enemy. For example, great tits (*Parus major*) showed more aversive responses to animated butterflies with a pair of large eyespots than those without, and such eyespots were more effective than modified, less mimetic, but equally contrasting patterns [28]. Although several studies have supported the eye mimicry hypothesis [e.g., 16, 28, 29], many conspicuous patterns other than eyespots, such as dots and stripes, likely deter attacks from predators as well [30–33]. Some field experiments with artificial prey have supported the conspicuousness hypothesis, demonstrating survival rates for both conspicuous (eyespot and non-eyespots) pattern prey stimuli were higher than control prey stimuli [30, 31, 34]. Such discrepancies might have arisen from differences in experimental design between studies, such as the size, number, and shape of the presented pattern stimuli or the bird species used as subjects in the experiments [12, 35]. However, there has been no systematic attempt to synthesise and compare earlier studies quantitatively.

Here, we conduct a systematic review with meta-analysis to synthesise empirical evidence on the intimidating effects of eyespots and the factors that contribute to predator avoidance responses towards them. To examine the two hypotheses above, we ask three interrelated questions. First, we examine whether conspicuous patterns, namely eyespots and non-eyespot patterns (i.e., conspicuous patterns other than eyespots), influence bird responses or prey survival in a manner that increases the success of predator avoidance. Second, we test whether pattern resemblance to eyes (eye-like shape) is the key to predator avoidance (which differentiates the eye mimicry hypothesis from the conspicuousness hypothesis). Third, we examine what factors promote bird response and increase prey survival, such as pattern size and the number of patterns (i.e., eyespots and non-eyespots; Fig. 1).

Materials and Methods

We preregistered our methods and planned analyses before data extraction and analysis in Open Science Framework (https://osf.io/ymwvb; [36]). We referenced and followed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses;[37]) and PRISMA-EcoEvo (Preferred Reporting Items for Systematic reviews and Meta-analyses in Ecology and Evolutionary biology; [38]) for reporting this study (Table S1).

Search protocols

We used the PICO (Population, Intervention, Comparator, Outcome; Table 1) framework [39] to specify the scope of our research questions and to inform our literature searching and screening. We conducted a comprehensive literature search across multiple databases, including Scopus, ISI Web of Science, Google Scholar (for non-English studies), and Bielefeld Academic Search Engine (for unpublished theses; i.e., grey literature). We designed the search strings (see Table S2) to identify studies that used experimental methods to examine the effects of eyespot patterns on birds' predation behaviours. We did not set any temporal restrictions on the database searches. Additionally, we conducted backward and forward reference searches within the Scopus database using four key publications [12–15]. The strings were translated for searches in non-English languages, and search results were assessed by reviewers with expertise in the respective languages: AM for Japanese, ML for Polish and Russian, PP for Portuguese and Spanish, and YY for Simplified and Traditional Chinese. We limited Google Scholar searches to the top 100 results in each language, sorted by relevance. In cases of disagreement between the reviewers, discrepancies were discussed and resolved to reach a consensus. The screening process and results are shown in the PRISMA-like flowchart (Fig. 2a).

Eligibility criteria

We set specific criteria for including studies in our meta-analysis (according to our pre-registered protocol). Initial screening, including titles, abstracts, and keyword assessment for English-language bibliographic records, was conducted by AM and ML using Rayyan (https://www.rayyan.ai; [40]) following predefined inclusion criteria. Subsequently, AM and PP independently screened the full texts of studies that passed the initial screening. To be eligible, a study had to conduct experiments and provide data on bird behavioural responses or prey survival/attacked rates. We excluded studies solely involving non-avian predators, such as fish, insects, mammals, or other species. However, studies that included a mix of species from different taxonomic groups were allowed if the primary focus was on avian predation. In our analysis, we only considered research that presented both conspicuous and control (non-conspicuous) patterns as stimuli. We omitted studies using actual predator or human eyes as stimuli since we focused on understanding how eyespot patterns in butterflies and caterpillars, which are unlikely to resemble specific bird or vertebrate species eyes, affect predation avoidance [41]. We also excluded studies that used bright and contrasting patterns as control stimuli because such stimuli would prevent comparison with eyespot patterns or other conspicuous patterns. Furthermore, we focused only on studies that used real or artificial butterflies, moths, caterpillars, or a piece of paper as prey or presented stimuli. We also did not consider research that only investigated avian physiological responses to conspicuous patterns. In addition, we did not include studies that only assessed whether prey with eyespots or conspicuous patterns were less likely to be attacked by birds, based on wing or body damage alone, without including control stimuli. This is because it was not possible to quantitatively assess the effect of eyespots or other conspicuous patterns on predation avoidance without control stimuli.

Data collection

We extracted four types of information from each study. First, we collected citation information, such as title, author name, and publication year. Second, we gathered the details of the presented stimuli used in each experiment within studies: type of control pattern (plain neutral-coloured or camouflaged), type of treatment pattern (eyespots or non-eyespot patterns), pattern area (mm^2: area per shape comprising the pattern), total pattern area (mm^2: when multiple patterns exist on the presented stimulus, it denotes the total area of all patterns; for stimuli with single eyespot or distinct pattern, the value equals the pattern area), linear size of the pattern (mm: e.g., maximum diameter or length of pattern), number of shapes in pattern, total area of prey surface (mm^2: e.g., butterfly wings and caterpillar bodies), prey material type (i.e., whether a real butterfly or a complete imitation of a particular butterfly was used as prey), and prey shape type (a further subdivision of the former). For non-eyespot patterns, we also noted pattern shapes (e.g., circles, stripes, and triangles). In each study, bird responses to control and treatment pattern stimuli and prey survival/attacked rates when these patterns were present were reported. Bird responses contained a variety of measures, including the number of attacks and escape behaviours, latency to attack, latency to approach, and the proportion of birds attacking the presented stimuli. Henceforth, we refer to these measures and responses as 'predator avoidance'. Third, we obtained data for calculating effect sizes (e.g., mean, standard deviation or standard error, and sample size of control and treatment group) from plots using WebPlotDigitizer 4.6.0 (https://automeris.io/WebPlotDigitizer), detailed tables, texts, or raw data. In survival analysis plots, we extracted data at the point in time when the difference between the ‘survival’ or ‘attacked’ rates of the intervention and comparison groups was greatest as outcomes. Study design (i.e., whether experiments were done independently or dependently between the control and treatment group) was also recorded. Fourth, we gathered predator and prey information, specifically, the study species (common English name and scientific name) and predator diet type. In some cases, studies did not use a specific bird species as a predator or a specific lepidopteran species as prey. We contacted authors when such information was ambiguous or missing. When the paper did not report the pattern area and diameter of the treatment stimulus or the presented stimulus surface area, AM calculated or measured them from available images using ImageJ v.1.53i [42].

The dataset was originally divided into two parts. The first part involved the data from presenting eyespot patterns to avian predators and directly observing their responses (predator dataset). The sample size or unit of analysis in this part was based on the number of individual avian predators. The second part involved the data from using real or artificial abstract butterflies, moths, or caterpillars with eyespots or non-eyespot patterns as stimuli or prey, and observing their survival/attacked probabilities in the field (prey dataset). The sample size or unit of analysis in this part was based on the number of real or artificial abstract prey. However, we also used the combined dataset that included both predator and prey datasets, as detailed in the **“Meta-analysis and meta-regressions”** and **“Publication bias”** sections.

Effect size calculation

To obtain the effect size point estimates and sampling variances, we used the natural logarithm of the response ratio (lnRR) between the means of the treatment and the treatment control stimulus groups [43–45]. Positive lnRR values indicate heightened aversion in birds and enhanced prey survival, while negative lnRR values signify diminished bird aversion and increased prey mortality. The point estimate and sampling variance (var) of lnRR can be then calculated in:

$$lnRR = ln(\frac{M_T}{M_C}) \tag{1}$$

$$var(lnRR) = \frac{SD^2{}_T}{N_T M^2{}_T} + \frac{SD^2{}_C}{N_C M^2{}_C} - 2r\sqrt{\frac{SD^2{}_T}{N_T M^2{}_T}}\sqrt{\frac{SD^2{}_C}{N_C M^2{}_C}} \tag{2}$$

where M_T and M_C are mean responses of treatment and control groups (e.g., total frequency of attacking prey, latency of approach, or prey survivability), respectively. SD and N are (sample) standard deviations and sample size, respectively. The term, r is the correlation coefficient between responses of the two groups. Some of our eligible studies used the paired (dependent) study design where treatment and control samples originated from the same individuals, and sample sizes between the two groups were the same. None of these studies provided an estimate of r. Thus, when calculating our effect sizes, we assumed that this correlation was 0.5, which is conservative [46]. For the other studies that used independent study design, we set $r = 0$.

We note that our dataset included proportion (percentage) data (e.g., predator attack rate or prey survival probability), which are bounded at 0 (0%) and 1 (100%). Therefore, we transformed group means (M) and group standard deviations (SD) for proportion data using Equations (3) and (4) before applying (1) and (2) to calculate lnRR and the sampling variance:

$$f(M) = \ arcsine\ (\sqrt{M}) \tag{3}$$

$$SD(f(M)) = \sqrt{\frac{SD^2}{4M(1-M)}} \tag{4}$$

where f indicates a function, in our case, the arcsine transformation. The standard deviation (SD) related to this transformation was derived using the delta method before calculating lnRR and the sampling variance [47]. We have also assumed that the standard deviation was $SD(f(M)) = 1/\sqrt{8}$ if SD was not available.

Meta-analysis and meta-regressions

We used the *rma.mv* function from the package metafor v.4.4.0 [48] in R v.4.3.1 [49] for our analyses. We started by fitting multilevel, mixed-effect meta-analytic models to the predator and prey datasets. These meta-analytic models explicitly incorporated random factors, Study ID, Cohort ID (groups of the same subjects), and Shared control ID (indicating effect sizes sharing control groups) [50] along with Observation ID, fitted by the above function [48]. The model for the predator dataset included Species ID and a correlation matrix related to phylogenetic relatedness for the species as random factors [51]. This is because we had data on the bird species used in the experiment in the predator dataset, and we needed to control for phylogenetic relationships between birds. We also quantified the total I^2 (a measure of heterogeneity not attributed to sampling error [52]) and how much each random factor was explained (partial I^2), calculated by the *i2_ml* function from the package orchaRd v.2.0.0 [53]. After running both meta-analytical models, we found that phylogeny and Species ID did not need to be controlled for in the predator dataset, as their partial I^2 were zero ($I^2 = 0.00\%$). That is, these factors explained little heterogeneity between effect sizes.

Therefore, we merged predator and prey datasets (i.e., full dataset) without considering phylogenetic information and used them for the following models. We had, as random effects, Study ID, Cohort ID, Shared control ID, and Observation ID for our meta-analytic model using the full dataset. The Cohort ID and Shared control ID were removed from our subsequent meta-regressions because they both explained little heterogeneity (both partial $I^2 < 0.001\%$). This intercept-only (meta-analytic) model tested the conspicuous patterns (eyespots and non-eyespots) that affected predator avoidance (i.e., our first question).

Next, we tested whether eyespots and non-eyespot patterns differ in the magnitude and direction of the effect of elicited bird predator avoidance and what factors contribute to the deterring effects of conspicuous patterns. We performed uni-moderator meta-regression models with each of eight moderators: treatment stimulus pattern types (eyespots vs. non-eyespots), pattern area, the number of pattern shapes, prey material type, maximum pattern diameter/length, total pattern area, total area of prey surface, and prey shape type. We also ran a multi-moderator meta-regression model, including the first four of the eight variables mentioned in the uni-moderators, due to moderator correlations. We used log-transformed data for pattern area, total pattern area, total area of prey surface, and pattern maximum diameter/length in our analysis to normalise these moderators. We created all result plots in the *orchard_plot* and *bubble_plot* functions from the package orchaRd [53].

Publication bias

We used three approaches to assess the presence of publication bias in our study. First, we visually assessed the funnel plot asymmetry by examining the residuals from a meta-analytic model, which included all the random factors utilised in our study. These residuals were plotted against the precision of the effect sizes. Secondly, we performed an alternative method to Egger's regression. This method used the inverse of the effective sample size as a moderator within a multilevel meta-analytic model [54]. Third, we examined the possibility of time-lag bias by including publication year as a moderator in our multilevel meta-analytic model. Uni-moderator models were run for each inverse of the effective sample size and publication year, and a multi-moderator model was carried out with the full model including both inverse of the effective sample size and publication year as moderators.

Additions and deviations

We made two changes to the pre-registration: the addition of four new moderators and the removal of two moderators. The new moderators were pattern area, total pattern area, total area of prey surface, and prey shape types, although similar moderators were in the pre-registration such as the number of eyespots (patterns) and diameter of an eyespot (a pattern). These *post-hoc* decisions were taken to refine our initial moderators. We subsequently used them in our meta-regression analyses. We originally intended to include the broad outcome categories of predator avoidance measure as a moderator in the models, but the diversity of reported results made categorisation impossible. Therefore, we did not include it as a moderator. We also collected information on bird diet but decided not to include it. This decision was because six of the seven bird species in our study were omnivores, resulting in a lack of variability needed to detect diet effects in our data (for more details, please see **Results**).

Results

Screening outcomes and dataset characteristics

We obtained 270 effect sizes from 33 studies (164 experiments) for our analysis. The screening process and reasons for exclusion at the full-text screening stage are summarised in the PRISMA-like flowchart (Fig. 2a), with additional details available in Table S3, which comprises a list of included/excluded studies. Of the dataset, 68.9% of effect sizes came from eyespot presentation experiments (Fig. 2b). The remaining 31.1% of effect sizes came from non-eyespot pattern presentation experiments (Fig. 2b). The latter category encompassed various shapes, including circles (71.4%), rectangles (16.7%), diamonds (6.0%), complex patterns (combinations of circles and diamonds; 4.8%), and stripes (1.1%); 93.7% of the control stimuli used in these experiments involved the removal of the pattern used in the treatment stimuli; the remaining stimuli were camouflage patterns (6.3%). Prey shape type used for stimulus presentation varied from real or imitation of a particular butterfly (24.4%) to simply a piece of paper (21.5%) (Fig. 2b). The number of pattern shapes varied between studies from one to 11, but in most experiments, they were two (i.e., a pair of shapes; Fig. 2c). Additionally, we found that the size of these patterns, both area and maximum diameter/length, exhibited considerable variation across studies (Fig. 2c). The total area of the patterns and stimulus also varied widely (Fig. 2c). The studies reported responses to conspicuous pattern stimuli by seven bird species (Fig. 2d). Chickens (*Gallus gallus*) and common starlings (*Sturnus vulgaris*) were the most studied birds in our dataset. Apart from chickens (eight studies) and Eurasian blue tits (*Cyanistes caeruleus*; five studies), effect sizes were available from just one or two studies per species. Six of the seven species were omnivores, and one (yellow bunding; *Emberiza sulphurata*) was a granivore [55].

Does the presence of conspicuous patterns affect predator avoidance?

The overall mean effect size was statistically significant, showing a 21.86% (this percentage value is the back-transformed values of lnRR) increase in the probability of predator avoidance, such as higher prey survival rates or eliciting fewer attacks from birds (estimate = 0.20, 95% CI = [0.08, 0.31], $t_{[df = 268]} = 3.40$, $p = 0.0008$), in prey with conspicuous patterns than in prey without such patterns (Fig. 3a). Total heterogeneity across effect sizes was high ($I^2 = 96.50\%$); more specifically, observation ID (representing the within-study effect) accounted for the most heterogeneity, 79.88%, with study ID (representing between-study effect) accounting for the remaining 16.61%.

Is there a difference in predator avoidance between eyespots and conspicuous patterns?

There was no statistically significant difference between the effects of eyespots and non-eyespot patterns ($F_{[df1 = 1,\ df2 = 268]} = 0.33$, $p = 0.57$, $R^2 = 0.27\%$; Fig. 3b). On average, eyespot patterns resulted in 24.37% (estimate = 0.22, 95% CI = [0.08, 0.35], $t_{[df = 268]} = 3.17$, $p =$

0.002) and non-eyespot patterns in 17.11% (estimate = 0.16, 95% CI = [-0.02, 0.34], $t_{[df = 268]}$ = 1.71, p = 0.09) increases in predator avoidance compared with control stimuli, although this trend was not statistically significant for non-eyespots (Fig. 3b).

What factors promote predator avoidance?

Our uni-moderator meta-regression model with pattern area (individual shape area) showed that larger patterns were associated with an increase in predator avoidance (estimate = 0.11, 95% CI = [0.03, 0.19], $t_{[df = 268]}$ = 2.71, p = 0.007, R^2 = 8.56%; Fig. 4a). The total pattern area also promoted predator avoidance (estimate = 0.09, 95% CI = [0.004, 0.17], $t_{[df = 268]}$ = 2.07, p = 0.04, R^2 = 5.18%; Fig. S1a). Similarly, the maximum diameter/length of the pattern positively influenced predator avoidance (estimate = 0.19, 95% CI = [0.04, 0.35], $t_{[df = 268]}$ = 2.46, p = 0.01, R^2 = 6.62%; Fig. S1b). In contrast, an increased number of pattern shapes significantly reduced the effect of predator avoidance (estimate = -0.06, 95% CI = [-0.11, -0.008], $t_{[df = 268]}$ = -2.29, p = 0.02, R^2 = 2.46%; Fig. 4b). We found no significant effects of total prey surface area on predator avoidance (estimate = -0.03, 95% CI = [-0.15, 0.09], $t_{[df = 268]}$ = -0.48, p = 0.63, R^2 = 0.42%; Fig. S1c). Predator avoidance was not statistically significantly affected by differences in whether the presented prey looked like a real lepidopteran species ($F_{[df1 = 1, df2 = 268]}$ = 0.12, p = 0.72, R^2 = 0.13%). Both types of prey material (real/imitation and abstract butterfly) had similar positive trends (Fig. 3c), with the former increasing predator avoidance by 25.55% (estimate = 0.23, 95% CI = [0.03, 0.43], $t_{[df = 268]}$ = 2.24, p = 0.03) and the latter by 20.07% (estimate = 0.18, 95% CI = [0.04, 0.33], $t_{[df = 268]}$ = 2.44, p = 0.02). Further, when also considering prey type (Fig. S2), abstract and real butterflies significantly exhibited increased predator avoidance by 37.98% (estimate = 0.32, 95% CI = [0.11, 0.53], $t_{[df = 268]}$ = 3.04, p = 0.003) and by 25.40% (estimate = 0.23, 95% CI = [0.03, 0.42], $t_{[df = 268]}$ = 2.25, p = 0.03), respectively, but artificial abstract caterpillars (estimate = 0.07, 95% CI = [-0.18, 0.31], $t_{[df = 266]}$ = 0.53, p = 0.60) and artificial abstract prey (estimate = 0.01, 95% CI = [-0.35, 0.37], $t_{[df = 266]}$ = 0.06, p = 0.95) did not, respectively. When comparing each prey type (e.g., abstract butterfly vs. real butterfly), none of the differences was statistically significant (Fig. S2).

The multi-moderator (full) regression model showed that only pattern area positively affected predator avoidance (estimate =0.10, 95% CI = [0.009, 0.18], $t_{[df = 266]}$ = 2.16, p = 0.03; Table S4). Contrary to the uni-moderator regression model, the number of patterns showed no significant effects on predator avoidance, although the consistent trend remained (estimate = -0.05, 95% CI = [-0.11, 0.004], $t_{[df = 266]}$ = -1.84, p = 0.07; Table S4). The full model accounted for 8.33% of the variation in the dataset. The complete output of the multi-moderator model is displayed in Table S4.

Publication bias

The funnel plot showed no visual sign of funnel asymmetry (Fig. 5a). The meta-regression analysis, which included the square root of the inverse of the effective sample size, further supported this observation by showing that the effective sample size did not significantly predict the effect size values (estimate = -0.09, 95% CI = [-0.83, 0.65], $t_{[df = 266]}$ = -0.24, p = 0.81; Fig. 5b). There was no detectable trend suggesting that more recent publications consistently showed lower or higher effect size values, which would have indicated the presence of time-lag publication bias (estimate = −0.0008; 95% CI = [−0.01, 0.01], $t_{[df = 266]}$ = -0.12, p = 0.90; Fig. 5c). We obtained the same trends from multi-moderator meta-regressions (Fig. S3).

Discussion

Eyespots and non-eyespot patterns did not differ significantly in the magnitude of deterring effects (Fig. 3b). Avian predators showed similar avoidance responses to the conspicuous patterns compared to control ones (Fig. 3a). Specifically, larger pattern sizes played a crucial role in eliciting negative responses from birds (Fig. 4a). Further, negative responses from birds showed the tendency to decline with increasing pattern number: single patterns were likely more intimidating than a group of patterns (Fig. 4b). Taken together, our results support the conspicuousness hypothesis rather than the eye mimicry hypothesis.

Eye mimicry or conspicuousness hypothesis?

Overall, our meta-analysis showed that conspicuous patterns could increase predator avoidance by over 20%. Specifically, our results indicate that conspicuousness per se can be advantageous in avoiding bird predation (Fig. 3ab, Fig. 4). The evidence favouring the conspicuousness hypothesis comes mainly from a series of field experiments by Stevens and his colleagues [30, 31, 34]. They showed that both eyespots and non-eyespots improved the prey survival similarly compared to non-conspicuous patterns [30, 31, 34]. In addition, their research showed prey with more conspicuous patterns (i.e., large-size patterns) tended to survive more than others [30, 31, 34], and eye resemblance (e.g., number or pattern shapes) did not significantly affect the prey's survival [30, 31, 34]. Given that these pattern stimuli used in the experiments are rarely or never found in natural environments [34], the most parsimonious explanation for these results is neophobia or dietary conservatism in birds [56–58]. Both phenomena appear to diminish with habituation and/or learning. A few studies investigated such factors for intimidating effects, and they showed that repeated encounters made birds more habituated to eyespot patterns [16, 59, 60]. We need more systematic tests of bird habituation to vividly- or aposematic-coloured patterns to better understand the evolution and function of such patterns in Lepidoptera.

While our meta-analytic results favour the conspicuousness hypothesis, several empirical studies support the eye mimicry hypothesis. For example, De Bona et al. [28] found that a pair of eyespots of *Caligo martia* was as effective as true owl eyes and more efficient in eliciting predator avoidance responses than less mimetic but equally contrasting circles. Blut and Luau [61] created artificial eye-spotted prey with different similarities to the vertebrate eyes and checked their survival rates in a field experiment. They revealed that the prey with the most mimetic pattern had the highest survival rate [61]. Although studies on Lepidoptera larvae are relatively limited, caterpillar eyespots are considered part of snake mimicry [14]. Some research examined the benefit of eyespots by presenting artificial caterpillars (marked with eyespots and control) made from dyed pastry to wild birds and showed that eyespots improved survival [60, 62, 63]. Despite these convincing pieces of empirical evidence, our meta-analytic results showed that eye resemblance did not improve predator avoidance. If the eye mimic hypothesis was true, we would have seen a clear difference between studies investigating eyespots and non-eyespots.

However, we observed little heterogeneity among studies, despite finding high heterogeneity within individual studies. This finding implies that if each study followed similar experimental procedures within studies, our main result on predator avoidance would be more generalisable. The high within-study heterogeneity can be caused by varying stimulus characteristics contributing to the effect size variations, even in the same studies. Bird phylogenetic relatedness explained little heterogeneity in our predator dataset, but this may have occurred because a limited number of subject bird species (i.e., chickens, common starlings, Eurasian blue tits) dominated our dataset (Fig. 2d). While we cannot exclude the possibility of species differences in birds' responses to the conspicuous patterns, our analysis indicated that bird species identity did not explain the observed variation in predator avoidance.

We also note that conspicuous patterns can also be important for conspecific communication in butterflies, not just for avoiding predation [12, 64]. For example, eyespots on *Bicyclus anynana* are known to function as sexual signals. For example, males choose females depending on eyespot size and reflectance [65]. Regarding the non-eyespot patterns, males of *Heliconius cydno* and *H. pachinus* can recognise conspecific females by the bright colour of wing patches [66, 67]. Conspicuous patterns can also act as social signals in other taxa (e.g., birds: [68]), but this function remains unclear in butterflies. Therefore, the diversity of patterns on wings could be shaped by intra-specific and inter-specific communication. We should simultaneously consider the influence of anti-predator and sexual/social signalling functions on the evolution of butterfly conspicuous patterns [cf., 65, 69, 70].

What factors explain the observed heterogeneity?

The indicators of pattern size, including each pattern area (Fig. 4a), total pattern area (Fig. S1a), and maximum diameter/length (Fig. S1b), were the most important moderators of effect sizes, overall indicating that large patterns could promote predator avoidance. Notably, these size metrics were correlated, so they are not independent of each other. Several studies suggested that the pattern size difference is related to the difference in prey survival [21, 26, 30]. For example, eyespots larger than 6.0 mm may have a strong deterrent effect with increasing size [26], but such patterns may increase the visibility of lepidopterans, and their presence may increase predation rates as well [71]. Indeed, small conspicuous patterns tend to attract predators' attention, as explained by the deflection hypothesis [12, 72]. The effect may contribute to the observed negative overall effect sizes (Fig. 3, Fig. 4). Considering studies on *B. anynana* with eyespots with a deflecting effect (maximum diameter is about 5.0 mm; Table S5), a size of at least 6.0 mm is required to avoid predator approach. However, it is uncertain whether the effect would linearly increase with size or whether an optimal size exists. Although eyespot sizes on actual Lepidoptera may be restricted by their body or wing size ([e.g., 73], but see also [21]), it would be interesting to find a maximum threshold for patterns that promote predator avoidance responses in birds.

Among other moderators tested (prey material type, total pattern area, and prey shape type), the only moderator that seemed to explain heterogeneity was the number of patterns (Fig. 4b; yet it is likely inconclusive; see Table S4). Previous studies predominantly employed a single pattern or a pair of patterns, leading to limited variations. Nonetheless, our findings indicate that a single eyespot is equally or more effective than a pair of eyespots. Consequently, the resemblance to a pair of eyes, a crucial aspect of the eye mimicry hypothesis, may be optional for effective predator avoidance. Indeed, we should note that the presence of both eyes is unnecessary for birds to recognise their predators because birds may often see only one eye of their predators. To disentangle the two hypotheses, we recommend conducting the following experiments with two key features [30, 35, 74]: a set of stimuli that (1) have the same size (area or diameter/maximum length of each pattern or total pattern area) but with different numbers of patterns ranging from a few usually found in Lepidoptera to numerous patterns unlike those seen in them, and (2) are presented with the same number of patterns and the same size but different pattern shapes. Results from these experiments could deepen our current knowledge, allowing us to inch toward a more definitive answer.

Knowledge gaps and future opportunities

Along with other conspicuous patterns, eyespots are believed to deter bird predation, and our meta-analysis supports this function. However, five major gaps remain in the current literature and our knowledge. First, birds and humans likely perceive eye-like shapes differently based on the interspecific diversity of bird vision [75]. For example, most bird species can detect ultraviolet light, which is invisible to humans, and the ultraviolet reflection of the butterflies' eyespots may contribute to predator avoidance [e.g., 20, 22]. In addition, researchers can quantify and objectively evaluate conspicuousness, such as size and number, but the assessment of 'eye mimicry' remains subjective. Thus, it could be premature to conclude that eyespots on Lepidoptera resemble vertebrate eyes universally.

Second, some lepidopterans present conspicuous patterns to potential predators in combination with other elements, such as sounds and movements [13, 16, 17, 76, 77], presumably to emphasise the conspicuousness of the patterns. Most of the current literature does not take these effects into account in experiments, although some studies argue in favour or against their importance [e.g., 16, 17]. We should also consider how factors other than those constituting the pattern (e.g., colour, number, and size) are involved in the predator avoidance function of eyespots. The location of the butterfly's eyespot patterns varies from species to species as well; eyespots exist on the wings' ventral, dorsal, or both sides. Not only the dorsal eyespot patterns, which were used in most studies, but also the ventral eyespot patterns should be explored. In addition, we need to avoid presenting patterns unnaturally when using real butterflies in experiments. For example, many owl butterflies (family Caligo) have a pair of eyespot patterns on the ventral side. Their eyespots are usually visible to birds when the wings are closed and would not present side by side as in the eyes of the owl's frontal face.

Third, recent studies have shown that birds are sensitive to the gaze of other individuals and may respond more aversively when their gazes are directed at them [e.g., 78-80]. Skelhorn and Rowland [81] showed that the anti-predation effect may be further enhanced if the inner circle of the eyespot is in a more gazing-like position for subject birds.

However, further research is needed to investigate the importance of the position of the inner circle.

Fourth, as mentioned above, studies focusing on caterpillar eyespots are much more scarce compared to butterflies; Hossie and Sherratt [82] have shown similarities between caterpillars and snakes, but the response of birds to actual caterpillars has not been experimentally tested. Conversely, in butterflies, similarities between the eyespot patterns on wings and the eyes of birds of prey have not been investigated.

Finally, birds are generally considered as potential predators of butterflies and caterpillars. Although other taxa species, such as invertebrates [83–85], lizards [27, 86, 87], and rats [88–91], are also known to prey on lepidopterans, there are much fewer studies using non-avian species as predators. The effectiveness of eye mimicry versus being conspicuous may vary depending on the predator, and either one may be more effective depending on specific predator species. Therefore, we should expand the range of taxa used for experiments to get a better and more generalisable understanding of the eyespots' function and evolution in butterflies and caterpillars. Additionally, much of the research has been conducted in Europe and North America. Of the studies we included, only two were from other regions (India [115] and Singapore [26]). The empirical results may differ in areas with many species of lepidopterans with eyespot patterns [e.g., 41].

Knowing the effects of conspicuous patterns may contribute to creating a world where birds and humans can live more harmoniously. Both eyespots and conspicuous patterns have already been used to control birds, particularly in agriculture, although their effectiveness has been questioned [e.g., 92, 93]. Such uncertainty may reflect our limited understanding of why birds avoid eyespots and conspicuous patterns. Nevertheless, visual stimuli are less likely to harm birds or affect the natural environment than others (e.g., nest/egg destructions or toxic chemicals; reviewed in [94]). Therefore, when proven effective, they could be used for better pest control, population management and conservation [95].

Conclusion

We have shed light on a traditional but controversial research topic that has fascinated behavioural ecologists for decades. Our findings provide a better understanding of the evolution of signal designs, but also show that more work is needed to understand the function of the eyespot patterns in Lepidoptera, such as whether eyespot patterns evolved due to mimicry or conspicuousness.

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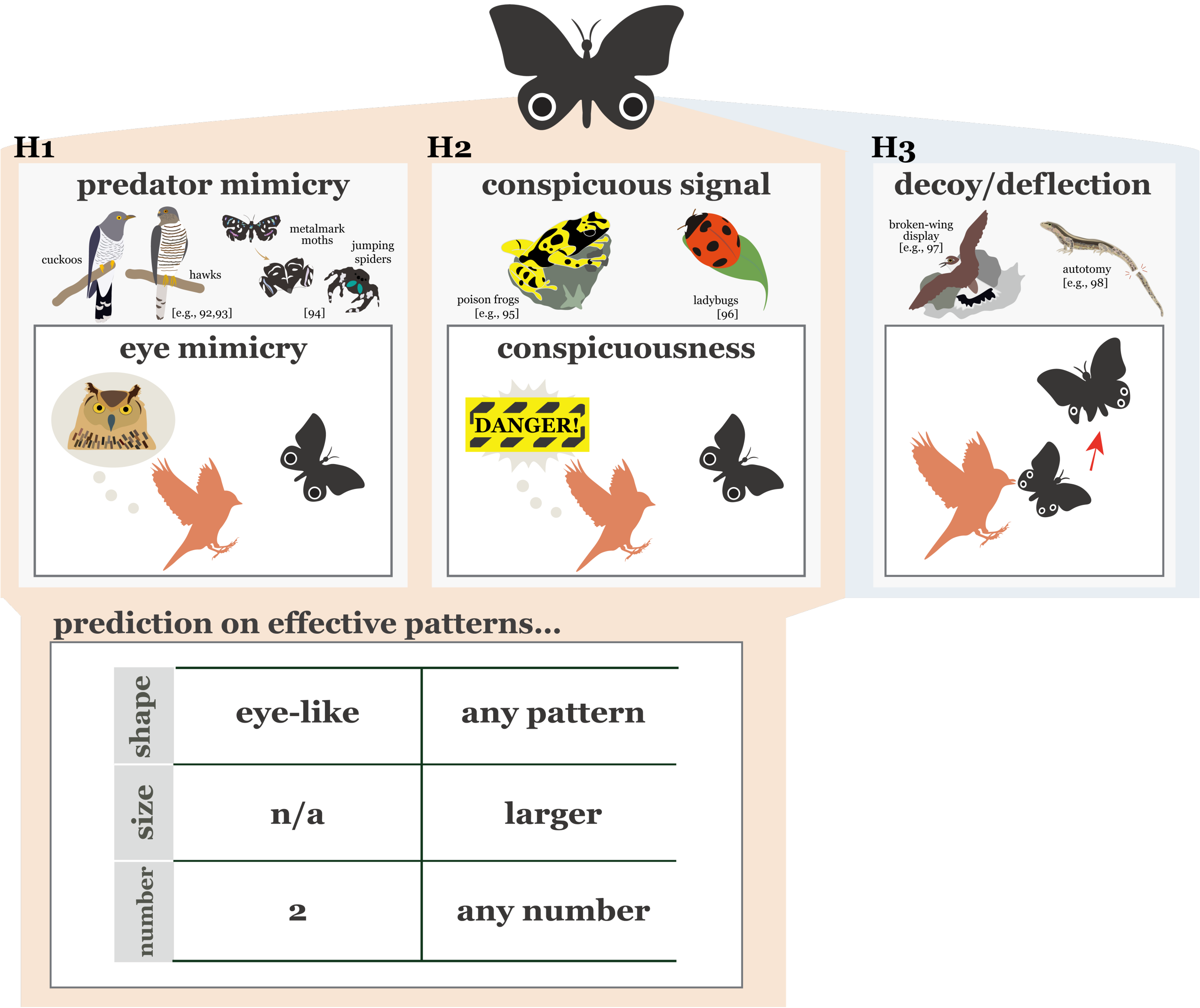
(doi:10.1093/beheco/arv135)

Figures and table



shape	eye-like	any pattern
size	n/a	larger
number	2	any number

Figure 1. A visual summary of three hypotheses that explain the predation avoidance function of eyespot patterns and the predictions that can be derived from these two hypotheses. The resemblance of eye spots to actual eyes is discussed through the predator mimicry hypothesis and the conspicuous signal hypothesis. The table shows the predictions derived from these two hypotheses.

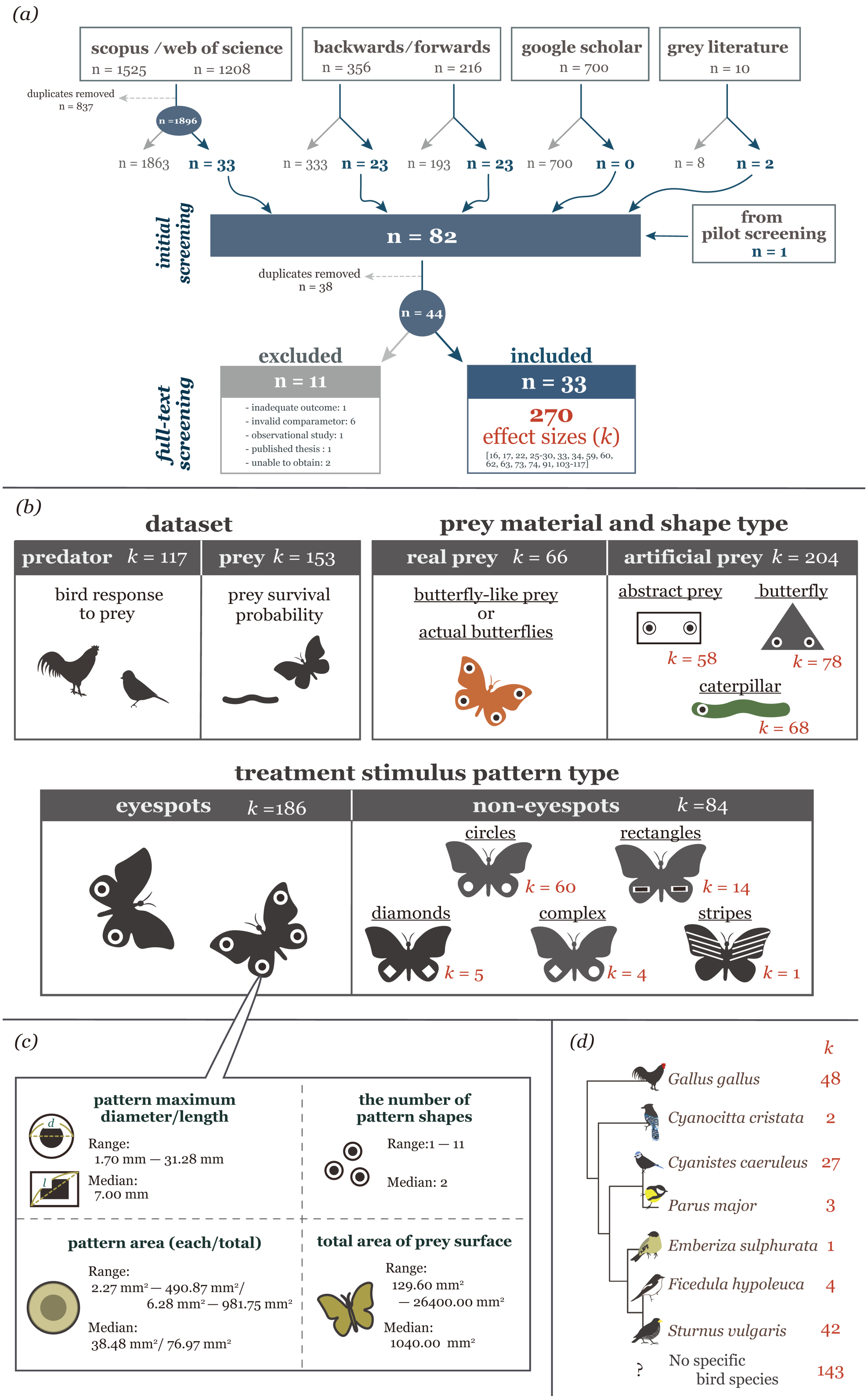


Figure 2. Overview of the dataset. (a) shows a PRISMA-like flowchart of the systematic literature search for the meta-analysis. (b) and (c) give details of the main moderators

examined in the meta-analysis. (d) provides the phylogenetic tree of bird species included in the meta-analysis, together with the sample sizes and number of effect sizes per species.

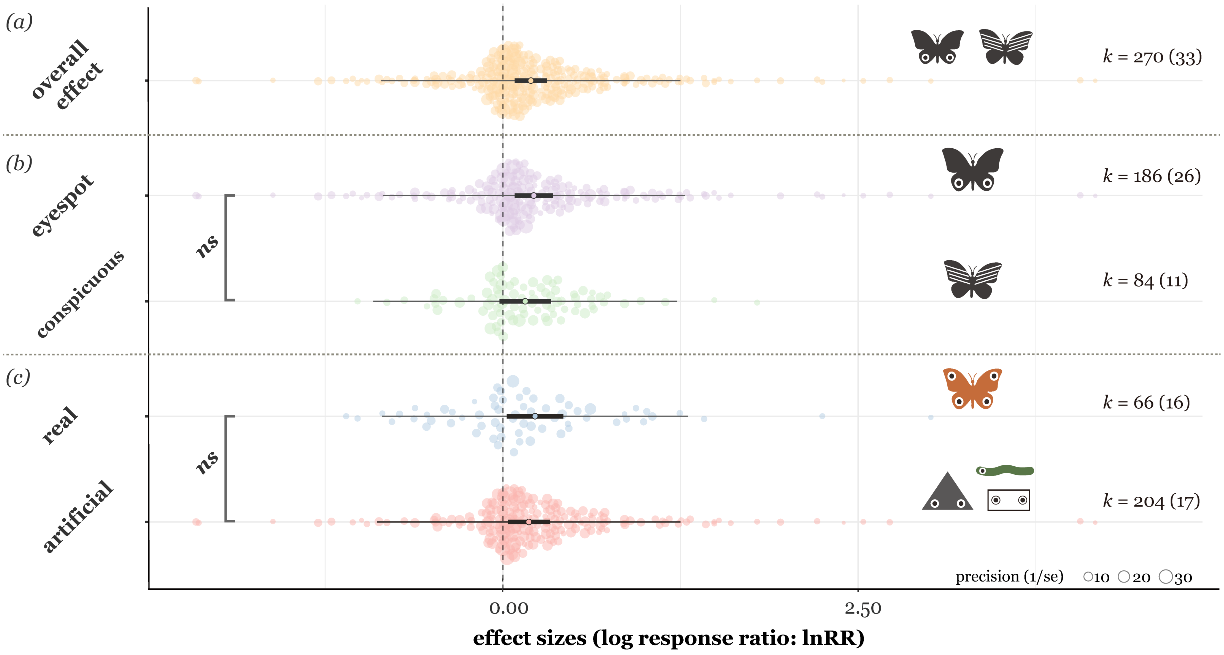


Figure 3. Mean effect sizes of (a) overall for all highly salient patterns, (b) effects split by experiments with eyespots versus conspicuous patterns, and (c) two prey types used in the experiments. Thick horizontal lines represent 95% confidence intervals, and thin horizontal lines represent 95% prediction intervals. The points in the centre of each thick line indicate the average effect size. *k* is the number of effect sizes used to estimate the statistics, followed by the number of studies in the brackets.

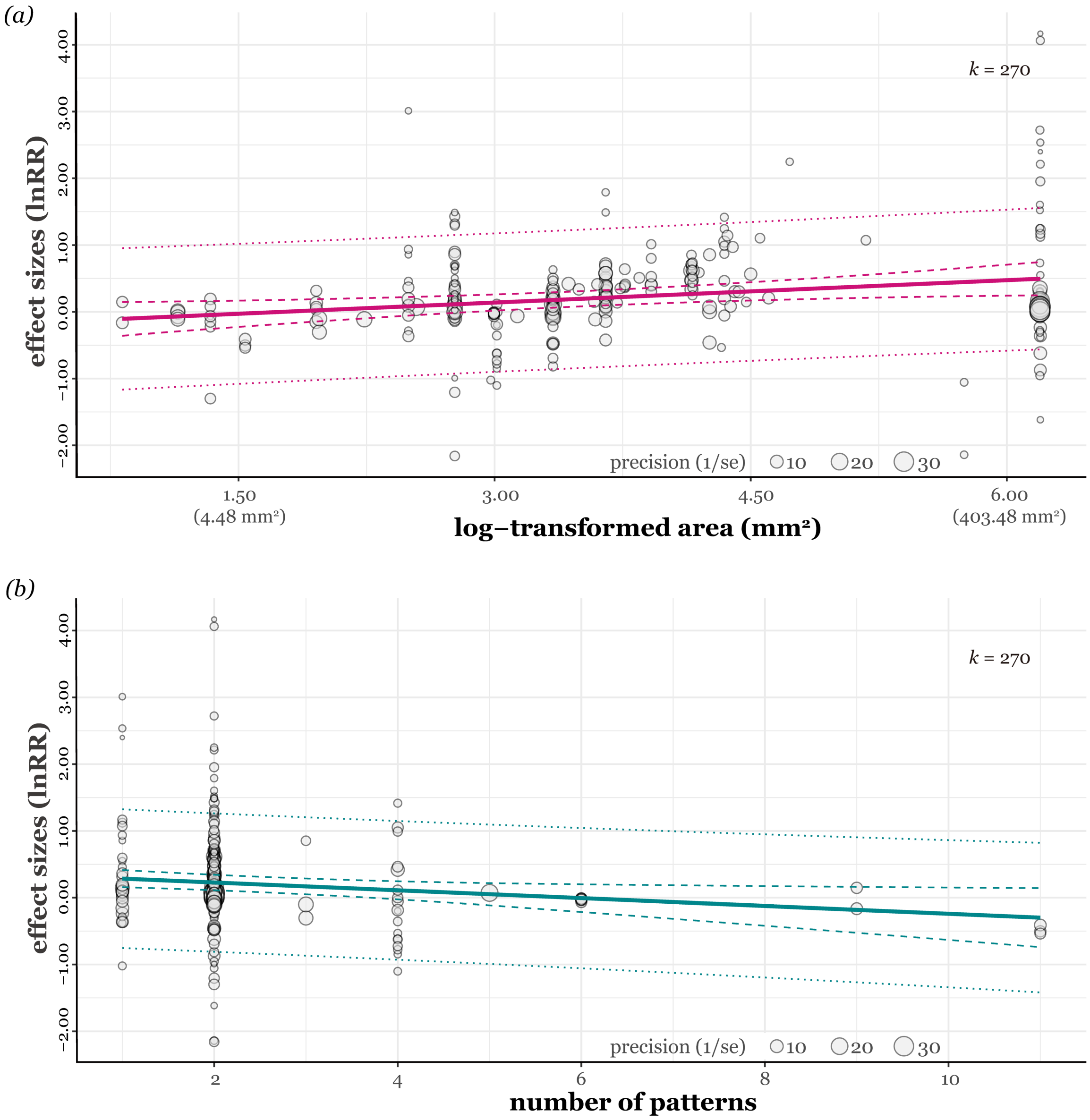


Figure 4. The relationships between (a) prey salient pattern area (log-transformed) and effect sizes and (b) number of prey salient patterns and effect sizes. Circle sizes are scaled according to precision, *k* represents the number of effect sizes. Each fitted regression line is shown as a coloured straight line, and 95% confidence and prediction intervals are shown as dashed and dotted coloured lines, respectively.

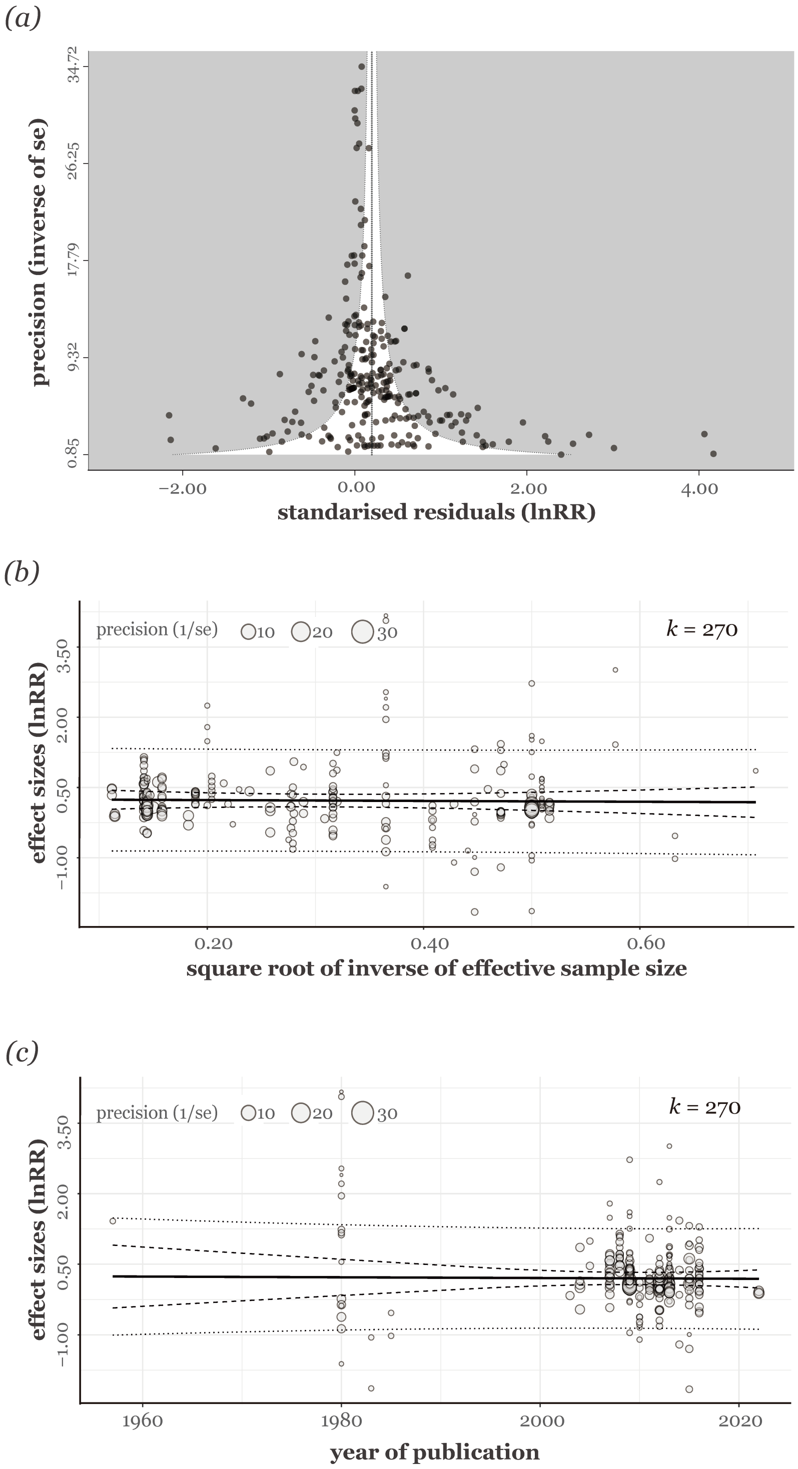


Figure 5. (a) Funnel plot using effect size and its inverse standard error. The relationship between effect sizes and (b) the square root of the inverse of effective sample size and (c) publication year. In (b) and (c), circle sizes are scaled accordingly to precision, and *k* represents the number of effect sizes. Each fitted regression line is shown as a straight line,

and 95% confidence and prediction intervals are shown as dashed and dotted lines, respectively.

Table 1. Descriptions of the population, Intervention, comparator, and outcome (PICO) used to define the scope of this study.

PICO	Description
Population	Birds as predators and butterflies, moths, caterpillars, and their models as prey
Intervention	Presenting eyespot or conspicuous pattern stimulus to birds
Comparator	Presenting stimulus that is neither eyespot nor conspicuous patterns
Outcome	Avian behavioural responses to eyespot or conspicuous pattern stimuli The probability of prey surviving or being attacked (for the stimuli)

Supplementary figures and tables

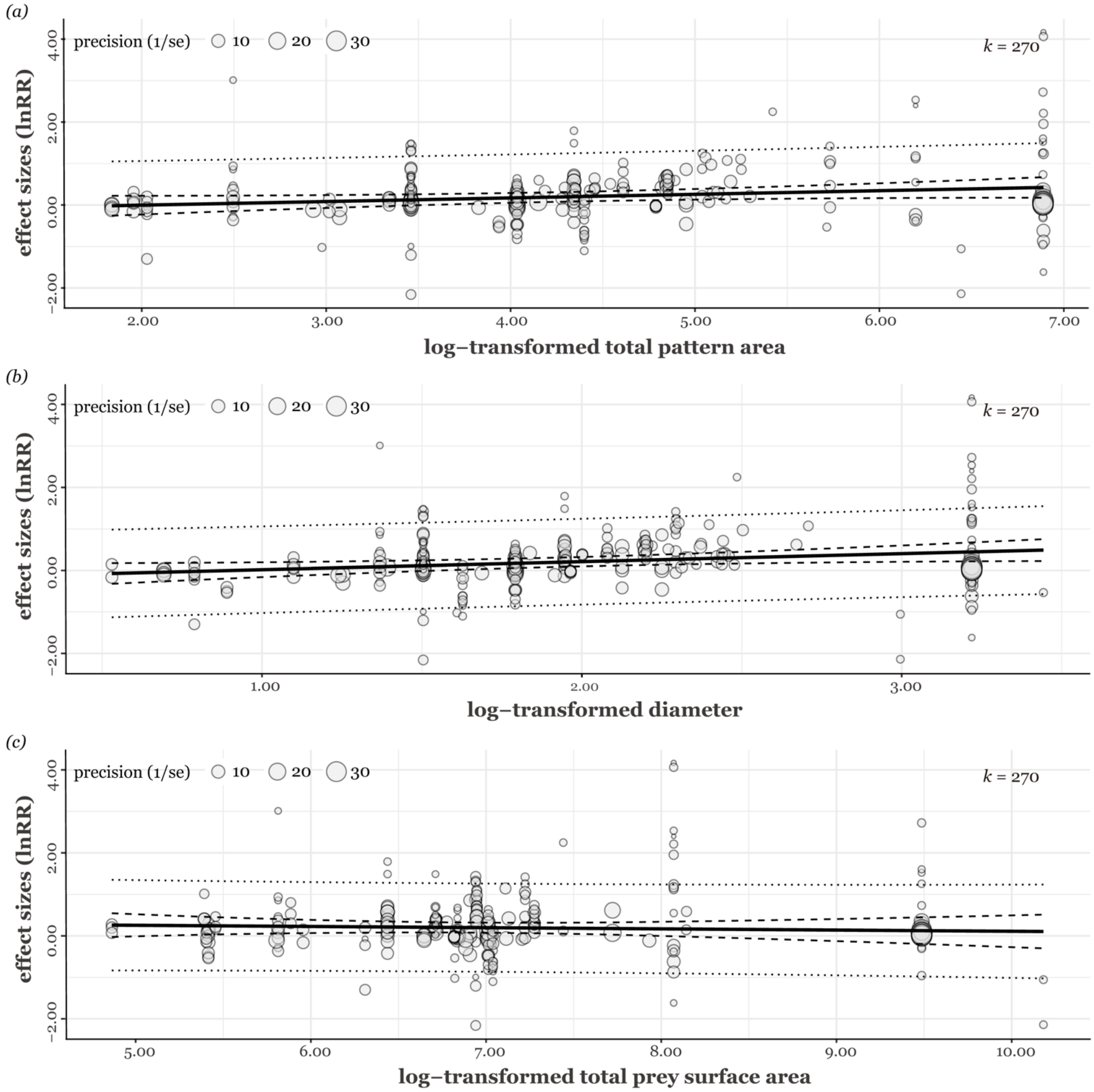


Figure S1. The relationships between (a) total pattern area, (b) pattern maximum diameter/length, and (c) total prey surface area and effect sizes. *k* shows the number of effect sizes. Each fitted regression line is shown as a solid straight line, and 95%confidence and prediction intervals are shown as dashed and dotted lines, respectively.

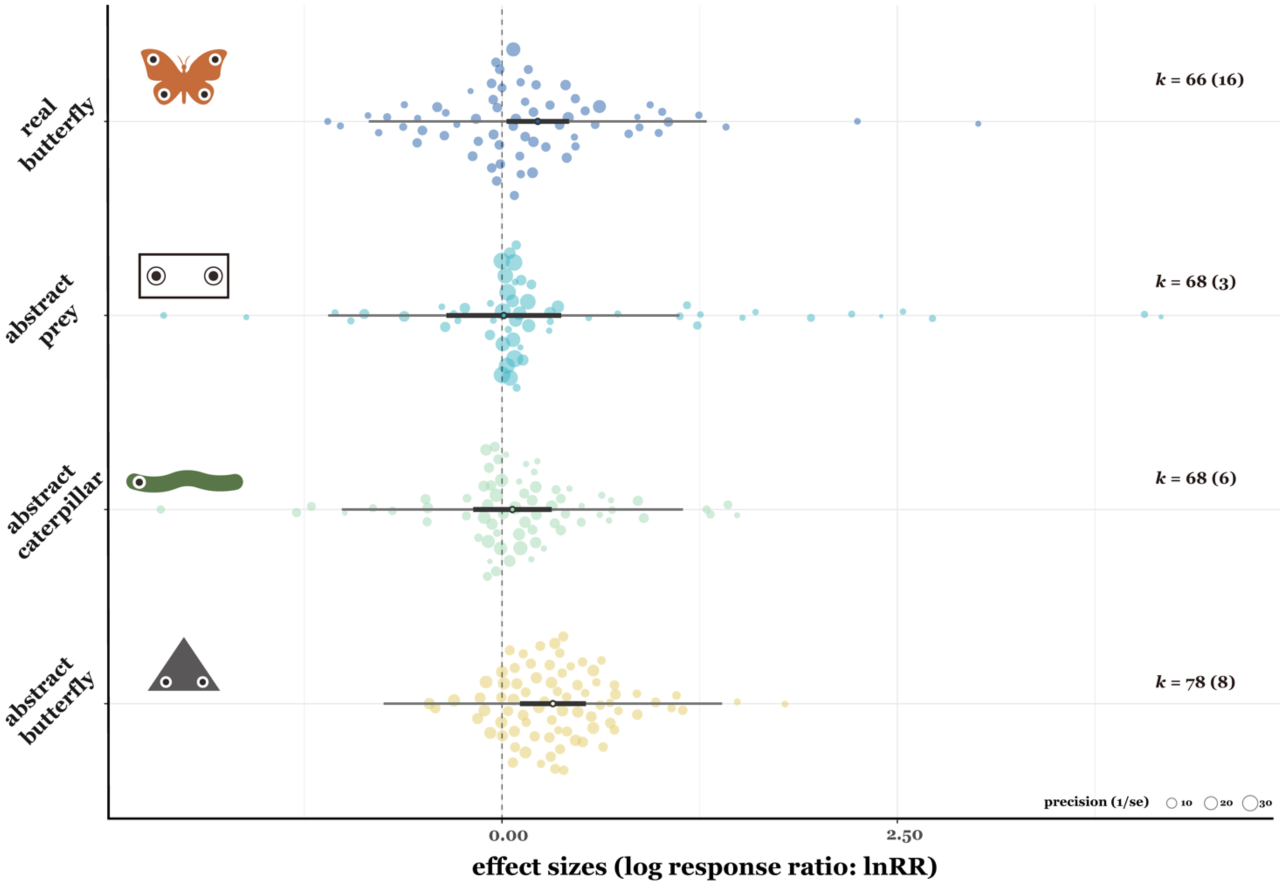


Figure S2. Mean effect sizes of total prey shape types. Thick horizontal lines represent 95% confidence intervals, and thin horizontal lines represent prediction intervals. The points in the centre of each thick line indicate the average effect size. *k* shows the number of effect sizes.

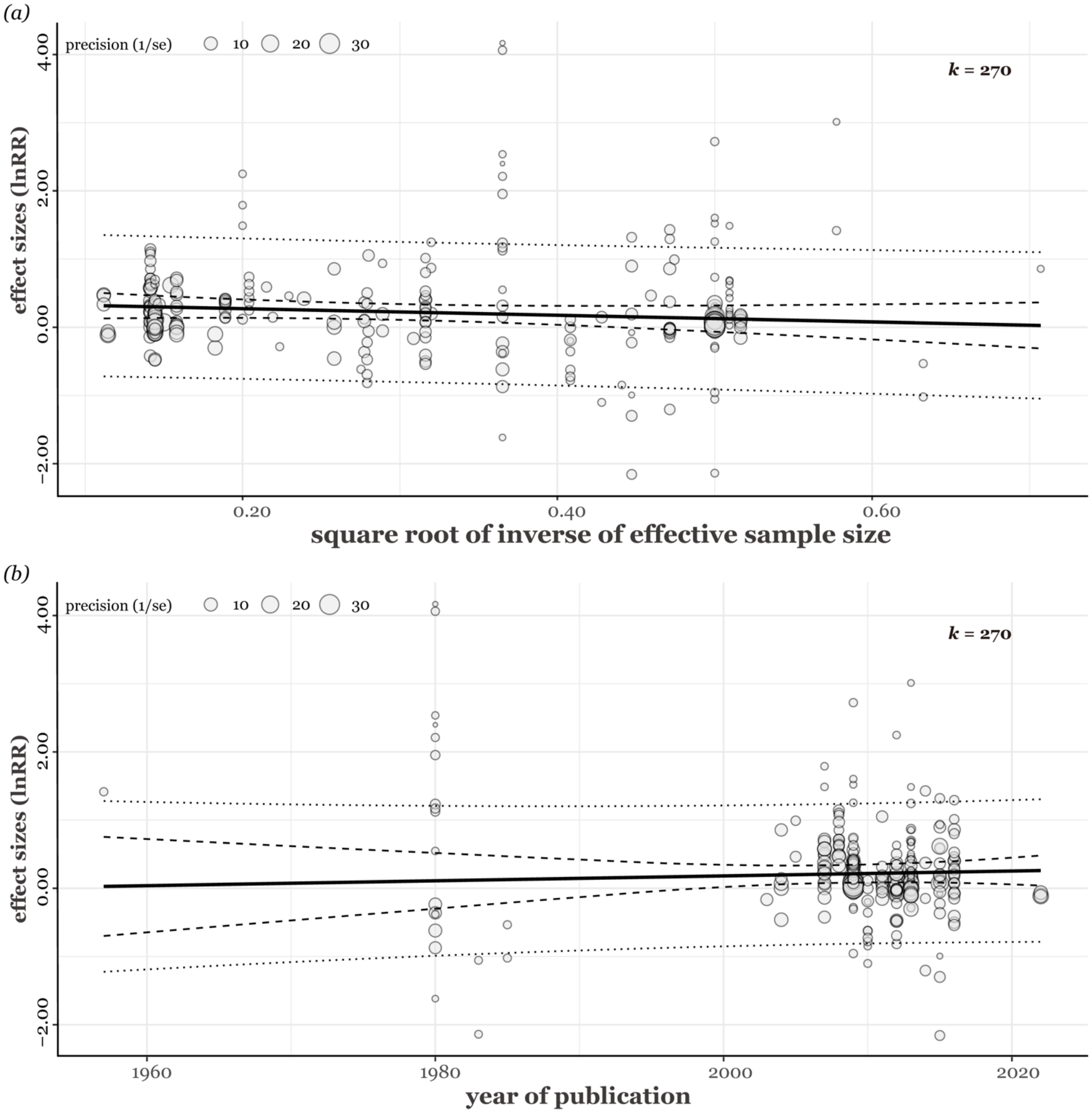


Figure S3. (a) relationship between effect size and the square root of the inverse of effective sample size, and (b) relationship between effect size and publication year. Both plots were based on the multi-moderator model. *k* shows the number of effect sizes. Each fitted regression line is shown as a solid straight line, and 95% confidence intervals and prediction intervals are shown as dashed and dotted lines, respectively.

Table S1. PRISMA-EcoEvo Checklist.

Checklist item	**Sub-item number**	**Sub-item**	**Reported by authors?**	**Notes**
Title and abstract	1.1	Identify the review as a systematic review, meta-analysis, or both	Yes	
	1.2	Summarise the aims and scope of the review	Yes	
	1.3	Describe the data set	Yes	
	1.4	State the results of the primary outcome	Yes	
	1.5	State conclusions	Yes	
	1.6	State limitations	Yes	
Aims and questions	2.1	Provide a rationale for the review	Yes	
	2.2	Reference any previous reviews or meta-analyses on the topic	Yes	
	2.3	State the aims and scope of the review (including its generality)	Yes	
	2.4	State the primary questions the review addresses (e.g. which moderators were tested)	Yes	
	2.5	Describe whether effect sizes were derived from experimental and/or observational comparisons	Yes	
Review registration	3.1	Register review aims, hypotheses (if applicable), and methods in a time-stamped and publicly accessible archive and provide a link to the registration in the methods section of the manuscript. Ideally registration occurs before the search, but it can be done at any stage before data analysis.	Yes	
	3.2	Describe deviations from the registered aims and methods	Yes	
	3.3	Justify deviations from the registered aims and methods	Yes	
Eligibility criteria	4.1	Report the specific criteria used for including or excluding studies when screening titles and/or abstracts, and full texts, according to the aims of the systematic review (e.g. study design, taxa, data availability)	Yes	

	4.2	Justify criteria, if necessary (i.e. not obvious from aims and scope)	Yes	
Finding studies	5.1	Define the type of search (e.g. comprehensive search, representative sample)	Yes	
	5.2	State what sources of information were sought (e.g. published and unpublished studies, personal communications)	Yes	
	5.3	Include, for each database searched, the exact search strings used, with keyword combinations and Boolean operators	Yes	
	5.4	Provide enough information to repeat the equivalent search (if possible), including the timespan covered (start and end dates)	Yes	
Study selection	6.1	Describe how studies were selected for inclusion at each stage of the screening process (e.g. use of decision trees, screening software)	Yes	
	6.2	Report the number of people involved and how they contributed (e.g. independent parallel screening)	Yes	
Data collection process	7.1	Describe where in the reports data were collected from (e.g. text or figures)	Yes	
	7.2	Describe how data were collected (e.g. software used to digitize figures, external data sources)	Yes	
	7.3	Describe moderator variables that were constructed from collected data (e.g. number of generations calculated from years and average generation time)	Yes	
	7.4	Report how missing or ambiguous information was dealt with during data collection (e.g. authors of original studies were contacted for missing descriptive statistics, and/or effect sizes were calculated from test statistics)	Yes	
	7.5	Report who collected data	Yes	
	7.6	State the number of extractions that were checked for accuracy by co-authors	No	

Data items	8.1	Describe the key data sought from each study	Yes	
	8.2	Describe items that do not appear in the main results, or which could not be extracted due to insufficient information	Yes	
	8.3	Describe main assumptions or simplifications that were made (e.g. categorising both 'length' and 'mass' as 'morphology')	NA: no assumptions or simplifications needed to be made	
	8.4	Describe the type of replication unit (e.g. individuals, broods, study sites)	Yes	
Assessment of individual study quality	9.1	Describe whether the quality of studies included in the systematic review or meta-analysis was assessed (e.g. blinded data collection, reporting quality, experimental *versus* observational)	No	
	9.2	Describe how information about study quality was incorporated into analyses (e.g. meta-regression and/or sensitivity analysis)	No	
Effect size measures	10.1	Describe effect size(s) used	Yes	
	10.2	Provide a reference to the equation of each calculated effect size (e.g. standardised mean difference, log response ratio) and (if applicable) its sampling variance	Yes	
	10.3	If no reference exists, derive the equations for each effect size and state the assumed sampling distribution(s)	Yes	
Missing data	11.1	Describe any steps taken to deal with missing data during analysis (e.g. imputation, complete case, subset analysis)	NA: there was no missing data	
	11.2	Justify the decisions made to deal with missing data	NA: there was no missing data	
Meta-analytic model description	12.1	Describe the models used for synthesis of effect sizes	Yes	
	12.2	The most common approach in ecology and evolution will be a random-effects model, often with a hierarchical/multilevel structure. If other types of models are chosen (e.g. common/fixed effects model, unweighted model), provide justification for this choice	NA: only (weighted) random-effects models were used	

Software	13.1	Describe the statistical platform used for inference (e.g. *R*)	Yes	
	13.2	Describe the packages used to run models	Yes	
	13.3	Describe the functions used to run models	Yes	
	13.4	Describe any arguments that differed from the default settings	Yes	
	13.5	Describe the version numbers of all software used	Yes	
Non-independence	14.1	Describe the types of non-independence encountered (e.g. phylogenetic, spatial, multiple measurements over time)	Yes	
	14.2	Describe how non-independence has been handled	Yes	
	14.3	Justify decisions made	Yes	
Meta-regression and model selection	15.1	Provide a rationale for the inclusion of moderators (covariates) that were evaluated in meta-regression models	Yes	
	15.2	Justify the number of parameters estimated in models, in relation to the number of effect sizes and studies (e.g. interaction terms were not included due to insufficient sample sizes)	Yes	
	15.3	Describe any process of model selection	Yes	
Publication bias and sensitivity analyses	16.1	Describe assessments of the risk of bias due to missing results (e.g. publication, time-lag, and taxonomic biases)	Yes	
	16.2	Describe any steps taken to investigate the effects of such biases (if present)	Yes	
	16.3	Describe any other analyses of robustness of the results, e.g. due to effect size choice, weighting or analytical model assumptions, inclusion or exclusion of subsets of the data, or the inclusion of alternative moderator variables in meta-regressions	Yes	
Clarification of *post hoc* analyses	17.1	When hypotheses were formulated after data analysis, this should be acknowledged.	NA: there were no hypotheses that were formed after data collection	
	18.1	Share metadata (i.e. data descriptions)	Yes	

Metadata, data, and code	18.2	Share data required to reproduce the results presented in the manuscript	Yes	
	18.3	Share additional data, including information that was not presented in the manuscript (e.g. raw data used to calculate effect sizes, descriptions of where data were located in papers)	Yes	
	18.4	Share analysis scripts (or, if a software package with graphical user interface (GUI) was used, then describe full model specification and fully specify choices)	Yes	
Results of study selection process	19.1	Report the number of studies screened	Yes	
	19.2	Report the number of studies excluded at each stage of screening	Yes	
	19.3	Report brief reasons for exclusion from the full text stage	Yes	
	19.4	Present a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)-like flowchart (www.prisma-statement.org).	Yes	
Sample sizes and study characteristics	20.1	Report the number of studies and effect sizes for data included in meta-analyses	Yes	
	20.2	Report the number of studies and effect sizes for subsets of data included in meta-regressions	Yes	
	20.3	Provide a summary of key characteristics for reported outcomes (either in text or figures; e.g. one quarter of effect sizes reported for vertebrates and the rest invertebrates)	Yes	
	20.4	Provide a summary of limitations of included moderators (e.g. collinearity and overlap between moderators)	Yes	
	20.5	Provide a summary of characteristics related to individual study quality (risk of bias)	Yes	
Meta-analysis	21.1	Provide a quantitative synthesis of results across studies, including estimates for the mean effect size, with confidence/credible intervals	Yes	

Heterogeneity	22.1	Report indicators of heterogeneity in the estimated effect (e.g. *I*2, *tau*2 and other variance components)	Yes	
Meta-regression	23.1	Provide estimates of meta-regression slopes (i.e. regression coefficients) and confidence/credible intervals	Yes	
	23.2	Include estimates and confidence/credible intervals for all moderator variables that were assessed (i.e. complete reporting)	Yes	
	23.3	Report interactions, if they were included	NA: no interactions were included	
	23.4	Describe outcomes from model selection, if done (e.g. R2 and AIC)	Yes	Please see the link provided in the Data Accessibility.
Outcomes of publication bias and sensitivity analyses	24.1	Provide results for the assessments of the risks of bias (e.g. Egger's regression, funnel plots)	Yes	
	24.2	Provide results for the robustness of the review's results (e.g. subgroup analyses, meta-regression of study quality, results from alternative methods of analysis, and temporal trends)	Yes	
Discussion	25.1	Summarise the main findings in terms of the magnitude of effect	Yes	
	25.2	Summarise the main findings in terms of the precision of effects (e.g. size of confidence intervals, statistical significance)	Yes	
	25.3	Summarise the main findings in terms of their heterogeneity	Yes	
	25.4	Summarise the main findings in terms of their biological/practical relevance	Yes	
	25.5	Compare results with previous reviews on the topic, if available	Yes	
	25.6	Consider limitations and their influence on the generality of conclusions, such as gaps in the available evidence (e.g. taxonomic and geographical research biases)	Yes	

Contributions and funding	26.1	Provide names, affiliations, and funding sources of all co-authors	Yes	
	26.2	List the contributions of each co-author	Yes	
	26.3	Provide contact details for the corresponding author	Yes	
	26.4	Disclose any conflicts of interest	NA: there were no conflicts of interest	
References	27.1	Provide a reference list of all studies included in the systematic review or meta-analysis	Yes	
	27.2	List included studies as referenced sources (e.g. rather than listing them in a table or supplement)	Yes	

Table S2. Search strings used for each database. We accessed Scopus, ISI Web of Science core collection, Google Scholar (*Japanese*, *Polish*, *Portuguese*, *Russian*, *Spanish*, *Simplified Chinese*, and *Traditional Chinese*) on 08/06/2023, and Bielefeld Academic Search Engine (BASE) on 26/06/2023. BASE was used as a source of grey literature. We conducted backward and forward reference searches for key review articles using Scopus on 19/06/2023. We modified search strings to collect studies to capture studies examining the effects of eyespot patterns on birds using experimental methods. Search strings were adapted to the structure of each database.

Database	Search strings
Scopus	TITLE-ABS-KEY (((eyespot* OR eye-spot* OR "eye spot*" OR eye-like* OR "eye like*" OR eye-mimic* OR "eye mimic*" OR "eye similari*" OR "predator* eye*" OR "eye similar*" OR concentric*) AND (attack* OR antipredator* OR anti-predator* OR aposematic* OR avoid* OR conspicuous* OR warn* OR fear* OR intimidat* OR predator-prey* OR butterfl* OR moth* OR bird* OR avian* OR caterpillar* OR prevent* OR aves OR passeri*)) AND NOT (fish* OR manti* OR lizard* OR bat* OR nano* OR health* OR patients OR women OR men OR children OR pediatric OR medic* OR hormon* OR genes OR magnet* OR valve* OR fluid* OR concrete OR beam* OR tissue* OR charge* OR energ* OR electro*))
ISI Web of Science	TS = (((eyespot* OR eye-spot* OR "eye spot*" OR eye-like* OR "eye like*" OR eye-mimic* OR "eye mimic*" OR "eye similari*" OR "predator* eye*" OR "eye similar*" OR concentric*) AND (attack* OR antipredator* OR anti-predator* OR aposematic* OR avoid* OR conspicuous* OR warn* OR fear* OR intimidat* OR predator-prey* OR butterfl* OR moth* OR bird* OR avian* OR caterpillar* OR prevent* OR aves OR passeri*)) NOT (fish* OR manti* OR lizard* OR bat* OR nano* OR health* OR patients OR women OR men OR children OR pediatric OR medic* OR hormon* OR genes OR magnet* OR valve* OR fluid* OR concrete OR beam* OR tissue* OR charge* OR energ* OR electro*))
BASE	eyespot* AND (avoid* predator* prevent* intimidat* mimi*) AND (ave* bird* passerine* butterfl* moth* lepidoptera caterpillar*) AND (experiment* stud*)
Google scholar	eyespot avoid|predator|prevention|intimidation|mimic aves|bird|passerine|butterfly|moth|lepidoptera|caterpillar experiment|study We translated the above English search string into *Japanese*, *Polish*, *Portuguese*, *Russian*, *Spanish*, *Simplified Chinese*, and *Traditional*

<table>
<tr><td></td><td><i>Chinese</i> for searching on Google Scholar.

<i>Japanese:</i>
目玉模様|眼状紋 忌避|捕食|防除|威嚇|擬態 鳥|鳴禽|蝶|蛾|鱗翅目|芋虫|幼虫 実験|研究
<i>Polish:</i>
oko|oczy skrzydla|wzor|plama
ochrona|unikanie|drapieżnik|zapobieganie|zastraszenie
ptak|motyl|gasienica|owad eksperyment|badania
<i>Portuguese:</i>
ocelo|”mancha ocelar”|“olhos falsos”|“falsos olhos”
evitar|predador|prevenção|intimidação
ave|pássaro|borboleta|mariposa|lagarta experimento|estudo
<i>Russian:</i>
глаз|глаза избегать|хищник|профилактика|запугивание
птица|бабочка|мотылек|Воробьинообразные|Чешуекрылые|Гусеница
эксперимент|изучать
<i>Spanish:</i>
ocelo|”ojos falsos”|”falsos ojos”
evitar|depredador|prevención|intimidación
ave|pájaro|mariposa|polilla|oruga experimento|estudio
<i>Simplified chinese:</i>
眼点 避免|捕食者|预防|恐吓|模仿 鸟类|鸟|雀|蝴蝶|蛾|鳞翅目|毛毛虫 实验|试验|学习
<i>Traditional chinese</i>:
眼點 避免|捕食者|預防|恐嚇|模仿 鳥類|鳥|雀|蝴蝶|蛾|鱗翅目|毛毛蟲 實驗|試驗|學習</td></tr>
</table>

Table S3. List of (a)included and (b) excluded studies at the full-text screening stage with exclusion reasons.

(a) included studies

title	year	authors	journal	doi
The Function of Eyespot Patterns in the Lepidoptera	1957	Blest, AD.	Behaviour	10.1163/156853956X00048
Reactions of male domestic chicks to two-dimensional eye-like shapes	1980	Jones, RB.	Animal Behaviour	10.1016/S0003-3472(80)80025-X
The Feeding Behaviour of Starlings (*Sturnus vulgaris*) in the Presence of 'Eyes'	1983	Inglis, IR., Huson, LW., Marshall, MB. and Neville, PA.	Zeitschrift für Tierpsychologie	10.1111/j.1439-0310.1983.tb02151.x
Butterfly wing markings are more advantageous during handling than during the initial strike of an avian predator	1985	Wourms, MK. and Wasserman, FE.	Evolution	10.1111/j.1558-5646.1985.tb00426.x
Significance of butterfly eyespots as an anti-predator device in ground-based and aerial attacks	2003	Lyytinen, A., Brakefieid, PM. and Mappes, J.	Oikos	10.1034/j.1600-0706.2003.11935.x
Does predation maintain eyespot plasticity in *Bicyclus anynana*?	2004	Lyytinen, A., Brakefield, PM., Lindström, L., and Mappes, J.	Proceedings of the Royal Society B: Biological Sciences	10.1098/rspb.2003.2571
Asymmetry in size, shape, and color impairs the protective value of conspicuous color patterns	2004	Forsman, A. and Herreträm, J.	Behavioral Ecology	10.1093/beheco/arg092
Prey survival by predator intimidation: an experimental study of peacock butterfly defence against blue tits	2005	Vallin, A, Jakobsson, S., Lind, J. and Wiklund, C.	Proceedings of the Royal Society B: Biological Sciences	10.1098/rspb.2004.3034
Field experiments on the effectiveness of 'eyespots' as predator deterrents	2007	Stevens, M., Hopkins, E., Hinde, W., Adcock, A., Connolly, Y., Troscianko, T. and Cuthill, IC.	Animal Behaviour	10.1016/j.anbehav.2007.01.031

The anti-predator function of 'eyespots' on camouflaged and conspicuous prey	2008	Stevens, M., Stubbins, CL. and Hardman, CJ.	Behavioral Ecology and Sociobiology	10.1007/s00265-008-0607-3
Conspicuousness, not eye mimicry, makes "eyespots" effective antipredator signals	2008	Stevens, M., Hardman, CJ. and Stubbins, CL.	Behavioral Ecology	10.1093/beheco/arm162
The protective value of conspicuous signals is not impaired by shape, size, or position asymmetry	2009	Stevens, M., Castor-Perry, SA. and Price, JRF.	Behavioral Ecology	10.1093/beheco/arn119
The function of animal 'eyespots': Conspicuousness but not eye mimicry is key	2009	Stevens, M., Cantor, A., Graham, J. and Winney, IS.	Current Zoology	10.1093/czoolo/55.5.319
Fixed eyespot display in a butterfly thwarts attacking birds	2009	Kodandaramaiah, U., Vallin, A. and Wiklund, C.	Animal Behaviour	10.1016/j.anbehav.2009.02.018
Can we use starlings' aversion to eyespots as the basis for a novel 'cognitive bias' task?	2009	Brilot, BO., Normandale, CL., Parkin, A. and Bateson, M.	Applied Animal Behaviour Science	10.1016/j.applanim.2009.02.015
Constant eyespot display as a primary defence-survival of male and female emperor moths attacked by blue tits	2010	Vallin, A., Sven J. and Christer W.	The Journal of Research on the Lepidoptera	10.5962/p.266504
Deflective effect and the effect of prey detectability on anti-predator function of eyespots	2011	Vallin, A. and Dimitrova, M., Kodandaramaiah, U. and Merilaita, S.	Behavioral Ecology and Sociobiology	10.1007/s00265-011-1173-7
Number of eyespots and their intimidating effect on naïve predators in the peacock butterfly	2011	Merilaita, S., Vallin, A., Kodandaramaiah, U., Dimitrova, M., Ruuskanen, S. and Laaksonen, T.	Behavioral Ecology	10.1093/beheco/arr135
The 'sparkle' in fake eyes - the protective effect of mimic eyespots in lepidoptera	2012	Blut, C., Wilbrandt, J., Fels, D., Girgel, EI.and Lunau, K.	Entomologia Experimentalis et Applicata	10.1111/j.1570-7458.2012.01260.x

Eyespots interact with body colour to protect caterpillar-like prey from avian predators	2012	Hossie, T.J. and Sherratt, T.N.	Animal Behaviour	10.1016/j.anbehav.2012.04.027
Anti-predator adaptations and strategies in the Lepidoptera	2012	de Wert, L.	Doctoral thesis	none
Bird attacks on a butterfly with marginal eyespots and the role of prey concealment against the background	2013	Olofsson, M., Jakobsson, S. and andWiklund, C,	Biological Journal of the Linnean Society	10.1111/bij.12063
Defensive posture and eyespots deter avian predators from attacking caterpillar models	2013	Hossie, TJ and Sherratt, TN	Animal Behaviour	10.1016/j.anbehav.2013.05.029
Revealed by conspicuousness: distractive markings reduce camouflage	2013	Stevens, M., Marshall, KLA, Troscianko, J., Finlay, S., Burnand, D. and Chadwick, SL.	Behavioral Ecology	10.1093/beheco/ars156
Eyespot display in the peacock butterfly triggers antipredator behaviors in naïve adult fowl	2013	Olofsson, M., Lovlie, H., Tibblin, J., Jakobsson, S. and Wiklund, C.	Behavioral Ecology	10.1093/beheco/ars167
The position of eyespots and thickened segments influence their protective value to caterpillars	2014	Skelhorn, J., Dorrington, G., Hossie, TJ. and Sherratt, TN.	Behavioral Ecology	10.1093/beheco/aru154
Predator mimicry, not conspicuousness, explains the efficacy of butterfly eyespots	2015	De Bona, S., Valkonen, JK., López-Sepulcre, A. and Mappes, J.	Proceedings of the Royal Society B: Biological Sciences	10.1098/rspb.2015.0202
Body size affects the evolution of eyespots in caterpillars	2015	Hossie, TJ., Skelhorn, J., Breinholt, JW., Kawahara, AY. and Sherratt, TN.	Proceedings of the National Academy of Sciences of the United States of America	10.1073/pnas.1415121112
What makes eyespots intimidating- the importance of pairedness Evolutionary ecology and behaviour	2015	Mukherjee, R. and Kodandaramaiah, U.	BMC Evolutionary Biology	10.1186/s12862-015-0307-3

On the deterring effect of a butterfly's eyespot in juvenile and sub-adult chickens	2015	Olofsson, M., Wiklund, C. and Favati, A	Current Zoology	10.1093/czoolo/61.4.749
Multicomponent deceptive signals reduce the speed at which predators learn that prey are profitable	2016	Skelhorn, J., Holmes, GG., Hossie, T.J. and Sherratt, TN.	Behavioral Ecology	10.1093/beheco/arv135
Attack risk for butterflies changes with eyespot number and size	2016	Ho, S., Schachat, SR., Piel, WH. and Monteiro, A.	Royal Society Open Science	10.1098/rsos.150614
The effectiveness of eyespots and masquerade in protecting artificial prey across ontogenetic and seasonal shifts	2022	Postema, EG.	Current Zoology	10.1093/cz/zoab082

(b) excluded studies

title	**year**	**authors**	**journal**	**doi**	**reason**
The effects of a tranquilliser on the reactions of domestic chicks to an aversive eye-like shape	1979	Jones, RB.	IRCS Medical Science	none	No full-text
Young domestic chicks avoid eye-like shapes	1980	JONES, RB	Applied Animal Ethology	10.1016/0304-3762(80)90037-1	No full-text
The startle responses of blue jays to Catocala (*Lepidoptera: Noctuidae*) prey models	1985	Schlenoff, DH.	Animal Behaviour	10.1016/S0003-3472(85)80164-0	Wrong outcome
Fearful symmetry: Pattern size and asymmetry affects aposematic signal efficacy	1999	Forsman, A. and Merilaita, S.	Evolutionary Ecology	10.1023/A:1006630911975	Invaild comparator
"An eye for an eye?" - On the generality of the intimidating quality of eyespots in a butterfly and a hawkmoth	2007	Vallin, A., Jakobsson, S. and Wiklund, C.	Behavioral Ecology and Sociobiology	10.1007/s00265-007-0374-6	Invaild comparator

Coincident disruptive coloration	2009	Cuthill, IC and Szekely, A	Philosophical Transactions of the Royal Society B-Biological Science	10.1098/rstb.2008.0266	Invaild comparator
Marginal eyespots on butterfly wings deflect bird attacks under low light intensities with UV wavelengths	2010	Olofsson, M., Vallin, A., Jakobsson, S. and Wiklund, C.	PLoS ONE	10.1371/journal.pone.0010798	Invaild comparator
Insect coloration as a defence mechanism against visually hunting predators	2011	Lyytinen, A.	Doctoral thesis	none	Published thesis
Effects of lepidopteran eyespot components on the deterrence of predatory birds	2015	Blut, C. and Lunau, K.	Behaviour	10.1163/1568539X-00003288	Invaild comparator
Antipredator behavior by a nesting hummingbird in response to a caterpillar with eyespots	2019	Marden, JH. and Pérez Carrillo, JF.	Ecology	10.1002/ecy.2582	Obseravational study
The Influence of the eyespots of peacock butterfly (*Aglais io*) and caterpillar on predator recognition	2020	Park, J. and Heo D	Open Science Journal	10.23954/osj.v5i2.2455	Invaild comparator

Table S4. Summary of a multi-moderator model including all moderators. The bold typeface is used when a 95% confidence interval (CI) does not contain zero; thus, it can be interpreted as an existing significant effect in predator avoidance.

	Estimate	95%CI
intercept	-0.06	(-0.50, 0.34)
Treatment stimulus	-0.02	(-0.19, 0.23)
Log-transformed area	**0.09**	**(0.009, 0.18)**
Number pattern	-0.05	(-0.11, 0.004)
Material type of prey: real	0.18	(-0.09, 0.45)

Table S5. Average maximum diameter of Eyespots on *Bicyclus anynana*. AM obtained the pictures from lepdata.org/photos/animals/ and https://data.nhm.ac.uk/ and measured the eyespot diameters. Raw data is https://ayumi-495.github.io/eyespot/.

Median	Range
3.41	1.82 – 5.04