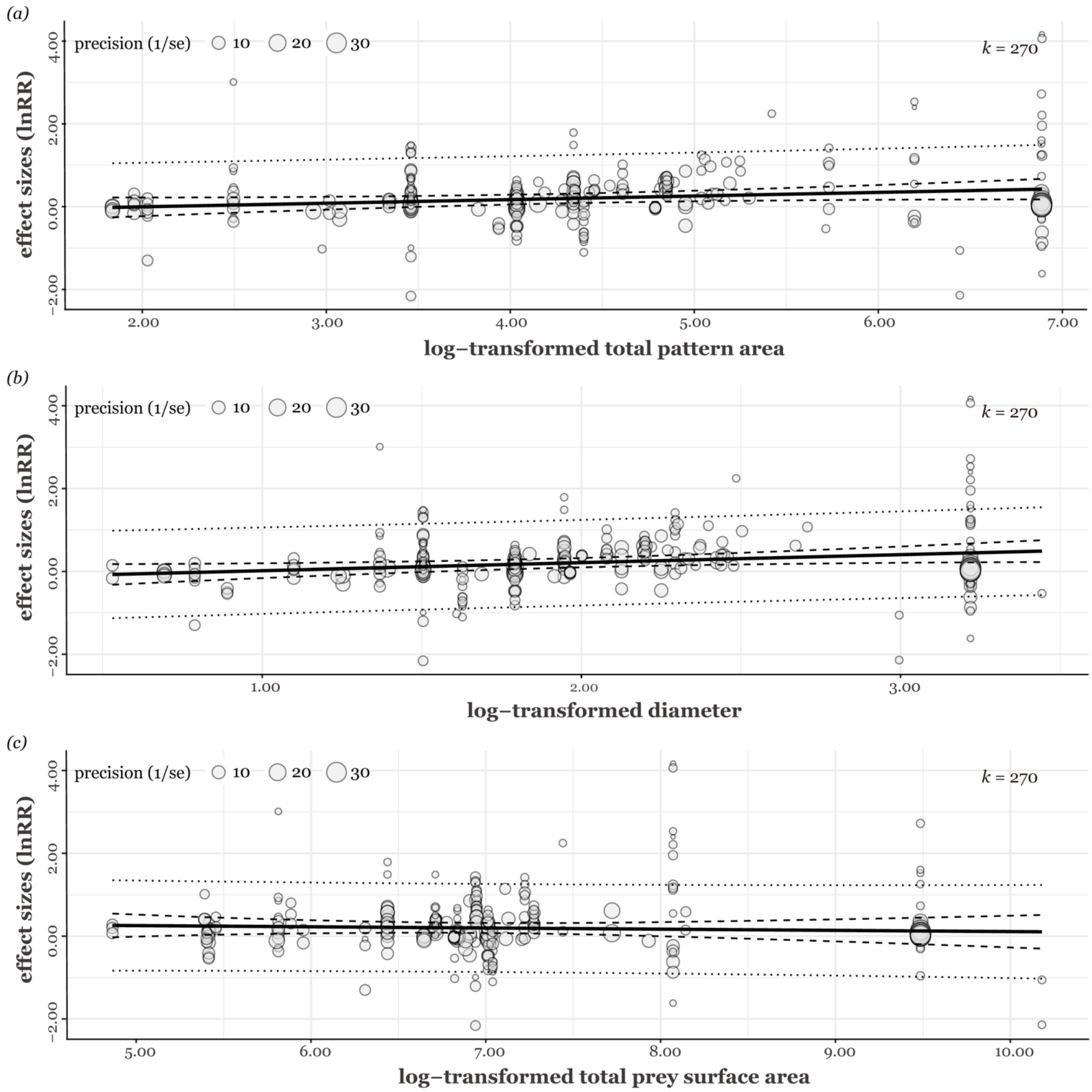

Figure S1. The relationships between (a) total pattern area, (b) pattern maximum diameter/length, and (c) total prey surface area and effect sizes. *k* shows the number of effect sizes. Each fitted regression line is shown as a solid straight line, and 95%confidence and prediction intervals are shown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

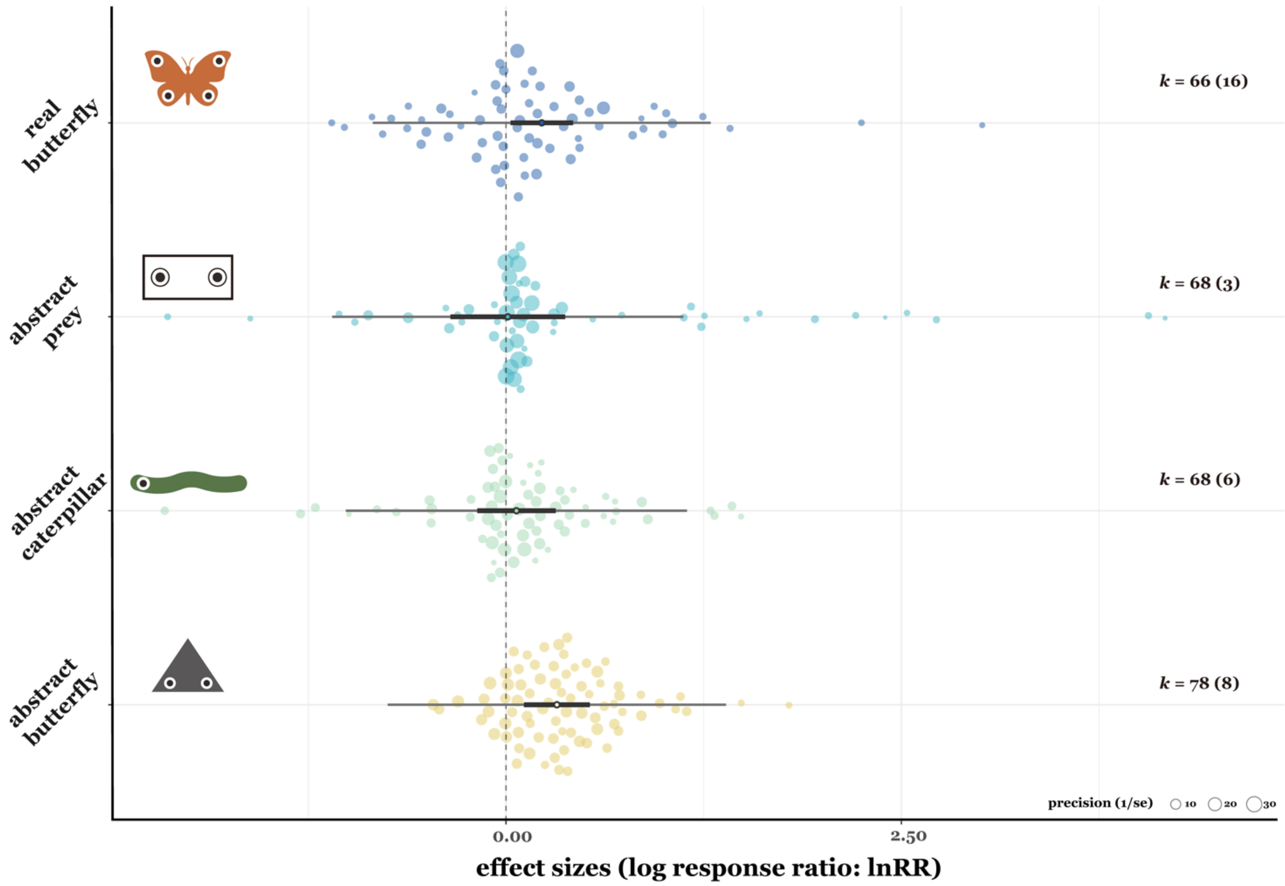

Figure S2. Mean effect sizes of total prey shape types. Thick horizontal lines represent 95% confidence intervals, and thin horizontal lines represent prediction intervals. The points in the centre of each thick line indicate the average effect size. *k* shows the number of effect sizes.

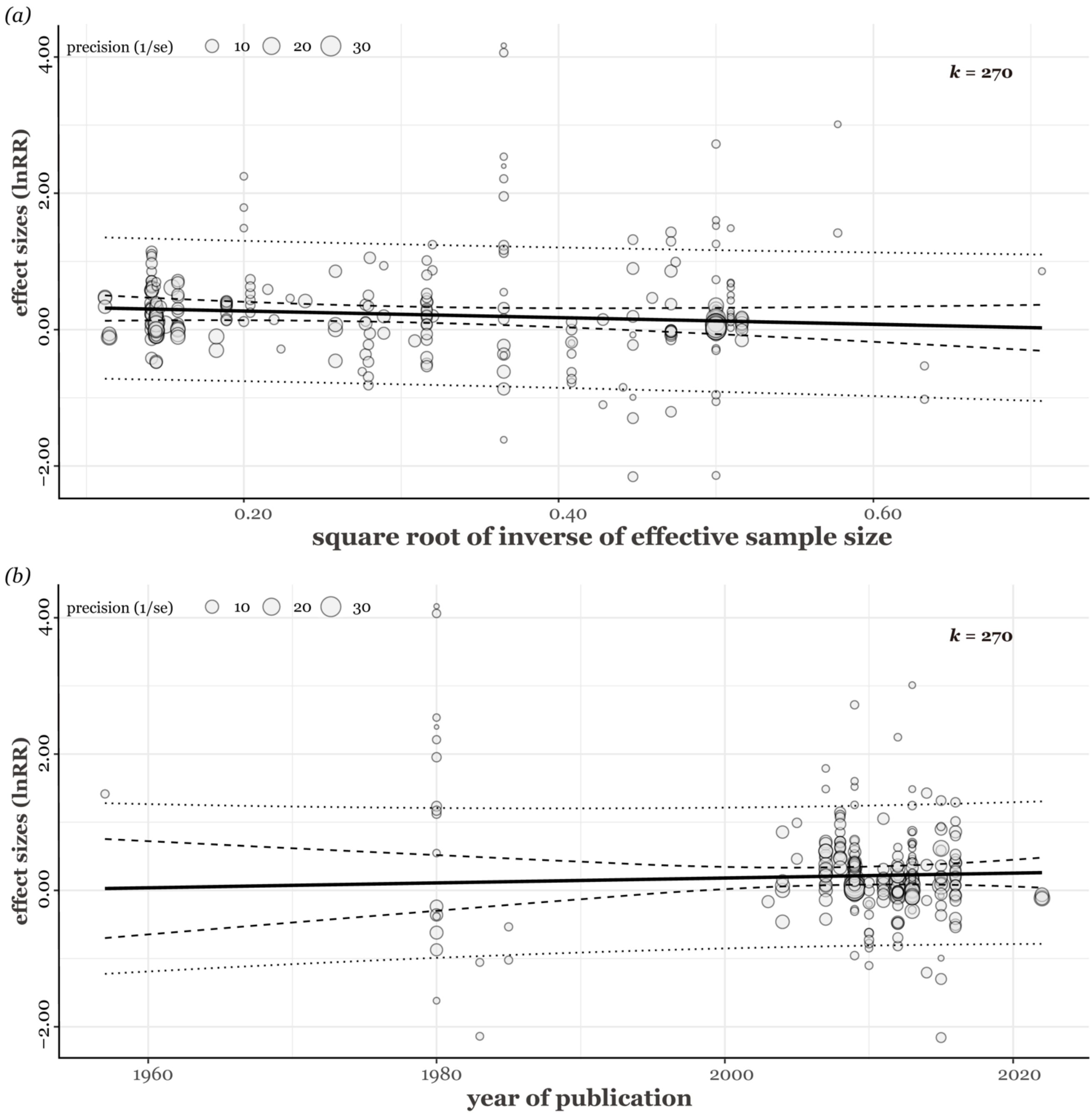

Figure S3. (a) relationship between effect size and the square root of the inverse of effective sample size, and (b) relationship between effect size and publication year. Both plots were based on the multi-moderator model. *k* shows the number of effect sizes. Each fitted regression line is shown as 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 were no missing data	
	11.2	Justify the decisions made to deal with missing data	NA: there were 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.	NA: only (weighted) random-effects models were used	

		common/fixed effects model, unweighted model), provide justification for this choice		
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 are no hypotheses that were formed after data collection	
Metadata, data, and code	18.1	Share metadata (i.e. data descriptions)	Yes	
	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	Yes	

		(PRISMA)-like flowchart (www.prisma-statement.org).		
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 scholor	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 Chinese* for searching on Google Scholar.

	Japanese: 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 *Polish:* oko|oczy skrzydla|wzor|plama ochrona|unikanie|drapieżnik|zapobieganie|zastraszenie ptak|motyl|gasienica|owad eksperyment|badania *Portuguese:* ocelo|”mancha ocelar”|“olhos falsos”|“falsos olhos” evitar|predador|prevenção|intimidação ave|pássaro|borboleta|mariposa|lagarta experimento|estudo *Russian:* глаз|глаза избегать|хищник|профилактика|запугивание птица|бабочка|мотылек|Воробьинообразные|Чешуекрылые|Гусеница эксперимент|изучать *Spanish:* ocelo|”ojos falsos”|”falsos ojos” evitar|depredador|prevención|intimidación ave|pájaro|mariposa|polilla|oruga experimento|estudio *Simplified chinese:* 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 *Traditional chinese*: 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

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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 Herretröm, 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