

Supplementary Material

Table S1

Outline of possible nonmutually exclusive functions of eyespots in lepidopteran caterpillars and the relevant literature

Possible function	Comments	Empirical work related to caterpillars	Additional references
Eye mimicry: eyespots protect prey because they resemble the eyes of dangerous predators (e.g. snakes)	Classic explanation and prevailing hypothesized function for eyespots on caterpillars, especially within the naturalist community	None, although Janzen et al. (2010) provide natural history observations and theoretical grounds to support this mechanism	Caterpillar eyespots: Bates 1862; Poulton 1890; Edmunds 1974; Janzen et al. 2010 Adult Lepidoptera: Stevens 2005; Kodandaramaiah 2011; Merilaita et al. 2011
Startle signal: eyespots are conspicuous markings that startle attackers	Markings scare off attackers because they are conspicuous and presented suddenly	None	With moth-like prey: Blest 1957; Cundy & Allen 1988 Reviewed in reference to eyespots: Stevens 2005; Kodandaramaiah 2011 General review: Ruxton et al. 2004 (Section 13.5)
Conspicuous signal: predators avoid attacking prey that possess conspicuous markings	Includes effects of ‘dietary conservatism’ and ‘neophobia’. It seems possible that birds avoid prey with eyespots simply because eyespots are unfamiliar	Shirota (1980) used captive birds to examine latency to attack <i>Bombyx mori</i> larvae affixed with various paper disc eyespots	Coppinger 1970; Schuler & Hesse 1985; Roper & Cook 1989; Roper 1990; Stevens 2005; Stevens et al. 2007, 2008a
Direction: eyespots direct attacks towards additional defences	Seems plausible for several species (e.g. <i>Papilio</i> spp., <i>Cerura vinula</i>). Blest (1957) suggested it specifically for papilionid larvae with osmeterium	Blest (1957) analysed where captive birds pecked mealworms painted with a single spot	Poulton (1890) details the spraying of irritant fluid from anterior end (near eyespots) of <i>C. vinula</i> larvae when touched
Deflection: eyespots deflect attacks away from vulnerable body parts (e.g. head capsule)	Refuted by Janzen et al. (2010) on the grounds that attack to any part of a caterpillar’s body would be lethal. However, reducing the probability of strikes to a caterpillar’s true head could have some benefit	None	Janzen et al. 2010 Evidence in adult Lepidoptera reviewed by: Stevens 2005; Kodandaramaiah 2011 General review: Ruxton et al. 2004 (Ch. 13)

Possible function	Comments	Empirical work related to caterpillars	Additional references
Warning signal: eyespots act as a warning signal indicating unpalatability	Unlikely. Caterpillars with eyespots tend to be palatable. In the <i>Papilio</i> genus, aposematism is not linked to the presence of plant toxins (Prudic et al. 2007)	Evidence that eyespot caterpillars are palatable: Brower 1958; Leslie & Berenbaum 1990; Takagi et al. 1995	Poulton (1890) provides several anecdotes where eyespot caterpillars are eaten by birds
Distraction: eyespots increase crypsis by distracting attention from the true edge of prey	Small conspicuous markings reduce detection of prey by distracting predators' 'attention' away from the true outline of a prey item	None with caterpillars	Suggested by Thayer (1909) Tested with moth-like targets by Stevens et al. (2008b); not supported

Table S2

Insectivorous birds observed in each location during the associated field trail conducted over the summer of 2010 near Kemptville (Sites 1 and 2) and Oxford Mills (Site 3), ON, Canada

Bird species	Migration	Site 1			Site 3			Site 3		
		25 May	14 Jul	11 Aug	8 Jun	20 Jul	18 Aug	7 Jul	30 Jul	24 Aug
<i>Cardinalis cardinalis</i>	Resident									x
<i>Cyanocitta cristata</i>	Resident	x			x	x	x	x	x	x
<i>Poecile atricapilla</i>	Resident		x	x	x	x	x		x	x
<i>Sitta carolinensis</i>	Resident			x						
<i>Spinus tristis</i>	Resident	x	x			x			x	
<i>Agelaius phoeniceus</i>	Short–Medium				x					
<i>Bombycilla cedrorum</i>	Short–Medium									x
<i>Passerculus sandwichensis</i>	Short–Medium					x			x	
<i>Quiscalus quiscula</i>	Short–Medium				x			x		
<i>Sayornis phoebe</i>	Short–Medium				x				x	
<i>Spizella passerina</i>	Short–Medium	x			x			x		
<i>Turdus migratorius</i>	Short–Medium		x		x	x	x	x		x
<i>Vireo gilvus</i>	Short–Medium							x		
<i>Zonotrichia albicollis</i>	Short–Medium		x	x				x		x
<i>Dendroica pensylvanica</i>	Neotropical							x		
<i>Dendroica petechia</i>	Neotropical	x								
<i>Dumetella carolinensis</i>	Neotropical							x		
<i>Geothlypis trichas</i>	Neotropical	x	x	x					x	x
<i>Mniotilta varia</i>	Neotropical				x	x				
<i>Pheucticus ludovicianus</i>	Neotropical								x	
<i>Setophaga ruticilla</i>	Neotropical							x		



Figure S1. Dorsolateral view of an eyespot pastry caterpillar used in our experiment. Note that the pastry caterpillar in this photograph received a peck directly in the right eyespot.

Table S3

Number of each tree species used to pin pastry caterpillars in each location at each site

Site	Location	<i>Populus tremuloides</i>	<i>Populus deltoides</i>	<i>Betula papyrifera</i>	<i>Acer rubrum</i>
1	1	1	23	0	0
	2	1	23	0	0
	3	11	0	3	10
2	1	6	0	10	8
	2	12	0	12	0
	3	10	0	8	6
3	1	19	0	5	0
	2	8	2	4	10
	3	12	0	0	12

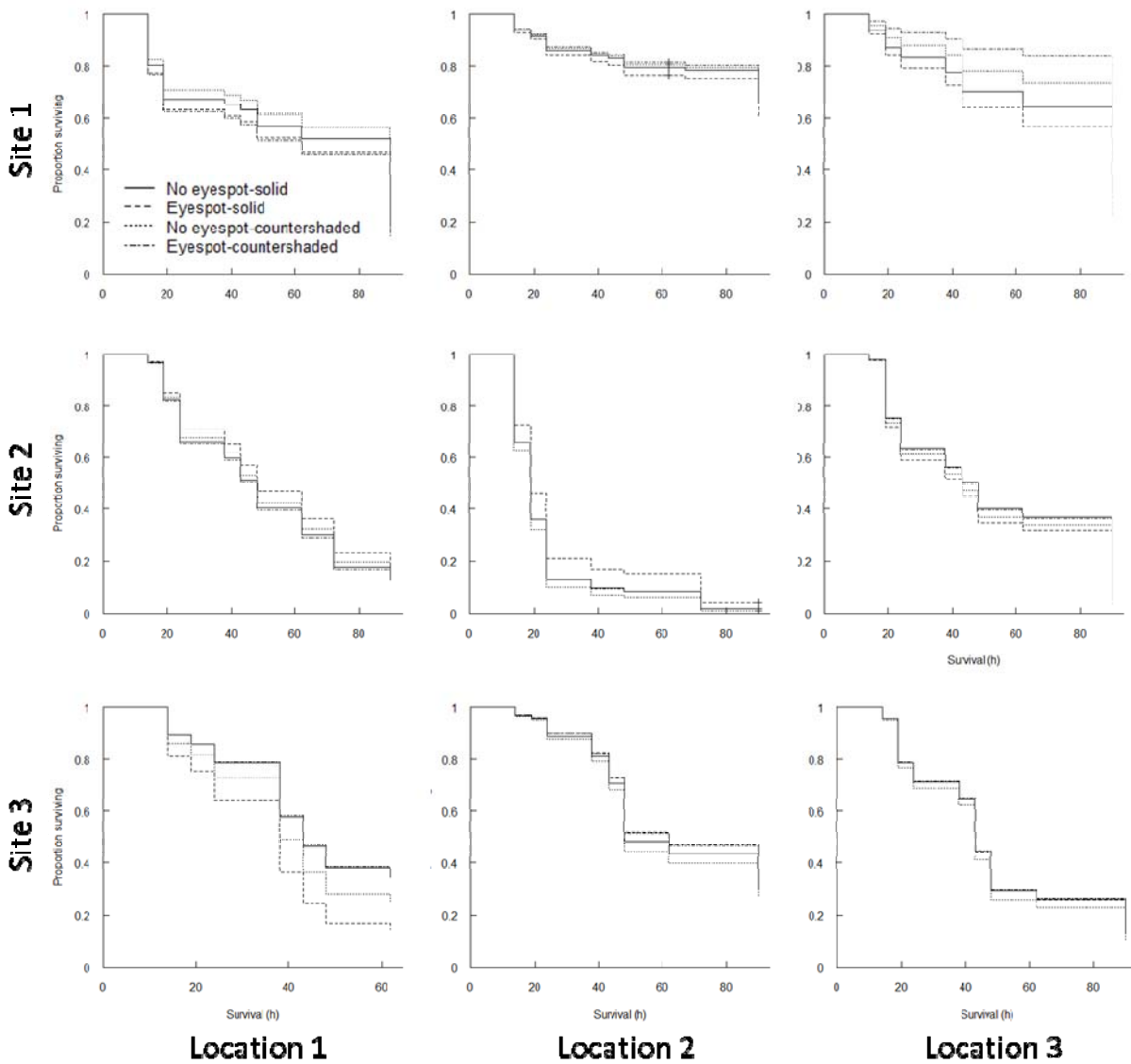


Figure S2. Fitted cumulative survival charts comparing the survival of four treatments of pastry caterpillars in each of the nine locations. Note that each chart is based on $N = 96$ baits and, for clarity, these survival curves are fitted without controlling for the tree species on which they were pinned.

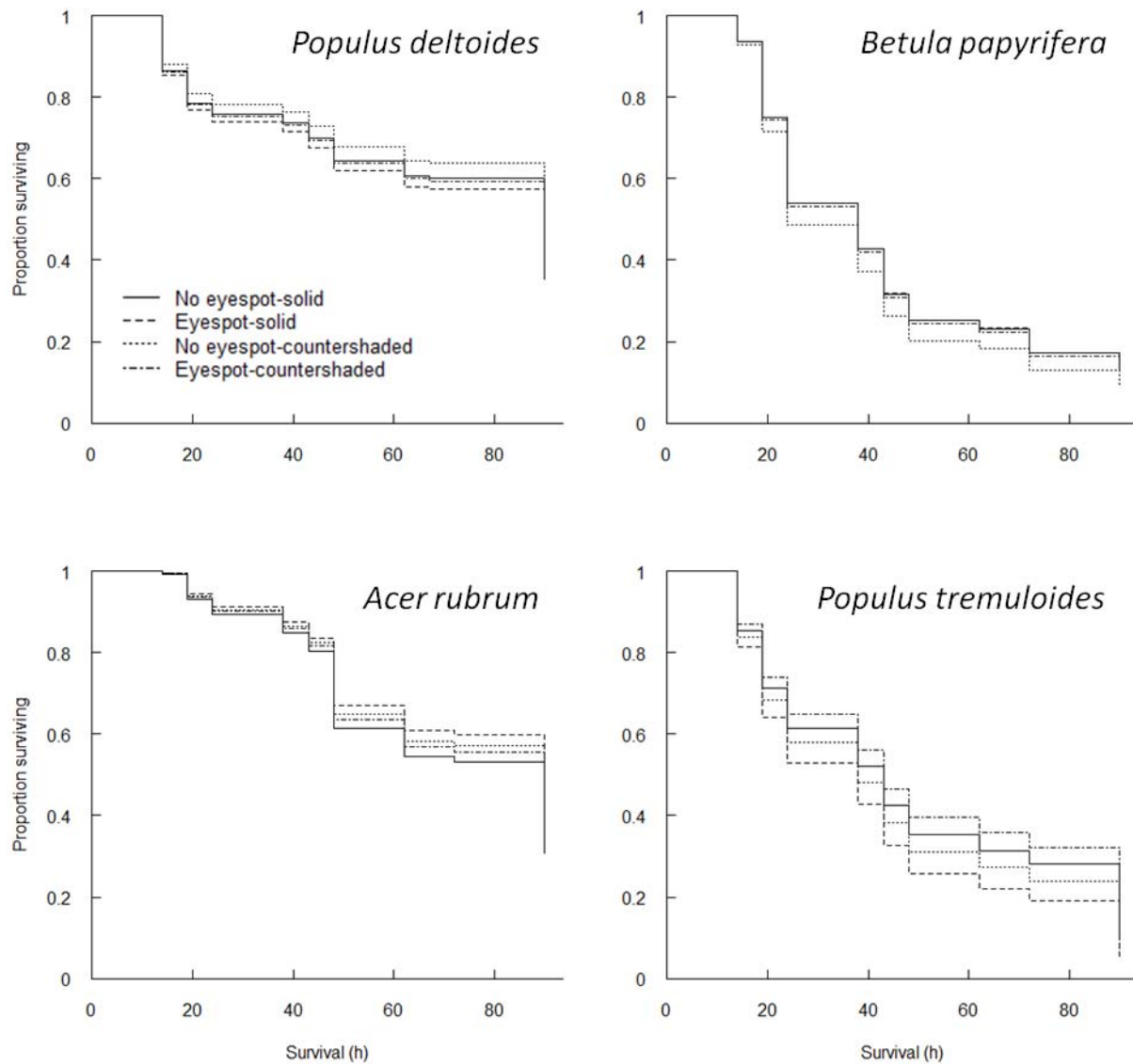


Figure S3. Fitted cumulative survival charts comparing the survival of four treatments of pastry caterpillars pinned to different tree species. Note that, for clarity, these survival curves are fitted without controlling for the location where the trials took place. Sample sizes also varied among the four charts: *Populus tremuloides*: $N = 320$; *Populus deltoides*: $N = 192$; *Betula papyrifera*: $N = 168$; *Acer rubrum*: $N = 184$. Note: with the exception of *P. tremuloides*, there was no significant difference in the survival rate of caterpillars among the four eyespot/colour treatments (*P. tremuloides*: overall Wald = 14.67, $df = 3$, $N = 320$, $P = 0.002$; *P. deltoides*: overall Wald = 3.1, $df = 3$, $N = 192$, $P = 0.38$; *B. papyrifera*: overall Wald = 2.01, $df = 3$, $N = 168$, $P = 0.57$; *A. rubrum*: overall Wald = 1.48, $df = 3$, $N = 184$, $P = 0.69$).

Table S4

Results from fitting Cox proportional hazards regression model of pastry caterpillar survival when missing prey were censored, stratified by location and tree species

	Coef	Exp(coef)	SE(coef)	Robust SE	Wald	<i>P</i>
Colour	0.108	1.114	0.1725	0.1347	0.802	0.4228
Eyespot	0.209	1.2324	0.1695	0.1275	1.639	0.1012
Colour×eyespot	-0.4559	0.6339	0.2465	0.1812	-2.516	0.0119

In this reanalysis, only prey recovered from the field with peck marks were counted as prey killed, whereas all prey that disappeared over the course of the trial were censored at the time they went missing. Caterpillars pinned to the same tree were clustered in the analysis, and the robust standard error was used to calculate the Wald statistic.

Table S5

Results from Akaike's Information Criterion (AIC) model selection used to determine the factors affecting the number and location of bird pecks on pastry caterpillars deployed in the field

Model	<i>df</i>	AIC	Δ AIC
section×eyespot + (1 individual)	13	2295.665	0
section + colour + (1 individual)	8	2331.238	35.573
section + eyespot + colour + (1 individual)	9	2332.568	36.903
section + (1 individual)	7	2333.523	37.858
section + eyespot + (1 individual)	8	2334.536	38.871
section + eyespot×colour + (1 individual)	10	2334.566	38.901
section×colour + (1 individual)	13	2335.619	39.954
(1 individual)	2	2782.861	487.196

(1|individual): individual as a random factor in the analysis. For marginality reasons, all associated main effects were included in the fitted model when a given interaction was specified.

Table S6

Summary output for the fixed effects from the best fit model explaining the abundance and distribution of pecks on pastry caterpillars with and without eyespots

	Estimate	SE	<i>z</i>	Pr(> <i>z</i>)	
(Intercept)	0.11251	0.08275	1.36	0.173949	
section2	-1.14244	0.14244	-8.02	1.06E-15	*
section3	-1.17274	0.14410	-8.139	4.00E-16	*
section4	-1.37684	0.15606	-8.823	2.00E-16	*
section5	-1.08445	0.13937	-7.781	7.19E-15	*
section6	-0.08963	0.10137	-0.884	0.376591	
Eyespots	-0.49519	0.13388	-3.699	0.000217	*
section2:eyespots	0.88867	0.20169	4.406	1.05E-05	*
section3:eyespots	0.29037	0.22627	1.283	0.19939	
section4:eyespots	-0.11863	0.27019	-0.439	0.66063	
section5:eyespots	-0.11376	0.24053	-0.473	0.636233	
section6:eyespots	0.78271	0.15374	5.091	3.56E-07	*

* $P < 0.05$.

Effects of Co-occurring Snakes on Peck Distribution

Snakes are commonly proposed as the model for caterpillars with eyespots (e.g. Bates 1862; Wagner 2005). Although the relationship between snake density and predation rate was not the central concern of our paper, we estimated the density of snakes observed in our study areas because it is conceivable that (potential) model density might have influenced predators' reactions to our prey. We were not deliberately focused on any one species of snake; however, only garter snakes, *Thamnophis sirtalis*, were observed, and they were recorded in each replicate location where we conducted our trials. Published work describing the specific diet of Ontario *T. sirtalis* is lacking, but studies of *T. sirtalis* conducted on Vancouver Island show that birds are occasionally included in their diet, typically nestlings (Gregory 1978, 1984). Weatherhead & Blouin-Demers (2004) summarized videotaped nest predation, which showed that *Thamnophis* species (including *T. sirtalis*) are indeed nest predators of passerine birds. Finally, Nice & Ter Pelkwyk (1941) observed that both a hand-reared and a wild male song sparrow, *Melospiza melodia*, displayed curiosity behaviour (e.g. hopping around, peck) when observing garter snakes, whereas a wild female song sparrow treated small garter snakes as nest enemies. Thus, garter snakes probably pose a legitimate threat, at least to nesting passerines.

In light of the above observations, it seemed possible that eyespot-directed pecks could represent hostile behaviour towards temperate snakes. We assessed this possibility by quantifying snake density and the extent of eyespot-directed attacks. We predicted that, if eyespot-directed pecks reflected the reaction of birds to temperate snakes, then we should observe more eyespot-directed attacks in field locations with greater snake density.

METHODS

Snake Observations

Occasionally, we observed *T. sirtalis* during checks of pastry baits. At each observation, the time and location was recorded. Using the cumulative time spent checking the survival of artificial prey and the total number of snake observations during this time, we were able to calculate a relative abundance of snakes in each of the nine locations (i.e. total number of observations/total time spent checking (in hours)). These values were clearly indicative of relative snake density in each location at the time when the associated field trials were running. Notably, this method parallels the line transect method employed by herpetologists, which efficiently detects reptiles in both short- and long-term studies (Hutchens & DePerno 2009).

Data Analysis

To test for an effect of snake density on the location of pecks, we generated a single ‘peck directionality’ variable by subtracting the number of pecks on the ‘tail’ end from the number of pecks on the ‘head’ end on pastry caterpillars with eyespots (i.e. values > 0 denote ‘head’-directed strikes). From this, we calculated the mean peck directionality for each location (i.e. $N = 8$ values, as the birds in one location left no pecked prey), and regressed these values against log-transformed snake density. A second linear regression tested whether snake density affected the proportion of prey with eyespots that had received pecks directly on their eyespots (e.g. Fig. S2). In both regression analyses, snake density was log transformed to achieve normality (and homogeneity) of residuals.

RESULTS

Thamnophis sirtalis was the only snake species observed over the course of the experiment. All snakes were observed on the ground along the transect of trees used in a given trial. We detected no significant relationship between snake density and either 'head'-directed strikes ($R^2 = 0.46$, $F_{1,6} = 5.01$, $N = 8$, $P = 0.066$; Fig. S4a), or the proportion of eyespot caterpillars pecked directly within the eyespot ($R^2 = 0.48$, $F_{1,6} = 5.56$, $N = 8$, $P = 0.056$; Fig. S4b), although in both cases the trend was of borderline statistical significance. The nonsignificant trend was for fewer head-directed or eyespot pecks to occur as snake density increased.

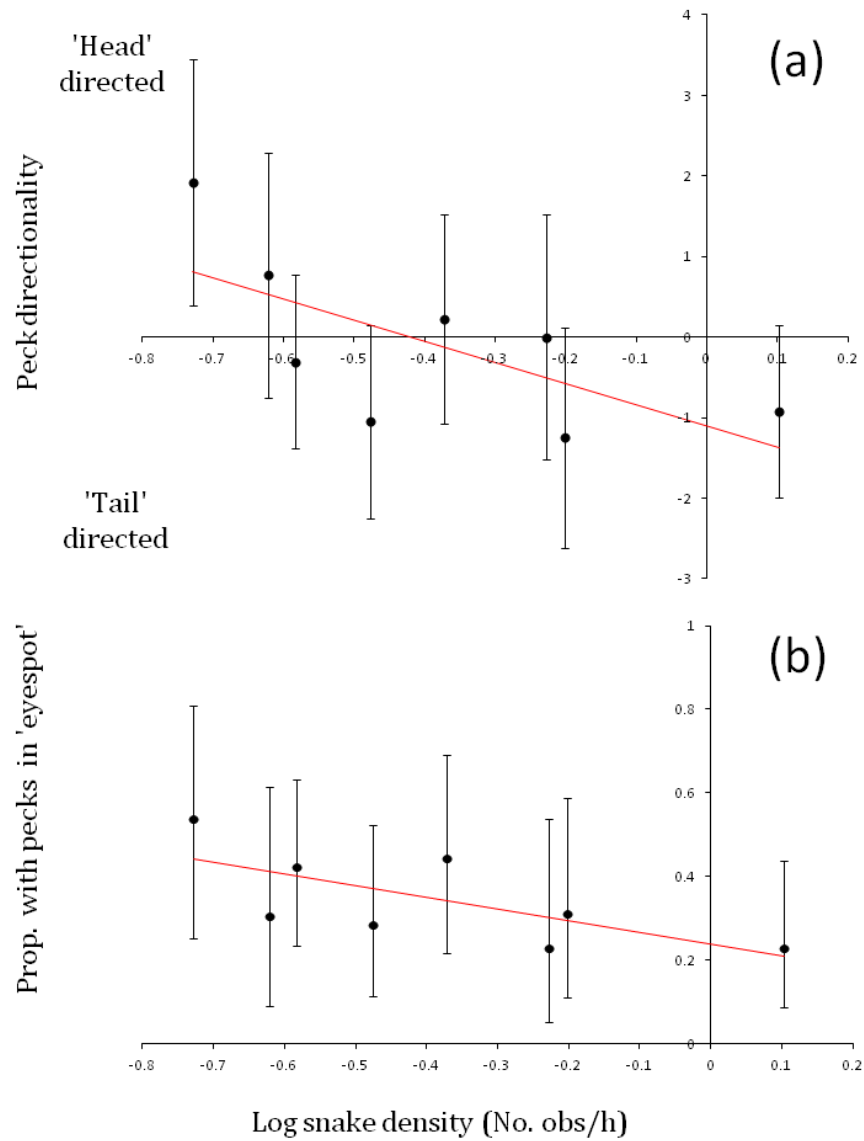


Figure S4. (a) Relationship between the mean directionality of bird pecks (number of ‘head’ pecks – number of ‘tail’ pecks) on pastry caterpillars with ‘eyespots’ and log-transformed snake density (number of snakes observed/h). Directionality values >0 indicate head directed pecks, and error bars represent 95% confidence limits for the mean directionality. (b) Relationship between log-transformed snake density and the proportion of pastry caterpillars with eyespots that received eyespot-directed pecks. Error bars represent 95% exact binomial confidence limits.

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