



Original Article

Mate detection in a territorial butterfly—the effect of background and luminance contrast

Martin Bergman,^{a,b} Nicolas Lessios,^a Brett M. Seymoure,^a and Ronald L. Rutowski^a

^aSchool of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA and ^bDepartment of Biology, Lund University, Sölvegatan 35, SE-223 62 Lund, Sweden

Received 27 November 2014; revised 16 February 2015; accepted 17 February 2015; Advance Access publication 3 April 2015.

Many animals search for potential mates or prey using a perch-and-sally strategy. The success of such a strategy will depend on factors that affect the observer's ability to detect a passing resource item. Intrinsic factors (e.g., eye structure and physiology) have received much recent attention, but less is known about effects on object detection in nature and extrinsic factors such as size, coloration, and speed of a passing object and the background against which the object is viewed. Here, we examine how background affects the detection of butterfly models by perched males of the butterfly *Asterocampa leilia* in the field. We test the hypothesis that male choice of perch site in nature will influence the contrast between the object and background against which it is viewed and that this will influence success in detecting the object. We also test the effect of contrast by manipulating the brightness of the object and presenting butterfly models of different reflectance (ranging from black to white). We found an effect of model luminance, with dark models being most likely to elicit a response regardless of background. Further, there was an effect of background type with models viewed against blue sky eliciting the highest response. Perceived luminance contrast correlates to behavior; highly contrasting objects are more frequently detected. This study expands our understanding of visual system performance and has implications for our understanding of the behavior and evolutionary ecology of perching species.

Key words: Lepidoptera, mate-locating behavior, perching, sexual selection, territoriality, vision.

INTRODUCTION

Many animals visually search for resources such as potential mates or prey using a perch-and-sally strategy. This strategy involves individuals waiting at some vantage point on the lookout for mates or prey passing by and then sallying out to intercept, investigate, and pursue any objects of potential interest. Perch-and-sally tactics are a widespread and conspicuous component of animal behavior in both invertebrates and vertebrates (Atkins 1980; Thornhill and Alcock 1983; Remsen and Robinson 1990). This strategy contrasts with patrolling (actively searching) as a way to locate mates or prey and the perch–patrol dichotomy in mate-locating behavior and hunting strategies is well established in the literature on insect behavioral ecology (Scott 1974; Ehrlich 1984; Rutowski 1991; Corbet 1999; Wiklund 2003; Corbet and May 2008).

Even though perching as a mate-locating tactic or hunting strategy is widespread, little research has been done to understand the factors that contribute to success in detecting, intercepting, and pursuing moving objects of interest. This is peculiar because the behavior is strikingly similar among all perching species, and success in this activity should have a huge effect on the performer's

fitness. A more comprehensive understanding of the proximate factors that influence male's success in mate searching or hunting, especially factors influencing visual mate detection, will give us more insight in the potential adaptive features of mate-locating behavior and mating system structure in insects.

Both *intrinsic* and *extrinsic* factors will influence a percher's visual detection of passing objects. Over the last few decades, our understanding of intrinsic factors, such as visual system performance, has increased due to studies of eye structure, optics, and electrophysiology (Land and Nilsson 2012). However, less attention has been given to the role of extrinsic factors such as object size, coloration, pattern and shape, the speed of the object, the properties of the background against which the object is viewed, perch location, and the positioning of the observer in relation to the object and its path. These factors will cause variation in ambient light levels, contrast between the object and the background and blurring effects, which are all known to be key elements in visual acuity and target detection (Land 1997; Rutowski 2003). Intrinsic factors studied in controlled laboratory environments have been the focus of attention in the last few decades. However, extrinsic factors are ideally examined under field conditions, where experiments are logistically more difficult and more difficult to control but yield results more directly relatable to what the animals are doing in nature. In species that use a perching strategy to find mates or prey, we expect

Address correspondence to M. Bergman. E-mail: martin.bergman@biol.lu.se.

strong selection on behavior to adapt to environmental, extrinsic factors in order to optimize success in mate searching or hunting.

One of the relatively few species that has been studied in the field to understand visual aspects of perching behavior is the Empress Leilia butterfly, *Asterocampa leilia*. Males of this nymphalid species perch in the morning from about 8 to 11 AM on or adjacent to the larval food plant, desert hackberry (*Celtis pallida*), and wait for females to pass by (Austin 1977; Rutowski and Gilchrist 1988). From his perch, the male flies out to investigate passing objects. Conspecific females are pursued and courted, whereas conspecific males are chased away from the perching site (Rutowski and Gilchrist 1988).

During the course of a morning's activity, males of *A. leilia* can adopt 2 different perching positions: 1) on the ground, next to a hackberry tree, or 2) on the hackberry tree at a height of about 0.85 m, the same height at which *A. leilia* females fly through the habitat (Rutowski 2000). Males start the morning perching on ground but shift to perching off ground, on hackberry trees as temperatures rise (Rutowski et al. 1991). The change of male's perch preference is driven by thermoregulatory issues; ground temperatures can exceed the tolerable thermal maximum for *A. leilia*, whereas temperatures are lower at 0.85 m, the typical perch height of *A. leilia* (Rutowski et al. 1994). Perch location influences the male's ability to detect passing objects. In a field study, Rutowski et al. (2001) presented butterfly models to perched males and found that males on ground were more likely to initiate pursuit of a passing model than those off ground. The body posture of males perched on the ground (body pitch upward about 30° and facing away from the sun) is different from that of males perched on the ground (body pitch slightly down, facing out of bush; Rutowski 2000). This difference in body posture with perch location means the equatorial acute zone of their eyes (Rutowski and Warrant 2002) is directed slightly differently relative to the likely flight path of conspecifics. However, this difference suggests that males on the ground should be less likely to detect conspecifics passing through the territory, contrary to what was observed.

Alternatively, Rutowski et al. (2001) argued that differences in the rate of response was most likely because males perching above ground are likely to view passing conspecifics against a relative dark background of nearby vegetation, whereas males perching on ground are likely to have the relatively bright and uniform blue sky as the background. Rutowski et al. (2001) crudely tested this hypothesis by increasing the flight height of the models to change the potential viewing background for perched males. And indeed, this change increased the proportion of males that responded to the models (Rutowski et al. 2001). However, the authors did not explore this issue further.

Other studies have also shown that background affects the detection of passing conspecifics or prey (Labhart and Nilsson 1995; Switzer and Eason 2000), but few studies have further investigated what properties of object and background are important. Animals sense motion of a passing target as temporally correlated changes in stimulation in sets of photoreceptors (Cronin et al. 2014). Hence, the luminance of a target and its contrast with the luminance from a background will affect the magnitude of such change in stimulation and thereby significantly affect visual detectability, where a high contrast increases the change in signal and thereby detectability (Buser and Imbert 1992). For an insect, this means that when luminance contrast between an object and its background is high, the viewer will be able to see that object from further away and, at a given distance, detect smaller objects (Mazokhin-Porshnyakov 1969;

e.g., Lehrer and Bischof 1995). Here, we experimentally explore the implications of object-background contrast in nature, with *A. leilia* as the subject. We investigate how background contributes to variation in luminance contrast between a passing object and the background against which it is viewed and how this will influence visual mate detection in nature. We further test the hypothesis that visual mate detection is influenced by contrast by manipulating the brightness and color of the object and consequently also manipulating contrast. We presented butterfly models of different reflectances (ranging from black to white) to perched males. We further, and more quantitatively than previously, tested the prediction that the type of background a male chooses by selecting a perch site in nature will influence the contrast of the object and that the choice of perch site thereby also will influence his success in detecting the object. We predicted that a perch site that creates a high contrast between the object and the background will increase detectability and perch sites with a low contrast between the object and the background will decrease detectability.

MATERIALS AND METHODS

Study site

The experiments were conducted in May–June and September–October 2013 in the upper Sonoran Desert in the Mazatzal Mountains of central Arizona, USA. The site, known as Round Valley (33.79784N, 111.4877W), is in the floodplain of Sycamore Creek and has been used in previous studies of *A. leilia* (Rutowski and Gilchrist 1988; Rutowski et al. 1991, 2001; Rutowski 2000). The larval foodplant of *A. leilia*, the desert hackberry tree (*C. pallida*), is abundant in this area.

Butterfly model presentation device and general experimental procedure

Butterfly models were presented to perched males with an apparatus that has been used in previous studies of mate detection in territorial butterflies (Rutowski et al. 2001; Bergman and Wiklund 2009) and consisted of 2 metal poles, positioned 8 m apart, and each with an attached pulley. The pulley on one of the poles was turned by a small electrical motor controlled by a portable remote control. A loop of thin (0.65 mm in diameter) nylon line was strung between the pulleys and a model attached with an 8-cm line to the loop. The attached butterfly model was presented to a male at a height of 0.85 m above ground. When the motor was activated, the model quickly accelerated to a speed of about 4.5 m/s. For each presentation, we measured the time it took for the model to get from one pole to the other, that is, to travel 8 m. We calculated the speed of the model and only recorded the male's response if the speed of the model was between 4 and 5 m/s, the typical flight speed for this species. All models were made of 2-mm-thick cardboard. The models were constructed to match the size (45 mm wingspread) and shape of an *A. leilia* with wings spread.

We used 4 different types of butterfly models hereafter referred to as *black*, *gray*, *white*, and *tan*, respectively. The black and gray models were painted with an acrylic paint (Model Master Acryl, ©The Testor Corp.), the white model consisted of white filter paper glued to the cardboard model, and the tan model consisted only of unpainted cardboard. The 4 butterfly models varied in their reflectance spectra (Figure 1) (for description of the measuring methods, see Reflectance section): the black, gray, and white butterfly models had a flat reflectance curve, where the black reflected least and

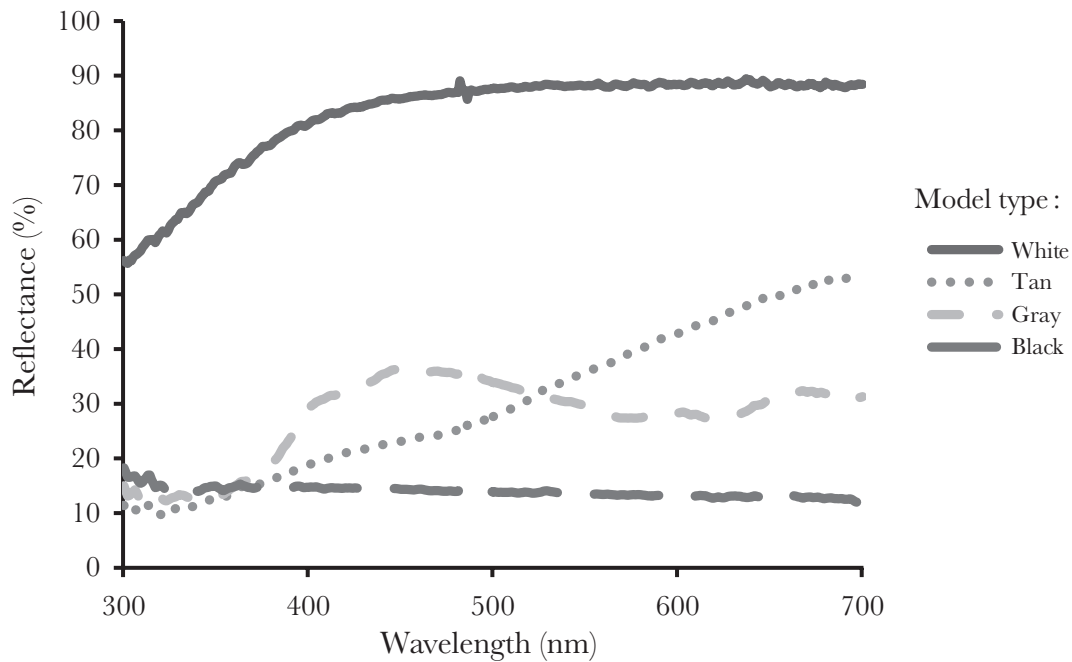


Figure 1

Reflectance spectra of the 4 butterfly models presented to perched males of *Asterocampa leilia*.

white reflected most light between 300 and 700 nm. The average reflectance spectrum of the tan butterfly model ($26.6 \pm 2.7\%$) was similar to the gray butterfly model ($27.6 \pm 1.3\%$) but had a shift toward longer wavelengths and thereby the coloration also matched the overall ventral wing surface reflectance of *A. leilia* females (Supplementary Figure S1). Further, tan model average reflectance ($26.6 \pm 2.7\%$) was not significantly different from female ventral hind wing average reflectance ($21.0 \pm 2.33\%$, *t*-test: $t_4 = 1.88$, $P = 0.13$).

Each model presentation proceeded as follows. We located a male in the field, perched either on the ground or on a hackberry tree, and set up the presentation apparatus near him. The flight path of the model was set up so that it was perpendicular to a line between the perching male and the midpoint of the model flight path. The distance between the midpoint of the flight path and the perching male was between 1 and 2 m for all presentations, which is a range of distances where males of *A. leilia* respond most frequently to passing objects of the size and shape presented (Rutowski et al. 2001). Once the apparatus was activated and the model moved from one pole to the other, we recorded the following information: 1) whether the male responded to the passing model by flying up and pursuing the model, 2) the direction that the male faced relative to north just before the model was presented, 3) the compass bearing of the flight path, 4) the compass bearing of a line from the perching male to the nearest point of the flight path, and 5) the perch height of the male.

To characterize the background against which the butterfly model was viewed by the male in a presentation, we took panoramic photos with a Casio EX-ZR100 camera of the model flight path from the vantage point of the male's perch (Figure 2). This was done for all presentations during the experiment.

Although the apparatus for presenting butterfly models was relatively mobile, the number of potential males available on any given day was limited in time and space (cf. Rutowski et al. 1996, 2001). To maximize data yield per unit effort, after setting up the

apparatus at a perching site we presented, in randomized order, as many of the 4 types of butterfly models as we could. However, we never presented the same model type to a specific male more than once. We were able to present all 4 model types to 78% of the males and conducted on average 3.51 presentations per individual male. In total, we presented models for 51 different males and we did 179 presentations.

Occasionally, due to high flight activity, resident males engaged in territorial contests with intruder males between different model presentations; these occurrences led to uncertainties of whether the same male was present for all model presentations at a given site. However, in territorial interactions, the original resident most often wins the fight and returns to the perch site (Rutowski and Gilchrist 1988; Kemp and Wiklund 2001) making a change in resident unlikely. Moreover, even though males of *A. leilia* rarely occupy a perch site for more than 1 day (Rutowski and Gilchrist 1988), we never presented models for males on the same perch site on subsequent days and we alternated experimental areas within Round Valley to avoid running the same male twice. However, sometimes the male left or changed perch site after taking off. Hence, some of the males did not experience all 4 models and the sample sizes might vary between groups. This also means that across the 4 model types, the observed responses were not completely independent. However, within any model type, all responses are to our best knowledge independent.

Analyses and statistical methods

To estimate the main background for each presentation, we used the images taken after each presentation. In the images, we overlaid the "flight" path of the model. Because earlier studies on *A. leilia* have shown that the male's ability to detect passing objects is strongly affected by distance and that perched males cannot detect a natural-sized gray butterfly model at a distance larger than 3 m (Rutowski et al. 2001), we excluded parts of the flight path that were further away than 3 m from the perched male. In the images,

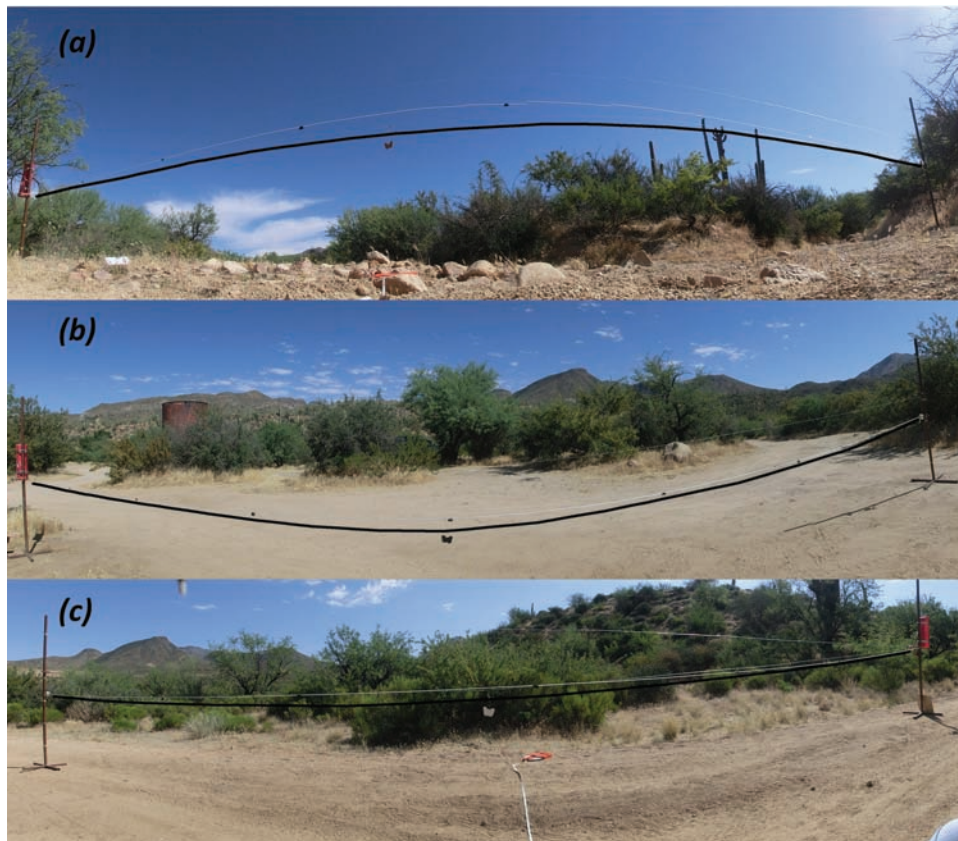


Figure 2

Background classification images taken after presentations of butterfly models to males of *Asterocampa leilia*. Each photo was taken from the exact spot the male was perched. The black line shows the flight path of the model. (a) A background consisting of mainly sky, (b) a background consisting of mainly sand, and (c) a background consisting of mainly vegetation.

we identified 3 different background categories against which the “flight” path was viewed: sky, vegetation, and sand. With ImageJ (<http://imagej.nih.gov/ij/>), we measured (in number of pixels) the proportion of the “flight” path seen against sky, vegetation, and sand, respectively, and the category with highest proportion was considered the main background for that presentation (Figure 2).

To analyze the effect of butterfly model type and background, we used a generalized estimating equation (GEE) with a binary logistic model. We used the response by the male (take-off or no take-off) as a binary dependent variable and butterfly model type with background as predictors. Because we have several presentations for the same male, male identity was used as a subject variable and model type was consequently treated as a within-subject variable. As the interaction between butterfly model type and background was nonsignificant, it was excluded from the model when testing for the main effects of model type and background. The GEE model was run in SPSS 22. For testing the differences in response between the 4 model types, we performed pairwise McNemar’s test between each combination of model type. Four model types resulted in 6 pairwise tests and the α -value was adjusted with a sequential Bonferroni correction.

Reflectance

We collected leaves from hackberry and mesquite trees (*Prosopis velutina*) along with sand and rocks from the field sites in Round Valley and transported them back to the lab. Vegetation reflectance measurements were taken immediately to ensure that the

vegetation retained its natural reflectance. We measured reflectance of 20 hackberry leaves from 4 different hackberry trees and 10 mesquite leaves from 2 different mesquite plants. We also measured 10 dishes of sand and each model used in the behavioral trials. For each plant, we measured reflectance 3 times on the upper and lower surfaces of leaves. To characterize the color of each model, we measured reflectance at 5 points on the model and averaged the spectra. We measured the ventral hind wing reflectance of 3 *A. leilia* females to confirm that the tan model was similar in coloration to female *A. leilia* individuals. The ventral surface of *A. leilia* is not homogenous; however, butterflies that employ a perch and sally mate location strategy are highly unlikely to have sufficient visual acuity to resolve specific internal pattern elements of potential mates, given they are a small, moving target relative to their field of view. With this in mind, we measured across the hind wing with a sampling area of 1 cm. To collect all reflectance spectra, we used a bifurcated reflectance probe (Ocean Optics, Dunedin, FL) with the collecting fiber connected to a spectroradiometer (USB2000, Ocean Optics) and the illuminating fiber connected to a Pulsed Xenon light source (Ocean Optics). All reflectance measurements were collected in a dark room with the probe positioned perpendicular to the target surface and were measured relative to a spectralon diffuse reflectance white standard (Labsphere, Inc., North Sutton, NH). The output of the spectroradiometer was collected with SpectraSuite software (Ocean Optics) and then raw spectral data from 300 to 700 nm were binned into 1-nm bins with CLR (Montgomerie 2008). Reflectance spectra were first averaged for

each plant and then all plants were averaged resulting in one average vegetation reflectance. Similarly, all sand reflectance spectra were averaged for an overall sand reflectance, and each butterfly model's reflectance spectra were averaged.

Irradiance

Light environment (ambient irradiance) measurements were collected on 7 November 2013 from 5 perching locations where butterfly models were presented in Round Valley. Irradiance measurements were used with reflectance to estimate the radiance from the models and the radiance from background as viewed by male *A. leilia* in behavioral trials. The measurements were made when the sky was clear from 10:00 AM to 12:30 PM. Ambient irradiance was measured separately for the butterfly models and background (i.e., sand, vegetation, and blue sky). We used a cosine-corrected irradiance probe (Ocean Optics) connected to a spectroradiometer (USB 2000, Ocean Optics) connected to a laptop running SpectraSuite software (Ocean Optics). The radiance of the butterfly models is determined by the ambient irradiance from the hemisphere in the direction of the perched male multiplied by the diffuse reflectance of the model. We aimed the cosine-corrected irradiance probe at where the viewer would be perched during a butterfly model presentation (Supplementary Figure S2). Hence, when taking the relevant irradiance measurements that contributed to butterfly model radiance (via reflection from the model), the probe was held at the position in the horizontal plane where the butterfly model would pass, at a distance of 1 m from the perch, and aimed at the hemisphere that includes the potential perching point of the male (Supplementary Figure S2). The radiance reflected from the background is determined by the light environment from the hemisphere facing the direction of the butterfly model and the perched male. When taking irradiance measurements that contribute to background radiance (via reflection), the probe was held at the position in the horizontal plane closest to the background type (sand or vegetation) and aimed at the hemisphere that includes the butterfly model (Supplementary Figure S2). Because males can perch either on the hackberry tree or on the ground in front of the tree, both positions were used for taking the irradiance measurements. Because males perched on hackberry exhibit a wide range of orientations, with no significant mean vector in relation to sun azimuth or point of compass in the field (Rutowski et al. 1991), the 5 perch site locations selected were focused on hackberry trees oriented 90° relative to sun azimuth (99 ± 44°). To characterize backgrounds of blue sky, we took irradiance measurements of blue sky background that was relevant to the orientation of males perched on ground (Rutowski et al. 1991), the probe was held at the position of perched males, and aimed at blue sky, 180° from sun azimuth. As with reflectance spectra, we binned the irradiance data into 1-nm bins from 300 to 700 nm using CLR (Montgomery 2008). Irradiance was then converted to photon flux units relevant for vision (photons/cm²/s).

Visual system model

We modeled *A. leilia* luminance contrast by calculating achromatic contrast using a von Kries term that assumes receptor adaptation (sensu Morehouse and Rutowski 2010). Insects have been found to detect motion using luminance contrast from a single spectral photoreceptor type with peak absorption ($\lambda_{R_{max}}$) in the green region (Briscoe and Chittka 2001). Therefore, we incorporated a visual pigment with $\lambda_{R_{max}}$ of 530 nm found in a related nymphalid species, *Vanessa cardui* (Briscoe et al. 2003)—photoreceptor absorbances are unknown for *A. leilia*—and a rhodopsin template (Govardovskii

et al. 2000) into our model. We then calculated luminance contrasts between butterfly models and backgrounds according to a log linear model of photoreceptor quantum catch with a von Kries transformation for receptor light adaptation:

$$q_G = \ln \left(\frac{Q_G}{Q_{GB}} \right) \quad (1)$$

where q_G is the quantum catch of a green photoreceptor with peak sensitivity at 530 nm.

$$Q_G = \int R(\lambda) I(\lambda) A_G(\lambda) d\lambda \quad (2)$$

$$Q_{GB} = \int R_B(\lambda) I(\lambda) A_G(\lambda) d\lambda \quad (3)$$

$R(\lambda)$ is the averaged reflectance from a target, R_B is the averaged reflectance from a background type, $I(\lambda)$ is the relevant ambient irradiance (photons/cm²/s), and $A_G(\lambda)$ is the modeled photoreceptor absorbance. We integrated these equations from 300 to 700 nm. All background types are either diffusely reflecting surfaces (sand and vegetation) or diffuse light sources (blue sky), allowing direct comparisons. The calculated luminance contrasts between various butterfly models and backgrounds were compared to the response patterns of males in the field in order to test the prediction that high contrast should result in a high probability of males responding.

RESULTS

Effect of butterfly model type

Across presentations, regardless of background and perch locations, model type strongly affected the probability that a male responded by leaving his perch to chase the model (Figure 3A and Table 1). Eighty-seven percent of the males responded when the black model was presented but only 26% responded to presentations of the white model. Fifty-two percent of the males responded to the gray model and 60% responded to the tan-colored model. The response to the black model was significantly higher than the response to the 3 other model types (Table 2). Likewise, the response to the white model was significantly lower than all other models (Table 2). However, there was no difference in male response to gray- and tan-colored models (Table 2).

Effect of perch location

Male perch location also affected the male's probability of responding to the presented models. As reported in Rutowski et al. (2001), males that were perched on the ground were more likely to respond to the presented models than males perched on a hackberry tree (Figure 3B; chi-square test: $\chi^2 = 7.87$, $P = 0.005$). This was true for all 4 model types but seemingly stronger in the tan-colored model (Figure 3B). When the tan-colored model was presented for males perching on ground, 86% of the males responded. When the tan-colored model was presented for males perching off ground, significantly fewer males, only 44%, responded by sallying after the model (Fisher's Exact test: $P = 0.017$).

Effect of background

In this study, we have described the background type as 1 of 3 discrete categories: sky, sand, or vegetation. In 54% of the presentations, the male viewed the passing model against a background that consisted of 90–100% of a single background type. The background never contained less than 50% of a single background

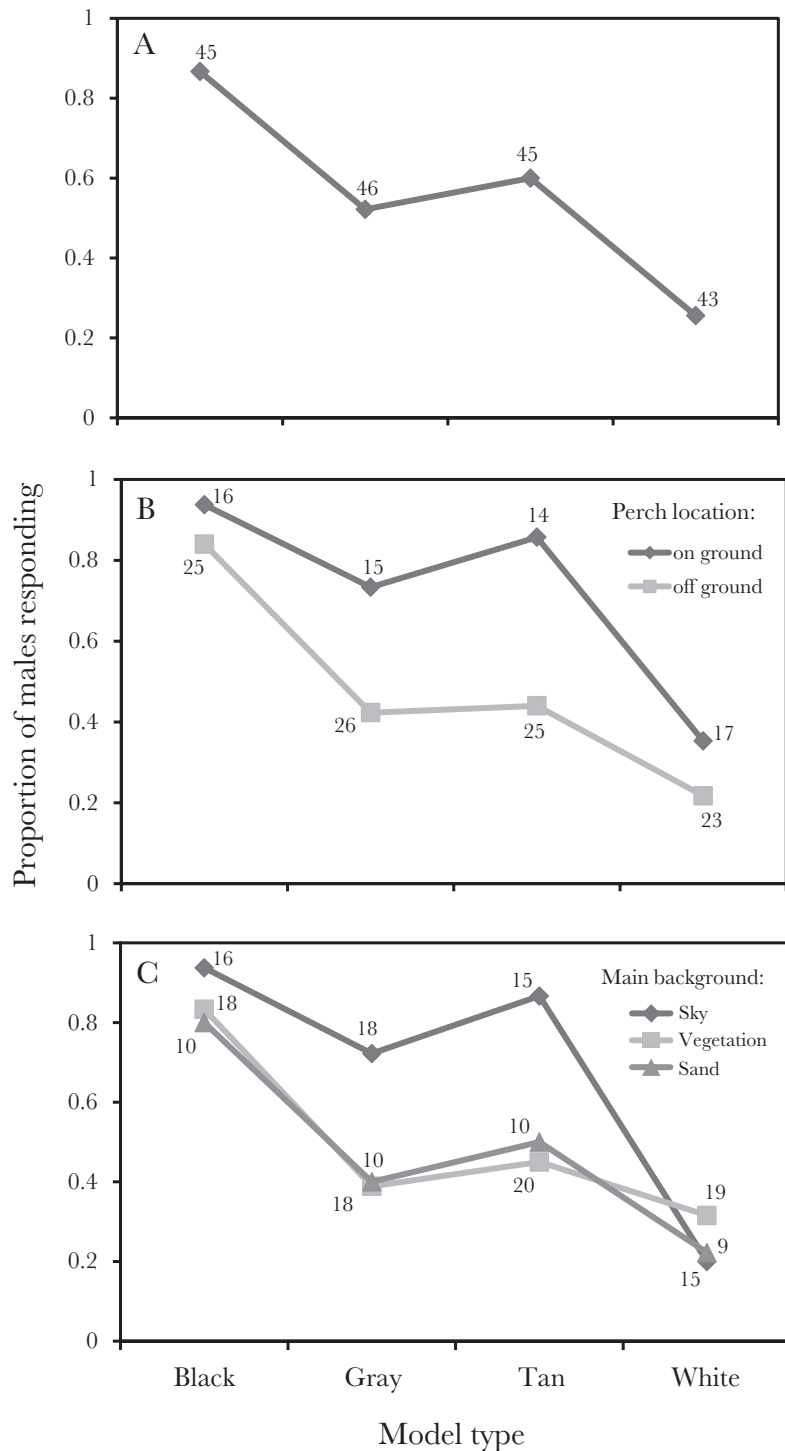


Figure 3

The relationship between the proportion of perched males responding as a function of model type: (A) for all presentations, (B) for all males as a function of perch location, and (C) for all males as a function of main background type. For all graphs, the number adjacent to each data point is the number of presentations with that model type.

type. We find an overall effect of the main background type on the probability that males would respond to the presented models (Figure 3C and Table 1). Males that viewed the models against a background of mostly sky were more likely to respond (87%) than males that viewed the model with vegetation (45%) or sand (50%) as the background (Figure 3C). There was no interaction between butterfly model and background type (Table 1). The strength of the

background effect differed slightly between model types but was strongest for the tan-colored model (Figure 3C).

Luminance contrast

The luminance contrast between the presented butterfly model and the background varied with model type. The contrast was highest against all backgrounds for the black model. The contrast

Table 1

The results from a GEE showing the effect of model type and background on the probability of getting a response from a perched male to a presented butterfly model

Factor	df	Wald χ^2	P
Model type	3	28.53	<0.001
Background type	2	7.51	0.023
Model type \times background type	6	5.83	0.44

Table 2

The result of 6 pairwise McNemara's tests between 4 butterfly model types

	Model type			
	Black	Gray	Tan	White
Model type				
Black		$\chi^2 = 12.07$, $P < 0.001$	$\chi^2 = 10.08$, $P = 0.0015$	$\chi^2 = 21.04$, $P < 0.001$
Gray			$\chi^2 = 0.57$, $P = 0.45$	$\chi^2 = 6.72$, $P = 0.0095$
Tan				$\chi^2 = 10.32$, $P = 0.0013$
White				

After correction of the alpha values with a sequential Bonferroni, all pairwise tests are significant, except the difference between the gray and the tan model, which is not significant.

was lower for the other 3 models with small differences between the models (Figure 4A). However, the white model differed from the other 3 models in the directionality of the contrast. The white model often had a higher luminance than the background, whereas the tan, grey, and black models most often were darker than the background. Consequently, the absolute contrast between the white model and the background was similar to the contrast between the grey model and the background, and the tan model and the background, but with an essential difference in the directionality of the contrast. Nevertheless, the contrast values were largely correlated with the behavioral responses for 3 of the 4 models (Figure 4A). The most contrasting butterfly model (black) generated the highest response from males, whereas the models with a lower contrast generated a lower behavioral response from males.

Luminance contrast also varied with background. Here, the contrasts between models and background are greatest when the background is blue sky than when the background is sand or vegetation (Figure 4B). Again the pattern of change in contrast with background parallels the behavioral data where across model types males responded most frequently when blue sky was the background than when sand or vegetation was the background (cf. Figure 3C). But again, there is little difference in absolute contrast between the white model and the 3 background types (Figure 4B), but reversal in contrast directionality. When the white model is viewed against the vegetation or the sand, it had a higher luminance than the background.

DISCUSSION

Our results show that both the brightness of an object as well as the background against which it is viewed strongly affects male probability of detecting the object and initiating a pursuit flight. Perched males are most likely to detect and pursue dark objects such as

the black models when they pass by. Moreover, objects were most likely to be detected when viewed against a bright uniform background such as blue sky compared with a background of vegetation or sand. Our modeling of perceived brightness contrast between the presented butterfly models and the background correlates with the behavioral data, at least for 3 of the 4 butterfly models. Hence, we argue that the effect of model brightness and background type can be largely explained by differences in achromatic luminance contrast and that a high contrast between object and background facilitates visual detection of small fast moving objects. However, the response to white objects is far lower than would be expected by our measured luminance contrast.

Characterization of background

We acknowledge a few potential sources of error in our measurement techniques. First, we did not determine the background against which a male was viewing the model at the moment he responded. However, not knowing this is mitigated by 2 features of the background data. First, in 42% of all presentations from the male's perspective, there was only one background type, sky, sand, or vegetation, along the model flight path. Second, in cases where there were mixtures of backgrounds, there was often one background type that dominated the scene. Also, when there was a mixture of backgrounds, the response from the males was not significantly different than when there was only a single background (Supplementary Table S1). Hence, we contend that using the main background is a good estimation of what the background was at the point the male responded.

Second, our background categories gloss over complexity within categories. Vegetation is especially likely to be a matrix of bright and dark parts. The result would be that the luminance contrast between a passing object and the background would shift repeatedly and quickly when viewed by the male. It is unknown how this would affect visual detection. However, the effect of this variation is mitigated by the fact that the acuity of male eyes is such that this variation will not be resolved, especially when the background is several meters or more away. The perceived luminance variation will effectively be averaged, resulting in a fairly constant luminance contrast of a passing object. Switzer and Eason (2000) found that in a territorial dragonfly, intruders viewed against distant vegetation were more readily detected than intruders viewed against near vegetation.

Lastly, our field irradiance measurements used to calculate this contrast may not have captured accurately all of the background light that was present. Specifically our measurements omitted the skylight, a major extrinsic factor in desert ecosystems, which would contribute to background luminance. Further, the light contributing to background luminance comes from a larger field of view than is represented by measuring vector irradiance, which by necessity is restricted to the hemisphere in which a cosine-corrected collector is directed. Nevertheless, the effect of scattered background light is predicted not only to increase perceived luminance contrast of objects that have less luminance than the background (black, tan, and gray models) but also to decrease the perceived contrast of objects that have a higher luminance than the background (white model). This would explain why the measured luminance contrast of the white target is higher than predicted by behavioral responses, and the contrast for the 3 other models is slightly lower than predicted by the behavioral responses, as seen in Figure 4A. Further, we find that the deviation is larger when the background was sand, or vegetation (Figure 4B), which could be explained due

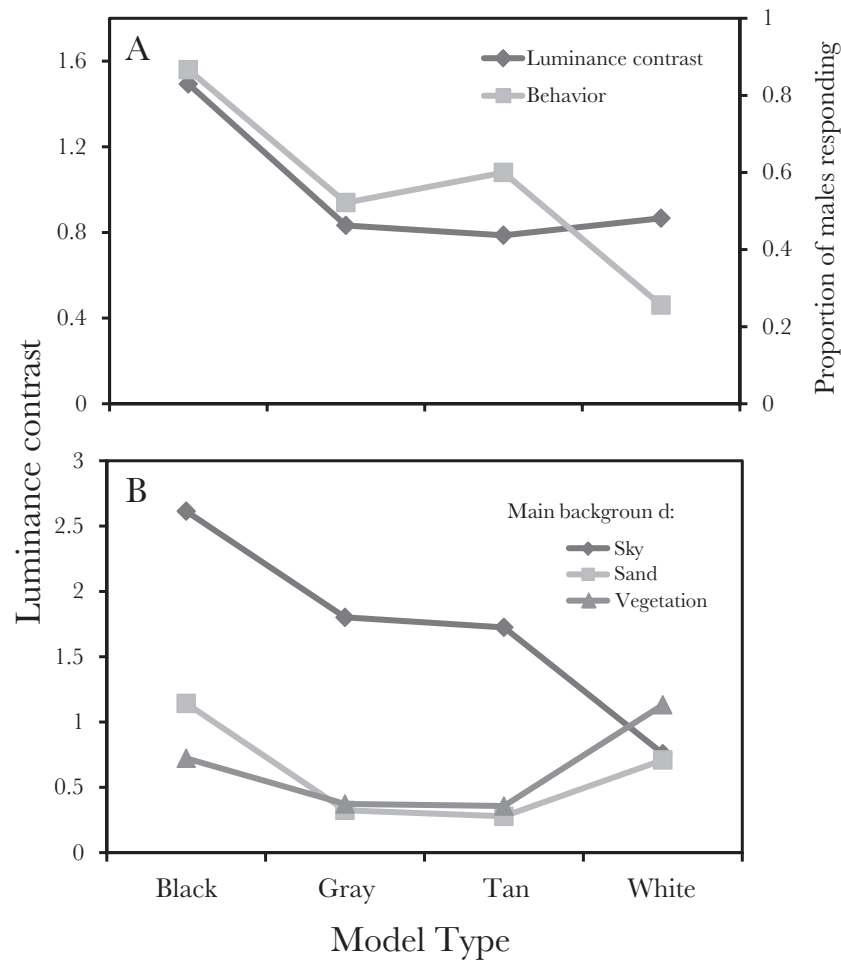


Figure 4

(A) The relationship between the proportion of perched males responding as a function of model type for all presentations (right y axis) and luminance contrast as a function of model type (left y axis). (B) Luminance contrast as a function of model type and background.

to a stronger effect of scattered skylight. As overall levels of direct illumination decrease, the proportion of scattered skylight contributing to ambient illumination is higher.

Visual modeling and luminance contrast

By using physiological models of color processing, we explored the role that perceived luminance contrast between object and background might play in explaining these results. The models we used are built on several assumptions. First, the model assumes that the spectral sensitivity of *A. leilia* is similar to that of *V. cardui*. Second, the model assume that *A. leilia* mainly use their green receptor for target detection. It is well known that bees use their green receptor for target detection (Giurfa and Vorobyev 1997, 1998), but it is still largely unknown how this works in butterflies. However, which visual pathway (chromatic or achromatic) is used for target detection in bees depends on the angle subtended by the target. If a target subtends an angle above 30° , color vision is used for target detection and recognition. But if a target is small and close to detection limits (subtends less than 5° in the visual field), detection is guided by the long wavelength photoreceptor class alone (Giurfa and Vorobyev 1997, 1998). The long wavelength photoreceptor is used to see the intensity differences between the object and the background, a mechanism also referred to as “green contrast” (Giurfa et al. 1996). Behavioral studies also suggest that butterflies

use achromatic vision and target-background intensity contrast for some behavioral tasks, such as landing on flowers (Koshitaka et al. 2011). In our study, we did not find any difference in the males’ response to the gray model and the tan-colored model (Table 2 and Figure 3A). The gray- and the tan-colored models are similar in brightness but differ in spectral properties (Figure 1). The similarity in response to the gray and tan model may indicate that *A. leilia* also uses an achromatic rather than a chromatic visual pathway to detect small passing objects. Based on this and considering that the butterfly models at the distance presented in our study subtended less than 5° in the perched male’s visual field, we conclude that males use only their long wave receptor for detection of the presented models. Hence, given that the assumptions made by our visual model are correct, we can infer that perched males of *A. leilia* mainly use luminance and not color contrast to detect flying females and other males.

Contrast directionality and response to white objects

The comparison of the behavioral data and the measured luminance contrast between the butterfly models and the background show that the white model deviates from the correlation between behavior and measured luminance contrast. As mentioned above, it is likely that our natural irradiance measurements were unable to

accurately capture all of the background light that was present and that we thereby underestimate the background luminance perceived by the perched males. However, there is also a small possibility that there is an additional, biological explanation for this mismatch. Two other studies, where artificial butterfly-shaped models have been presented to perched butterfly males in the field, reported a very low response to white objects (Stride 1957; Tinbergen et al. 1972). The species studied were both nymphalids (*Hipparchia semele* and *Hypolimnas misippus*) and Stride (1957) proposed an adaptive explanation to the low response to white objects. Because perching behavior involves costly approach flights and investigation of essentially anything that comes into the visual field, a mechanism that allows discrimination would be an advantage for the male. Approach inhibition based on brightness of the object would avoid pursuit of butterflies in the family Pieridae, a family with many species of white butterflies (Stride 1957). Even though an underestimation of background luminance seems likely to explain the low response to white models in *A. leilia*, it is not impossible that there is a general approach inhibition toward white objects in perching species and that this could be the result of undescribed visual system response that detect the direction of contrast rather than only the magnitude and affects a male's decision to take off and pursue a passing object.

CONCLUSIONS

Studies like the one presented here that examine how extrinsic factors such as background affect visual detection under natural settings complement previous studies on intrinsic factors, that is, how visual systems work and how morphological and physiological features affect visual performance. By taking the experiment to the field, we could test hypotheses about how natural variation and real environmental factors affect visual system performance. Studies like this are also important to understand the behavioral and evolutionary ecology of a perch-and-sally strategy. Males of *A. leilia* can affect their success at detecting and pursuing passing females by choosing a perch site that facilitates this task. By studying this in wild males found on their natural perch sites, we can understand the variation in real environmental factors and how this will structure selection for choosing preferred perch sites. In an earlier study, Bergman and Wiklund (2009) studied visual mate detection in the speckled wood butterfly (*Pararge aegeria*), a species in which males defend mating territories in large sunspots on the forest floor (Davies 1978; Wickman and Wiklund 1983). Bergman and Wiklund (2009) showed that males enhance their chance of visually detecting passing females by preferring to perch in large sunspots over small ones. In this system, the background varies little between perch sites, but the level of ambient illumination in the sunspot facilitates visual detection of passing females, presumably because a female entering the sunspot will be illuminated by the sun, appear brighter, and thereby contrast more with the dark forest background. Further, a female passing a male sitting in a large sunspot will be illuminated by the sun for a longer time than a female passing a male in a small sunspot and thereby more likely to be detected. Similar to *A. leilia*, males of *P. aegeria* can increase the chance of viewing a passing female at a high contrast to the background by the choice of perch site. We argue that this and other extrinsic factors have large effects on the success of perch-and-sally tactics and ultimately on the evolution of perch site preferences.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behco.oxfordjournals.org/>

FUNDING

This study was supported by a grant from the Swedish Research Council to M.B. N.L. was supported by the National Science Foundation Graduate Research Fellowship under grant no. DGE-0802261.

We thank Almut Kelber and Cole Gilbert for useful comments on this manuscript. We thank Etienne Martin for assistance in the field work.

Handling editor: Bob Wong

REFERENCES

- Atkins MD. 1980. Introduction to insect behavior. New York: Macmillan Publishing.
- Austin GT. 1977. Notes on the behavior of *Asterocampa leilia* (Nymphalidae) in southern Arizona. *J Lepid Soc.* 31:111–118.
- Bergman M, Wiklund C. 2009. Visual mate detection and mate flight pursuit in relation to sunspot size in a woodland territorial butterfly. *Anim Behav.* 78:17–23.
- Briscoe AD, Bernard GD, Szeto AS, Nagy LM, White RH. 2003. Not all butterfly eyes are created equal: rhodopsin absorption spectra, molecular identification, and localization of ultraviolet-, blue-, and green-sensitive rhodopsin-encoding mRNAs in the retina of *Vanessa cardui*. *J Comp Neurol.* 458:334–349.
- Briscoe AD, Chittka L. 2001. The evolution of color vision in insects. *Annu Rev Entomol.* 46:471–510.
- Buser P, Imbert M. 1992. *Vision*. Cambridge (MA): The MIT Press.
- Corbet PS. 1999. *Dragonflies: behavior and ecology of Odonata*. Ithaca (NY): Combstock Publishing.
- Corbet PS, May ML. 2008. Fliers and perchers among Odonata: dichotomy or multidimensional continuum? A provisional reappraisal. *Int J Odonatol.* 11:155–171.
- Cronin TW, Johnsen S, Marshall NJ, Warrant EJ. 2014. *Visual ecology*. Princeton (NJ): Princeton University Press.
- Davies NB. 1978. Territorial defence in the speckled wood butterfly (*Pararge aegeria*): the resident always wins. *Anim Behav.* 26:138–147.
- Ehrlich PR. 1984. The structure and dynamics of butterfly populations. In: Vane-Wright RI, Ackery PR, editors. *The biology of butterflies—symposium of the royal entomological society of London*. London: Academic press. p. 25–40.
- Giurfa M, Vorobyev M. 1997. The detection and recognition of color stimuli by honeybees: performance and mechanisms. *Isr J Plant Sci.* 45:129–140.
- Giurfa M, Vorobyev M. 1998. The angular range of achromatic target detection by honey bees. *J Comp Physiol A.* 183:101–110.
- Giurfa M, Vorobyev M, Kevan P, Menzel R. 1996. Detection of coloured stimuli by honeybees: minimum visual angles and receptor specific contrasts. *J Comp Physiol A.* 178:699–709.
- Govardovskii VI, Fyhrquist N, Reuter T, Kuzmin DG, Donner K. 2000. In search of the visual pigment template. *Vis Neurosci.* 17:509–528.
- Kemp DJ, Wiklund C. 2001. Fighting without weaponry: a review of male-male contest competition in butterflies. *Behav Ecol Sociobiol.* 49:429–442.
- Koshitaka H, Arikawa K, Kinoshita M. 2011. Intensity contrast as a crucial cue for butterfly landing. *J Comp Physiol A.* 197:1105–1112.
- Labhart T, Nilsson DE. 1995. The dorsal eye of the dragonfly *Sympetrum*: specializations for prey detection against the blue sky. *J Comp Physiol A.* 176:437–453.
- Land MF. 1997. Visual acuity in insects. *Annu Rev Entomol.* 42:147–177.
- Land MF, Nilsson DE. 2012. *Animal eyes*. New York: Oxford University Press.
- Lehrer M, Bischof S. 1995. Detection of model flowers by honeybees: the role of chromatic and achromatic contrast. *Naturwissenschaften.* 82:145–147.
- Mazokhin-Porshnyakov GA. 1969. *Insect vision*. New York: Plenum Press.

- Montgomerie R. 2008. CLR, version 1.05. Kingston (Canada): Queen's University.
- Morehouse NI, Rutowski RL. 2010. In the eyes of the beholders: female choice and avian predation risk associated with an exaggerated male butterfly color. *Am Nat.* 176:768–784.
- Rensen JV Jr, Robinson SK. 1990. A classification scheme for foraging behaviour of birds in terrestrial habitats. *Stud Avian Biol.* 13:144–160.
- Rutowski RL. 1991. The evolution of male mate-locating behaviour in butterflies. *Am Nat.* 138:1121–1139.
- Rutowski RL. 2000. Postural changes accompany perch location changes in male butterflies (*Asterocampa leilia*) engaged in visual mate searching. *Ethology.* 5:453–466.
- Rutowski RL. 2003. Visual ecology of adult butterflies. In: Boggs CL, Watt BW, Ehrlich PR, editors. *Butterflies—ecology and evolution taking flight*. Chicago (IL): University of Chicago press. p. 9–25.
- Rutowski RL, Demlong MJ, Leffingwell T. 1994. Behavioural thermoregulation at mate encounter sites by male butterflies (*Asterocampa leilia*, Nymphalidae). *Anim Behav.* 48:833–841.
- Rutowski RL, Demlong MJ, Terkanian B. 1996. Seasonal variation in mate-locating activity in the desert hackberry butterfly (*Asterocampa leilia*; Lepidoptera: Nymphalidae). *J Insect Behav.* 9:921–931.
- Rutowski RL, Dickinson JL, Terkanian B. 1991. Behavior of male desert hackberry butterflies, *Asterocampa leilia* (Nymphalidae) at perching sites used in mate location. *J Res Lepid.* 30:129–139.
- Rutowski RL, Gilchrist GW. 1988. Male mate-locating behavior in the desert Hackberry butterfly, *Asterocampa leilia* (Nymphalidae). *J Res Lepid.* 26:1–12.
- Rutowski RL, McCoy L, Demlong MJ. 2001. Visual mate detection in a territorial male butterfly (*Asterocampa leilia*): effects of distance and perch location. *Behaviour.* 138:31–43.
- Rutowski RL, Warrant EJ. 2002. Visual field structure in the Empress Leilia, *Asterocampa leilia* (Lepidoptera, Nymphalidae): dimensions and regional variation in acuity. *J Comp Physiol A.* 188:1–12.
- Scott JA. 1974. Mate-locating behaviour of butterflies. *Am Midl Nat.* 91:103–117.
- Stride GO. 1957. Investigations into the courtship behaviour of the male of *Hypolimnna misippus* L. (Lepidoptera, Nymphalidae), with special reference to the role of visual stimuli. *Br J Anim Behav.* 5:153–167.
- Switzer PV, Eason PK. 2000. Proximate constraints on intruder detection in the dragonfly *Perithemis tenera* (Odonata: Libellulidae): effects of angle of approach and background. *Ann Entomol Soc Am.* 93:333–339.
- Thornhill R, Alcock J. 1983. *The evolution of insect mating systems*. Cambridge (MA): Harvard University Press.
- Tinbergen N, Meeuse BJD, Boerema LK, Varossieau W. 1972. The courtship of the grayling *Eumenis* (= *Satyrus semele* (L.)) (1942). In: Tinbergen N, editor. *The animal in its World*. London: Allen & Unwin. p. 197–249.
- Wickman PO, Wiklund C. 1983. Territorial defence and its seasonal decline in the speckled wood butterfly (*Pararge aegeria*). *Anim Behav.* 31:1206–1216.
- Wiklund C. 2003. Sexual selection and the evolution of butterfly mating systems. In: Boggs CL, Watt BW, Ehrlich PR, editors. *Butterflies—ecology and evolution taking flight*. Chicago (IL): University of Chicago press. p. 67–90.