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How Females of *Lucihormetica verrucosa* (Blattodea, Blaberidae) Might See the Pronotal Spots of Their Mates

Wie Weibchen von *Lucihormetica verrucosa* (Blattodea, Blaberidae) die Pronotum-Flecken ihrer Partner sehen könnten

Jana Beckert, Hartmut Greven & Klaus Lunau

Zusammenfassung: In Wahlversuchen haben wir Hinweise dafür gewonnen, dass die Weibchen der Leuchtschabe *Lucihormetica verrucosa* Männchen mit roten Pronotumflecken, d.h. mit einem hohen Carotinoidgehalt der Epidermis, die unter der transparenten Pronotum-Cuticula liegt, attraktiver finden als Männchen mit weißen oder gelben Flecken, deren Epidermis keine oder nur wenige Carotinoide enthält. Wenn man davon ausgeht, dass die Balz bei *L. verrucosa* zumindest partiell visuell gesteuert wird und dass diese Schabenart als nacht- oder dämmerungsaktive Schabe „rotblind" ist, aber über UV- und Grünrezeptoren verfügt, stellt sich die Frage, wie die Weibchen die roten Flecken wahrnehmen. Eine Analyse der Fleckenfarbe mit Hilfe der RGB-Skala zeigt, dass die roten Flecken einen geringeren Grünanteil haben. Dies könnte die Weibchen befähigen, die unterschiedlich gefärbten Flecken zu unterscheiden und so indirekt Auskunft über die Qualität des Partners zu erhalten.

Schlüsselwörter: Leuchtfleckschabe, Carotenoide, Partnerwahl

Summary: Based on choice experiments we found some evidence that males of the cockroach *Lucihormetica verrucosa* with red pronotum spots, i.e. with a high carotenoid content in the epidermis underlying the transparent spot cuticle, are more attractive to females than males with white or yellow spots, whose epidermis does not contain or contains very little amounts of carotenoids. Assuming that courtship of *L. verrucosa* is at least in part visually triggered and that this nocturnal or crepuscular species is "red-blind", but has UV and green receptors, the question arises how females might perceive the red spots. An analysis of the spot colour using the RGB scale showed that the red spots have a lower amount of reflected green light, which may enable females to differentiate between variously coloured spots to gain indirectly information about the quality of their mates.

Key words: Glow spot cockroach, carotenoids, mate choice

1. Introduction

Males of glow spot cockroaches (genus *Lucihormetica*) are characterized by two distinct white, yellow or red spots on the pronotum. Earlier studies preferably on *Lucihormetica verrucosa* have shown (1) that the colour of these spots may vary between red, yellow and white depending on the carotenoid content of diet (e.g. Fritzsche 2013; Beckert et al. 2017), (2) that the carotenoids are stored mainly in the epidermis below the transparent cuticle of the spots (see Greven et al., in prep.) instead of within the fat body as previously suggested (Greven & Zwanzig 2013; Beckert et al. 2017), (3) that these carotenoids are exclusively α- and β-carotenoids (see Greven et al., in prep.), (4) that the spots reflect UV-light (Zompro & Fritzsche 1999; Vršanský et al. 2012; Vršanský & Dušan 2013; Beckert et al. 2017), (5) that the intensity of this UV reflection decreases with increasing red colouration (Beckert et al. 2017), and (6) that white but not red spots fluoresce under UV-light (Beckert et al. 2017).

Because of the diet-dependent colouration and the particular significance of carotenoids for animals serving as visual and colouring pigments, antioxidants and hormone precursors (e.g. Blount & McGraw 2008; Heath et al. 2013), it has been concluded that the red colouration of the spots may represent a condition-dependent character giving males (intrasexual selection) and/or females (intersexual selection) information about the quality of their rivals or mates (Greven & Zwanzig 2013). In addition, based on the assumption that glow spot cockroaches are bioluminescent, it was speculated they might mimic poisonous bioluminescent click beetles (see Vršanský et al. 2012; Vršanský & Dušan 2013; for critical remarks on bioluminescence in glow spot cockroaches see especially Merritt 2013; see also Greven & Zwanzig 2013; Beckert et al. 2017).

All these suggestions have not been tested experimentally up to now. In the present note we report on mate choice experiments giving some indirect evidence that "red" males of *L. verrucosa* appear more attractive to females than "white" males and discuss, how this might be possible, assuming that *L. verrucosa* as other nocturnal cockroaches do not have red photoreceptors, but have UV and green receptors (e.g. Mazokhin-Porshnyakov & Cherkasov 1985; Mote & Goldsmith 1970; Koehler et al. 1987; Heimonen et al. 2016).

2. Material and methods

Animals came from a breeding stock of the Biology Department of the Heinrich-Heine University Düsseldorf. Cockroaches were held in various plastic containers at 20-25 °C and were fed either with carotenoid-enriched or carotenoid-poor diet leading to males with red spots ("red" males) or males with yellow or white spots ("white" males) (for details see Beckert et al. 2017). To obtain virgins females were separated immediately after the imaginal moult and were reared without males.

2.1. Experimental procedures

Two experiments were carried out. In experiment I one virgin female was confronted with one male (10 tests with 10 males (I-X) and 10 virgin females (*I-X*)) (Tab. 1).

In experiment II one virgin female could choose between two males (10 tests with 20 males (XI-XXX) and 10 virgin females (*XI-XX*)) (Tab. 1).

The males were taken from different breeding boxes, to exlude that they did know each other before the test. Prior to each experiment, the length (front margin of the pronotum to the genital opening) of each male was measured using a caliper gauge. Care was taken to use similarly sized males. To distinguish the animals, they were marked on the wings with a white Edding pen.

In each experiment the cockroaches were filmed over 30 min from above in an "arena" (plastic box with a base area of 14 x 7 cm) with an Iphone 6S (Apple) attached to a tripod. The videos were analysed with the free program "VLCmedia player". As a measure of the male's attractiveness we used the time (indicated by the time code) the female needed from the start of the test up to climb the male, i.e. the shorter this period, the more attractive was the male.

2.2. Colour analysis of pronotum spots

The spots of all males that proved to be attractive to the females were analysed by means of RGB values using single images from the videos and the tool "colour pipette"(radius 4) of the image-editing program GIMP 2.8.22 (GNU Image Manipulation Program, www.gimp.org).

For that, the "arena" was divided into five slightly overlapping subareas and one photo (from the video sequence) was taken from

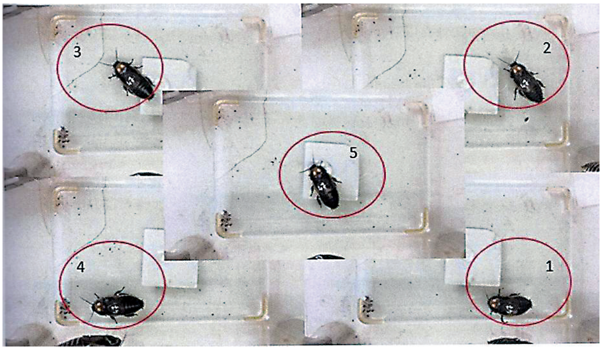

Fig. 1: Division of the experimental arena in five subareas (red circles) to assess colouring of the pronotal spots. For further explanations see text.
Abb. 1: Aufteilung der Arena in fünf Areale (rote Kreise) zur Beurteilung der Farbe der Pronotumflecken. Weitere Erklärungen im Text.

each male in each subarea resulting in five images per male. In the case a male did not stay in a given subarea during the test, he was photographed in the nearest distance from this area. Care was taken that the males were aligned with the upper left corner of the arena in the direction of the light source (Fig. 1).
To calculate the colours of the pronotum spots we used the (additive) RGB system that describes a colour through its components, i.e. the primary colours red, green and blue. The RGB values indicate the portions of these colours, red, green and blue (Byers 2006; www.itwissen.info). From the five images of both spots per male RGB-values were calculated for the primary colours and from the two averages obtained per male another mean was formed.

2.3. Photography

With the help of false colour photography we simulated the "cockroach-specific" view on the spots. We used a Panasonic Lumix GH-1 (Kadoma, Japan) modified for false colour photography and an Ultra-Achromatic-Takumar 1:4.5/85 mm lens and various filters (UV/IR-Cut filter for normal photos under a daylight lamp (OSRAM Lumilux, Cool Daylight L58W/865, Munich), U-filter and UV/IR-Cut filter under a UV-torsch (MTE U303, Singapore) for UV and fluorescence photos. Normal photos were disassembled into the individual colour channels (red, green, blue) and reassembled with the UV-photo according to the supposed receptor equipment of cockroaches (for technical details see Lunau & Verhoeven 2017).

2.4. Statistics

The correlation between the attractiveness of the males and the colour of the pronotum spot was shown using Spearman's rank correlation (www.socscistatistics.com). For this purpose, attractiveness of the males (see above) was sorted in ascending order (1-17) (see Tabl. 3), compared with the values calculated with the RGB model (see above) and checked for correlation.

3. Results

3.1. Mate choice and attractiveness

Experiment I (1♂:1♀): From the 10 males five were climbed by the female and reached the final mating position (Tab. 1).
Experiment II (2♂♂:1♀): From the 20 males 12 males were climbed; six males reached the final mating position (Tab. 1).
Thus, 17 males were available for colour analysis.

Tab. 1: Composition of the experimental groups. Left: in pairs (1♂ : 1♀); right: in groups of three (2 ♂♂ : 1♀). Bold numbers = males climbed up by a female; bold numbers underlined = males in final mating position.

Tab. 1: Zusammensetzung der Versuchsgruppen. Links: paarweise (1♂ :1 ♀); rechts: in Dreiergruppen (2♂♂ : 1♀). Fette römische Zahlen = Männchen, die von einem Weibchen bestiegen wurden; unterstrichene, fette Zahlen = Männchen, die die endgültige Paarungsstellung erreicht hatten.

1♂ : 1♀	♂	♀	**2♂♂ : 1♀**	♂	♀
	I	*I k*		XI + XII	*XI*
	II	*II*		XIII + **XIV**	*XII*
	III	*III*		**XV** + XVI	*XIII*
	IV	*IV*		**XVII** + **XVIII**	*XIV*
	V	*V*		**XIX** + **XX**	*XV*
	VI	*VI*		**XXI** + XXII	*XVI*
	VII	*VII*		**XXIII** + **XXIV**	*XVII*
	VIII	*VIII*		**XXV** + **XXVI**	*XVIII*
	IX	*IX*		XXVII + XXVIII	*XIX*
	X	*X*		**XXIX** + XXX	*XX*

Tab. 2: RGB-values of 17 males and colour of the pronotal spots after the subjective impression of the observer.

Tab. 2: RGB-Werte von 17 Männchen and Farbe der Pronotumflecken nach dem subjektiven Eindruck des Betrachters.

♂♂	Blue	Green	Red
XIV (white)	74.6	78.9	84.3
XIX (red)	34.3	43.9	79.3
XX (orange)	32.6	58.5	82.9
XXI (red)	38.4	48.9	76.9
XXV (red)	26.5	35.2	79.0
XXIII (red)	29,9	43	78.7
XVIII (white)	67.6	78.8	81.2
IV (white)	71.3	83.5	84.6
VII (yellow)	34.8	57.8	88.6
XXVI (yellow-brown)	22.6	34.8	54.3
VI (yellow)	33.7	66.4	83.8
XV (yellow)	39.4	75	86.7
XXIV (yellow)	50.4	84.6	93.5
III (white)	71.5	79.2	83.4
XVII (white)	71.1	79.5	82.9
XXIX (white)	76.2	83.7	88.3
I (red)	20.7	34.5	71.3

The time a female needed to climb the first time on the male varied between 01:30 and 24:02 min (see Tab. 3).

3.2. Colour analysis

In the RGB analysis "Red" had always the highest value in all males. The distribution of the load of each channel changes according to the colour of the spots. Compared to green and blue spots, red spots have relatively high "Red" values. White spots reveal high values for all colours and spots with an intermediate colour (yellow, orange) have low "Blue" values, but high "Red" and "Green" values (Tab. 2).

Tab. 3: 17 males used in the choice experiments ranked after the time passed until being climbed by the female (A, B, C), and increasing blue (D), green (F) and red (H) values (see Tab. 2) each followed by the corresponding rank for the time passed until being climbed by the female (E, G, I). Statistical analysis according to Spearman's rank correlation; $p < 0.05$ = significant; see Tab. 2)
Tab. 3: 17 Männchen aus den Wahlversuchen geordnet nach der Zeit, die bis zum Aufsteigen des Weibchens verstrichen war (A, B, C), nach zunehmenden Blau- (D), Grün- (F) und Rot-Anteilen (H) (vgl. Tab. 2) jeweils gefolgt vom zugehörigen Rang nach der Zeit, die bis zum Aufsteigen des Weibchens verstrichen war (E, G, I). (vgl. Tab. 2). Statistische Analyse mit Spearman's Rangkorrelationskoeffizient; $p < 0.05$ = signifikant) (vgl. Tab. 2).

A	B	C	D	E	F	G	H	I
Males	Time (min)	Rank Time	Rank Blue	Corr. Rank Time	Rank Green	Corr. Rank Time	Rank Red	Corr. Rank Time
XXIV	01:30	1	I	6	I	6	XXVI	8
XXIII	02:05	2	XXVI	8	XXVI	8	I	6
XXV	05:24	3	XXV	3	XXV	3	XXI	12
XIX	05:25	4	XXIII	2	XXIII	2	XXIII	2
VII	06:39	5	XX	9	XIX	4	XXV	3
I	07:44	6	VI	14	XXI	12	XIX	4
XVIII	07:44	7	XIX	4	XX	9	XVIII	7
XXVI	07:49	8	VII	5	VI	14	XVII	16
XX	08:29	9	XXI	12	VII	5	XX	9
IV	08:57	10	XXIV	1	XXIV	1	III	15
XXIX	10:14	11	XV	13	XVIII	7	VI	14
XXI	10.39	12	XVIII	7	XIV	17	XIV	17
XV	12:09	13	XVII	16	III	15	IV	10
VI	15:47	14	IV	10	XVII	16	XXIV	1
III	16:11	14	III	15	IV	10	XXIX	11
XVII	19: 37	16	XIV	17	XXIX	11	VII	5
XIV	24:02	17	XXIX	11	XV	13	XV	13
			r=0.57843; **p=0.015**		r=0.56618; **p=0.01782**		r=0.25; **p=0.33317**	

There was a significant negative correlation between the males' attractiveness and its "Blue" value (Spearman's rank correlation: $p = 0.015$; $r = 0.57843$) and "Green" value ($p = 0.01782$, $r = 0.56618$). There are no significant results concerning "Red" values ($p = 0.33317$, $r = 0.25$) (Tab. 3).

4. Discussion

The results of the present study suggest that females of *Lucihormetica verrucosa* (1) prefer males with red spots, and (2) recognize the red spots by their blue and green components and by the fact that they do not reflect UV.
Ad 1: When taking the time a female needed to climb the male for the first time as measure of attractiveness, females appeared to favour "red" males. Cockroaches have several traits that might attract the mates, i. e. short- or long-distance pheromones, dominance, size and secretions of the male's tergal glands (see Gemeno & Schal 2004; Bell et al. 2007). Precopulatory and copulatory behaviour of *L. verrucosa* correspond to the "type I mating behaviour". After meeting (more or less coincidentally) and mutual palpating, the male presents his dorsal surface by rising the wings, the female climbs on his back to palpate him and to nibble on the tergal glands (if present at all). Then the male backs up clasping her genitalia, the female rotates 180° and mates reach their final linear opposed position (see Greven & Zwanzig 2013). Climbing on the male and nibbling on his back can be interpreted as sign of interest, which, however, does not necessarily lead to copulation. In our experiments not in all cases couples reached the final mating position. However, in all cases coupling (clasping and/or final position) was relatively short, either interrupted by the mates themselves or by the observer, e.g. when the final position was reached only towards the end of the 30 min experimental

Fig. 2: „Red", „yellow", and „white" male of *Lucihormetica verrucosa*. Above: Conventional photo. Below: The same males from the "cockroach-perspective" (kindly created by Maryam Masrouri on the basis of the UV-photo, and the 2x green components of the conventional photo).
Abb. 2: „Rotes", „gelbes" und „weißes" Männchen von *Lucihormetica verrucosa*. Oben: Normalaufnahme. Unten: Aus „schabenspezifischer" Sicht (freundlicherweise erstellt aus dem UV-Photo und 2x den Grünkomponenten aus der Normalaufnahme von Maryam Masrouri).

observation time. Successful copulations usually take more than 20 min (Greven & Zwanzig 2013). With regard to the first phase of courtship, i.e. the encounter of the mates and mutual antennal contact, visual cues may be involved, whereas after climbing on the male other criteria become important for the female.

Ad 2. The properties of the visual system in glow spot cockroaches are unknown. In other cockroach species, such as the nocturnal *Periplaneta americana*, the visual system is highly adapted to low light levels (e.g. Heimonen et al. 2016). This species (Mote & Goldsmith 1970; Paul et al. 1986), *Blatta orientalis* (Mazokhin-Porshnyakov & Cherkasov 1985) and *Blattella germanica* (Koehler et al. 1987) possess only UV and green types of photoreceptors. Assuming that also the largely nocturnal cockroaches of the genus *Lucihormetica* (see also Zompro & Fritzsche 1999; Vršanský, et al. 2012; Dittrich et al. 2015) have only these two types of photoreceptors, our results suggest that the blue and green ranges of wavelengths provide significant information about the colour of the pronotal spots. The green type of photoreceptor possesses a maximal sensitivity around 500 nm (Mote & Goldsmith 1970; Paul et al. 1986; Mazokhin-Porshnyakov & Cherkasov 1985; Koehler et al. 1987) and is thus most probably sensitive to perceive this information. The amount of reflection in the UV range of wavelengths (Beckert et al. 2017) could be an additional source of information to distinguish between differently coloured individuals. In addition, after illuminating the spots with UV-light, fluorescence appears in the green range of wavelengths and thus could improve the signal in the green range of wavelengths. Altogether, the cockroaches may be able to receive information about the pronotal spot colour using their green receptors suggesting that they distinguish differently red males through the presence of green in the reflection spectrum of the spots; red spots may be seen as dark gray. False-colour photography shows that the white spots are much more conspicuous for cockroaches than the red ones (Fig. 2). In our choice experiments females preferred males with a lower value of green in the spots, which corresponds to the most heavily red spot colour for the human eye. Further analysis shows a significant correlation between the amount of green reflection and the suspected attractiveness of the males.

Generally, we think that the colour of the pronotum spots is of particular significance for glow spot cockroaches despite their likely red-blindness. The relevance of the pronotum spots is underlined by their exposed location, and their strong dependence on the diet. This has previously led to the assumption the spots might be important for intra- and/or intersexual selection to inform females about the quality of potential mates and males about the quality of rivals via their carotinoid content (discussed in detail by Greven & Zwanzig 2013). This will require that the cockroaches may differentiate between "white" and "red" males, which, as shown above, appears possible indirectly with cockroach-subjective conspicuous spots being a stain. Importance of the spots for intra- and/or intersexual selection does not exclude other functions. Although not explicitely mentioned in the literature, natural enemies of the glow spot cockroaches are certainly various amphibians, squamates, birds and mammals, but it is hard to believe that these spots act as an aposematic signal for these predators (see also Vršanský et al. 2012). The assumption that glow spot cockroaches mimic the poisonous click beetle *Pyrophorus nocticula* that has similar truly bioluminescent pronotal spots (Vršanský et al. 2012), is ill founded, all the more as true bioluminescence, i.e. production and emission of light through chemical means, appears still unsubstantiated in glow spot cockroaches (see the discussion in Vršanský et al. 2012; Vršanský & Dušan 2013; Merritt 2013; Greven & Zwanzig 2013). However, during the day glow spot cockroaches might appear similar to predatory animals as bioluminescent click beetles, since then the bioluminescent spots are not active.

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BSc Jana Beckert
Prof. Dr. Klaus Lunau
Institut für Sinnesökologie
Department Biologie
Heinrich-Heine-Universität Düsseldorf
Universitätsstr. 1
D-40225 Düsseldorf
E-Mail: Klaus.Lunau@hhu.de

Prof. Dr. Hartmut Greven
Department Biologie
Heinrich-Heine-Universität Düsseldorf
Universitätsstr. 1
D-40225 Düsseldorf
E-Mail: grevenh@uni-duesseldorf.de