RESEARCH NOTE

Incidence of the thermal transition in the range of 45-5°C in chromatophores present in the wings of *Schistocerca americana* [version 1; peer review: 1 approved, 2 approved with reservations]

María Belén Cañizares [1], Nathaly Naranjo[1], Bence Mátyás [2]

[1]Universidad Central del Ecuador, Cayambe, Ecuador
[2]Grupo de Investigación en Ciencias Ambientales (GRICAM), Universidad Politécnica Salesiana, Quito, Ecuador

v1 **First published:** 27 Feb 2019, **8**:223 (https://doi.org/10.12688/f1000research.18314.1)
Latest published: 27 Feb 2019, **8**:223 (https://doi.org/10.12688/f1000research.18314.1)

Abstract
The variation of the color intensity of the chromatophores present in the wings of *Schistocerca americana* was analyzed by exposing 31 specimens to thermal transitions within the range of 45 - 5 °C. The adult specimens were collected using a mini-terrarium of dimensions 40x40x30 cm. As a substrate, a layer of soil, stones, and finally a layer of grass were used along with branches of bushes and leaves; hydroponic lettuce, cabbage and the grass were used as food for the specimens. Optical microscopy of the wings of the insects was used for live observation without coverslips or contrasting substances. At 45°C, degradation of color intensity was observed in the chromatophores present in the wings. At 5°C, chromatophores intensify their color to brownish-black. This temperature was the extreme minimum that *S. americana* could tolerate. We found negative correlation between the temperature and the degree of darkness (R2 = 0.8038). Our results are in accordance with a previously published study in which *Phaulacridium vittatum* was examined, as the decrease of temperature caused darkening color change in melanin-type chromatophores. The present investigation can be considered as the first initial study of its kind for *S. americana*, in terms of examining the changes in the color intensity of the chromatophores present in the wings caused by thermal transition under laboratory conditions.

Keywords
Schistocerca americana, Thermal transition, Chromatophores, Melanism, Pigmentary coloration

Open Peer Review

Referee Status: ? ? ✓

	Invited Referees 1	2	3
version 1 published 27 Feb 2019	? report	? report	✓ report

1 **Ana Danitza Peñafiel-Vinueza**, Pontificia Universidad Católica del Ecuador, Ecuador

2 **David Terán**, University of Queensland, Australia
Universidad Técnica de Ambato, Ecuador

3 **Daniel A. Lowy**, VALOR HUNGARIAE Budapest, Hungary
NOVA University, USA

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Bence Mátyás (bmatyas@ups.edu.ec)

Author roles: Cañizares MB: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Supervision, Visualization, Writing – Original Draft Preparation; **Naranjo N**: Data Curation, Investigation, Methodology, Project Administration, Validation, Visualization, Writing – Original Draft Preparation; **Mátyás B**: Conceptualization, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

How to cite this article: Cañizares MB, Naranjo N and Mátyás B. **Incidence of the thermal transition in the range of 45-5°C in chromatophores present in the wings of *Schistocerca americana* [version 1; peer review: 1 approved, 2 approved with reservations]** F1000Research 2019, **8**:223 (https://doi.org/10.12688/f1000research.18314.1)

First published: 27 Feb 2019, **8**:223 (https://doi.org/10.12688/f1000research.18314.1)

Introduction

Melanism, the occurrence of darker pigmentation in specimens within species is well-known in insects[1,2]. It is also known that chromatophores to those cells present in the animal integumentary system containing pigments, and these cells respond to hormonal factors and/or neuronal (response to stimuli) factors[2]. There are several studies focusing on why melanism has evolved, and a hypothesis about this is known as thermal melanism hypothesis[2,3]. This states that at low temperatures, darker insects are at an advantage compared to light ones because they warm up more quickly. There are several studies that support the thermal melanism hypothesis related to butterflies[3–5] and hoverflies[6].

Insects have a chemical or pigmentary coloration represented mainly by carotene and melanin, which are found in the cuticle, hypodermis or sub-hypodermis[7]. In the exocuticle, it is more common to find the variants of carotene and melanin as yellow, brown and black colorings, and in the hypodermis as yellow, red, green and orange colorings[8]. Adult *Schistocerca americana* have wings with large brown spots (melanin) on a light-colored background[9]. The study of the Arthropoda phylum and its Insecta class is highly wide-spread, however, the majority of the studies regarding grasshoppers' behavior under different temperature conditions, focus on habitat selection only[10,11].

The main objective of this study was to analyze the effect of thermal transition on the intensity of the color of the chromatophores present in the wings of the species *S. Americana.* This study was inspired by the observation in the literature[3–6] that temperature has a direct effect on change and/or color variations of the chromatophores located in other species and phyla of the Animalia kingdom.

Methods

Specimen collection

A total of 31 adult *S. americana* were collected by hand according to Harris *et al.* 2013[3] in Tumbaco- Quito Ecuador (GPS coordinates: 0°13'19.1"S 78°22'18.2"W) in May, 2017. No permits were needed for collecting the specimens considering that the S. *americana* does not appear in the red list (IUCN), and the sampling site is not located in a protected area. Nevertheless, prior consultation was carried out with the local authorities (Mayor's office and Ministry of Environment of Ecuador). All efforts were made to ameliorate any suffering to the animals following the protocol described by the Ecuadorian code of practice for the care and use of animals for scientific purposes of the Ministry of Environment of Ecuador.

Housing of specimens

Three terrariums were used in the experiment: one with the dimensions 40x40x30 cm and two with the dimensions 70x70x50 cm (Figure 1A). The first terrarium was used for the collection of specimens in the and the others of greater longevity for the creation of an adaptation habitat and for experimentation, respectively. As a substrate, a layer of soil (approx. 4cm) collected in the same location, one layer of stones (approx. 2 cm), and finally a layer of grass (approx. 2cm) was used along with branches of bushes and leaves (Figure 1A). A plastic mesh was used to cover the terrariums. Hydroponic lettuce, cabbage and the grass were used as food for the specimens.

Thermal transition

To ensure adaption from nature to laboratory conditions, 25°C was applied for 7 days before the heat treatments. To observe the color changes, in the laboratory, the specimens were exposed to temperatures of: 45°C, 40°C, 35°C, 30°C, 25°C and 5°C for which a system of five 100w lightbulbs were used as a source of heat. Every two days a light bulb was switched off in order to ensure the decrease of temperature. For low temperature (5°C) all the lights were turned off in the system and dry ice was placed directly on the substrate.

Data collection

For the microscopic observation, the specimens were sacrificed at -4°C, and the primary wings were removed from the insects

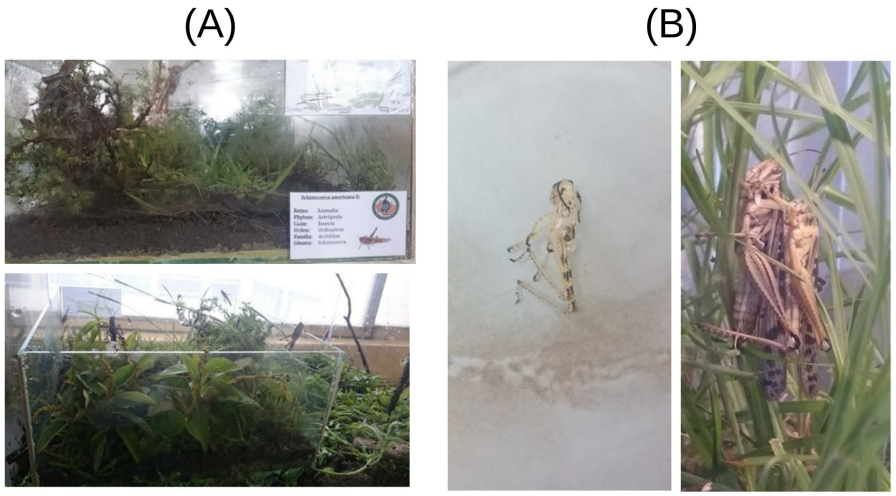

Figure 1. Terrariums used in the study and evidence of adaption. (**A** top) Mini terrarium for collection; (**A** bottom) habitat for the specimens during the experiment; (**B** left) Evidence of adaptation, molt; (**B** right) Evidence of adaptation, reproduction.

using entomological tweezers, avoiding tearing them, at room temperature according to the method by Paredes *et al.*[12]. Optical microscopy (Carl Zeiss, model: Laboval 4) was used for live observation of the color change without coverslip or contrast.

For color interpretation, we used hexadecimal classification by selecting the wing spot areas using iVinci Express v.4.6 on a 32 scale where the degree of darkness is interpreted in percentage using 0to255 software tool (see the color codes used in the analysis in *Underlying data*[13]). Linear regression was applied for analyzing the correlation between the temperature and the degree of darkness in Microsoft Office 365.

Results and Discussions

Adaption to the terrarium environment was considered successful as after a one-week period the specimens began molting (Figure 1B left) and reproducing (Figure 1B right).

At 45°C, degradation of color intensity can be observed in chromatophores present in the wings (Figure 2A). At 40°C chromatophores of the melanin-type begin to darken (Figure 2B); however, between the temperature of 45°C and 40°C there are no huge differences in color change. At 35°C chromatophores of the wings take a light brown color, intensify their color (Figure 2C). At 30°C the chromatophores turn dark brown, indicating an increase in their color intensity (Figure 2D). A temperature of 25°C corresponds to the optimum temperature for the normal development of the species in the region[14] (Figure 2E). This is the reference color of the chromatophores used for all analysis. At 5°C chromatophores of the melanin-type intensify their color to brownish-black (Figure 2F). This temperature was the extreme minimum that *S. americana* could tolerate.

Temperature, precipitation and solar radiation are the meteorological elements that most affect the distribution, rate of growth, reproduction, migration and adaptation of insects[15]. The thermal melanism in tests with *Phaulacridium vittatum* and its exposure to the heat of lights within the range 300 to 700 nm represented an intensification in its color with a variation in percentage of between 2.49% to 5.65%[3]. The majority of the studies that examine the thermal melanism have focused on species with very distinct color morphs representing a wide range in melanism.

We found negative correlation between the temperature and the degree of darkness (R2 = 0.8038). Our results are in accordance with a previously published study[3] in which *P. vittatum* was examined - a decrease of temperature caused darkening color change in melanin-type chromatophores.

Conclusions

The present investigation can be considered as the first initial study of its kind for *Schistocerca americana*, in terms of examining the changes in the color intensity of the chromatophores present in the wings caused by thermal transition under laboratory conditions. The color change can be considered as an indicator of a reaction to the increase or decrease of temperature[2,3] as the clear surface (in this case the wings) reflects the radiation emitted by the lightbulbs and thus absorb less heat, while in the case of a decrease in temperature, the wings absorb more heat.

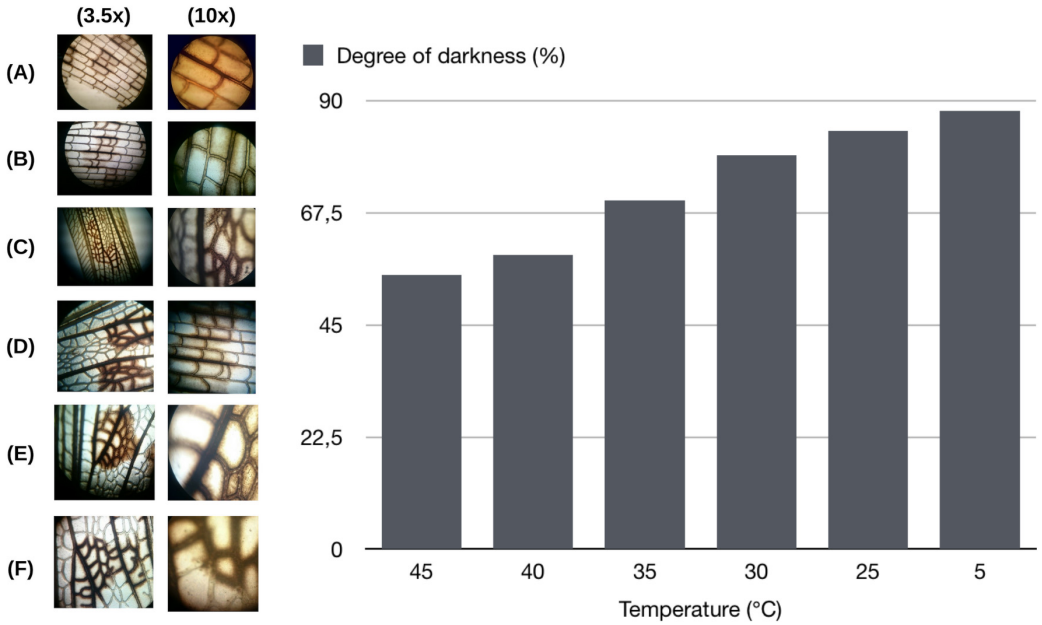

Figure 2. Results of the incidence of the thermal transition in the range of 45–5°C in chromatophores present in the wings of *Schistocerca americana*. Chromatophores (melanin) at a temperature of (**A**) 45 ºC; (**B**) 40 ºC; (**C**) 35ºC; (**D**) 30ºC; (**E**) 25 ºC; (**F**) 5ºC. Magnifications: 3.5 x (left), and 10x (right).

Data availability

Underlying data

Figshare: Microscopy photos of the wings. https://doi.org/10.6084/m9.figshare.7749404.v1[16]

Figshare: Raw data for Figure 2. https://doi.org/10.6084/m9.figshare.7749395.v1[13]

Grant information

The author(s) declared that no grants were involved in supporting this work.

References

1. Majerus MEN: **Melanism: Evolution in Action.** Oxford University Press. 1998.
Reference Source
2. Sadava D: **Vida, la ciencia de la Biología.** Octava ed., Buenos Aires: Médica Panamericana. 2009.
Reference Source
3. Harris R, McQuillan P, Hughes L: **A test of the thermal melanism hypothesis in the wingless grasshopper *Phaulacridium vittatum*.** *J Insect Sci.* 2013; **13**(1): 51.
PubMed Abstract | Publisher Full Text | Free Full Text
4. Clusella-Trullas S, van Wyk JH, Spotila JR: **Thermal melanism in ectotherms.** *J Therm Biol.* 2007; **32**(5): 235–245.
Publisher Full Text
5. Lewis JE: **Temperature Induced Seasonal Melanism in the Wings of *Copaeodes minima* (Lepidoptera: Hesperiidae).** *Fla Entomol.* 1985; **68**(4): 667–671.
Publisher Full Text
6. J Holloway G, Marriott CG: **Colour Pattern Plasticity in the Hoverfly, *Episyrphus balteatus*: The Critical Immature Stage and Reaction Norm on Developmental Temperature.** *J Insect Physiol.* 1998; **44**(2): 113–119.
PubMed Abstract | Publisher Full Text
7. Karl I, Geister TL, Fischer K: **Intraspecific variation in wing and pupal melanization in copper butterflies (Lepidoptera: Lycaenidae).** *Biol J Linn Soc.* 2009; **98**(2): 301–312.
Publisher Full Text
8. De la Cruz Lozano J: **ENTOMOLOGÍA, MORFOLOGÍA Y FISIOLOGÍA DE LOS INSECTOS.** Palmira: Universidad Nacional de Colombia. 2006.
Reference Source
9. Continente: **[En línea].** 2014.
Reference Source
10. Bonello S, Piskorowski K, Spotts A: **The effects of habitat color and surface temperature on habitat selection of various *Melanoplus* grasshopper color morphs.** University of Michigan Biological Station. 2017.
Reference Source
11. Ahnesjo J, Forsman A: **Differential Habitat Selection by Pygmy Grasshopper Color Morphs; Interactive Effects of Temperature and Predator Avoidance.** *Evol Ecol.* 2006; **20**(3): 235–257.
Publisher Full Text
12. Paredes E, Márquez G, Juárez MP, *et al.*: **Quimiotaxonomía de *Triatoma longipennis* por Análisis de Hidrocarburos Cuticulares.** INVURNUS. 2008; **3**(2): 3–10.
Reference Source
13. Cañizares MB, Naranjo N, Mátyás B: **Raw data for Figure 2.** *figshare.* Dataset. 2019.
http://www.doi.org/10.6084/m9.figshare.7749395.v1
14. Murriagui Ibarra M: **EXPANSIÓN URBANA Y DEMANDA DE TRANSPORTE PÚBLICO DE BUSES: CASO DE ESTUDIO PARROQUIAS DE CUMBAYÁ, TUMBACO Y PUEMBO.** [En línea]. 2016.
Reference Source
15. Retana J: **Relación entre algunos aspectos climatológicos y el desarrollo de la langosta centroamericana *Schistocerca piceifrons* piceifrons en el Pacífico Norte de Costa Rica durante la fase cálida del fenómeno El Niño-Oscilacion Sur (ENOS).** Instituto Metereológico Nacional. 2000; **7**(2): 73–87.
Reference Source
16. Cañizares MB, Naranjo N, Mátyás B: **Microscopy photos of the wings.** *figshare.* Figure. 2019.
http://www.doi.org/10.6084/m9.figshare.7749404.v1

Open Peer Review

Current Referee Status: ? ? ✓

Version 1

Referee Report 15 April 2019

https://doi.org/10.5256/f1000research.20033.r47128

✓ **Daniel A. Lowy** [1,2]

[1] VALOR HUNGARIAE Budapest, Budapest, Hungary

[2] NOVA University, Alexandria, VA, USA

The authors report their study on the variation of color intensity of the chromatophores present in the wings of *Schistocerca americana.* For this, 31 specimens were subjected to thermal transitions within a range of decreasing temperatures, from 45 to 5°C. Darkening and intensifying of color was revealed by optical microscopy of the insect wings.

The reviewer believes that this study represents a novelty and is of interest to the scientific community. The authors' approach may have an impact in the monitoring of local climate changes. The manuscript is well written, has a good flow, and its English style is good. The observations listed below are intended to serve for further improving the quality of the paper.

Observations regarding the content:

1. Please, add to the Abstract, as well, the following statement of the Conclusion: "The color change can be considered as an indicator of a reaction to the increase or decrease of temperature as the clear surface (in this case the wings) reflects the radiation emitted by the light bulbs and, therefore, absorb less heat, while at decreasing temperature, the wings absorb the more and more heat."

2. Please add to the Introduction: "It is believed that the detected color in natural environment may become an indicative of local climate change." The reviewer believes that instructing school students to survey the color of insects may represent a statistical means to reveal possible micro changes in climate.

3. Three additional references would be welcome: (i) at the end of section on Specimen collection: Reference (or web site) for the protocol of the Ministry of Environment of Ecuador; (ii) for iVinci Express v.4.6. (iii) for 0to225 software.

4. Housing of specimens – the second sentence is incomplete: "...the collection of specimens in the (???) and the others of greater longevity...etc."

Suggestions for style improvements:

Introduction:

- The following sentence reads difficultly: “It is also known that chromatophores (to those) OF cells present in the animal integumentary system containing pigments, (and these cells) respond to hormonal factors and/or neuronal (response to stimuli) factors[2].” Please, delete the words in parentheses and add "OF".

- Last sentence of paragraph 1: "insects are at (an) advantage...because they ABSORB SOLAR LIGHT MORE EFFICIENTLY, SO THAT THEY warm up more quickly." Please, delete the words in parentheses and add the capitalized part for more accuracy.

- Paragraph 3: ...by the observation REPORTED in the literature (3-) etc.

Thermal transition:

- The temperature was set to 25°C (rather than "was applied")...were exposed to DECREASING temperatures of 45°C, 40°C, etc.

Results and Discussions:

- ...at 45°C and 40°C there are no (huge) SIGNIFICANT differences etc. At 35°C chromatophores of the wings become light brown, intensifyING their color (rather than "intensify").

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Analytical chemistry

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 09 April 2019

https://doi.org/10.5256/f1000research.20033.r45936

?

David Terán [1,2]
[1] School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Qld, Australia
[2] Universidad Técnica de Ambato, Ambato, Ecuador

Incidence of the thermal transition in the range of 45-5°C in chromatophores present in the wings of *Schistocerca Americana*

The manuscript presented by Cañizares and co-authors reports the effect of temperature (radiant heat) in wing chromatophores of *S. americana*. There are other publications about this topic, and the present manuscript is in accordance of reported results. However, the manuscript requires addressing of different issues to be accepted. The suggestions are the following:

Introduction:

- The citations 11-12 could have a peer review source.
- The description of the natural environment of *S. americana* is missing. This information will enrich the description of the hypothesis of the manuscript.

Material and methods:

- The description of house-keeping is not clear.
- The range of temperatures is between 45-5˚C however there is a gap between 25-5˚C.
- There is no statement about how the temperature was controlled.
- There is no statement of how long the *S. Americana* was kept at certain conditions.

Results and discussion:

- It's true that "Temperature, precipitation and solar radiation are the meteorological elements that most affect the distribution, rate of growth, reproduction, migration and adaptation of insects" as stated in paragraph 3 lines 1-3. However, are the authors sure that they can mention this in a very short time of exposure in their experiment?
- The last paragraph about the negative correlation requires more discussion; as it is the most interesting part of the paper.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Biology, ecology, medical entomology, biochemistry, structural biology, drug discovery

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Referee Report 14 March 2019

https://doi.org/10.5256/f1000research.20033.r45030

? **Ana Danitza Peñafiel-Vinueza**
Laboratorio de Genética Evolutiva, Facultad de Ciencias Exactas y Naturales, Pontificia Universidad Católica del Ecuador, Quito, Ecuador

The presented research note addresses the effect of temperature on melanin pigment of wing chromatophores of *S. americana* by manipulating radiant heat. Despite the results found being in accordance with previous studies, there are a number of issues with the methods and results that need to be clarified. I present more specific comments, as follows:

Introduction:

- It would be helpful to have more information about the ecology and distribution of *S. americana*, to enrich the introduction and to correlate with the conclusions.

Methods:

- Housing of specimens: "first terrarium used for the collection of specimens in the (_) and the others…" – I think you wanted to state “the first terrarium used for the collection of specimens in the field”, if this is right and since the specimens were collected by hand, I understand that this first terrarium was used for carrying the specimens from the field to laboratory.

- Thermal transition: Not clear why, if thermal transition ranged from 45° to 5°C, the temperatures tested did not include the ranges from 20° to 10° and instead went directly from 25° to 5°C. Since the distribution of *S. americana* includes places where the temperature occurs in the range that is missing, testing these temperatures could complete the overview of thermal effects from the whole 45° to 5°C range. Otherwise, please clarify and state your reasons.

- If temperature is induced by manipulating radiant heat to reach the range 45° to 25°C, why did you use dry ice to induce the 5°C temperature? The methodology used in the laboratory should be homogeneous, otherwise it can increase variability in results.

- Data collection: How many wings were analyzed for each treatment? The number should be stated here, it seems that only one wing was analyzed for each treatment.

Results and Discussions:

- The statement “the majority of the studies that examine the thermal melanism have focused on species with very distinct color morphs representing a wide range in melanism” (third paragraph, line 7-9). Is taken almost exactly as is stated by Harris *et al.* (2013[1]) (reference #3 in your manuscript) in their conclusions.

- A discussion about how the negative correlation between temperature and pigmentation that you have found, relates with the adaptation to the environment where *S. americana* lives.

References

1. Harris RM, McQuillan P, Hughes L: A test of the thermal melanism hypothesis in the wingless grasshopper Phaulacridium vittatum.*J Insect Sci*. 2013; **13**: 51 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
No

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Entomology, Ecology, Genetics, Taxonomy

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.