ELECTRONIC SUPPLEMENTARY MATERIAL 3

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Correlation between the observer's assignment and the real colour of grasshoppers

To estimate the blackness of Azure sand grasshoppers (*Sphingonotus azurescens*) in the field, we compared each individual to a 100-level grey scale (Figure S2). To determine the validity of this method, we retrospectively examined the correlation between the observer's assignment and the real colour (brightness) of grasshoppers using a sample of 32 adult individuals originating from the same population.

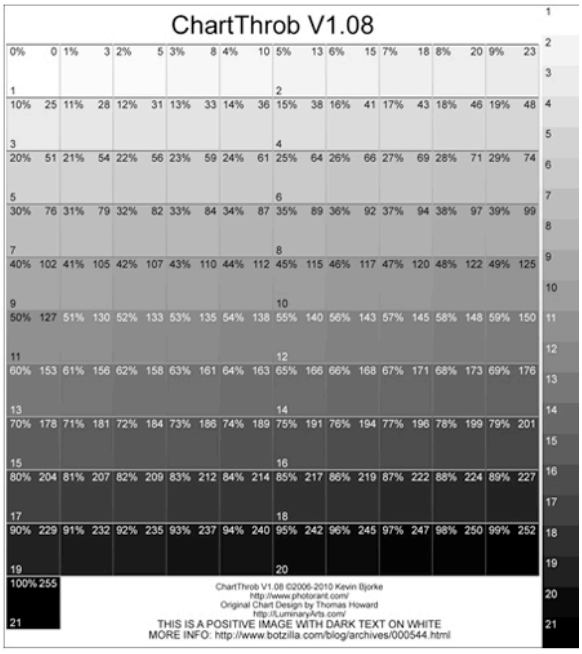

Figure S2. Grayscale chart used for visual determination of grasshopper colour (% blackness) in the field, as generated using the tool 'ChartThrob' of Adobe Photoshop (https://www.adobe.com/).

To measure the colour of these individuals objectively, we took standardized digital photographs from a fixed distance with a Pentax K-r camera mounted on a tripod and equipped with a Pentax 18-55mm zoom lens and a dual flash with diffusers. For all pictures, we used fixed camera settings (55-mm zoom, f = 14, shutter speed = 1/50, ISO = 200) and flash settings inside a closed box covered internally with black velvet. Following Hochkirch et al. (2008), individuals were immobilized by pressing them down with a clean and transparent plastic lid into a Petri dish filled with cotton wool, such that the dorsal part of

their pronotum was parallel to the front of the camera lens. In addition, we used an 18% grey card as a reference reflectance standard for subsequent image correction.

For colour measurements, we defined a fixed diamond-shaped polygon in the posterior part of the pronotum, which is representative of overall grasshopper colour. We then measured the brightness of this area as the percentage of reflectance (with respect to the reflectance standard) by extracting linearized and normalized images of RGB from the RAW format files with the software ImageJ (Schneider et al. 2012) and the Mica Toolbox version 1.11, following Troscianko & Stevens (2015). Because a human determined darkness of grasshoppers in the field, brightness was calculated for the spectral sensitivity of humans (Hofer et al. 2005). This measurement of brightness of the sample of 32 grasshoppers (by A.B-V) was done independent with respect to darkness measurements using the 100-level grey scale (by A.S-F).

RESULTS

There was a significant correlation between the observer's assignment and the objectively measured brightness of grasshoppers (spearman rank correlation test, $r_s = -0.614$, $P = 0.0002$), supporting the validity of the method used to estimate individual colouration in the field (Figure S3).

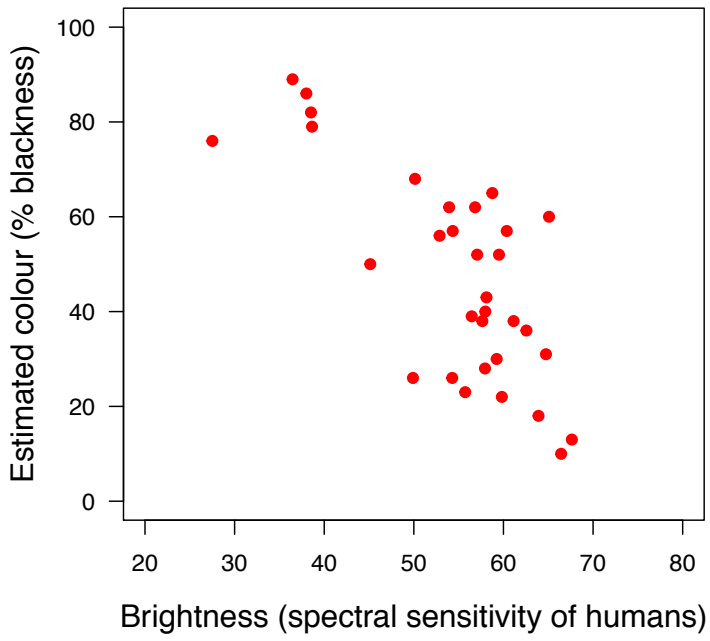

Figure S3. Relationship between estimated colour of grasshoppers through visual scoring of % blackness and measured brightness from standardized digital photographs.

References

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