Phenotypic plasticity in color without molt in adult grasshoppers of the genus *Sphingonotus* (Acrididae: Oedipodinae)

Juan Ramon Peralta-Rincon[1], Graciela Escudero[1], Pim Edelaar[1]

1 Universidad Pablo de Olavide, Seville, Spain.

Corresponding author: *Pim Edelaar* (edelaar@upo.es)

Academic editor: *Corinna Bazelet* | Received 19 December 2016 | Accepted 23 April 2017 | Published 28 June 2017

http://zoobank.org/66D9F013-B62E-4817-9A58-AA4FCE3A2905

Citation: Peralta-Rincon JR, Escudero G, Edelaar P (2017) Phenotypic plasticity in color without molt in adult grasshoppers of the genus *Sphingonotus* (Acrididae: Oedipodinae). Journal of Orthoptera Research 26(1): 21–27. https://doi.org/10.3897/jor.26.14550

Abstract

Homochromy (i.e. that individuals have a similar color as their environment) is frequent in grasshoppers, and probably functions to reduce detection by potential predators. Nymphs of several soil-perching grasshopper species are known to show color changes during development that increase homochromy, with color being determined with each molt. While this is well documented for young individuals, the only color change in response to the environment that has been recorded for adult grasshoppers of these species is an overall darkening of the individual when exposed to dark surfaces. Whether grasshoppers can also adaptively change color hue is relevant for our understanding of the evolution of locally adapted crypsis. We therefore exposed two groups of adult grasshoppers to a bluish-gray substrate or a reddish-brown substrate, and recorded their color over time. Quantitative digital image analysis showed that adult soil-perching grasshoppers remained capable of adapting to changes in the color of their surroundings through a plastic response. Compared to nymphs, the changes are not as strong and much slower. We suggest that color change in adults occurs through the ongoing deposition of melanins, with eumelanin making individuals more bluish-gray and pheomelanin making individuals more reddish-brown. The fact that color change is possible but slow supports that other mechanisms, such as habitat choice or selective predation, may also play a role in adapting local populations to substrate color. In addition, the ability of these grasshoppers to produce different melanins in response to the environment supports a previous suggestion that they might be useful in the future development of animal models to study melanin-related diseases like melanoma and Parkinson´s disease.

Key words

homochromy, crypsis, color change, image analysis, habitat choice, pheomelanin, eumelanin

Introduction

Crypsis is a well known anti-predation mechanism observed in a wide variety of species. It is common among plant- and ground-dwelling insects such as mantises, phasmids, grasshoppers and bush crickets. Adaptive phenotypic plasticity (the ability of a single genotype to change its phenotype in res ponse to environment cues) is often key to optimizing crypsis in animals whose habitat is heterogeneous through space or time (Umbers et al. 2014, Valverde and Schielzeth 2015, Kang et al. 2016). For instance, for grasshoppers four types of color change have been recorded: green-brown morph switching, color pattern changes, hue shifting and blackening (Rowell 1972). While color pattern has often been found to be determined mostly by genes and maternal effects (Karlsson et al. 2009, Forsman 2011, Karpestam et al. 2012b), hue variation is thought to be driven to a great extent by an adaptive plasticity response to environmental cues, as the new overall color tends to match that of the subject's surroundings (Ergene 1955, Yerushalmi and Pener 2001, Valverde and Schielzeth 2015). There is also evidence of blackening in response to temperature and exposure to solar radiation as well as dark substrates (Forsman 2011, Karpestam et al. 2012a, Valverde and Schielzeth 2015). Green-brown morph determination and switching remains the less understood of the four types of color change but there is positive evidence for both genetic and environmental influences (Rowell 1972, Valverde and Schielzeth 2015). The environmental cues that are used for adaptive plasticity to enhance crypsis are often unknown, but effects of temperature, humidity and/or visual input have been recorded (Rowell 1972, Umbers et al. 2014, Valverde and Schielzeth 2015).

The family Acrididae (which contains amongst others the band-winged grasshoppers and locusts) is the most studied group of grasshoppers. This is partly because it is the largest and most widespread family, can be responsible for tremendous agricultural losses, and is used in many countries for human consumption. They often show homochromy, and those species that perch on the ground tend to strikingly resemble the color of their local substrate. In his revision of this family's variable coloration, Rowell (1972) proposed three non-exclusive causes for this local adaptation in color: differential predation, color change, and habitat selection. He deemed the first one immediately acceptable, and additive effects of the other two seemed logical to attain an almost perfect color match. However, studies regarding the color change in Acrididae grasshoppers have normally been conducted with nymphs or, at most, recently metamorphosed adults (Valverde and Schielzeth 2015) while fully developed imagoes, though indeed cryptic, have not received as much attention.

Apart from neglect of plasticity in adults, another problem with most earlier studies is that assessments of color change have been

done rather subjectively, either assigning individuals to discrete arbitrary color levels or comparing the subject's color to standard charts which do not allow true quantitative analyses. To overcome these limitations, when possible, it is preferred to use objective digital image analysis as a less biased approach (Stevens et al. 2007).

We have used this approach to study the color matching of azure sand grasshoppers, *Sphingonotus azurescens* (Rambur) (Orthoptera: Acrididae: Oedipodinae) colonizing distinctly colored novel urban habitats in Seville, Spain. While this species naturally occurs on open sand or clay soils with little to no vegetation, we have recently found them to locally also use abandoned man-made surfaces (sidewalks, bicycle paths, asphalt roads) for perching, feeding, courtship, and reproduction. We found that individuals using these pavements were consistently more cryptic on their local pavement than on other, adjacent pavements. This shows that these grasshoppers are able to adapt their color to fine scale environmental variation, even when it involves novel materials (asphalt, bricks, tiles) that historically they have not interacted with (Edelaar et al. in preparation). Given the degree of daily movement of these grasshoppers (on average 12 meter/day, Edelaar et al. in preparation), this observed fine scale population differentiation in color among pavements should quickly cease to exist, unless something helps to maintain color divergence. Surprisingly, previous studies show that this appears to be mainly due to habitat choice, since selective predation and color change appeared to be too weak and too slow, respectively, to explain the observed patterns (Edelaar et al. in preparation). With respect to color change, this conclusion rests on the observation that the response to a black background is limited and slow, and virtually nonexistent when a white background is used (Edelaar et al. in preparation, see also Ergene 1953). However, the different pavements do not only differ in darkness but also in hue, and it has not yet been tested whether changes in hue in response to background color are possible in adult azure sand grasshoppers, nor in any other grasshopper species.

There is an additional reason why color plasticity in these adult grasshoppers deserves a closer look. The reddish color displayed by azure sand grasshoppers has recently been reported to be related to the presence of pheomelanin, a pigment formerly thought to be restricted to vertebrates only. Interestingly enough, this pigment's pathway features mixed-type melanins arising from both dopamine and DOPA, a process that in vertebrates has only been reported for neuromelanin (Galván et al. 2015). This is important because of the link between neuromelanin and Parkinson's disease. Grasshopper (and maybe other insect) species with this kind of biochemical pathway may therefore play a role in the future investigation of this pigment's link to this important disease (Galván et al. 2015). Being able to change the production of pheomelanin in adult grasshoppers by a change in background might therefore be very useful.

For this variety of reasons, our main objective in this study was therefore to test whether this species of acridid grasshopper is still capable of changing in hue in response to background color, even after reaching full maturity.

Methods

Study subject.— The azure sand grasshopper is a ground dwelling species which predominantly inhabits little vegetated, xeric scrublands and grasslands and perches on the bare soil rather than on vegetation. They show a base coloration that varies from reddish-brown to bluish gray, and these colors can vary from being very pale to very dark. Color variation is continuous and there is no known green-brown polymorphism in this species. Their base color generally resembles that of the substrate surrounding the individual. They also show a variable pattern of dark markings, and a pale band halfway down the anterior pair of wings and the hind legs which helps to disrupt their outline and therefore provides additional crypsis (Fig. 1).

Figure 1. Example of an image taken for color measurement. The individual was held in place by the transparent plastic lid of the Petri dish to obtain a correct position. Brightness and hue were measured in the red diamond-shaped part of the thorax. The color of a small area of the background paper was also measured (red circle) as a reference gray standard to correct for lighting variation among images.

Nineteen individuals were collected in late August and early September 2013 in the vicinity of Dos Hermanas (Seville, Spain) and kept as a group for two months in one of two transparent plastic boxes (30 × 40 cm). These boxes were each filled with either "blue" (fine bluish gray gravel) or "red" (red-brown earth) substrate, in order to test if adult grasshoppers would change their color accordingly. Individuals were either assigned to the treatment "blue" (10 individuals - 8 males and 2 females) or "red" (9 individuals - 5 males and 4 females). Individuals were marked by writing a number on their anterior wing tips with a water- and light-resistant marker pen. Food was a mixture of dried wheat bran (45%), dried mosquito larvae (45%), and powdered milk (10%) (the species is omnivorous). Bottled mineral water was available in the form of a gel (ReptiGel). Ambient light was provided by regular fluorescent ceiling lamps from 8:00 to 20:00 hours, and heat was provided from below using heating mats for terrariums in order to keep ambient temperature between 35 and 40 degrees Celsius.

Image production and color measurement.— At the beginning of the experiment and for the duration of the treatments, we regularly took photographs of each individual to subsequently measure its color (Fig. 1). The experiment was terminated after 49 days, when

most individuals had died of old age. In order to create comparable images, all photographs were taken using the same setup: a Pentax K-r DSLR camera with a dual lamp Mecablitz 15MS-1 diffuser macro flash, mounted on an 18-55mm Pentax kit lens. The lens was always locked at 55mm and the camera set to shoot in RAW format with fixed manual settings (f/14 aperture, 1/50 shutter speed, ISO 200). Each image featured a 90% reflectance gray paper background to control for white balance and flash intensity (Fig. 1). The grasshoppers were held in the same position (offering a full dorsal view, with the head pointing to the top of the image) and at the same distance from the lens by pressing them into a base of cotton wool with the transparent plastic lid of a Petri dish. A diamond-shaped zone from the posterior part of the grasshoppers' pronotum was used to measure color over time for each individual, as this area is flat and representative for overall individual color (Fig. 1).

Any color difference between grasshoppers from different treatments could also be due to soiling of the individuals with fine dust from the substrates. In fact, this happened in a first trial with finely mashed up red soil, so we restarted this treatment three weeks later with newly collected individuals from the same area using intact, natural pieces of red soil (which has caused a shift in the timing of sampling for each treatment and may account for differences in color between treatments at the beginning of the experiment, since older grasshoppers tend to be darker, see Fig. 2). We therefore thoroughly cleaned all subjects which were still alive when the experiment was finished and compared the color change between treatments ("Cleaned" dataset, N=5). To clean the grasshoppers, individuals were first frozen to rapidly kill them, and then rubbed with a piece of cotton dipped in water with dishwashing detergent and cleared under running water. Images were taken when the cleaned individuals where dry again. Few grasshoppers reached this cleaning stage due to the mortalilty inherent to working with older individuals, and only live grasshoppers could be tested since color changes rapidly after natural death.

Color was objectively measured using the color analysis features of the Mica Toolbox plugin (Troscianko and Stevens 2015, we used version 1.12 Mac) for imageJ (Schneider et al. 2012). Briefly, camera RAW image files were transformed into multispectral images using a Human cone-catch model. We used this model since measures with a photospectrometer showed that neither the grasshoppers nor the natural and urban substrates reflect UV radiation. The XYZ values obtained from these images were converted to the CIE-L*a*b* color space for easier interpretation of luminosity and hue variation. CIE-L*a*b* defines colors using 3 independent axes: L for lightness, a for green-to-red hue and b for blue-to-yellow hue. Higher L, a and b values describe a lighter, redder, and yellower color, respectively. Preliminary experiments indicated that color values were highly repeatable among images taken for the same individual on the same day, so in this study only one image was taken per individual per day.

Statistical analysis.— The statistical analysis software R (R Core Team 2015) and the packages lme4 (Bates et al. 2015), ggplot2 (Wickham 2016) and gridExtra (Auguie 2016) were utilized to visualize data, test our hypotheses and plot results.

To test for a differential change in color between individuals exposed to the distinctly colored soils, a linear mixed model was fitted to the "Change over time" measurements on each CIE-L*a*b* axis. This model contained Substrate (red or blue), Sex,

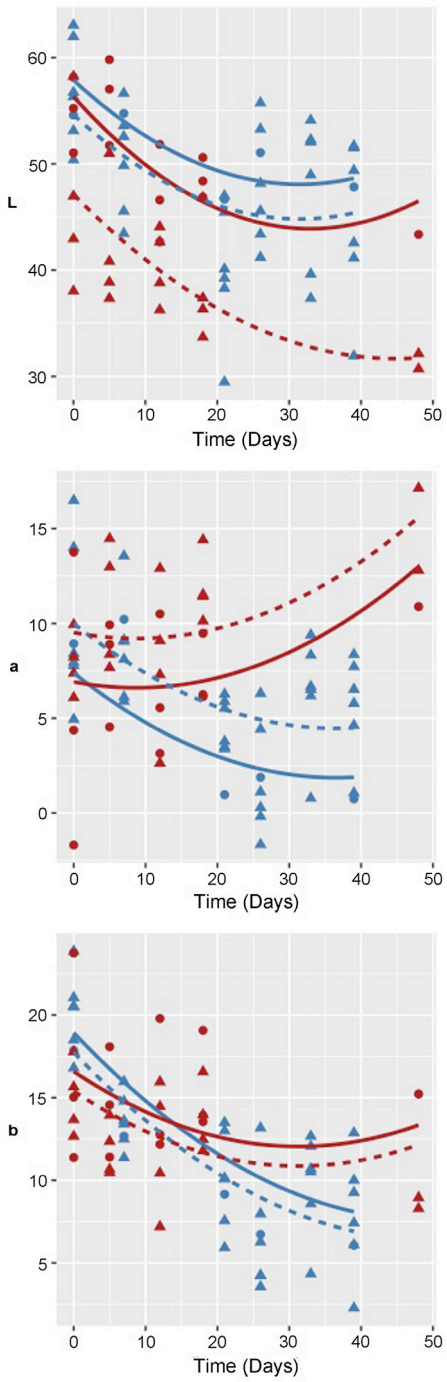

Figure 2. Average changes in color over time (days since the start of each treatment) for adult azure sand grasshoppers kept on bluish-gray stones (blue symbols and lines) or reddish-brown soil (red symbols and lines). Color is expressed in the three independent dimensions of the CIE-L*a*b* space (see text). Lines show the predicted values for each CIE-L*a*b dimension according to a fitted model containing Time, Substrate, Sex, Time:Substrate and sqTime; we excluded the subject random effect for better visualization. Continuous lines and circles are for females while dashed lines and triangles are for males. The "Red Earth" treatment had to be restarted using grasshoppers from the same field location but captured later in time. As a result, the timing of data collection is out of phase between treatments, and L is initially slightly lower for "Red Earth" individuals (since the species gets darker with age).

Time (since the beginning of the experiment, in days), Squared time (to fit non-linear change) and the Time:Substrate interaction as fixed effects. This interaction tests our main hypothesis that any color change over time depends on the substrate. As a random effect, we added Subject (individual identity) to correct for the repeated nature of the data.

To check that any differential color changes were not due to soiling, we also fitted similar linear mixed models to the "Cleaned" dataset. This model containing Substrate (the treatment), Status (living at the beginning versus dead + cleaned at the end), sex and Substrate:Status interaction as fixed effects and Subject again as a random effect.

The statistical support for any effects on color was determined by comparing the AIC value of our full model to that of a similar model lacking the studied effect. Given a set of statistical models that try to explain the same data, the AIC (Akaike Information Criterion) gives an estimation of the relative quality of each one of them, based on likelihood and with a penalty for including more parameters. The lower the AIC value of a model, the stronger the support for that particular model. For comparison with more traditional ways of testing for statistical significance, the AIC difference is presented with a p-value obtained by a loglikelihood-ratio test that compared the same two models as described above.

Results

Regarding the color change of live grasshoppers over time, substrate: color showed a non-significant effect on grasshopper lightness (DAIC=0.04, p=0.16, c^2=1.96, df=1), and no differential change in lightness over time (Fig. 2). In contrast, hue values were influenced by the substrate on which individuals were kept (Fig. 2): the Time:Subtrate interaction received considerable statistical support for both the green-red axis a (DAIC=-11.9, p<0.001, c^2=13.9, df=1) and the blue-yellow axis b (DAIC=-8.4, p=0.001, c^2=10.4, df=1). Individuals kept on bluish stones turned to a duller, more desaturated color while the ones kept on the red earth became relatively more red and yellow colored.

Regarding the color change of grasshoppers after freezing and cleaning, we also found strong statistical supports that substrate color influenced adult grasshopper color, although the patterns were somewhat different to those for live grasshoppers (Fig. 3). The interaction Substrate:Status was strongly supported for L (DAIC=-8.02, p=0.002, c^2=10.02, df=1), for a (DAIC=-16.08, p<0.001, c^2=18.08, df=1) and for b (DAIC=-17.33, p<0.001, c^2=19.34, df=1). Compared to the start of the experiment, and after being frozen, grasshoppers kept on red earth turned darker, a bit redder and a bit less yellow, a pattern that is very similar to the live grasshoppers. In contrast, grasshoppers kept on blue stones became much darker, redder, and more blue, a pattern that is quite different from the live grasshoppers. This conforms to our visual assessments (see Fig. 4): they showed a much darker, purplish color after being frozen and cleaned.

Discussion

Our results show that color change in response to the color of the substrate is not restricted to nymphs but also occurs in fully developed *S. azurescens* imagoes (Figs 2-4). These changes are similar in appearance to those we have seen in nymphs of this species (Edelaar et al. 2017, pers. obs.), as well as those reported for nymphs in other Acrididae species (Rowell 1972). Individuals changed their color in such a way that they increased resemblance (at least to the human eye) to that of the substrate on which they were kept. It is therefore likely that the observed color change is due to direct visual input, and functions to increase crypsis and reduce predation rate under field conditions, as suggested before (Rowell 1972, Cox and Cox 1974, Edelaar et al. 2017). As far as we know, such adaptive changes in color hue have not been recorded before for adult grasshoppers, only for nymphs.

Apart from changes in hue, we also observed changes in lightness, with individuals in both treatments becoming darker over time. This is in agreement with previous experiments we have performed with this species (Edelaar et al. 2017, pers. obs.). These have shown that nymphs are capable of adaptive plasticity in darkness and can become darker or paler, depending on the rearing substrate (and even more so when exposed to simulated predation risk: Edelaar et al. 2017), while adults generally turn darker over time, but more so when kept on a dark substrate. Whether the observed darkening in this experiment is due to aging or due to substrate matching is not known, but it is clear that adults can change color over time.

Exactly how this is done cannot be addressed with our data, but a likely explanation given the combined results and observations is that pigments can still be deposited in the cuticle of adults, but cannot be removed afterwards. This would explain why a medium gray adult placed on a white background will virtually stop darkening over time, but it will not become paler (i.e. a poor cryptic coloration persists), whereas nymphs can become paler when they molt. The color changes we observed in response to the different substrates are then likely due to the deposition of different pigments: more brownish ones when kept on brown soil, and more gray ones when kept on gray stones. In both treatments, such deposition of additional pigments to change an individual´s hue is in line with the observed general darkening. That several pigments are involved is also hinted at by the distinct color differences observed after freezing and cleaning: apparently the effects of freezing differed between the individuals from the different treatments, because the procedure was identical for both groups and the color change for individuals kept on gray stones from darkish gray towards more violet would not be expected if the color difference was simply due to external pollution. Moreover, the individuals from this same experiment were subsequently used for pigment analysis (Galván et al. 2015). That analysis not only reported one of the first demonstrations of pheomelanin (the brown version of melanin) in non-vertebrates, but it also found that the grasshoppers kept on brown soil were rich in pheomelanin, while the grasshoppers kept on gray stones were rich in eumelanin (the black version of melanin) (see Galván et al. 2015, online Supporting Information). Hence, at least part of the changes in color we saw in response to manipulated substrate color can be explained by the presence (and probably *de novo* deposition) of the different chemical forms of melanin.

A noteworthy observation is that the color changes seem to become slower as time passes (Fig. 2). This might be because grasshoppers were reaching the coloration they aimed for, but alternatively the ability to adaptively change color decreases with age or with the amount of pigment already deposited. Our experience with this species is that, although similar in direction and overall appearance, the adaptive color change is remarkably slower in adults than it is in nymphs. This has behavioral and ecological implications. Given that adults are around for a longer time than nymphs, and that (larger, flying) adults are more mobile than nymphs, one would expect that adults come into contact with a greater range of differently colored substrates.

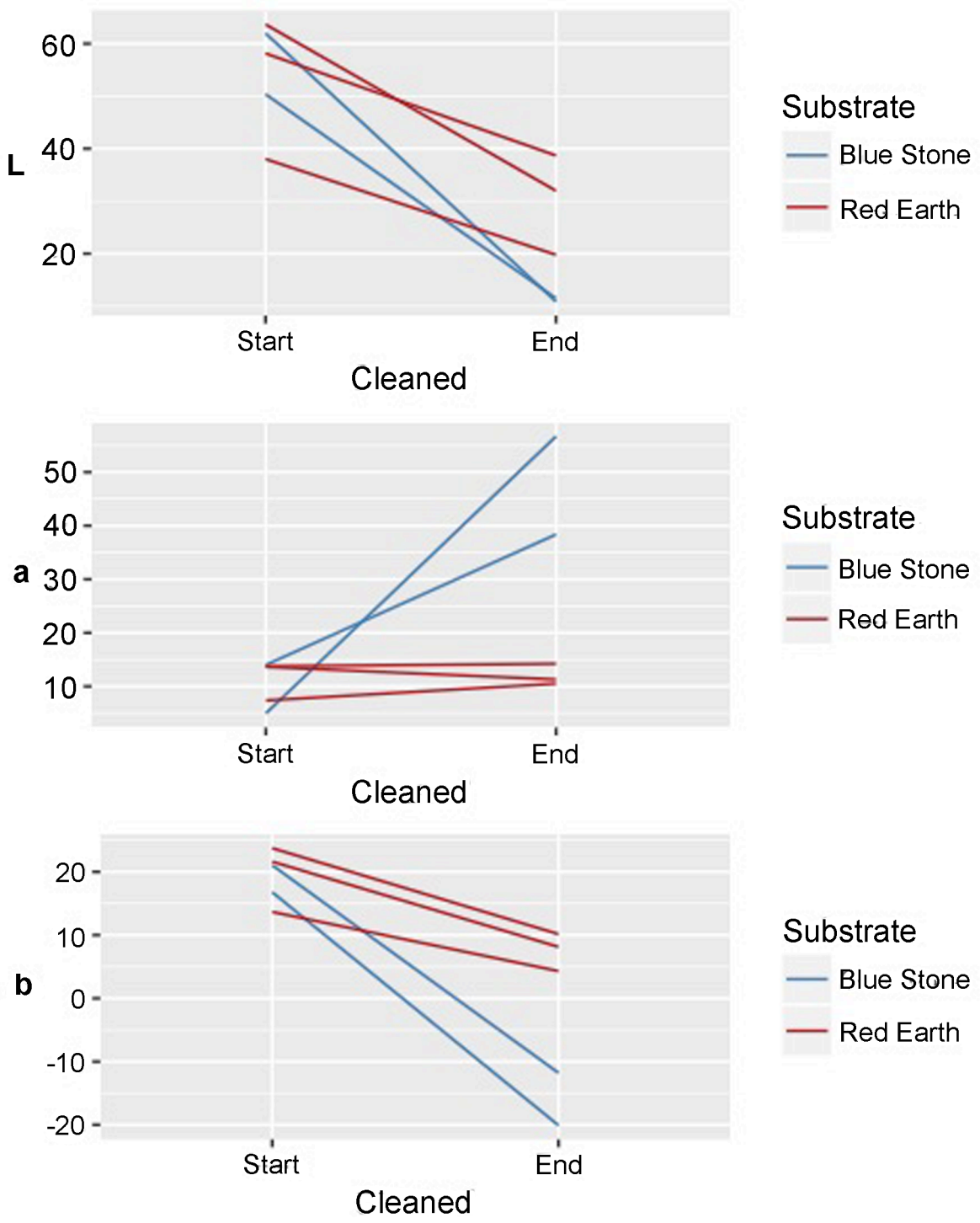

Figure 3. Individual changes in color in the final surviving azure sand grasshoppers when kept on bluish-gray stones (blue lines, N = 2) or reddish-brown soil (red lines, N = 3). The comparison is between the same individuals at the beginning of the experiment and once frozen and cleaned at the end. Color is expressed in the three independent dimensions of the CIE-L*a*b* space (see text).

Yet local populations of adult grasshoppers are typically quite well matched in color to their local environments, even if these environments are very close to each other in space. Edelaar et al. (in preparation) describe a situation in which grasshoppers living on differently colored, adjacent pavements (e.g. dark asphalt roads compared to pale sidewalks) are locally adapted in color, despite the fact that the observed degree of movement (on average 12 meter/day) would predict a homogenization of

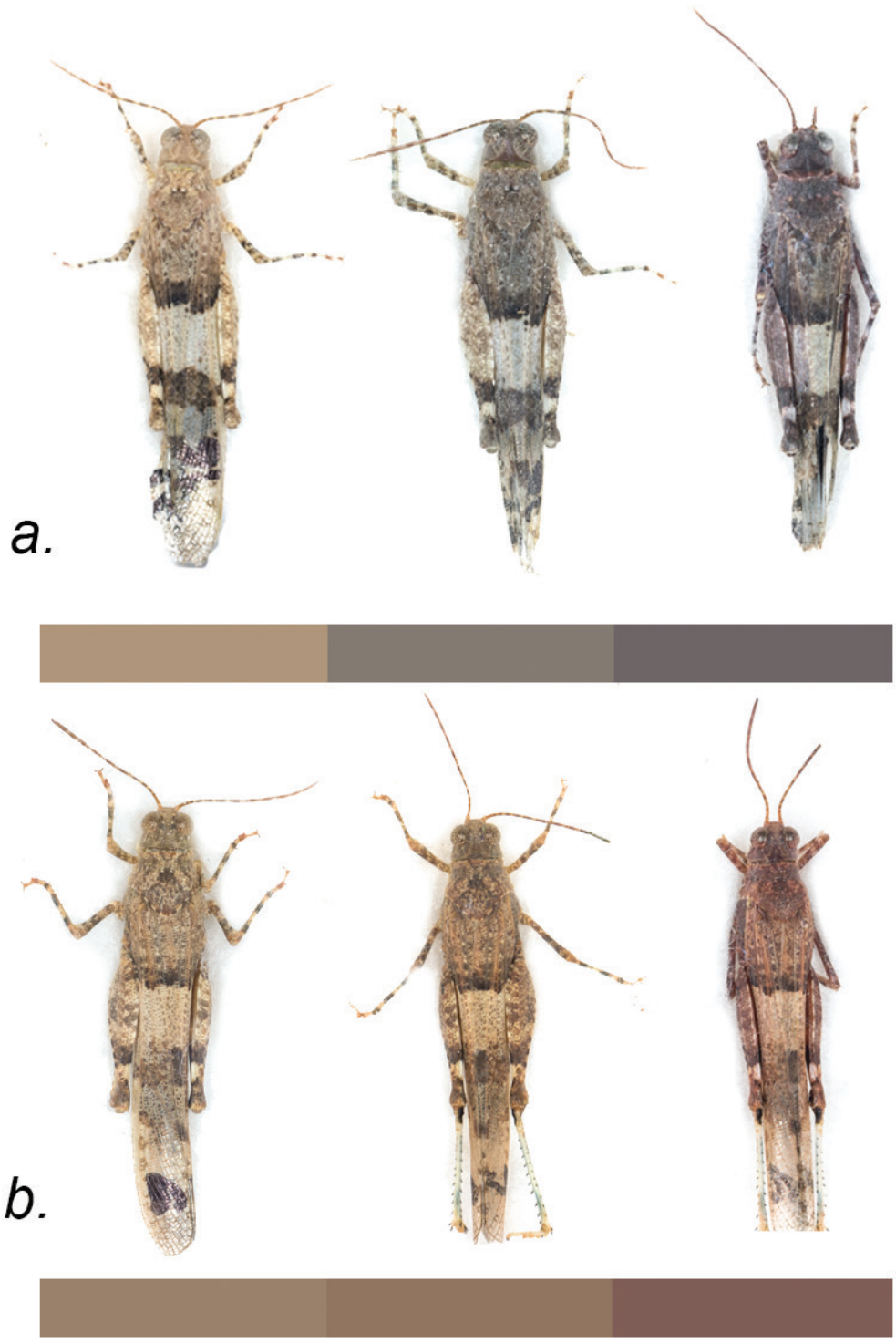

Figure 4. Change in color over time for two example individuals. From left to right: at the start of the experiment, 7 weeks later, and after freezing and cleaning at the end of the experiment. Individual **a**. was kept on blue-gray substrate and individual **b**. was kept on reddish-brown substrate. For visualization, the bars under the images show the average dorsal color as captured by the CIE-L*a*b* values measured for each individual at each point in time.

color across pavements in one day. The fact that the plastic color change as reported here is adaptive but relatively slow supports the conclusion that this local adaptation is in fact mostly driven by the grasshoppers´ selection of the environment, with individuals choosing their perching pavement as a function of their own color in order to increase crypsis.

The plasticity in the production of pheomelanin in grasshoppers (both nymphs and adults) might be relevant for future applied studies,

because in humans pheomelanin is associated with increased risk of melanoma in the epidermis (Mitra et al. 2012) and to Parkinson's disease in the brain (Spencer et al. 1998). Finding pheomelanin and understanding its chemical pathways in invertebrates may allow the development of new animal models for these diseases (Galván et al. 2015). Having a simple manipulation technique available to vary the production of pheomelanin (placing individuals on brown soil) would add to the versatility of such a model.

References

Auguie B (2016) gridExtra: miscellaneous functions for "grid" graphics. http://CRAN.R-project.org/package=gridExtra

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using {lme4}. Journal of Statistical Software 67: 1–48. https://doi.org/10.18637/jss.v067.i01

Cox GW, Cox DG (1974) Substrate color matching in the grasshopper *Circotettix rabula* (Orthoptera: Acrididae). Great Basin Naturalist 10: 60–70.

Edelaar P, Baños-Villalba A, Escudero G, Rodríguez-Bernal C (2017) Background colour matching increases with risk of predation in a colour-changing grasshopper. Behavioral Ecology (early online). https://doi.org/10.1093/beheco/arx016

Ergene S (1953) Homochromer Farbwechsel ohne Häutung bei Heuschrecken auf schwarzen Untergrund. Zoologische Jahrbucher 74: 437–624.

Ergene S (1955) Weitere Untersuchugen über Farbanpassung bei Oedaleus decorus. Zeitschrift fur vergleichende Physiologie 37: 226–229. https://doi.org/10.1007/BF00298311

Forsman A (2011) Rethinking the thermal melanism hypothesis: Rearing temperature and coloration in pygmy grasshoppers. Evolutionary Ecology 25: 1247–1257. https://doi.org/10.1007/s10682-011-9477-7

Galván I, Jorge A, Edelaar P, Wakamatsu K (2015) Insects synthesize pheomelanin. Pigment Cell & Melanoma Research 28: 599–602. https://doi.org/10.1111/pcmr.12397

Kang C, Kim YE, Jang Y (2016) Colour and pattern change against visually heterogeneous backgrounds in the tree frog *Hyla japonica*. Scientific Reports 6: 22601. https://doi.org/10.1038/srep22601

Karlsson M, Johansson J, Caesar S, Forsman A (2009) No evidence for developmental plasticity of color patterns in response to rearing substrate in pygmy grasshoppers. Canadian Journal of Zoology 87: 1044–1051. https://doi.org/10.1139/Z09-097

Karpestam E, Merilaita S, Forsman A (2012a) Reduced predation risk for melanistic pygmy grasshoppers in post-fire environments. Ecology and Evolution 2: 2204–2212. https://doi.org/10.1002/ece3.338

Karpestam E, Wennersten L, Forsman A (2012b) Matching habitat choice by experimentally mismatched phenotypes. Evolutionary Ecology 26: 893–907. https://doi.org/10.1007/s10682-011-9530-6

Mitra D, Luo X, Morgan A, Wang J, Hoang MP, Lo J, Guerrero CR, Lennerz JK, Mihm MC, Wargo JA, Robinson KC, Devi SP, Vanover JC, D'Orazio JA, McMahon M, Bosenberg MW, Haigis KM, Haber DA, Wang Y, Fisher DE (2012) An ultraviolet-radiation-independent pathway to melanoma carcinogenesis in the red hair/fair skin background. Nature 491: 449–453. https://doi.org/10.1038/nature11624

R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org/.

Rowell CHF (1972) The variable coloration of the Acridoid grasshoppers. Advances in Insect Physiology 8: 145–198. https://doi.org/10.1016/S0065-2806(08)60197-6

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9: 671–675. https://doi.org/10.1038/nmeth.2089

Spencer JP, Jenner P, Daniel SE, Lees AJ, Marsden DC, Halliwell B (1998) Conjugates of catecholamines with cysteine and GSH in Parkinson's disease: possible mechanisms of formation involving reactive oxygen species. Journal of Neurochemistry 71: 2112-2122. https://doi.org/10.1046/j.1471-4159.1998.71052112.x

Stevens M, Párraga CA, Cuthill IC, Partridge JC, Troscianko TS (2007) Using digital photography to study animal coloration. Biological Journal of the Linnean Society 90: 211–237. https://doi.org/10.1111/j.1095-8312.2007.00725.x

Troscianko J, Stevens M (2015) Image Calibration and Analysis Toolbox - a free software suite for objectively measuring reflectance, colour and pattern. Methods in Ecology and Evolution 6: 1–32. https://doi.org/10.1111/2041-210X.12439

Umbers KDL, Fabricant SA, Gawryszewski FM, Seago AE, Herberstein ME (2014) Reversible colour change in Arthropoda. Biological Reviews 89: 820–848. https://doi.org/10.1111/brv.12079

Valverde JP, Schielzeth H (2015) What triggers colour change? Effects of background colour and temperature on the development of an alpine grasshopper. BMC Evolutionary Biology 15: 168. https://doi.org/10.1186/s12862-015-0419-9

Wickham H (2016) ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics. https://CRAN.R-project.org/package=ggplot2

Yerushalmi Y, Pener MP (2001) The response of a homochrome grasshopper, *Oedipoda miniata*, to the dark-colour-inducing neurohormone (DCIN) of locusts. Journal of Insect Physiology 47(6): 593–597. https://doi.org/10.1016/s0022-1910(00)00156-6