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Influence of light intensity and substrate color on dorsal gray color change in *Phrynocephalus helioscopus* and *Phrynocephalus grumgrzimailoi*

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ABSTRACT

Body coloration is a functional adaptation that increases reproductive success or decreases the predation risk in animals. To understand the effect of light and substrate on coloration, we investigated the dorsal gray change in *Phrynocephalus helioscopus* and *Phrynocephalus grumgrzimailoi* maintained in habitats with a different light intensity and different substrate. We measured their gray gradient under different light and substrate conditions to establish the functional meaning of color change. We found that (1) in these two species, the gray level of dorsal gray decreased when they were housed on a light substrate with a high light intensity, (2) they increased their dorsal gray color when they were housed on a dark substrate with a low light intensity, and (3) *P. helioscopus* became darker than *P. grumgrzimailoi* with increasing light intensity but became brighter as the substrate darkened. These results imply that light intensity and substrate treatments induced strong color change responses. *Phrynocephalus helioscopus* used light and *P. grumgrzimailoi* used substrate as their main color change cues. *Phrynocephalus grumgrzimailoi* was likely to actively avoid predation while *P. helioscopus* has a thermoregulatory adaptation. We suggested that both light and substrate are important for color changing and camouflage capability in these lizards.

1. Introduction

Body coloration is an adaptive response to a number of selection pressures, such as isolation between sympatric species, defense against predators (Endler, 1978; Cooper and Greenberg, 1992; Merilaita and Lind, 2005; Stuart-Fox and Moussalli, 2008), endurance of "wear and tear" in harsh environments, reproduction (Norris, 1967; Butcher and Rohwer, 1989; Collins et al., 2000), and social interactions (Ross, 1995; Stuart-Fox et al., 2006a; Stuart-Fox and Moussalli, 2008). Species capable of physiological color change may alter their coloration in response to external conditions, and many species have independently evolved the ability to modify their body color over a period of seconds or minutes. These rapid changes are generally associated with intraspecific communication (Adamo and Hanlon, 1996; O'Connor et al., 1999), background matching (Osorio and Vorobyev, 1997), thermoregulatory purposes (Brown and Sandeen, 1948), and light intensity (Vroonen et al., 2012).

Color changes have been studied extensively in many different taxa, such as cephalopods (Whiteley et al., 2011), insects (Parkash et al., 2009), arachnids (Llandres et al., 2013), crustaceans (Thurman, 1988), fish (Hanlon et al., 1999; O'Connor et al., 1999; Mäthger et al., 2003), amphibians (King et al., 1994), and reptiles (Norris and Lowe, 1964; Stuart-Fox and Ord, 2004; Stuart-Fox et al., 2006a; b; Vroonen et al., 2012). Physical color change has been described in Agamidae lizards, and it is related to thermoregulation, background matching, and communication, and most of them are able to take spectrophotometric readings to characterize their dorsal body coloration (Madsen and Loman, 1987; Zucker, 1994a; b; Stuart-Fox and Ord, 2004; Stuart-Fox et al., 2006a; b; Zaidan and Wiebusch, 2007; Stuart-Fox and Moussalli, 2008, 2009; Vroonen et al., 2012). However, the co-effect of light and substrate background color on color change of the sand lizard remains unknown.

In general, lizards in arid habitats usually adopt specific strategies to survive the harsh environment, such as crypsis, the ability to match the

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surrounding substrate, and conceal itself (Luke, 1989; Rosenblum, 2006). Two Agamid lizards, the sunwatcher toadhead agama *Phrynocephalus helioscopus* and spotted toadhead agama *Phrynocephalus grumgrzimailoi*, are common lizards in the Junggar Basin, a landlocked arid region in eastern Central Asia. In this study, we used *P. helioscopus* *P. grumgrzimailoi* to investigate the effects of light intensity and substrate color on their body color. We hypothesized that when they are exposed to an external stimulus, they use the same factor as a cue to change their body color. To confirm or reject this hypothesis, we aimed to establish the functional meaning of color change and quantify dorsal coloration by measuring the gray gradient during manipulating experiments with different light and substrate conditions.

2. Materials and methods

2.1. Ethics statement

All animals in this study were maintained under animal research protocol IOZ-2015 that was approved by the Animal Welfare Ethics Review Committee of the Institute of Zoology, Chinese Academy of Sciences and animals were cared for in accordance with the principles and guidelines of the Animal Welfare Ethics Review Committee of the Institute of Zoology, Chinese Academy of Sciences, and the Chinese Wildlife Management Authority.

2.2. Study area and animals

We conducted our experiment in a monitoring station in the Kalamaili Nature Reserve (KNR) (88°30′–90°03′E, 44°36′–46°00′N), which is located in the northeastern Junggar Basin in Xinjiang, China. Altitude ranges from 600 to 1200 m above sea level and an arid continental climate is prevalent in this area. The mean annual temperature is 4°C-6°C (average of 20.5 °C in July and −18 °C in January), which is lower compared with the deserts in Central Asia but higher than that in the northern Mongolia Plateau (Xu et al., 2012a,b). Given the arid conditions of the area, water in KNR is extremely limited, and while the average annual precipitation is 159.1 mm, the average annual evaporation is 2090 mm (Chu, 2008). *Phrynocephalus helioscopus* and *P. grumgrzimailoi* were caught in sand dunes with plant communities dominated by *Allium polyrhizum*, *Anabasis salsa*, *Artemisia desertorum*, *Atraphaxis frutescens*, *Calligonum mongolicum*, *Ceratocarpus arenarius*, *Ceratoides latens*, *Haloxylon ammodendron*, *Reaumuria songarica*, and *Stipa glareosa* (Xu et al., 2012a,b).

Phrynocephalus helioscopus inhabits open arid regions of moderate elevation in Central and Western Asia. This species typically inhabits open areas with scattered rocks and bushes, and it relies largely on crypticity to escape detection (Clark and Clark, 1973; Clark et al., 1966). They feed on small invertebrates such as beetles and ants (Clark et al., 1966). *Phrynocephalus grumgrzimailoi* is another dominant species in eastern Central Asia (Ananjeva et al., 2011; Zhao et al., 1999) where it inhabits desert, farm, and salty soil regions (Zhao et al., 1999). As there is no difference in dorsal color in male and female *P. helioscopus* and *P. grumgrzimailoi* (Fu et al., 2013), we did not differentiate between females and males when we collected them.

2.3. Experimental procedure

The experiment was carried out in a test cage (100 × 50 × 50 cm) with two compartments and light could shine through the cage (Fig. 1). Five cages were set up as the treatment groups with different substrates: (1) white paper (represents a very bright substrate), (2) yellow sand (represents a natural substrate), (3) gray rock (represents occasional conditions), and (4) rock and sand (represents a natural substrate).

Before the experiment, 10 *P. helioscopus* were placed in one compartment and 10 *P. grumgrzimailoi* were placed in the other one. Cages were then carried to a fixed position exposed to sunlight. A photograph was taken every hour with an OLYMPUS SP-565UZ camera (resolution 2560 × 1920 pixels). Photographs were taken from a distance of about 0.5 m and focused on the dorsum to provide a grayness standard index (Fig. 2). We took a photo three times to minimize variation. Some individuals escaped from the cage due to strong winds, and specimens were caught and the experiment was restarted. Escape data are still included as these data are valid.

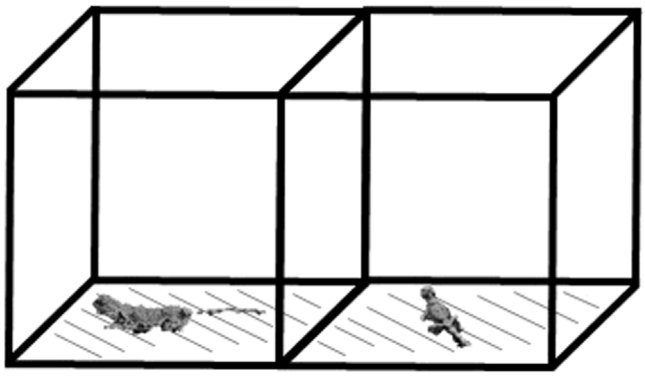

Fig. 1. Schematic diagram of the test cage used to determine the influence of light intensity and substrate color on dorsal gray change in *Phrynocephalus helioscopus* and *Phrynocephalus grumgrzimailoi*. The transparent surface indicates light exposure and the gray diagonal zone is the background (substrates are the same in both compartments during every experiment.) See text for details.

Many other methods are used to measure the dorsal color of a lizard. Compared with other color correction methods, the gray gradient has various advantages. First, many color samples with information of different intensity values can be obtained. This helps to improve the accuracy of the color correction. Second, we can acquire a whole range of intensity from 0 to 255. Captured images of the bar function as a basis of the color sensitivity properties (Jung and Ho, 2009). A dorsal gray gradient of *P. helioscopus* and *P. grumgrzimailoi* and the substrate were determined using a gray gradient bar and Photoshop Cs4 (©1990–2008, Adobe Systems Incorporated). We converted the photo from RGB to grayscale mode in Photoshop. The value of dorsal color (R_1), background color (R_2), region 25 standard index (S_{25}), and region 100 standard index (S_{100}) were then read. Light intensity was measured with a portable light meter (TES-1330A), and grayness was calculated using the following formula.

$$\text{Grayness value} = 100 - (100 - 25)*(R_i - S_{100})/(S_{25} - S_{100}) \ (i = 1, 2)$$

Successive tests ran from 09:00 to 23:00 when the light intensity was close to zero. Each experiment with a different background treatment lasted about 2–3 d, and each experiment was separated by an interval of at least one night roosting to return to the normal state.

2.4. Data analysis

Data analyses were performed using SPSS v. 20 (Armonk, NY: IBM Corp.). Because all data were not normally distributed (one sample Kolmogorov–Smirnov test, $P < 0.05$), we used nonparametric tests (Kruskal–Wallis test and Spearman correlation) to assess the variables. For our main analysis of differences in dorsal gray coloration of *P. helioscopus* and *P. grumgrzimailoi* by light intensity, the light intensity value was divided into six groups: Group A, 0; Group B, 0–10; Group C, 10–100; Group D, 100–1000; Group E 1000–10000; and Group F, > 10000. The gray value in the range 0–100 was divided into 10 groups: Group I, 0–10; Group II, 10–20; Group III, 20–30; Group IV, 30–40; Group V, 40–50; Group VI, 50–60; Group VII, 60–70; Group VIII, 70–80; Group IX, 80–90; and Group X, 90–100. We also assessed the relationship between dorsal gray and substrate gray by a Spearman correlation. The dorsal: substrate gray ratio was analyzed with