ARTICULATA 2019 34: 71–79 BIOLOGIE

A high percentage of brown colour *Tettigonia viridissima* when reared in the laboratory

Howon Rhee

Abstract

The coloration of *Tettigonia viridissima* is mostly green. However, among animals reared in the laboratory, a high percentage of brown individuals was discovered. This unusual coloration is discussed favouring the idea of multiple reasons.

Zusammenfassung

Heuschrecken der Art *Tettigonia viridissima* sind in der Natur fast immer grün. Aber wir haben verhältnismäßig viele braune Individuen gefunden, wenn wir die Art im Labor vom Nymphenstadium bis zum erwachsenen Tier aufgezogen haben. Eine derartige Farbvariation war bisher unbekannt. Die Ursachen der neuen gefundenen Farbvariation werden diskutiert. Möglicherweise sind viele Faktoren beteiligt.

Introduction

Due to ecological selection, morphological phenotypic plasticity is crucial for many animal species (BOND 2007, BERGSTROM & DUGATKIN 2012). Many orthopteran species perform cryptic coloration following this strategy (GWYNNE 2001, BENTON 2012). *Tettigonia viridissima* is a large and widely distributed European bush-cricket species (RHEE 2013, GRZYWACZ et al. 2017). As the scientific name "*viridissima*" (= very green) and the common name "The great green bush-cricket" imply, the species has typically and mostly a green body coloration in their natural environment (DETZEL 1998, SCHLUMPRECHT & WAEBER 2003, PFEIFER et al. 2011, BENTON 2012). Yellow individuals (form *flava,* NEDELKOV 1908) are rare in nature (DETZEL 1998, PFEIFER et al. 2011) and are sometimes hypothesised as a kind of incomplete albinism (WILLIG 1969, WIGGLESWORTH 1971, WIGGLESWORTH 1972, PFEIFFER et al. 2012), i.e. the missing of (a) certain pigment(s). During rearing animals for an experiment, many green nymphs sampled from nature changed surprisingly into fully brown body colour with pinkish abdomen sequentially after each moulting in the lab. This coloration was not found in the natural environment where green individuals were very common. In the laboratory, many individuals were closer to the brown morph observed from nymphs and just moulted adults. In this paper, I will describe the continuous colour variation from green to brown of *Tettigonia viridissima* in the laboratory and discuss possible reasons.

Material and methods

33 males fifth (from seven) instar nymph of *Tettigonia viridissima* were sampled in a meadow nearby Fachhochschule Bielefeld from 20th May 2018 to 3rd June 2018 (Fig. 1). All individuals were green when they were captured (Fig. 1). All animals were reared individually in plastic beakers containing of three short wood chopsticks for moulting and resting (Fig. 2). Each beaker was covered by a very soft nylon mosquito nets from one Euro shop (Fig. 2). The distance between beakers was fixed and they were standing close to each other. During the rearing, two small to medium sized house crickets nymphs (*Acheta domesticus*) or brown silent crickets nymphs (*Gryllus assimilis*) bought from Fressnapf pet shops, and four *Potentilla aurea* flowers from nature were given as meal once every two days. The size of crickets fed depended on the status of the instars of the nymphs because younger instars could not feed and hunt well on relatively large food crickets, compared to the older instars. The temperature of the chamber was maintained at 25 °C (humidity unknown). The light was controlled for a duration of 12:30 Light and 11:30 Dark. Water was sprayed on the net of the cage once per week, and once a day only for the animals in the moulting stage detected by thickness of wing and mandibular dehiscence.

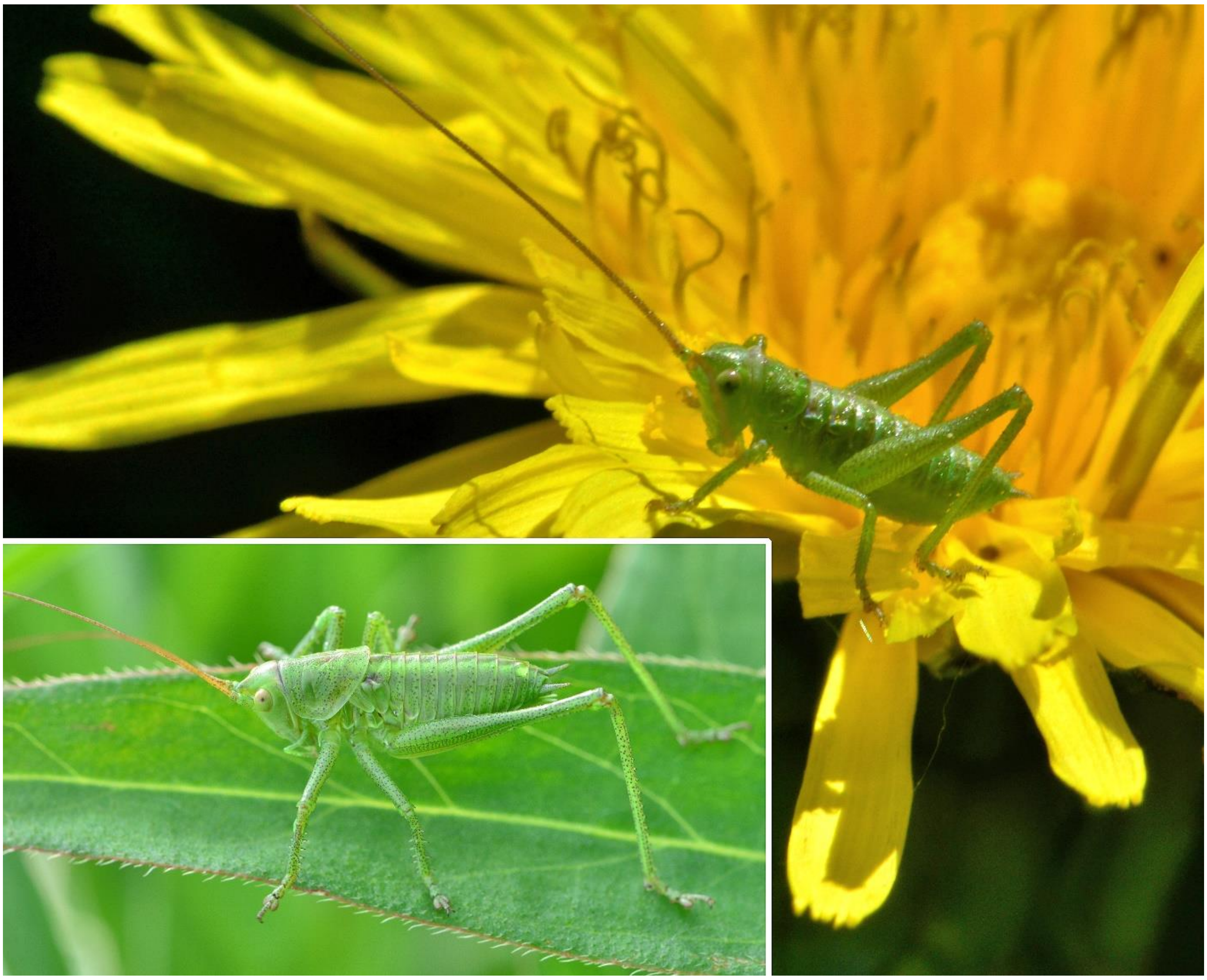

Fig. 1: A 1st nymph instar *Tettigonia viridissima* from 6th May, 2018 in Bielefeld, Germany and green 5th nymph instar *Tettigonia viridissima* male (inset), 20th May, 2018 in the field in Bielefeld, Germany.

Fig. 2: a) A beaker cage for individuals of *Tettigonia viridissima*; b) Rearing condition of *Tettigonia viridissima* in the lab until the adult stage.

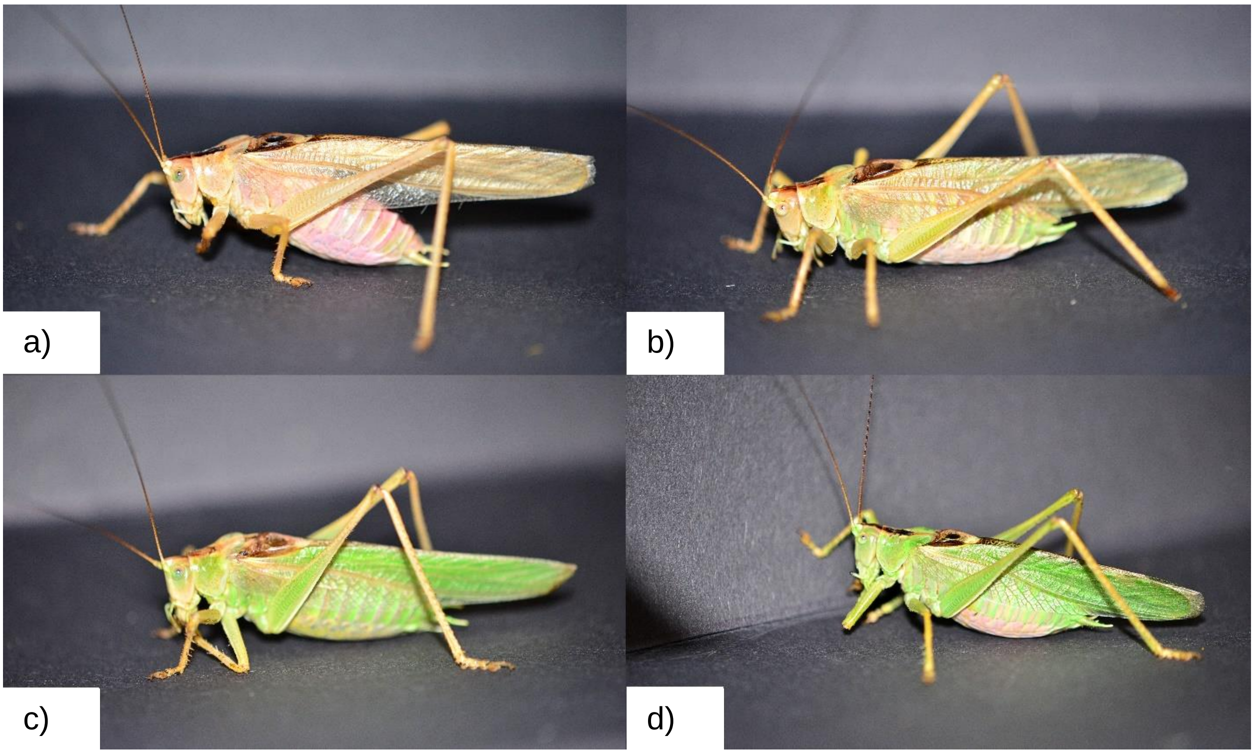

Fig. 3: The colour variation of *Tettigonia viridissima* in the laboratory population a) Brown individual; b) Greenish brown individual; c) Brownish green individual; d) Green individual.

All individuals moulted to adults from 7th June 2018 to 23rd June 2018. In order to define the continuous colour variation of the species in the lab, the numbers from 0 (brown) to 3 (green) were used for each individual. 29 individuals from the 33 samples were used to score the variation because some individuals died due to moulting failure during the rearing. The scoring was conducted on the 27th June

2018 when all adults were relatively young (4-20 days old). The scores were defined to include all colour variation and these were translated into identifiers during the data analysis. Brown (score 0) indicates that the body colour of an individual is full brown and sometimes, with some green flakes or purple on the abdomen (Fig.3a). Greenish brown (score 1) is that the body is mostly brown but there are some green flakes on the pronotum and hind legs and some parts of fore wings (Fig.3b). Brownish green (score 2) means that there are few brown flakes on the pronotum and all fore and middle legs with only hind tibia are brown, but fore wings and hind femora are green (Fig. 3c). Green (score 3) is that animals are full green and there are sometimes with only slightly yellowish markings on the tibias of the legs (Fig. 3d). In order to avoid sensory colour bias for the illustration pictures of the figure 3, an artificial black box for the animals and two books for the height for the box were used with the same position and the same time of the same day at my office. All pictures were taken using a Nikon D-7000 with 85 mm micro lens with 1/125 shutter speed and F 4 for the aperture with fixed power of the flesh light. The bar chart in the figure 4 was made from R Studio-program (R Core Team 2016).

Results

During rearing, most animals changed continuously their coloration from green to brown stepwise with every moult (not documented by photos). Among adults, brown was the most dominant colour (13 out of 29 individuals; 44.8%) and greenish brown was next with 9 out of 29 individuals (31%) (Fig. 4). Green was the rarest colour in the population with 3 from 29 individuals (10.3%) and 4 individuals were brownish green, which was 13.8% within the population (Fig. 4). Thus, 22 from 29 individuals (75.9%) were close to brown, while only 7 individuals from the total sample (24.1%) were close to green (Fig. 4).

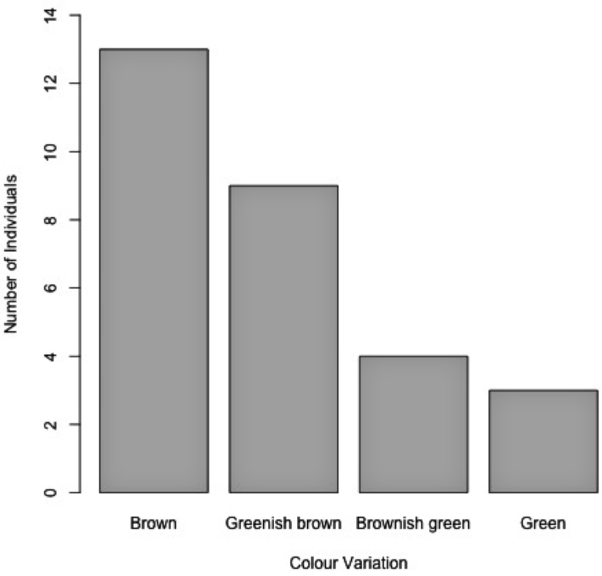

Fig. 4:
The number of individuals for each colour variation in the studied sample (n=29).

Discussion

Brown individuals of *Tettigonia viridissima* were found more frequently than green individuals in the laboratory, although all nymphs were green in nature during the sampling before they were reared in the lab. In the field, no brown nymphs and adult animals were observed. Consequently, this colour variation might be considered as a kind of morphological phenotypic plasticity. Genetic reasons resulting from missing pigments can be excluded – the animals could synthesise green pigments before capture. Brown animals together with green animals are found in many species of Orthoptera (DETZEL 1998, BENTON 2012) and also among tettigoniids. Here most examples are found among conocephalids (ODA & ISHII 2001), phaneropterids (ERGENE 1956) and tettigoniines (CHERRILL & BROWN 1991). Additionally, blackish brown individuals have been discovered in natural populations of the closely related species, *Tettigonia orientalis* complex in Japan (ICHIKAWA et al. 2006, MURAI & ITO 2011). The brown body colour may be explained under several hypothesis.

(1) Phenotypic variation in coloration may result from population density

Some Orthopteran species have different colour polymorphism at different density, especially in many locust species (SWORD 1999, NIJHOUT 2010, SONG et al. 2017, BENTON 2012). Hence, the density could affect the coloration of the species based on morphological phenotypic plasticity. Even though we reared all individuals individually with separate beakers, the animals may have detected the density of the population with the sense of odour. HAMOUDA et al. (2011) observed that a high percentage of yellow solitary form of *Locusta migratoria* was found when they were reared at relatively high density. *Schistocerca gregaria* gregarious nymphs reared at high density have aposematic coloration (SWORD et al. 2000, ERNST et al. 2015). Swarms of *Tettigonia viridissima* were discovered in the past (HARZ 1957, cited e.g. by INGRISCH & KÖHLER 1998, SCHLUMPRECHT & WAEBER 2003). However, there is no information about morphological traits of individuals during the swarm. Consequently, there is the possibility that brown individuals are a result of gregarious phase of Tettigonia viridissima.

(2) Environmentally induced green-brown dimorphism

Many Orthopteran species change their body coloration depending on the environmental condition (DEARN 1990, OWENS 2010, BENTON 2012). Thus, three possibilities (BENTON 2012) were discussed below.

Indirect: Adaptation to colour of surrounding

The individuals in the lab were not surrounded by green plants so the animals probably could have realised visually that such trait (brown coloration) is more beneficial than the trait found in the nature. According to PFEIFFER et al. (2011), who referred to an old study (FOERSTER 1846), yellow individuals of the species were difficult to distinguish from ripe corns, while green individuals were easy to detect from such background and green individuals tended to live on green plants more than yellow individuals. INGRISCH (1999) stated that the reason of a high proportion of green individuals of *Pseudorhynchus acuminatus* was that they probably succeeded to escape from visual detection of predators with the cryptic coloration during rainfalls in tropical forests. ROWELL (1971) showed that many grasshoppers have visual ability to percept colours of environment surrounding them. GILLS (1982) stated that *Circotettix rabula* in Colorado, the United Stated of America could choose the place resembling with their body coloration using visual ability. Consequently, *Tettigonia viridissima* could have visual ability to detect the colour of the environment surrounding them.

Direct: Temperature effect

WILLIG (1969) stated that individuals of *Carausius morosus* reared at 28 ºC were between pale beige and dark brown, while individuals of the species reared at 18 ºC became pale green. SEVGILI et al (2018) stated that melanism of insects affects their body temperature a lot because dark colour absorbs solar energy directly and quickly. In nature, the temperature relates to the light condition and these are less independent each other. However, in my study, the temperature was constant and controlled but the strength of light is relatively lower than nature. Consequently, the relationship between light condition and temperature is independently in the laboratory condition. Thus, just only temperature itself would be less likely to affect the coloration of *Tettigonia viridissima*.

Direct: Dryness/moisture

OKAY (1953) showed that the colour of nymphs in *Acrida spp*, *Locusta migratoria migratorioides* and *Schistocerca gregaria* changed depending on the humidity of plants food. LYMBERRY (1992) showed that *Mygalopsis marki* in Australia had higher percentage of brown individuals in dry and autumn population than in wet and spring population. Hence, the same could have been happened if different populations of *Tettigonia viridissima* (Mediterranean population vs Northern European population) react differently. Namely, the dry condition of the laboratory could influence the colour variation of the species. This could be tested by rearing animals under different conditions with different populations. If this hypothesis is right, due to evolutionary adaptation, *Tettigonia viridissima* lost colour polymorphism quite recently with their habitat and the current climate. Hence, the genetical polymorphism is still maintained, even though the genes indicating different colour morphs are not expressed in nature usually.

(3) Properties of food

In *Tetrix subulata*, dietary difference among colour morphs have been described (KARPESTEM & FORSMAN 2011). Thus, possibly, the dietary choice can affect the coloration of the species. INGRISCH (1976) and INGRISCH & KÖHLER (1998) showed that *Tettigonia viridissima* feeds on high diversity of plants and animal species. However, since we controlled the food, choice was very limited. According to own observation during the rearing, a related species, *Gampsocleis sedakovii obscura,* changed the colour from green to brownish during the growth when the species was caught in nature and reared them in the cage with limitation of food choice. INGRISCH (1999) stated that physiological mechanism of colour change in Orthoptera could be based on different foods showing with seasonal variation. Consequently, from these reasons, it is possible that a specific food component necessary for the green coloration (humidity of plants or particular species of plants or/ and flowers or/and other insect species) was lacking in our experiment. Therefore, the food effect could be studied in the future.

(4) Combination of factors

The coloration of many species in Orthoptera do not depend on just one single factor but the multiple environmental effects may influence coloration of a species. DEARN (1990) stated that colour polymorphism of grasshoppers is determined from humidity contents of food, population density and temperature. If the temperature is relatively high then either humidity is relatively wet or dry (temperature +

humidity), if the humidity is dry, wet food will be clumped only in restricted areas so the density of the species will be increased (dry + population density), otherwise, they will consume relatively dry food from this environmental condition (dry + dry food). Only individuals with high density feeding on *Ptelea spp* plants have aposematic coloration in nymphs of *Schistocera emarginata* (SWORD 1999). WIGGLESWORTH (1972) cited ROUSSEL (1966) that corpora allata of *Gryllus bimaculatus* inhibited black pigmentation of individuals during the experiment. Possibly, the food and the population density could affect the hormone whether it expresses to inhibit melanism or not. Consequently, even though the animals were reared in the laboratory, the coloration of *Tettigonia viridissima* is possibly not simple to analyse from just only one single reason. All factors described above could influence coloration of the species and possibly unequally.

Acknowledgement

I would like to thank for Prof. Dr. Klaus Reinhold (University Bielefeld, Germany) giving a chance to do this project. And I would also thank for Dr. Klaus-Gerhard Heller (Magdeburg, Germany) for comments and intensive discussion on the manuscript. I also thank for Dr. Steve Ramm (University Bielefeld, Germany) for some discussions and helping to use R Studio-program and also Bahar Patlar (University Bielefeld, Germany) for helping for the program. I also sincerely thank Kathleen Urchek (Greenwood Wildlife Rehabilitation centre, Colorado, The United States of America) for some English comments.

Author:
Howon Rhee
Fakultät für Biologie, Abt. Evolutionsbiologie, Universität Bielefeld
Morgenbreede 45
33615 Bielefeld
Germany
E-mail: howon.rhee@uni-bielefeld.de

References

BENTON, T. (2012): Grasshoppers & Crickets. – Collins, London; 532 pp.

BERGSTROM, C.T. & DUGATKIN, L.A. (2012): Evolution (International Student Edition). – W.W.Norton & Company. Inc, New York; 677 pp.

BOND, A.B. (2007) The evolution of color polymorphism: crypticity, searching images, and apostatic selection. – Annual Review of Ecology, Evolution and Systematics 38: 489-514.

CHERRILL, A.J. & BROWN, V.K. (1991): Variation in coloration in *Decticus verrucivorus* (Linneaus, 1758) (Orthoptera: Tettigoniidae) in southern England. – Entomologist’s Gazette 42: 175-183.

DEARN, J.M. (1990): Color pattern polymorphism. – In *Biology of Grasshoppers*, ed. RF Chapman, A Joern, pp. 517–49. New York: Wiley

DETZEL, P. (1998): Die Heuschrecken Baden-Württembergs. – Ulmer, Stuttgart; 580 pp.

ERGENE, S (1956): Farbanpassungen bei *Tylopsis liliifolia* Larven. – Zeitschrift für vergleichende Physiologie 38: 315-316.

ERNST, U., VAN HIEL, M., DEPUYDT, G., BOERHAN, B., DE LOOF, A. & SCHOOFS, L. (2015): Epigenetics and locust life phase transitions. –The Journal of Experimental Biology 218: 88-99.

FOERSTER, A. (1846): Zur Berichtigung der VON BARTELS in Mayen mitgetheilten entomologischen Notizen. – Verhandlungen des Naturhistorischen Vereins der preussischen Rheinlande und Westfalens 3: 37-63.

GILLS, J.E. (1992): Substrate colour matching cues in the cryptic grasshopper *Circotettix rabula rabula* (Rehn & Hebard). – Animal Behaviour 30: 113-116.

GRZYWACZ, B, HELLER, K.-G., WARCHALOWSKA-SILWA, E., KARAMYSHEVA, T.V. & CHOBANOV, D.P. (2017): Evolution and systematics of Green Bush-crickets (Orthoptera:Tettigoniidae: *Tettigonia*) in the Western Palaearctic: testing concordance between molecular, acoustic, and morphological data. – Organisms Diversity & Evolution 17 (1): 213-228.

GWYNNE, D.T. (2001): Katydids and Bush-Crickets Reproductive Behaviour and Evolution of the Tettigonidae. – Cornell University Press, New-York, U.S.A.; 317pp.

HAMOUDA, A.B, TANAKA, S., HAMOUDA, M.H.N., BOUAIN, A. (2011): Density-dependent phenotypic plasticity in body coloration and morphometry and its transgenerational changes in the migratory locust, *Locusta migratoria*. – Journal of Entomology and Nematology 3 (7): 105-116.

HARZ, K. (1957): Die Geradflügler Mitteleuropas. – Jena, Gustav Fischer, 494 S.

ICHIKAWA, A., KANO, Y., KAWAI, M., TOMINAGO, O. & MURAI, T. (2006): Orthoptera of the Japanese Archipelago in Color. – Hokkaido University (Orthopterological Society of Japan), Hokkaido; 687 pp+CD (in Japanese).

INGRISCH, S. (1976): Vergleichende Untersuchungen zum Nahrungsspektrum mitteleuropäischer Laubheuschrecken (Saltatoria: Tettigoniidae) – Entomologische Zeitschrift 86: 217-224.

INGRISCH, S. (1999): Farbwechsel einer adulten Schiefkopfschrecke (Ensifera: Tettigoniidae, Copiphorini) – Entomologische Zeitschrift,109 (6): 246-249.

INGRISCH, S. & KÖHLER, G. (1998): Die Heuschrecken Mitteleuropas. – Westarp Wissenschaften Verlagsgesellschaft mbH, Magdeburg; 460pp.

KARPESTAM, E. & FORMAN, A. (2011) Dietery differences among colour morphs of pygmy grasshoppers revealed by behavioural experiments and stable isotopes – Evolutionary Ecology Research 13: 461-477.

LYMBERY, A.J. (1992): The environmental control of colouration in a bushcricket, *Mygalopsis rnarki* Bailey (Orthoptera: Tettigoniidae). – Biological Journal of the Linnean Society 45: 71-89.

MURAI, T & ITO, I. (2011): Field guide to the Orthoptera of Japan. – Hokkaido University, Hokkaido; 449 pp (in Japanese).

NEDELKOV, N. (1908): [Second contribution to the entomological fauna of Bulgaria]. – Periodichesko spisanie na Bulgarskoto knizhovno druzhestvo v Sofia, 68 [1907] (5/6): 411-436 (In Bulgarian).

NIJHOUT, H.F. (2010): Molecular and physiological basis of colour pattern formation. – Advances in Insect Physiology 38: 219-265.

ODA, K. & ISHII, M. (2001): Body color polymorphism in nymphs and adults of a katydid, *Conocephalus maculatus* (Orthoptera: Tettigoniidae) – Applied Entomology and Zoology 36 (3): 345-348.

OKAY, S. (1953): Formation of green pigment and colour changes in Orthoptera. – Bulletin oft Entomological Research 44 (2): 293-315.

OWENS, N. (2010) The chameleon grasshopper. – The Norfolk Natterjack 111: 10-11.

PFEIFFER, M.A., NIEHUIS, M. & RENKER, C. (2011): Die Fang- und Heuschrecken in Rheinland-Pfalz. – Gesellschaft für Naturschutz und Ornithologie Rheinland-Pfalz, Mainz; 677 pp.

RHEE, H. (2013): Disentangling the distribution of *Tettigonia viridissima* (Linnaeus, 1758) in the eastern part of Eurasia using acoustical and morphological data. – Articulata 28 (1/2): 103-114.

ROUSSEL, J.P. (1966): Role des corpora allata sur la pigmentation de *Gryllus bimaculatus* DeG. – Journal of Insect Physiology 12: 1085-1082.

ROWELL, C.H.F. (1971): The variable coloration of the acridoid grasshoppers. – Advanced in Insect Physiology 8: 145-198.

SEVGILI, H., SIRIN, D., HELLER, K-G. & LEMONNIER-DARCEMONT, M. (2018): Review of the *Poecilimon (Poecilimon) zonatus* species group and description of new species from Turkey with data on bioacoustics and morphology (Orthoptera: Phaneropterinae). – Zootaxa 4417 (1): 1-60.

SCHLUMPRECHT, H. & WAEBER, G. (2003): Heuschrecken in Bayern. – Ulmer, Stuttgart; 515 pp.

SONG, H., FOQUEST, B., MARINO-PEREZ, R. & WOLLER, D.A. (2017): Phylogeny of locusts and grasshoppers reveals complex evolution of density-dependent phenotypic plasticity. – Scientific Reports 7(6606).

SWORD, G.A. (1999): Density-dependent warning coloration. – Nature 397: 397.

SWORD, G.A, SIMPSON, S.J., EL HADI, M. & WILPS, H. (2000) Density-dependent aposematism in the desert locust. – Proceedings of the Royal Society London B. 267: 63-68.

WIGGLESWORTH, V.B (1971): Das Leben der Insekten. – 383 S., Lausanne.

WIGGLESWORTH, V.B (1972): The principles of insect physiology (7th ed). – Chapman & Hall, 827pp.

WILLIG, A. (1969): Die Carotinoide und der Gallenfarbstoff der Stabheuschrecke, *Carausius forosus* und ihre Beteiligung an der Entstehung der Farbmodifikationen – Journal of Insect Physiology 15: 1907-1927.