A reddish-brown mutant in the desert locust, *Schistocerca gregaria*: phase-dependent expression and genetic control

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Abstract

A reddish-brown (RB) mutant of the desert locust, *Schistocerca gregaria* and its genetic control are described. Hatchlings with reddish-brown patterns (RB strain) were found among normal individuals with black patterns (normal strain) in a laboratory colony. The color patterns were similar in the two strains, but the intensity of the melanization appeared weaker in the former. Hatchlings of this mutant could be distinguished visually from normal individuals under crowded conditions only, because they all became green without developing dark patterns under isolated conditions. Under crowded conditions, the difference in the darkness of the body coloration diminished as nymphs grew bigger but persisted throughout their life, including the adult stage. Reciprocal crosses between the mutant and normal strains under crowded conditions produced normal phenotypes only, indicating that the RB phenotype was recessive to the normal phenotype. In the F₂ generation, the normal and RB phenotypes appeared in a ratio of 3 : 1, indicating that the RB phenotype is inherited in a simple Mendelian fashion and the RB gene is autosomal. These results suggest that the expression of the RB phenotype is phase-dependent and the RB gene might regulate the intensity of melanization.

Key words: Body color mutant; genetic control; locusts; phase polyphenism; *Schistocerca gregaria*

INTRODUCTION

Body color mutation occurs in various orders of insects (Fuzeau-Braesch, 1985). For example, albino mutants, which look normal in every respect except for their body color, have been reported occasionally in locusts and grasshoppers (Faure, 1932; Hunter-Jones, 1957; Putnam, 1958; Verdier, 1965; Boutheier, 1966; Nolte, 1971; Hasegawa and Tanaka, 1994). They have been used to investigate sperm competition (Hunter-Jones, 1960; Zhu and Tanaka, 2002; Tanaka and Zhu, 2003) and the role of body color in the formation of aggregation and group walking (marching) behavior (Gillett, 1973). An albino strain of the migratory locust, *Locusta migratoria* L., has been used to screen for the presence of a pigmentotropin in various insects (Tanaka, 1993, 2000c, 2006). In the desert locust, *Schistocerca gregaria* Forskål (Hunter-Jones,

1957) and *L. migratoria* (Nolte, 1971; Hasegawa and Tanaka, 1994), albinism is a recessive trait controlled by a single Mendelian unit. A black mutant which remains almost black even under isolated conditions has been reported for *S. gregaria*, and is recessive to the normal pigmented phenotype (Volkonsky, 1938). Another dark mutant in which black body coloration appears only in the adult stage is also controlled by a recessive gene in this locust (Yerushalmi et al., 2000).

Locusts show body-color polyphenism depending on the population density and other environmental factors (Faure, 1932; Hunter-Jones, 1958; Stower, 1959; Uvarov, 1966; Pener, 1991; Tanaka, 2004; Lester et al., 2005; Maeno and Tanaka, 2007). In *S. gregaria* and *L. migratoria*, solitary nymphs occurring at a low population density assume a cryptic or camouflaged green or brown body color matching their habitat background

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color. At a high population density, on the other hand, gregarious nymphs display a conspicuous yellow or orange background body color with intensive black patterns. Albino locusts of either species do not develop the gregarious body coloration and become whitish under crowded conditions. Under isolated conditions, on the other hand, they are either whitish or green in color (Hunter-Jones, 1957; Verdier, 1965; Hasegawa and Tanaka, 1994).

Body-color polyphenism also occurs in locust hatchlings (Hunter-Jones, 1958; Tanaka and Maeno, 2006, 2008; Maeno and Tanaka, 2007; Simpson and Miller, 2007). In *S. gregaria*, dark-colored hatchlings are produced by crowd-reared female adults, whereas green hatchlings are produced by isolated-reared female adults, although hatchlings between the two forms are sometimes produced together with these two forms. During a study of phase polyphenism in *S. gregaria*, light-colored hatchlings were found in a crowd-reared laboratory colony and developed reddish-brown patterns instead of black patterns. Therefore, this mutant, which will be referred to as the RB mutant, can be separated visually from normal individuals. The present study was carried out to characterize this mutant and to investigate its genetic control by crossing the RB mutant and the normal strain.

MATERIALS AND METHODS

Insects and rearing conditions. The normal strain of *S. gregaria* has been described previously (Tanaka and Yagi, 1997; Maeno and Tanaka, 2004). The RB mutant strain was established in 2006 from reddish-brown nymphs that were found in the normal strain maintained for more than 40 generations in Ohwashi laboratory. Nymphs and adults were kept in groups of approximately 100 individuals in large cages (42×22×42 cm) at 32±1°C, LD 16:8 h and 50–70% relative humidity, as described previously (Maeno et al., 2004). They were fed leaves of orchard grass and cabbage together with wheat bran. Some crowd-reared locusts were reared individually in small cages (28×15×28 cm) after adult emergence except for <1 day for mating.

Assessment of body coloration. To evaluate the darkness of the body color at hatching, head luminance was measured according to the method of

Tanaka (2003). Briefly, hatchlings immobilized on ice were photographed using a scanner (Epson ES 2000, Japan) after each nymph was placed on one side on the glass table of the scanner. Head luminance was measured using the histogram function of Photoshop 7.0 (Adobe Systems Incorporated, San Jose, CA). In *S. gregaria*, luminance values of hatchlings were significantly larger in green phenotypes than in crowded normal (black) phenotypes (Maeno and Tanaka, 2007).

Observations of exuviae. Exuviae derived from crowd-reared 1st stadium nymphs were collected from RB mutants and normal individuals. A piece of exuviae taken from the pronotum area was placed on a slide glass with a drop of glycerin, covered with a cover glass and observed under a microscope (OLYMPUS BX50, Japan) equipped with a digital camera (OLYMPUS DP50, Japan).

Crosses. To determine the genetic control of the RB trait, two experiments were carried out. Newly emerged female adults of each strain were separated from males and reared in a group of about 30 individuals in a large cage. Twenty-five days after adult emergence, females were individually transferred from the large cages to small cages in which each female was paired with a sexually mature male (ca. 3 weeks old). Female adults constantly kept with a male as pairs produce hatchlings characteristic of gregarious forms just as when they are kept with many males (Hunter-Jones, 1958). Thus, they can be regarded as crowd-reared locusts. On the other hand, females isolated immediately after adult emergence produce green progeny, as do solitary locusts (Hunter-Jones, 1958). In the first experiment, reciprocal crosses were made between the RB mutant and normal strains. In the second experiment, F₁ individuals were crossed to obtain F₂ generations or backcrossed to the pure-bred RB mutant or normal strain to determine the proportions of different body-color phenotypes. In most crosses, 5–10 pairs were used and 5 egg pods were collected from each pair. All egg pods were incubated at 32±1°C for hatching, which normally occurred in 2 weeks. Hatchling body coloration was recorded after 6 h of hatching based on the darkness of the body coloration. It is known that egg pods deposited by crowd-reared females sometimes produce a few green hatchlings characteristic of solitary forms together with dark-colored hatchlings (Hunter-Jones, 1958; Tanaka and

Fig. 1. Body color of normal (A, C, E, G and I) and reddish-brown (RB) mutant individuals (B, D, F, H and J) of *Schistocerca gregaria*. A and B, hatchlings derived from isolated-reared adults; C and D, hatchlings derived from crowd-reared adults; E and F, pronotum portion of exuviae of 1st stadium nymphs; G and H, third stadium nymphs under crowded conditions; I and J, adults reared under crowded conditions. Note that the dark patterns are lighter in the RB mutants than in normal hatchlings when produced by crowd-reared adults.

Maeno, 2006, 2008). In this study, such individuals also appeared from some egg pods (<2%), but they were not included in the analysis.

RESULTS

Body coloration of the RB strain

Hatchlings of the RB mutant strain were indistinguishable from those of the normal strain when they were produced by female adults that had been reared in continuous isolation, except for a short time for mating (Fig. 1A and B). In hatchlings produced by crowd-reared parents, the dark patterns of RB mutants looked similar to those of normal individuals, but the intensity of the melanization was weaker in the former than in the latter (Fig. 1C and D). No significant difference was found in head luminance between hatchlings of the two strains derived from isolated-reared locusts (Scheffé's test; $p > 0.05$; Fig. 2). In hatchlings produced by crowd-reared locusts, on the other hand, the luminance values were significantly higher in the RB mutant strain than in the normal strain (Scheffé's test; $p < 0.05$; Fig. 2). The color of exuviae was also

Fig. 2. Average luminance values for the head of normal or reddish-brown mutant hatchlings derived from either isolated- or crowd-reared adults of *Schistocerca gregaria*. Vertical lines indicate SD. Different letters on the histograms indicate significant differences at 5% (Scheffé's test). $n = 30$ each.

lighter in the former than in the latter (Fig. 1E and F). The differences in the intensity of melanization between the two strains became gradually obscure as nymphs grew bigger under crowded conditions,

and no conspicuous difference was detected at the 3rd nymphal stadium, although they were still visually distinguishable on close examination (Fig. 1G and H). In the adult stage, the intensity of melanization, especially on the wings, was lighter in the RB mutant than in the normal strain (Fig. 1I and J).

Crosses

All hatchlings obtained from each purebred strain under crowded conditions showed a phenotype similar to their parents (Fig. 3A and B). All F_1 hatchlings from reciprocal crosses between the two strains showed the normal phenotype (Fig. 3C and D). These results apparently indicated that the RB phenotype was recessive to the normal phenotype.

Figure 4 summarizes the results in the F_2 generation and backcrosses to the purebred strains. The results indicated that crosses between F_1 adults gave rise to values that were not significantly different from the expected ratio (3 : 1) by the law of segregation by Mendel (Fig. 4A and B; χ^2 -test; $p > 0.05$). In backcrosses of F_1 locusts to RB mutants, the frequencies of RB and normal progeny were almost equal (Fig. 4C and D; χ^2 -test; $p > 0.05$), indicating that the RB mutation is autosomal. On the other hand, backcrosses to the normal strain produced only normal phenotypes (Fig. 4E and F). These results indicated that the RB phenotype is controlled by a single Mendelian unit.

DISCUSSION

The present study demonstrated that the RB mutant of *S. gregaria* can be distinguished from normal individuals only when they express dark patterns characteristics of gregarious forms under crowded conditions, but the difference is most distinct at the 1st nymphal stadium (Fig. 1). In the normal strain, hatchlings were black when their mothers were reared under crowded conditions (Hunter-Jones, 1958). Under isolated conditions, both RB mutants and normal females produced green progeny in the present study. In albino mutants, hatchlings produced by crowd-reared females completely fail to express black patterns, characteristic of normal gregarious forms, and assume a whitish body color, but those produced by isolated-reared females may develop a green color (Hunter-Jones, 1957), just like normal and RB mutants.

Fig. 3. Proportions of normal (N) and reddish-brown (R) phenotypes obtained from purebred parents (A and B) and from crosses between normal and reddish-brown (RB) mutant strains (C and D) of *Schistocerca gregaria*. In each cross the female parent is listed first. Numbers in parentheses indicate n .

Fig. 4. Proportions of normal and reddish-brown phenotypes obtained from F_1 crosses (A and B) and from backcrosses (C–F). In each cross, the female parent is listed first. Numbers in parentheses indicate n .

Differences in color between RB mutants and normal individuals were also observed in the exuviae (Fig. 1). The present results were based on visual colors, and we know little about the pigments involved. However, that the exuviae is stained by pigments may indicate that they are probably melanins, although whether the observed difference between the two strains is caused by different melanin pigments or different concentrations of the same melanin is yet to be determined. It is interesting that both the enzyme and substrate for melanin

formation are present in an albino strain of *S. gregaria*, but melanization does not occur (Malek, 1957). In the RB mutant of *S. gregaria*, the capacity to express normal melanization might be suppressed or lacking.

Body color mutation is not a rare phenomenon in locusts. As mentioned, the albino phenotype is recessive to the normal phenotype in *S. gregaria* (Hunter-Jones, 1957) and *L. migratoria* (Nolte, 1971; Hasegawa and Tanaka, 1994). The present study demonstrated that the RB phenotype of *S. gregaria* is also recessive to the normal (pigmented) phenotype and is controlled by a single Mendelian unit (Figs. 3 and 4). While the genetic background of each color mutant in locusts has received much attention, no information is available about the genetic relationships between different color mutants. It would be interesting to examine such relationships by crossing different mutants.

How and when the RB mutant appeared in our colony is not known. Because it is controlled by a recessive gene, it is possible that it had been present before 2006 when it was first noticed in our colony.

A neuropeptide, [His⁷]-corazonin, plays an important role in controlling body-color polyphenism in locusts (Tanaka, 2001, 2006). Injection of this neuropeptide into green nymphs characteristic of solitary forms induces black patterns characteristic of gregarious forms in *L. migratoria* and *S. gregaria* (Tawfik et al., 1999; Tanaka, 2000a, b, 2001). There is a possibility that the RB mutant is caused by a mutation related to the synthesis of this neuropeptide or its receptor system. Another possibility is that the RB mutant might be a mutation related to the expression of enzymes responsible for normal pigmentation. Further studies on the pigments and the possible involvement of [His⁷]-corazonin would be important for understanding the cause of the less intense pigmentation in the RB mutant.

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REFERENCES

- Bouthelier, M. A. (1966) Modifications des pigments (omochromes et ptérides) en relation avec la mutation albino chez *Locusta migratoria cinerascens* Fabr. (Orthoptères, Acrididae). *C. R. Hebd. Seanc. Acad. Sci. Ser. D, Sci. Nat.* 262: 1480–1483.
- Faure, J. C. (1932) The phases of locusts in South Africa. *Bull. Entomol. Res.* 23: 293–405.
- Fuzeau-Braesch, S. (1985) Color changes. In *Comprehensive Insect Physiology Biochemistry and Pharmacology. Vol. 9. Behaviour* (G. A. Kerkut and L. I. Gilbert, eds.). Pergamon Press, Oxford, pp. 549–589.
- Gillett, S. D. (1973) The role of integumental colour pattern in locust grouping. *Anim. Behav.* 21: 153–156.
- Hasegawa, E. and S. Tanaka (1994) Genetic control of albinism and the role of juvenile hormones in pigmentation in *Locusta migratoria* (Orthoptera, Acrididae). *Jpn. J. Entomol.* 62: 315–324.
- Hunter-Jones, P. (1957) An albino strain of the desert locust. *Nature* 180: 236–237.
- Hunter-Jones, P. (1958) Laboratory studies on the inheritance of phase characters in locusts. *Anti-Locust Bull.* 29: 1–32.
- Hunter-Jones, P. (1960) Fertilization of eggs of the desert locust by spermatozoa from successive copulations. *Nature* 185: 336.
- Lester, R. L., C. Grach, M. P. Pener and S. J. Simpson (2005) Stimuli inducing gregarious colouration and behaviour in nymphs of *Schistocerca gregaria*. *J. Insect Physiol.* 51: 737–747.
- Maeno, K. and S. Tanaka (2004) Hormonal control of phase-related changes in the number of antennal sensilla in the desert locust, *Schistocerca gregaria*: possible involvement of [His⁷]-corazonin. *J. Insect Physiol.* 50: 855–865.
- Maeno, K. and S. Tanaka (2007) Effects of hatchling body colour and rearing density on body colouration in last stadium nymphs of the desert locust, *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae). *Physiol. Entomol.* 32: 87–94.
- Maeno, K., T. Gotoh and S. Tanaka (2004) Phase-related morphological changes induced by [His⁷]-corazonin in two species of locusts, *Schistocerca gregaria* and *Locusta migratoria* (Orthoptera: Acrididae). *Bull. Entomol. Res.* 94: 349–357.
- Malek, S. R. A. (1957) Sclerotization and melanization; two independent processes in the cuticle of the desert locust. *Nature* 180: 237.
- Nolte, D. J. (1971) Two pleiotropic albino mutations. In *Proceedings of the 4th Congress of the South African Genetic Society* (Pretoria, 1970). South African Genetic Society, Pretoria, pp. 36–38.
- Pener, M. P. (1991) Locust phase polymorphism and its endocrine relations. *Adv. Insect Physiol.* 23: 1–79.
- Putnam, L. G. (1958) Albinism in the migratory grasshopper

- per, *Melanoplus bilituratus* (Wlk.). *Nature* 182: 1529.
- Simpson, S. J. and G. A. Miller (2007) Maternal effects on phase characteristics in the desert locust, *Schistocerca gregaria*: a review of current understanding. *J. Insect Physiol.* 53: 869–876.
- Stower, W. J. (1959) The color patterns of hoppers of the desert locust *Schistocerca gregaria* (Forskål). *Anti-Locust Bull.* 32: 1–75.
- Tanaka, S. (1993) Hormonal deficiency causing albinism in *Locusta migratoria*. *Zool. Sci.* 10: 467–471.
- Tanaka, S. (2000a) The role of [His⁷]-corazonin in the control of body-color polymorphism in the migratory locust, *Locusta migratoria* (Orthoptera: Acrididae). *J. Insect Physiol.* 46: 1169–1176.
- Tanaka, S. (2000b) Hormonal control of body-color polyphenism in *Locusta migratoria*: interaction between [His⁷]-corazonin and juvenile hormone. *J. Insect Physiol.* 46: 1535–1544.
- Tanaka, S. (2000c) Induction of darkening by corazonins in several species of Orthoptera and their possible presence in ten insect orders. *Appl. Entomol. Zool.* 35: 509–517.
- Tanaka, S. (2001) Endocrine mechanisms controlling body-color polymorphism in locusts. *Arch. Insect Biochem. Physiol.* 47: 139–149.
- Tanaka, S. (2003) Effects of temperature and [His⁷]-corazonin on the body darkening in *Locusta migratoria*. *Physiol. Entomol.* 28: 290–297.
- Tanaka, S. (2004) Environmental control of body-color polyphenism in the American grasshopper, *Schistocerca americana*. *Ann. Entomol. Soc. Am.* 97: 293–301.
- Tanaka, S. (2006) Corazonin and locust phase polyphenism. *Appl. Entomol. Zool.* 41: 179–193.
- Tanaka, S. and K. Maeno (2006) Phase-related body-color polyphenism in hatchlings of the desert locust, *Schistocerca gregaria*: Re-examination of the maternal and crowding effects. *J. Insect Physiol.* 52: 1054–1061.
- Tanaka, S. and K. Maeno (2008) Maternal effects on progeny body size and color in the desert locust, *Schistocerca gregaria*: examination of a current view. *J. Insect Physiol.* 54: 612–618.
- Tanaka, S. and S. Yagi (1997) Evidence for the involvement of a neuropeptide in the control of body color in the desert locust, *Schistocerca gregaria*. *Jpn. J. Entomol.* 65: 447–457.
- Tanaka, S. and D.-H. Zhu (2003) Phase-related differences in mating strategy of a locust (Orthoptera: Acrididae). *Ann. Entomol. Soc. Am.* 96: 498–502.
- Tawfik, I. A., S. Tanaka, A. De Loof, L. Schoofs, G. Baggerman, E. Waelkens, R. Derua, Y. Milner, Y. Yerushalmi and M. P. Pener (1999) Identification of the gregarization-associated dark-pigmentotropin in locusts through an albino mutant. *Proc. Natl. Acad. Sci. USA* 96: 7083–7087.
- Uvarov, B. P. (1966) *Grasshoppers and Locusts*. Vol. 1. Cambridge University Press, Cambridge. 481 pp.
- Verdier, M. (1965) Mutation albinos de *Locusta migratoria*. I. Origine et description (C.S.). *Bull. Soc. Zool. Fr.* 90: 493–501.
- Volkonsky, M. A. (1938) Une mutation mélanique de *Schistocerca gregaria* Forsk. Obtenue en élevage. *C. R. Soc. Biol. Paris* 127: 254–256.
- Yerushalmi, Y., H. Abu-Hilal and M. P. Pener (2000) A “dark-adult” mutation of *Schistocerca gregaria* (Forsk.). *J. Orthoptera Res.* 9: 41–43.
- Zhu, D.-H. and S. Tanaka (2002) Prolonged precopulatory mounting increases the length of copulation and sperm precedence in *Locusta migratoria* (Orthoptera: Acrididae). *Ann. Entomol. Soc. Am.* 95: 370–373.