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Effect of Temperature on Pupal Pigmentation and Size of the Elytral Spots in *Coccinella septempunctata* (Coleoptera: Coccinellidae) from Four Latitudes in Japan

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Coccinella septempunctata populations collected from different latitudes in Japan were reared for the first time at three constant temperatures and the size of the central elytral spot was measured. At 20°C the elytral spot size increased with latitude. It was significantly greater in the Sapporo population, 43°N, than in the Tsukuba and Kagoshima populations, which are at latitudes 36 and 32°N, respectively, and smallest in the Iriomote population at latitude 24°N. In all populations the elytral spot increased in size when reared at lower temperature. This tendency was very strong in the Sapporo population. The pigmentation of pupae increased with decrease in temperature, thus they were mostly orange at 30°C and dark brown at 20°C. In agreement with previous reports by DOBZHANSKY and SASAJI, it is assumed that all three populations from the main Japanese islands belong to *C. septempunctata brucki*. In the Iriomote population, the spot was small and little affected by rearing temperature; this suggests that this population may belong to the subspecies *C. septempunctata septempunctata*. The contrast between earlier and later reports on the occurrence of the two subspecies in the Ryukyu Islands is discussed in relation to the dense ship transport from the main Japanese Islands after 1972.

Key words: *Coccinella septempunctata*, elytral spot, pupal pigmentation, latitude, temperature

INTRODUCTION

The geographical variation in the size of the spots on the elytra of *Coccinella septempunctata* cannot be attributed to variations in climate alone, although some authors have proposed that the trends in spot size are correlated with temperature and particularly with humidity (HONĚK, 1996). In this regards, it is of interest to clarify to what extent the elytral pattern of individuals from particular localities can be modified by rearing temperature.

To determine the degree to which genetic constitution and temperature interact in determining the elytral patterns of ladybirds, individuals from several latitudinally remote populations of *C. septempunctata* were reared at several constant temperatures and the size of their central elytral spots was measured. Another aim was to describe the effect of rearing temperature on the colour of the pupae of *C. septempunctata*, as the earlier report is vague on this point (HODEK, 1958).

MATERIALS AND METHODS

Insects. Adults of *C. septempunctata* were collected from four lowland localities in Japan: Sapporo (SPR) on Hokkaido Island at latitude 43°N, Tsukuba (TKB) on Honshu Island at 36°N, Kagoshima (KGS) on Kyushu Island at 32°N and Iriomote Island (IRI) in the Ryukyu Islands at 24°N (Fig. 1). Progenies were reared from egg to adult at 20°C, 25°C and 30°C to determine the effect of temperature on the size of the elytral spot. In addition, individuals from the SPR population were reared under long (16L:8D) and short day (12L:12D) conditions. Coccinellids were fed on aphids (mainly *Acrythosiphon pisum*). As there was no significant difference in the spot size of adults reared at these two daylengths the data are not presented. Adults were also sampled from České Budějovice (49°N), Czech Republic, on Sept. 8, 1994 and Naha (26°N), Japan, on March 20, 1993.

Measurement of elytral spot. As the relative sizes of the elytral spots, on and between the elytra of an individual, are the same, only the central elytral spot on the right elytrum of each beetle was measured with a micrometer attached to a binocular (Fig. 2). The index described below was used for expressing the relative size of the elytral spot, where the value of the spot width (b) was multiplied by the spot length (c) and then by 100; this was then divided by the square of the elytral length (a).

$$\text{Elytral spot index} = b \cdot c \cdot 100/a^2$$

DOBZHANSKY used a different formula for $38.0 \cdot c$ (DOBZHANSKY, 1933; DOBZHANSKY and

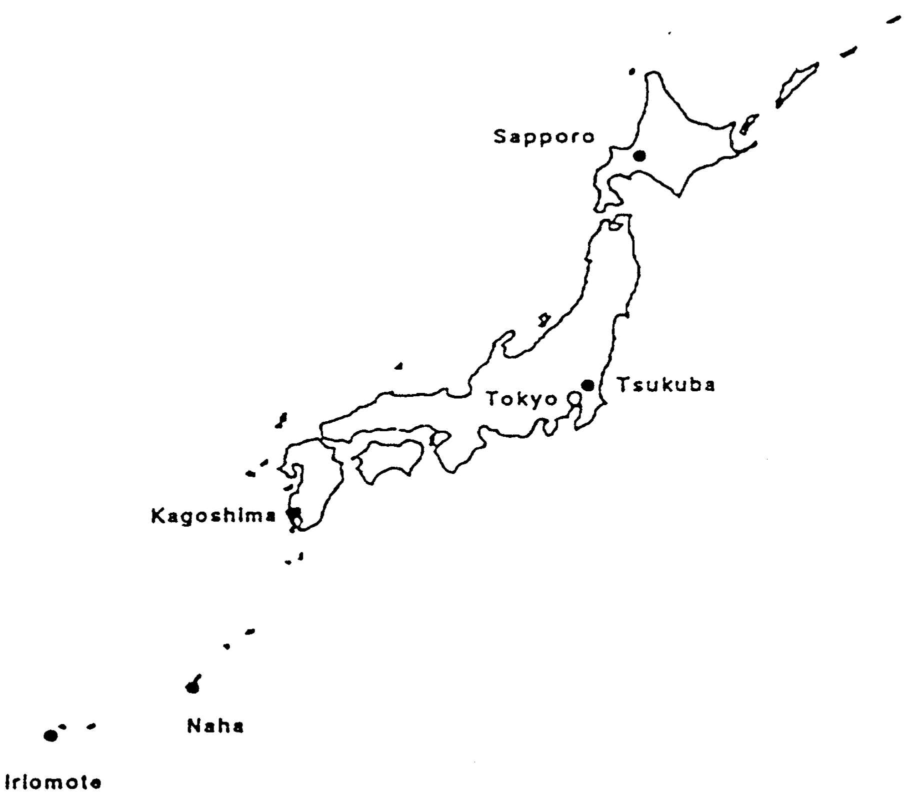

Fig. 1. Geographical localities of the five populations of *C. septempunctata* in Japan used in this study.

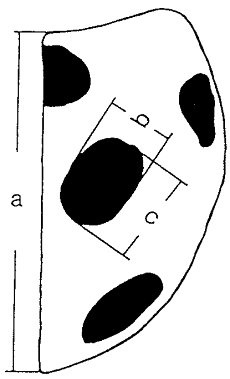

Fig. 2. Scheme of elytran spots of *C. septempunctata* and parameters for measuring spot size. Spot size was obtained by the formula: $b \cdot c \cdot 100/a^2$.

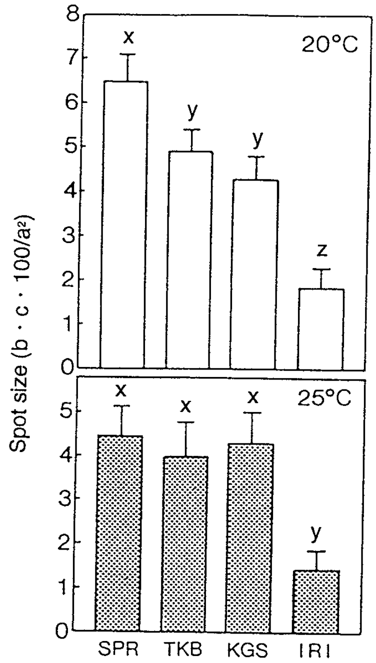

Fig. 3. Comparison of spot size in four populations of *C. septempunctata* reared at 20°C (above) and 25°C (below). Means followed by the same letter in each column are not significantly different ($p > 0.05$) by KRUSKAL-WALLIS test ($n = 20$–30). Vertical lines indicate SD.

SIVERTZEV-DOBZHANSKY, 1927). We developed our formula to include a measure of body size which was found to correlate with the spot size ($r = 0.691$; $df = 73$; $p < 0.0001$, TKB population). In other words, spot size is positively correlated with body size.

RESULTS

Size of elytral spots

When the beetles were reared at 20°C, the size of the central elytral spot was significantly greater in the descendents of the beetles collected from SPR than those from TKB and KGS, and that from IRI had the smallest spot size (Fig. 3). The results indicated that the spot size increased with latitude. When the beetles were reared at 25°C the latitudinal trend was less conspicuous.

The size of the elytral spot changed with rearing temperature within each population. For three populations, excluding the KGS population, the spot size was greatest at the lowest temperature (Fig. 4). This tendency was most conspicuous in the SPR population. In the IRI population the spot was quite small and was the same size as that of *C. septempunctata septempunctata* from south Bohemia, central Europe (CZ) (Fig. 5). Samples collected from the Okinawa mainland (NAH) (latitude 26°N), indicate that the population there is made up of two subspecies of *C. septempunctata* because the range of their spot size partly covered the value (1–2) of the samples of *C. septempunctata septempunctata* from central Europe.

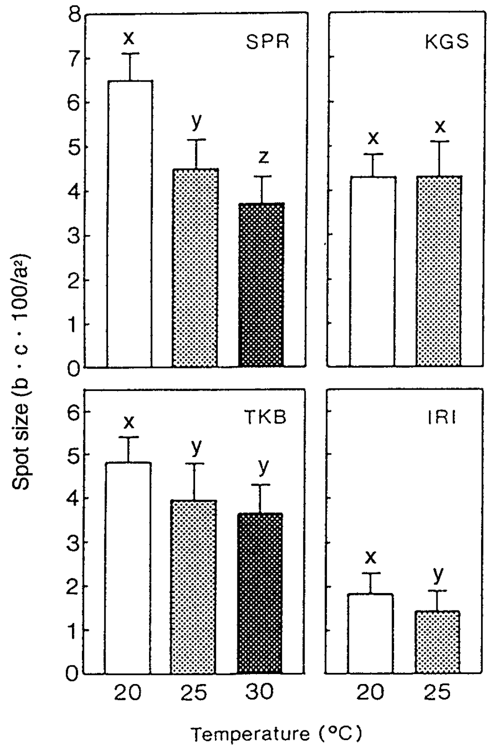

Fig. 4. Modification of spot size in each population at different rearing temperatures. Means followed by the same letter in each column are not significantly different ($p > 0.05$) by KRUSKAL-WALLIS test (SPR, TKB) or *U*-test (KGS, IRI) ($n = 20$–30). Vertical lines indicate SD.

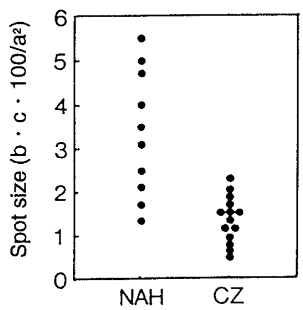

Fig. 5. Spot size of Naha (NAH) and Czech (CZ) populations of *C. septempunctata* sampled from the field.

Pupal colour

We determined the relationship between pupal coloration and imaginal elytral spot size. When reared at 20°C the background colour of pupae was almost black, while at 30°C it was almost orange (Fig. 6). At 25°C the pupae were of intermediate coloration. When reared at 20°C even pupae of the IRI population, which has the smallest elytral spots, were as black as those of the SPR population which has the largest elytral spots. This indicates that pupal and larval (data not shown) coloration is independent of elytral spot size.

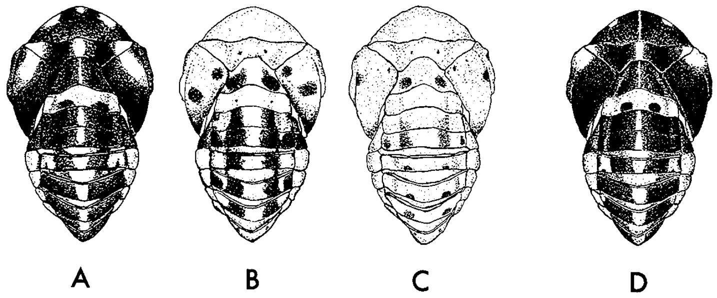

Fig. 6. Pigmentation of pupae reared at different temperatures. A, B and C: Sapporo population, D: Iriomote population. A and D: reared at 20°C; B: at 25°C; C: at 30°C.

DISCUSSION

Size of elytral spots in Japanese populations

We found a latitudinal trend in the size of the central elytral spots in Japanese populations of *C. septempunctata*. When reared at 20°C, the spot size differed significantly among different geographic populations and increased with latitude (Fig. 3). It has thus been experimentally documented over a latitudinal range of almost 20° from Hokkaido to Ryukyu that the size of the central elytral spot in *C. septempunctata* has a genetic basis. It is also likely that the climatic conditions of the regions studied (probably not only temperature, but also humidity) have been important in determining the evolution of coloration in these beetles. This study did not attempt to discriminate the variation between these two components, discussed by MAJERUS (1994). It is also possible that the differences between the populations would be greater if coccinellids were less mobile and the populations as a consequence more genetically isolated. It has previously been reported that the size of the elytral spot in *C. septempunctata* is exceptionally large in Kamchatka (56.1), Saghalien (56.0), and Japan (66.2) (DOBZHANSKY, 1933; DOBZHANSKY and SIVERTZEV-DOBZHANSKY, 1927). The value given for Japan is very similar to the figure presented here for Sapporo (65.5) at 20°C (The values in parentheses were calculated by Dobzhansky's formula: central elytral spot index $= 38.0 \cdot c$).

C. septempunctata on Ryukyu islands

All three of our populations from the main Japanese Islands (Hokkaido, Honshu, Kyushu) show large spots, indicating that they belong to the subspecies *C. septempunctata brucki*. The IRI population in a tropical climate had much smaller spots (which appeared to be inert to temperature impact) (Fig. 4) than the KGS population, although the temperatures at Kagoshima are also high (annual mean of daily maximum and minimum: 21.1, and 12.8°C, respectively). The IRI population appears to belong to *C. septempunctata septempunctata* since the spots are similar in size to those of the same subspecies from central Europe (Fig. 5), where the weather is similar to Hokkaido. The wide range of variation in spot size in the NAH population from the main Okinawa island indicates a mixture of both subspecies (Fig. 5).

Probably due to the size of the elytral spot, SASAJI (1971) reported that the population from the Ryukyu Islands (including the Okinawa main island and Iriomote Island) belonged to *C. septempunctata septempunctata*. However, according to LEEPER (1976), the Ryukyu population belongs to *C. septempunctata brucki*. The important increase in transport between

Okinawa and the Japanese main islands after the end of the American occupation of the Ryukyu Islands in 1972 might explain the conflicting reports by these two authors. The present results support this assumption.

Effect of temperature on spot size

The temperature at which the beetles were reared affected spot size in nearly all samples of the four populations; the size decreased with increases in temperature.

Many workers (DOBZHANSKY and SIVERTZEV-DOBZHANSKY, 1927; HONĚK, 1996; MAJERUS, 1994) have suggested that temperature and humidity might be important for elytral pigmentation. In this study we did not modify humidity. However, the relative humidity is modified by temperature, thus, our results were perhaps partly influenced also by different relative humidities.

Pigmentation in larvae and pupae

When reared at low temperatures, both larvae and pupae were heavily pigmented. This may be adaptative because heavily pigmented larvae/pupae can absorb more solar radiation, and as a consequence have a higher body temperature and faster development than lightly pigmented ones (HODEK, 1958; TANAKA, 1995). In *C. septempunctata* the larval and pupal pigmentation did not depend on the origin of the sample (population or subspecies) but on the rearing temperature (Fig. 6). Although the pigmentation of the elytral spot was also affected by rearing temperature, the effect was not as great as that observed in larvae and pupae.

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REFERENCES

DOBZHANSKY, T. (1933) Geographical variation in lady-beetles. *Am. Nat.* **67**: 97–126.

DOBZHANSKY, T. and N.P. SIVERTZEV-DOBZHANSKY (1927) Die geographische Variabilitat von *Coccinella septempunctata*. *Biol. Zentralbl.* **47**: 556–569.

HODEK, I. (1958) Influence of temperature, rel. humidity and photoperiodicity on the speed of development of *Coccinella 7-punctata* L. *Čas. Čs. Spol. Entomol.* (*Acta Soc. Entomol. Čechoslov.*) **55**: 121–141 (in Czech with English summary).

HONĚK, A. (1996) Variability and genetic studies. In *Ecology of Coccinellidae* (I. HODEK and A. HONĚK, eds.). Kluwer Acad. Publ., Dordrecht, pp. 33–60.

LEEPER, J.R. (1976) A review of the Hawaiian Coccinellidae. *Proc. Hawaii Entomol. Soc.* **22**: 279–306.

MAJERUS, M.E.N. (1994) *Ladybirds*. Harper Collins Publishers, London, 367 pp.

SASAJI, H. (1971) *Coccinellidae. Fauna Japonica.* Academic Press of Japan, Tokyo, 340 pp.

TANAKA, K. (1995) Colouring of pupae in *Harmonia axyridis*. *Insectarium* **32**: 14–17 (in Japanese).