

Maternal Influence and Temperature Effects on the Body Size of the Ladybird Beetle, *Cheilomenes sexmaculata* (FABRICIUS, 1781) (Coleoptera, Coccinellidae)

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Abstract The ladybird beetle, *Cheilomenes sexmaculata* (FABRICIUS, 1781), exhibits a remarkable elytral colour polymorphism composed of black and red, and a substantial variation in body size. Over the past 100 years, this ladybird species has spread out into western and central Japan because of climate warming; meanwhile, small, dark morph types have become the dominant phenotype. To determine whether the change in body size can be attributed to the temperatures experienced at growth, we investigated the effect of temperature at various developmental stages, from the first instar larva to the pupal stage, in a polymorphic population from Osaka, Japan. Female individuals of three different elytral morph types were collected from the wild population, and hatchlings from each female were equally divided into three groups and reared at constant temperatures: 20°C, 25°C, and 30°C. Our findings suggest that the body size of F1 adults was determined by genetic factors, not by growth temperatures. Moreover, the body size of the F1 population did not affect the F1 morph types. Therefore, the dominance of small dark morph types in many areas of Japan has genetic basis.

Keywords: elytral colour, morphs, growth temperature, maternal body size.

Introduction

The expression of phenotypic traits in organisms is dependent on both genetic factors and plastic responses to the environment. In regard to the relationship between body size and temperatures in ectotherms, these often exhibit a smaller body size and a faster growth rate when reared at higher temperatures. This phenomenon is called “the temperature-size rule” (e.g., ATKINSON, 1994; ANGILLETTA & DUNHAM, 2003). Although this thermal life-history plasticity has been observed in phylogenetically widespread invertebrates, many exceptions exist (ATKINSON, 1994, 1995; KLOK & HARRISON, 2013).

When examining the body size variations across geographical regions, the body size usually increased with latitude, a phenomenon called “Bergmann cline” (BERGMANN, 1847). This cline was applied to endotherms early, however, ectotherms including insects have later been showed a similar tendency based on field surveys (ALPATOV, 1929; BRYANT, 1977; ARNETT & GOTELLI, 1999; ROBINSON & PARTRIDGE, 2001). Nonetheless, many species show an inverse tendency (e.g., KLOK & HARRISON, 2013). In order to consider the proximal mechanisms for this cline, it is important to distinguish the following two factors: (1) the variation of genotypic frequencies among local populations; and (2) the plastic response of phenotypes, resulting from the environmental changes occurring along latitudinal gradients (IRIE, 2010). When considering the factors that might influence the plastic phenotypical response (2), “the temperature-size rule” may be key to explain the “Bergmann cline” mechanism: due to diverse environmental conditions, the influence of temperature might be especially significant (IRIE, 2010).

The ladybird beetle, *Cheilomenes sexmaculata* (FABRICIUS, 1781), is a common predator of aphids, and is widely distributed from intermediate to equatorial latitudes in Asia (SASAJI, 1971). Over

the past 100 years, this ladybird species has expanded its distribution in Japan from Kyusyu (33°N) to Kantô-Hokuriku (36°N) in response to climate warming (KAWAKAMI *et al.*, 2014). During this process, the frequency of melanic morph types increased, while the body size decreased in newly inhabited regions compared to the former range of distribution (KAWAKAMI *et al.*, 2015). Consequently, body size and morph types in this species show the geographical cline: the number of smaller and darker morph types increases with higher latitudes, why dark types tending to be smaller than light types in the field. It was reported that morph type expression was not related to temperature experienced during immature periods in this ladybird (KAWAKAMI *et al.*, 2018).

In this study, we investigated the variation in body size in *C. sexmaculata*. In order to do so, we field-caught females from a wild population in Osaka, Japan, and evaluated whether growth temperatures were correlated with the phenotypic expression of different body sizes by rearing the eggs deposited by these females at different temperatures. Subsequently, we examined the relationship between genetics and the phenotypic expression of the body size by evaluating the effects of both maternal size and growth temperatures on the F1 colour morph type. Finally, we evaluated whether the body size was morph type-dependent by examining the relationships between F1 morph types and their body sizes.

Materials and Methods

Cheilomenes sexmaculata is known to have 20 phenotypes of elytral polymorphs, including nigra, and types A to U, in which the degree of the elytral light and red portion varies from nigra (completely dark type), and type A (minimum light) to type U (maximum light) (SASAJI & AKAMATSU, 1979; MATSUKA *et al.*, 1985; KAWAKAMI *et al.*, 2013; Fig. 1). Among the populations of *C. sexmaculata* (34°36'N) observed in Osaka, only the morph types from nigra to "I" have been reported (KAWAKAMI *et al.*, 2013).

To examine the influence of growth temperatures on the determination of body sizes, seven female adults of *C. sexmaculata* (morph types: type A: three individuals; type B: two indiv.; type G: two indiv.) were collected in Nagai Park (34°36'N, 135°31'E), Osaka City, central Japan (Honshu) on April 11 and 13, 2004. The adults were transported to the laboratory, where they were individually placed in a transparent laboratory dish (95 mm in diameter, 20 mm in height). They were kept under a photoperiod of 16L : 8D (long photoperiod) at 25°C, and were provided with large quantities (leftovers were found daily) of *Aphis craccivora* KOCH, 1854 reared using seedlings of soybean (*Vicia faba* LINNAEUS) as the host plant. Eggs were obtained from the adult ladybirds, and the first instar larvae hatched from each batch were divided into three equivalent groups, and reared at constant temperatures of 20°C, 25°C, and 30°C, under a photoperiod of 16L : 8D, and with ample amounts of *A. craccivora*.

The body size of the emerged F1 adults, i.e., the length from the vertex of the head to the apex of elytra, were measured individually using slide calipers to the nearest 0.01 mm. To investigate plastic factors that might influence the variation in body size, the mean body sizes of the F1 group was compared between the three growth temperatures in both sexes. Next, to examine genetic factors, the mean body sizes were calculated for both sexes, and from each mother. The relationship between the F1 body sizes, growth temperatures, and the maternal body size were analysed using a two-way analysis of variance (ANOVA) in both sexes. The experiment was restricted to F1 individuals whose mother produced six or more larvae. Males and females of the F1 were born from four and six mothers, respectively. Finally, to examine the relationship between the body sizes and morph types of the F1 population, body sizes were tallied in relation to morph types for both sexes, and were analysed

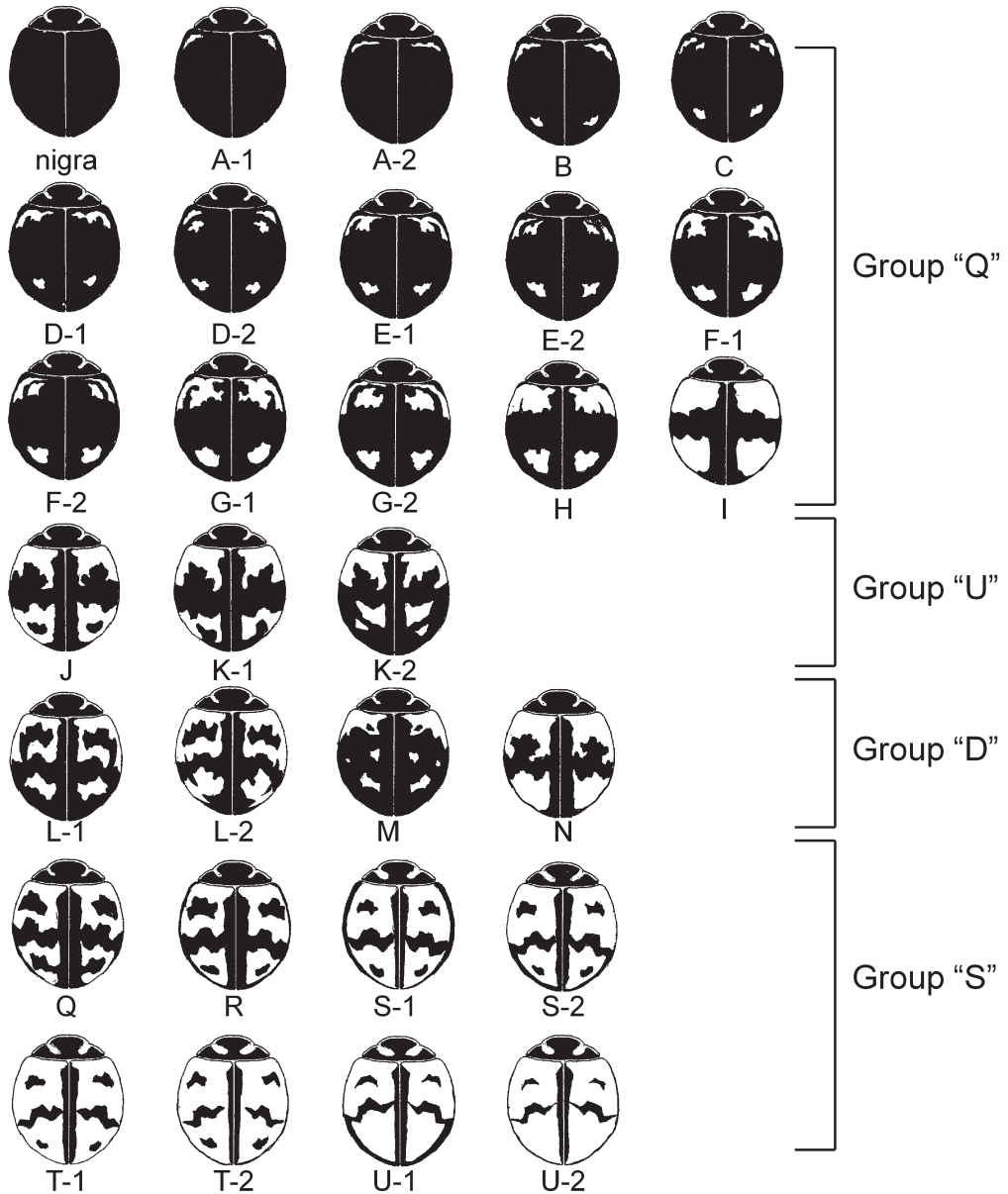


Fig. 1. Elytral morph types of *Cheilomenes sexmaculata* (FABRICIUS) reproduced from KAWAKAMI *et al.* (2013).

using a one-way analysis of variance (ANOVA). We used JMP 11 Discovery Software for all statistical analyses (SAS, 2013).

Results and Discussion

Oviposited eggs from field-caught adult females ($n = 7$) were reared to 135 individual F1 adults (58 males and 77 females). No significant difference was observed between the mean adult body size

Table 1. Results of a two-way ANOVA on the effects of family (maternal morph type) and temperature on body size.

| Variable | d.f. | SS | F | P |
|----------------------|------|------|------|--------|
| <i>Female</i> | | | | |
| Family | 5 | 48.1 | 4.63 | 0.0013 |
| Temperature | 2 | 5.9 | 0.57 | 0.5677 |
| Family × Temperature | 10 | 11.8 | 1.13 | 0.3548 |
| <i>Male</i> | | | | |
| Family | 3 | 22.7 | 5.94 | 0.0018 |
| Temperature | 2 | 5.2 | 1.35 | 0.2698 |
| Family × Temperature | 6 | 4.7 | 1.22 | 0.3143 |

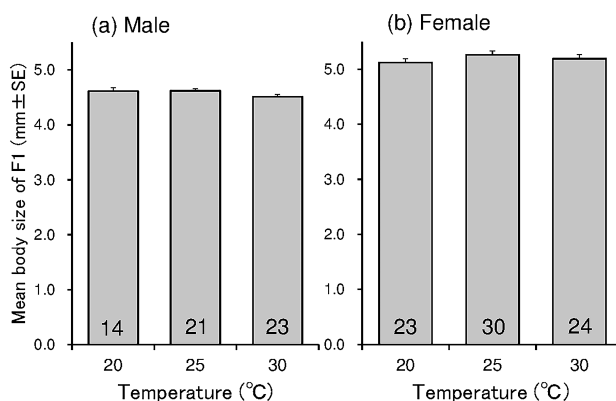


Fig. 2. The mean body size of F1 for (a) male and (b) female reared at 20°C, 25°C, 30°C. Vertical bars represent the standard error (SE). The numbers in each column represent the sample size.

of the F1 population and the temperatures at which they were grown in either sex (Table 1 & Fig. 2 a, b). The body sizes of each mother's F1 offspring were distributed within narrow ranges, regardless of mothers' size (Fig. 3 a, b). Namely, the body size of F1 individuals was significantly correlated to that of their mothers (maternal family; Table 1) rather than to growth temperatures. There was no significant difference in either sex between the F1 morph types and the F1 body sizes (Fig. 4 a, b; Male: $F = 0.1871$, $df = 4$, $P = 0.9441$; Female: $F = 0.8215$, $df = 5$, $P = 0.5385$).

This study clarified that the variation in body size of *Cheilomenes sexmaculata* is independent on growth temperature and is based on genetic factors instead (Table 1 & Figs. 2 & 3). However, the reason why smaller individuals tend to be dark morph types rather than light types in the field may be neither genetic correlation nor pleiotropism but be a subset of traits selected in the field, because no relationship was found between the morph types and the body sizes of F1. For instance, larger dark morph types may have a higher mortality rate than smaller individuals. In general, ladybird species with a larger body size tend to express light morph types in order to prevent overheating, because a larger body size can absorb more radiant energy than a smaller body size (BRAKEFIELD & WILLMER, 1985; STEWART & DIXON, 1989).

As an example of intraspecific variation in response to temperatures in Coccinellidae, it was reported that the body size in *Chilocorus nigritus* (FABRICIUS, 1798) decreased as the temperatures increased from 22°C to 34°C (PONSONBY, 2009), but this tendency differs from our study on *C. sexmac-*

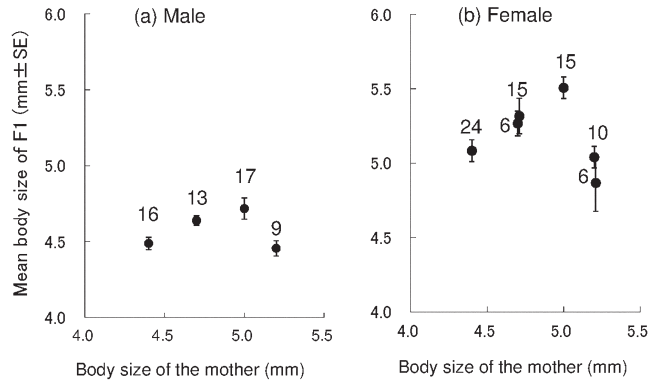


Fig. 3. The mean body size of F1 for (a) male and (b) female in relation to body size of the mother. Vertical bars represent the standard error (SE). The numbers on each bar represent the sample size.

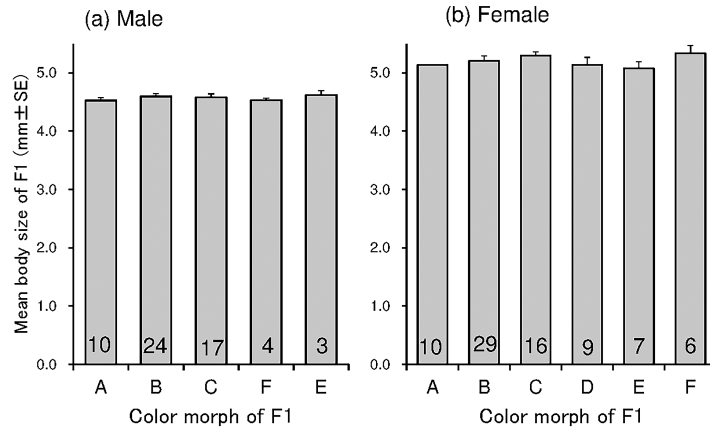


Fig. 4. The mean body size for (a) male and (b) female in each F1 colour morph. Vertical bars represent the standard error (SE). The numbers in each column represent sample size.

ulata. Moreover, females of ladybirds are generally larger and heavier than males (e.g., OMKAR *et al.*, 2009; UNGEROVÁ *et al.*, 2010, which is similar to that in *C. sexmaculata* (Figs. 2–4). However, regarding the influence of the growth temperature on body size, it is difficult to separate it from the effect of prey consumption and/or prey quality (NEDVĚD & HONĚK, 2012). Therefore, further studies are needed on whether prey consumption changed according to temperatures and whether availability of prey aphids varies with temperatures to conclude the causation of the present result.

As a plastic response of the body size to long-term change of temperature, the possibility that body size reduces because of a response to global warming has been proposed (DAUFRESNE & SAHUP, 2009). To date, it was indicated that the global warming caused the shift of species toward higher altitudes and latitudes (e.g., BEAUGRAND *et al.*, 2002) and the seasonal shifts in life cycle events (e.g., WALTHER *et al.*, 2002) of ectotherms. DAUFRESNE *et al.* (2009) reported that reduced body size of ectotherms among species, populations and communities are the third universal ecological response to the global warming in aquatic systems.

This study indicates that temperature did not play a role for body size reduction during range expansion in *C. sexmaculata*. As a possible factor, for instance, it is suggested that small body size may confer a survival advantage to adult insects because of the efficient use of radiant heat in higher latitudes with weaker radiance and shorter sunlight time than lower latitudes. Hence, the range expansion of the small types of this species to higher latitudes may have been driven by positive selection (KAWAKAMI *et al.*, 2015).

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要 約

河上康子・山崎一夫・大橋和典：ダンダラテントウ（鞘翅目テントウムシ科）における体サイズに生育温度が与える影響。——ダンダラテントウ *Cheilomenes sexmaculata* (FABRICIUS) は鞘翅に斑紋型多型をもち、かなりの体サイズ変異を示す。本種は気候温暖化により過去 100 年以上にわたり日本の西部と中央部へ分布を拡大した。その間に小型で暗色の型が優占的になった。そこで、本種の体サイズは生育温度に影響を受けて変化するかを室内飼育実験により調査した。野外から大阪個体群の 3 タイプの斑紋型をもつ雌個体を採集した。それぞれの雌個体が産卵した卵塊が孵化した直後、1 齢幼虫を均等に 3 つに分け、20°C、25°C、30°C で飼育した。その結果、子世代の体サイズは飼育温度の影響を受けず、どの母親個体に由来するか依存していた。また子世代の体サイズは子世代の斑紋型と相関していなかった。そのため、日本の多くの地域でみられる小型で暗色の型には遺伝的な基盤があると考えられた。

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