Increase in dark morphs and decrease in size during a range extension of *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae)

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Abstract. The ladybird beetle *Cheilomenes sexmaculata* (Fabricius) is widely distributed throughout the intermediate latitudinal and equatorial regions. It exhibits elytral colour polymorphism with a geographical cline showing increased prevalence of melanic morphs with increasing latitude. Over the past 100 years, this species has extended its distribution towards higher latitudes in Japan because of climatic warming. In this study, long-term changes in the frequency of different morphs and adult body size of this species during this range extension were analyzed based on a survey of specimens in collections. First, we examined specimens collected from areas that were colonized (34–36°N) during the period of range extension (1949–1989) and when it ceased (1990–2005). Chronologically, both the proportions of melanic morphs increased and body size decreased significantly. Next, specimens were examined from newly colonized regions and the former range of distribution (32–34°N); these specimens were collected during period when range extension had ceased. In newly colonized regions, the frequency of melanic morphs was higher and the body size was smaller than in the former range of distribution. These two findings indicate that the smaller, melanic morphs may have undergone a positive-selection driven range extension to higher latitudes.

INTRODUCTION

Insects that are distributed widely are known to exhibit various changes in their life history traits in response to spatial and temporal variations in habitat (Masaki, 1974). Consequently, variation in an assortment of intraspecific geographical traits, such as diapause (e.g. Danilevsky, 1965; Masaki, 1967; Danks, 1987), cold hardiness (Chen & Kang, 2004), body size (e.g. Sota et al., 2000; Stillwell et al., 2007) and morph frequency (Sloggett & Honěk, 2012) are reported. Furthermore, in the process of range extension, changes in insect phenotypes are recorded that are associated with adapting to the newly colonized areas (e.g. Grill et al., 1997).

For many insect species a range extension from low to high latitudes has been reported in recent years. Examples include the Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Boman, 2008), the great mormon *Papilio memnon* (Yoshio & Ishii, 2001) and the southern green stinkbug *Nezara viridula* (L.) (Musolin, 2007; Yukawa et al., 2007; Tougou et al., 2009). Three potential mechanisms driving this extension have been proposed: (1) climatic warming (Yoshio & Ishii, 2001); (2) changes in the climatic adaptive capability of the insect (Holt, 2003); and (3) a combination of both (1) and (2) (Gomi, 1997; Logan et al., 2003). In the cases of (2) and (3), changes in traits that are associated with adapting to changes in climate may occur during the process of northward extension. Nevertheless, few studies have investigated chronological changes in traits based on a comprehensive survey of specimens over time and across geography. Studies of the relationship between trait change and northward extension, therefore, may provide insight into the invasion and subsequent spread of non-native insects in response to climate change.

The lady beetle *Cheilomenes sexmaculata* is a common predator of aphids that is widely distributed throughout the mid-latitudes and equatorial regions (Sasaji, 1971). This species is polymorphic in terms of its elytral colour, with both melanic and non-melanic morphs (Sasaji & Akamatsu, 1979; Kawakami et al., 2013). It exhibits 22 elytral colour phenotypes (Fig. 1; nigra, types A–U; Sasaji & Akamatsu, 1979; Matsuka et al., 1985), and the proportion of red elytral colouration increases regularly from type A to U (Kawakami et al., 2013). Among them, types nigra–I (Fig. 1) are distributed from Kyushu to Honshu (31–36°N) in Japan (Kawakami et al., 2013). Geographical variation in the incidence of the different morphs is recorded, with the proportion of melanics increasing with latitude (Kawakami et al. 2013). Over the past 100 years, this species has extended its distribution from 32–34°N to 34–36°N on the main Japanese islands in response to climate warming (Kawakami et al., 2014); however, this geographical extension apparently ceased in the 1990s (Kawakami et al., 2014).

In this study, we focused on an analysis of two changes in phenotype, frequencies of different morphs and body size, in specimens collected during the period of northward range extension. First, in the newly colonized areas (34–36°N) we determined whether the frequencies of the different morphs and body size changed during the period of range extension and when it ceased. Second, we examined specimens collected after the range extension ceased (after 1990), to determine whether the frequencies

of different morphs and body sizes differed in the newly colonized areas and in the former range of distribution. In addition, we determined the variation in body size in the different morphs of specimens in collections. Herein we describe the phenotypic changes recorded and discuss the selective pressures driving these phenomena, with emphasis on climatic factors.

MATERIAL AND METHODS

Long-term changes in morph frequency and adult body size during the period of range extension

C. sexmaculata extended its distribution towards higher latitudes between 1920 and 1990, after which range extension ceased in Japan (Kawakami et al., 2014). To investigate long-term changes in morph frequency and adult body size during range extension, we examined specimens that were collected from the newly colonized areas (34–36°N, 208 sites) during the period of range extention (1949–2005). We examined 442 specimens (181 males, 261 females), and the localities at which they were collected and dates (as noted on specimen labels), morph type (nigra to I), sex, and body sizes were documented. Adult body length was measured from the vertex of the head to the apex of the elytra using a slide caliper with an accuracy of 0.01 mm.

The records for the specimens in the collections were subsequently divided into those collected in three consecutive periods: the early period of range extension (1949–1959), the latter period of range extension (1960–1989) and the period when range extension had ceased (1990–2005). The frequencies of the different morphs in the three periods were determined. The dataset was analyzed using tests for independence. Next, the relation between body size and the years in which they were collected (1949–2005) was analyzed using regression analysis and ANOVA.

Latitudinal variations in morph frequency and adult body size during the period when range extension had ceased

To determine the latitudinal variation in morph frequencies and adult body size during the period when range extension had ceased we examined specimens collected between 1990 to 2005 at 32–34°N (15 sites; 39 males, 44 females), where *C. sexmaculata* originally occurred prior to 1940, and at 34–36°N (78 sites; 107 males, 165 females), in the newly colonized area. Morph, sex and size of the specimens were recorded and aggregated, and the results for those collected at 32–34°N were compared with that for those collected at 34–36°N. The frequencies of the different morphs were analyzed using tests for independence and mean body size using ANOVA.

Variation in the body sizes of the different morphs

To determine the variation in the body sizes of the different morphs, we examined specimens collected between 1921 and 2005 at 32–36°N (273 sites; 267 males, 380 females). Morph, sex and size of the specimens were recorded and the morph categorized into one of four categories (nigra and A, B, C, and D to I). This dataset was analyzed using ANOVA followed by Tukey's multiple comparison method. We used JMP 11 Discovery (SAS Institute Inc., Cary, NC, USA) to carry out all the analyses.

Specimens used in these analyses were obtained from the National Museum of Nature and Science, Osaka Museum of Natural History, Museum of Nature and Human Activities Hyogo, Kurashiki Museum of Natural History, Ehime University Museum and Kyushu University Museum, as well as from the private collections of several researchers.
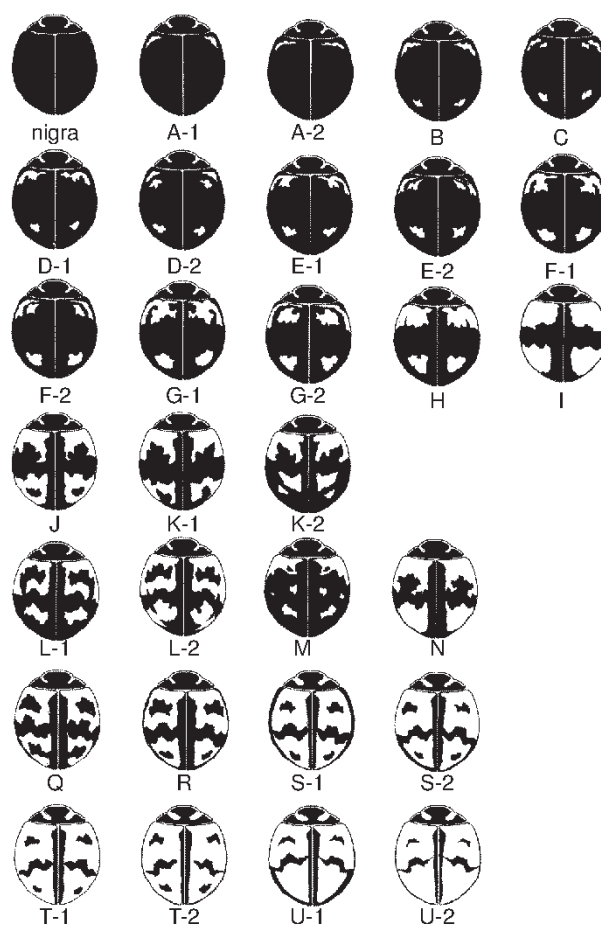

Fig. 1. Phenotypes of elytral colour morphs (nigra to U) of *Cheilomenes sexmaculata*, modified from Kawakami et al. (2013).

RESULTS

Morph frequency and adult body size of *C. sexmaculata* collected during different periods of the range extension

In areas newly colonized by *C. sexmaculata* (34–36°N), the frequencies of the different morphs in both sexes differed significantly in the different periods of range extension (1949–1959 vs. 1960–1989, Fig. 2; male: df = 3, χ^2 = 7.9879, P = 0.0463, female: df = 3, χ^2 = 8.3270, P = 0.0397). In particular there was a clear decrease in the D–I morphs (Fig. 2). Female body size decreased significantly ($R = -0.3685$, $F = 40.5380$, $P < 0.0001$) and male body size tended to decrease ($R = -0.1251$, $F = 2.8304$, $P = 0.0942$) from 1949–2005.

Latitudinal variation in morph frequency and adult body size during the period when range extension ceased

There was a significant difference in the morph frequency of specimens collected during the period when range extension had ceased (1990–2005) in the newly colonized areas (34–36°N) and those in the area of the original ladybird distribution prior to the 1940s (32–34°N) (Fig. 3; male: df = 3, χ^2 = 25.3653, $P < 0.0001$, female: df = 3, χ^2 = 12.2335, $P = 0.0066$). That is, the D–I morph frequency
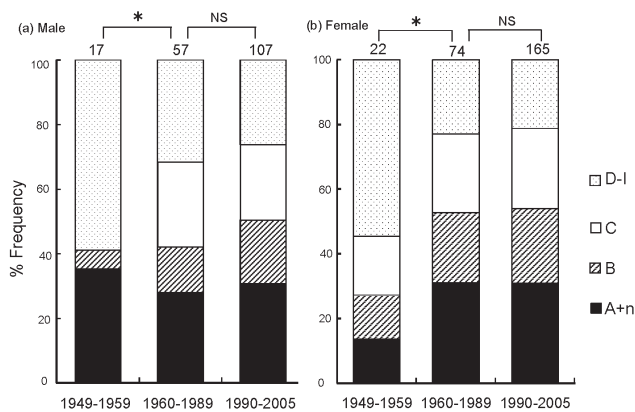
Fig. 2. Percentage frequencies of the different elytral morphs of *Cheilomenes sexmaculata* collected at 34–36°N in the three periods 1949–1959, 1960–1989 and 1990–2005. The numbers above each column are the sample sizes. Morphs (n = nigra to I) are described in Fig. 1. Statistically significant at *: $P < 0.05$.

was lower at 34–36°N than at 32–34°N (Fig. 3).The mean body size of adults of both sexes collected at 34–36°N was significantly smaller than of those collected at 32–34°N and males were significantly smaller than females (Fig. 4; latitude: $F = 49.0283$, $P < 0.0001$, sex: $F = 74.7156$, $P < 0.0001$, latitude × sex: $F = 1.5500$, $P = 0.2140$) .

Morph-dependent variation in body size

Comparison of the mean body sizes of four morphs (nigra and A, B, C, and D to I) revealed that in both sexes D to I morphs were significantly larger than in the other three morphs (Fig. 5; male: df = 3, $F = 13.4088$, $P < 0.0001$, female: df = 3, $F = 12.4624$, $P < 0.0001$).

DISCUSSION

Many ladybird species exhibit elytral colour polymorphism that is associated with varying degrees of fitness associated with the degree of melanism of the morphs (Sloggett & Honěk, 2012). Consequently, differences in the frequencies of morphs may reflect geographical and/or seasonal variation (Sloggett & Honěk, 2012), i.e. melanics may have a selective advantage over non-melanics because

Fig. 4. The mean body size of male and female *Cheilomenes sexmaculata* collected at 32–34°N and 34–36°N, during 1990–2005. The numbers in each column are the sample sizes. Vertical bars are the standard errors (SE). Statistically significant at **: $P < 0.01$.

of their increased absorption of solar radiation (Kryltsov, 1956; Brakefield & Willmer, 1985), particularly when they breed and feed during periods of lower temperature and increasing irradiance (Sloggett & Honěk, 2012). Consequently, there are higher frequencies of melanic morphs in regions with lower temperatures or irradiance (e.g. Benham et al., 1974; Muggleton et al., 1975).

In recent years, global climate change has significantly influenced the life histories of many insect species (e.g. Musolin, 2007; Robinet & Roques, 2010). Although morph frequencies in ladybirds may have been altered by climate change, few long-term trends in morph frequencies have been documented. For example, the morphs of *Adonia variegata* (Goeze) in South Slovakia were similar in 1937 and 1992 (Strouhal, 1939; Balthasarová, 1950; Sloggett & Honěk, 2012). Similarly, those of *Harmonia axyridis* Pallas populations in the Asian Far East province of Russia remained stable from 1966 to 1987 (Kholin,
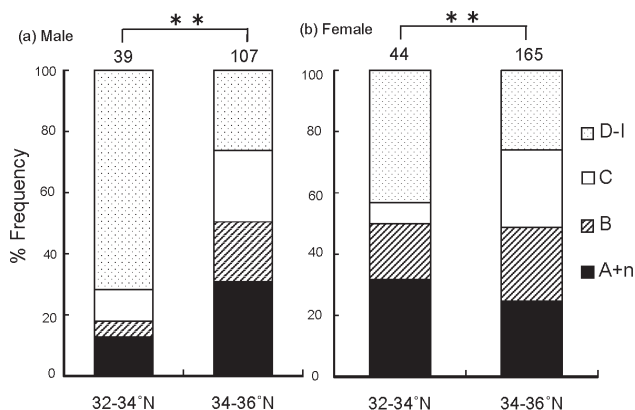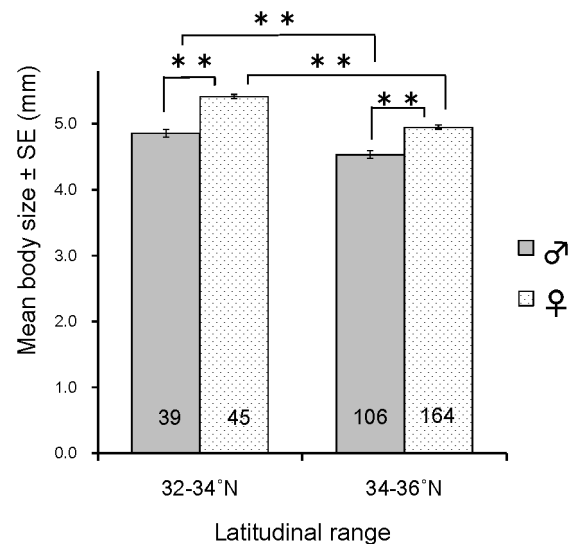

Fig. 3. Percentage frequencies of the different elytral morphs of *Cheilomenes sexmaculata* collected at 32–34°N and 34–36°N, during 1990–2005. The numbers above each column are the sample sizes. Morphs (n = nigra to I) are described in Fig. 1. Statistically significant at **: $P < 0.01$.
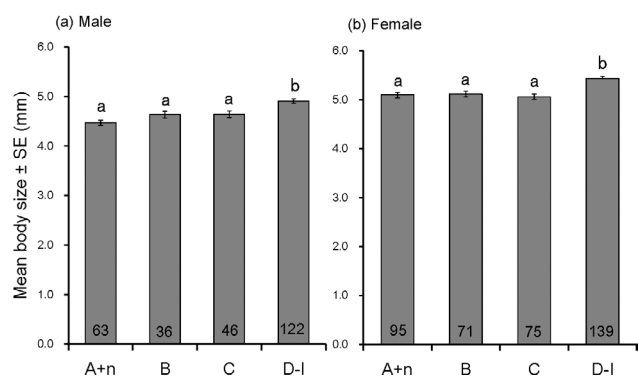

Fig. 5. The mean body size of four morphs of *Cheilomenes sexmaculata* collected at 32–36°N during 1921–2005. Morphs (n = nigra to I) are described in Fig. 1. The numbers in each column are the sample sizes. Vertical bars are the standard errors (SE). Different letters above columns indicate a significant difference, $P < 0.01$.

1988, 1990). In Vladivostok, however, melanics of *H. axyridis* increased between the 1920s (Dobzhansky, 1933) and 1948–1981 (Bogdanov & Gagalchy, 1986), for unknown reasons (Sloggett & Honěk, 2012). The morph frequency in *Hippodamia variegata* (Goeze) populations in Slovakia, exhibited slight but undirectional change from 1937 to 2011, during which the annual temperature increased by approximately two degrees (Honěk et al., 2012).

In this study, melanics (types nigra to C) of *C. sexmaculata* increased during periods when they spread northwards (Fig. 2), indicating that melanism may confer increased fitness at higher latitudes. Consequently, when the extension in range ceased, it is likely the frequency of melanics increased in the newly colonized areas (higher latitudes) compared to that in the original range of distribution (lower latitudes) (Fig. 3).

Alternatively, they show phenotypic plasticity in which morph frequencies respond to environmental conditions such as rearing temperature. For example, elytra pigmentation in *H. axyridis* (Sakai et al., 1974), *Coccinella septempunctata* Linnaeus (Okuda et al., 1997), *Epilachna vigintioctomaculata* Motschulsky (Kawabe, 1950) and *E. chrysomelina* (Fabricius) (Timofeeff-Ressovsky, 1941) is alternately suppressed and enhanced by rearing at high and low temperatures, respectively. However, in preliminary studies the morphs of *C. sexmaculata* were not affected by rearing temperature (Ohashi et al., unpubl. data); therefore, phenotypic plasticity is unlikely to account for the results recorded here.

In addition to the changes in morph frequencies, the mean adult body sizes of both sexes of this species decreased during periods of northward extension (Fig. 4). Generally, adult body size in most insect species is determined by genetic factors (Dingle, 1984; Fox et al., 1999) and/or environmental conditions experienced during larval growth (Gullan & Cranston, 1994). In predatory ladybirds, adult body size is susceptible to environmental conditions, and determined by: (1) quality and quantity of the prey available during the larval stages (e.g. Blackman, 1965; Honěk, 1983; Obrycki & Orr, 1990; Orr & Obrycki, 1990); and (2) temperature or solar radiation that determine the period suitable for growth or overwintering success (Nedvěd & Honěk, 2012; Osawa, 2001).

As stated above, a possible factor determining the decrease in mean body size recorded during northward extension of this species is a lack of available prey during the larval stages at higher latitudes (34–36°N). The adult body sizes of *C. sexmaculata* are known to be positively correlated with the quantity of larval prey (Okamoto, 1978). However, this species is a polyphagous predator, which feeds on at least 33 species of aphids (Hukusima & Kouyama, 1974; Sugiura & Takada, 1998), 26 of which are widely distributed in Japan (31–40°N) (Moritsu, 1983). Furthermore, the species diversity of aphids increases with latitude in temperate regions (Dixon et al., 1987; Hamilton & Brown, 2001).

Because of these reasons, it is likely that the variation in adult body size in *C. sexmaculata* is influenced by variation in temperature or solar radiation. One possible explanation is that the temperatures experienced during larval growth determine the variation in adult body size. However, unlike many other insect species, adult body size in ladybirds is poorly correlated with rearing temperature (Nedvěd & Honěk, 2012); for example, in *C. sexmaculata*, rearing temperature either has no effect on adult body size (Ohashi et al., unpubl. data) or only a marginal effect (Kawauchi, 1979). Furthermore, in *H. axyridis*, the largest adult body sizes are recorded when their larvae are reared at low temperatures (Nedvěd et al., 2013). It is known that in the past 100 years the distribution of *C. sexmaculata* has extended towards higher latitudes in Japan because of climate warming, i.e. the 15°C annual mean temperature isotherm has moved northwards (Kawakami et al., 2014). Therefore, the temperature in the newly colonized areas is similar to that in the previous distributional range and appears to have little effect on body size variation. In contrast, the amount of solar radiation at higher latitudes was less than that at lower latitudes during the range extension. The lower solar radiation at high latitudes might limit the amount of time suitable for growth throughout the year. This may lead to a shortened larval period, resulting in a decrease in adult body size (Blanckenhorn & Demont, 2004). Consequently, mean body size may have decreased during the northward extension in range.

Furthermore, in both males and females, the mean body size of pale coloured morphs is larger than that of melanics (Fig. 5). Moreover, as the frequency of pale morphs has decreased with range extension, there was a corresponding decrease in mean body size of this species. Thus, when the extension ceased, the mean body size of *C. sexmaculata* at the higher latitudes, which consisted of a lower frequency of pale morphs, was smaller than that at the lower latitudes, where there are higher frequencies of pale morphs. Large coccinellid species are not melanic, because the temperature of melanic morphs would frequently rise to deleterious levels (Stewart & Dixon, 1989). Similarly, in *Adalia bipunctata*, the body temperature of melanic morphs can swiftly increase when exposed to solar radiation, and large melanics have the highest risk of overheating (Brakefield & Willmer, 1985). These findings suggest that melanism and small body size may confer a selective advantage on adult insects in terms of their efficient use of radiant heat. Hence, the range extension in to higher latitudes of the small melanic morphs of this species may have been driven by positive selection.

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