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ELYTRAL POLYMORPHISM PATTERN IN APHIDOPHAGOUS LADYBIRDS, *COCCINELLA SEPTEMPUNCTATA* AND *CHEILOMENES SEXMACULATA* (COLEOPTERA: COCCINELLIDAE)

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ABSTRACT

We have collected and analysed variations and fusion patterns in elytral spots/stripes in the two aphidophagous ladybird beetles *i.e.,Coccinella septempunctata* (L.) and *Cheilomenes sexmaculata* (F.) in the central Uttar Pradesh. Collected specimens were well-ordered according to the elytral spot fusion. Eight morphs of *C. septempunctata* and twelve morphs of *C. sexmaculata* were collected from agriculture fields and were analysed for percent melanisation and elytral spot variation. M-1 and C-1 were considered typical forms, as they were frequent throughout the year. M-9 to M-11 were more frequent during November to March and the morph M-12 was less frequent. In *C. septempunctata* the morphs C-4 to C-7 were more frequent. The evaluation of polymorphism may help to understand the relationships between environmental conditions and traits of organisms. To date, no studies have examined the polymorphism of these ladybirds in the central plains of Uttar Pradesh, India.

KEY WORDS : Elytral polymorphism, Aphidophagous ladybirds, *Coccinella septempunctata, Cheilomenes Sexmaculata*

INTRODUCTION

For many years, researchers have investigated the intraspecific differences in the elytral colour patterns of ladybird beetles (Coleoptera: Coccinellidae) (Majerus, 1998; Khormizi *et al*., 2016; Majerus, 2016; Gautier *et al*., 2018; Rutkowski *et al*., 2019; MK *et al*., 2022). Polymorphism is an intriguing occurrence that can aid in our understanding of evolution (Graipel *et al*., 2019; Cosentino & Gibbs, 2022). Melanism in lepidopterans (Van't Hof *et al*., 2013), coleopterans (Hodek *et al*., 2012), including dipterans (Leblanc *et al*., 2015) is directly associated with body colour, temperature regulation, solar irradiation resistance, drought conditions endurance, and durability against abrasion. Melanism gives *Biston betularia* (L.) a benefit against predators through crypsis, imitation, and camouflage (Majerus, 1998; Rowland *et al*., 2022). Unlike many other polymorphisms, the emergence of a melanic population within a species is a noticeable change. Furthermore, melanism occasionally develops with respect to other evolutionary changes. According to Brakefield & De Jong (2011) and Clusella-Trullas & Nielsen (2020), non-melanic species inhabit in temperate conditions, while melanic individuals are frequently found in colder climates and their degree of melanisation varies with latitude and altitude. Seasonal changes, in addition to local differences, affect the prevalence of melanics in a population (Osawa & Nishida, 1992). The studies on ladybird elytral polymorphism have ranged from geographic distribution, seasonal incidence, behavioural variations, ecological and physiological costs, genetic basis and many more (Honek *et al*., 2017; Kudo & Hasegawa, 2022). The impact of colour patterns on ladybird thermoregulation has received the attention of many researchers (Sibilia *et al.,* 2018; Rosa & Saastamoinen, 2020). Even though many aspects have been associated with this diversity in colour patterns, climate conditions, such as temperature, solar irradiation, and humidity have a major impact (Roulin, 2014). In the highly polymorphic ladybird, *Harmonia axyridis* (Pallas), an increase in melanics and a fall in non-melanics were considered to be connected to a warmer environment in Japan (Murakami *et al*., 2019). However, Honek *et al*. (2020), found no

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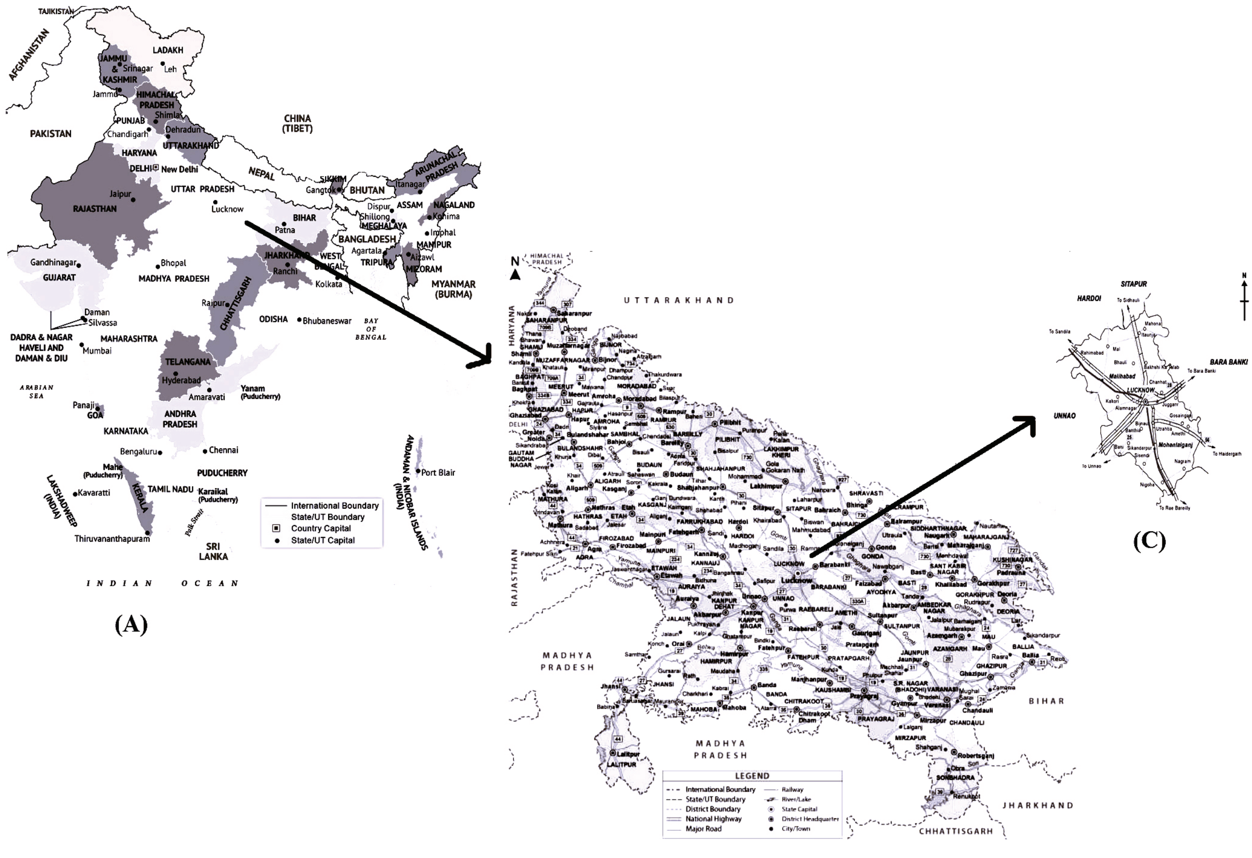
correlation between temperature and elytral pattern occurrence in Europe or any changes in the invasive *H. axyridis* morph frequencies. The darker morph of *Adalia bipunctata* (Linnaeus, 1758) ladybirds benefit from low temperatures because its darker colour enhances quicker absorption of UV, which increases foraging activity and reproduction rate (Okuda *et al*., 1997). Likely, many diverse variables that might support polymorphisms in ladybird colour patterns may modify trends connected to climate change. Phenotypic plasticity has also been reported in spot size with higher temperatures (Okuda *et al*., 1997). In addition to adult ladybirds, larvae and pupae also express paler colouration when temperature increases due to the plastic influence of temperature (Brakefield & Willmer, 1985; Zhang *et al*., 2020). Populations of the *A. bipunctata* have demonstrated industrial melanism (Okuda *et al*., 1997), or the decline in non-melanic species in industrial areas. Thus, it indicates that many ladybirds, including non-polymorphic ones, may have the ability to change their colour pattern in order to protect against forthcoming climatic changes and air pollution from industry (de Jong & Brakefield, 1998).

Several studies have reported various ladybird species from central Uttar Pradesh (Omkar & Bind, 1993; Omkar & Bind, 1995; Omkar & Pervez, 1999; Omkar & Pervez, 2020; Omkar & Ahmad, 2000; Pervez, 2004) including that of eastern Uttar Pradesh (Chaudhary & Singh, 2012; Singh *et al*., 2018) and western Uttar Pradesh (Kumar *et al*., 2017). Recently, seven morphs of *C. septempunctata* were recorded (Bhaisare & Chaudhary, 2022) in an ecosystems area, in Amarkantak, Madhya Pradesh India. Uttar Pradesh is largely an agriculture rich zone but polymorphism studies have been completely lacking in this area. Polymorphism in ladybird species may be affected by the humid subtropical environment, which includes hot, dry summers from late March to June and cool dry winters from mid-November to February. Although having a rich agricultural production and favourable climate, little is known about the prevalence of different morphs of predaceous ladybird beetles in this region.

MATERIALS AND METHODS

Collection of specimens

The study included samples taken from various places



(B)

Fig. 1: (A) India (B) Uttar Pradesh (C) All the sampling sites are displayed on map.

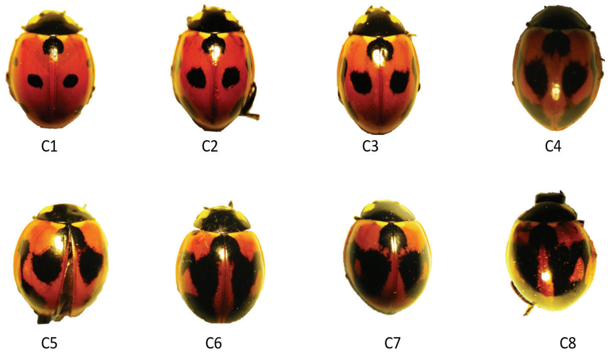


Fig. 2A: 8 morphs of *C. septempunctata* are marked as C1 (typical form), C2, C3, C4, C5, C6, C7, C8.

Fig. 2B: Elytral pattern in all the morphs is marked in grey scale.

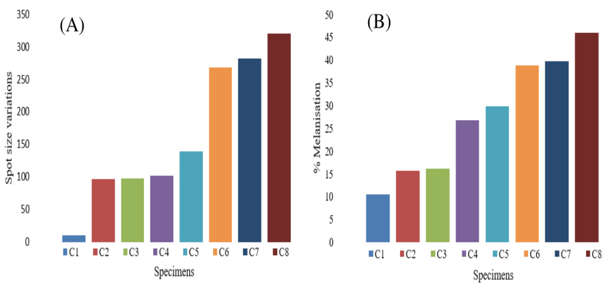


Fig. 2C: Bar diagram illustrate the elytral size variation (A) spot size variations (B) melanisation (in %) of *C. septempunctata.*

around the 100 km radius of Lucknow of the central plain biogeographic zone of Uttar Pradesh (Fig. 1). The ladybird beetles were observed and collected according to the occurrence in summers (35°C to 45°C), April to September and winters (10°C to 25°C), from October to March. Net sweeping method was used for species collection followed by active searching. All the morphs of *C. septempunctata* and *C. sexmaculata* were stored in a deep freezer at -80°C.

Elytral pattern analysis

Elytra was separated from all the specimens and pictures were taken using a Magnus MSZ-B1 stereo zoom microscope individually. Further, all the images were analysed with ImageJ software to depict the melanisation and fusion of spot patterns. For each elytral image, we manually selected a particular area of the image. The required area was selected with a rectangular shape. Scale was set according to the image area for elytral spot area analysis. Thresholding methods with default settings were used to select specific regions of each image. The data was subsequently represented using a bar diagram.

RESULTS

Elytral polymorphism

Coccinella septempunctata (L.)

We found eight separate morphs, each with unique elytral colour patterns and unique seven-spot fusion patterns of *C. septempunctata* (Fig. 2A). Morph C-1 (Fig. 2B), of *C. septempunctata* is a typical form, having distinct seven spots, as it was found ubiquitous throughout the year. In C-2 (Fig. 2B), spots were larger than in C-1, there was no fusion of any spot. Lateral spots in C-3 (Fig. 2B) were fused in a dumbbell shape. However, two spots near the suture line remain consistent with their appearance as in C-1. The scutellar spot is notably free and prominent. C-4 (Fig. 2B), scutella and sutural spots on the elytra were slightly merged. A very thin line connects both of the lateral dots on each elytron. C-5 (Fig. 2B), both scutellum and sutural spots are merged to some extent. Lateral spots were fused in a dumbbell shape. C-6 (Fig. 2B), spots around the edge of the sutural line and scutella spot were clearly merged. The coalesced spots nearly form a triangular shape. C-7 (Fig. 2B), sutural and lateral spots were merged and form a distinct triangular shape, while the scutella spot was slightly connected with a thin line to the sutural spots. C-8 (Fig. 2B), spots present near the sutural line and lateral are coalesced and produce a triangular shape. The scutellum spot is clearly merged with the sutural spot.

Cheilomenes sexmaculata(F.)

Twelve different morphs of *C. sexmaculata* were found in the collection. Based on the elytral spot or stripe patterns,

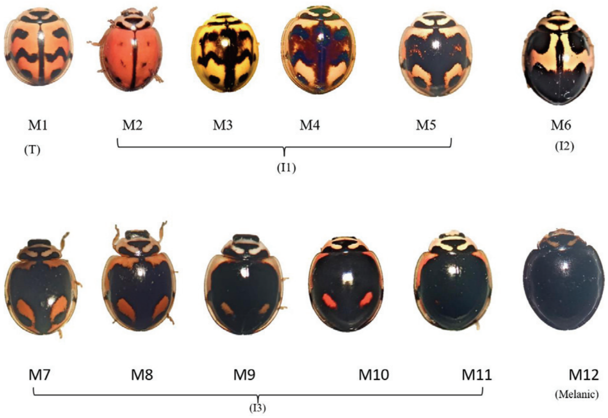


Fig. 3A: All the 12 morphs of *C. sexmaculata* are denoted with M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11 and M12 respectively. T=typical form, I1= intermediate 1, I2=intermediate 2, I3=intermediate 3.

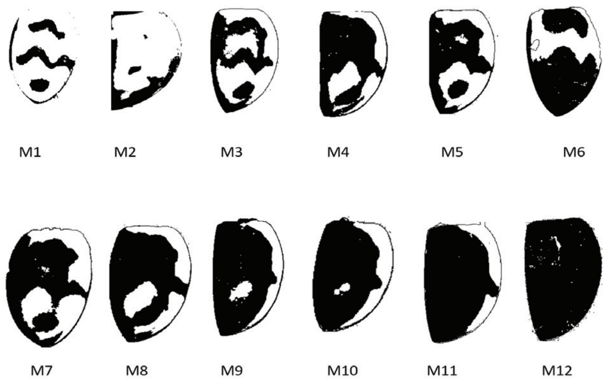


Fig. 3B: *C. sexmaculata* elytral pattern are marked in grey scale.

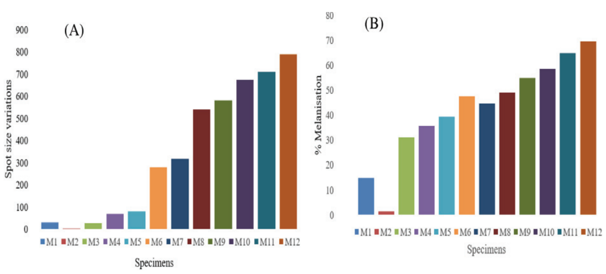


Fig. 3C: Bar diagram illustrate the elytral size variation (A) spot size variations (B) melanisation(in %) of *C. sexmaculata.*

twelve distinct variants were identified and used in the study. Twelve distinct morphs are arranged and represented by their elytral patterns in Fig. 3A.

M-1 represents a typical form of *C. sexmaculata*, characterised by a zig-zag pattern with two stripes and spots at the posterior ends. M-2 has an indistinct zig-zag pattern and is also pale in colour. Melanisation increases in M-3, M-4 and M-5, which have slight differences in their zig-zag patterns. M-2, M3, M4 and M-5 are intermediate-1 (I1) melanic forms. M-6 is the intermediate-2 (I2) and M-7, M-8, M-9, M-10 and M-11 are categorised as the intermediate-3 (I3) forms. M-12 represents the melanic form (Fig. 3B).

Elytral spot variations

All the generated data were depicted in a bar diagram. For *C. septempunctata*, C-2 to C-4 (Fig. 2C (a)), have slight variations, while C-8 shows the highest spot variations. In contrast, for *C. sexmaculata* spot variation gradually increases, with the highest variation observed in M-12. M-4 and M-5 show slight changes (Fig. 3C (a)).

Melanisation of elytra

Fig. 2A includes all eight morphs of *C. septempunctata*. Bar diagram depicting the percent melanisation of all the morphs, indicating a gradual increase (Fig. 2C (b)). C-2, C-3, C-6 and C-7 have slight variations.Twelve morphs of *C. sexmaculata* were assessed for melanisation (Fig. 3C (b)). M-12 exhibited the highest percentage of elytral melanisation.

DISCUSSION

The present study suggests that the occurrence of intraspecific elytral polymorphism is affected by seasonal temperature variations. Frequency of morphs varies with seasonal temperatures in summers (35°C to 45°C) and winters (10°C to 25°C), optimum temperatures (25°C to 30°C) support more darker specimens but pure melanics were not found in any season. Despite establishing and regulating temperature conditions, it has been possible to obtain pure melanic morphs in the laboratory (Dubey *et al*., 2016). *Coccinella septempunctata* is a potential aphid predator in many agro ecosystems (Srivastava & Omkar, 2005; Guroo *et al*., 2017). A total of 8 morphs of *C. septempunctata* were reported in this study. C-4 to C-7 morphs were more frequent during the February, March and November months. M1(typical) and C1(typical) specimens were distributed ubiquitously. *Cheilomenes sexmaculata* was available in harsh summers (35°C to 45°C) and winters (10°C to 25°C). During the seasonal variations, it was noticed that specimens M-9 to M-11 morphs were more frequent rather than M-12. While seasonal frequency of different morphs of *C. septempunctata* and *C. sexmaculata* were highest during the February, March and November months. It supports earlier findings in *A. bipunctata* (Okuda *et al*., 1997; Majerus & Zakharov, 2000), *C. septempunctata* (Srivastava & Omkar, 2005) and *H. axyridis* that melanics have greater mating success at moderate and ideal temperatures (Osawa & Nishida, 1992; Seo *et al*., 2008; Wang *et al*., 2013). In many animal taxa, colour polymorphism has a substantial influence on an individual's fitness within a species (de Jager & Ellis, 2014; Dubey *et al*., 2016; Forsman, 2016). Particular colour variants may be much harder for predators to directly identify than others because they are more cryptic (Ruxton *et al*., 2019). Due to this, understanding of genetic diversity and its evolutionary significance is severely hampered, and it is difficult to establish a connection between genotype and phenotype.

This is the first official record of morphs of *C. septempunctata* and *C. sexmaculata* from the central plain, Lucknow, Uttar Pradesh. The presence of these morphs is likely dependent on multiple factors including inter-population cross-mating, the heritability of elytral colour, molecular research, and prey choice. Morphs are important to study cross-mating among the populations, heritability of elytral colour, molecular studies and prey preference. From the application point of view, it is important to study whether these morphs differ in their role in pest management. It is thus important to undertake more studies on morph distribution in India.

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