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Melanic Facial Patterns and their Significance in the Multicolored Asian Lady Beetle (*Harmonia axyridis*)

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Abstract

The relationship between the patterns present on the pronotum and the elytron of the multicolored Asian lady beetle *Harmonia axyridis* was investigated. In males elytron size was a significant predictor of pronotal intensity and in females the fraction of the pronotum covered in black was related to their elytral spot brightness. Other significant sex differences included females having more of their pronota covered by melanins and the pigments deposited there being more intense than in males. The potential significance of these sex differences is discussed as well as the potential for these signals to serve in a mate choice context.

INTRODUCTION

Much of the foundational work on pigment production and significance in animals was carried out in vertebrates and came to the broad conclusion that carotenoid-based colors (reds, yellows, and oranges) are more valuable indicators of health and vigor than the brown and black melanin-based colors (Hill and Brawner, 1998; McGraw and Hill, 2000; reviewed in McGraw 2006a, 2006b.) However, recent work has shed light on the fact that melanins can be significant indicators of health and vigor in insects (Dolenska, *et al.*, 2009; Blount *et al.*, 2012). This difference between the role of melanins in vertebrates and invertebrates may be due to the fact that, because of often narrow diets, insects are likely to have less reliable access to adequate quantities of melanin precursor compounds like tyrosine (Stoehr, 2006). In addition to differences in acquisition, insects utilize melanins in more diverse manners than vertebrates. For example, as in vertebrates, melanins are a component of the immune response in insects, but they are also often used in insects for the absorption of thermal energy via the deposition of the pigments across broad palettes like elytra. Recent work has shown the degree of melanism can vary to a large degree seasonally, with insects depositing greater amounts of melanins during winter months (Michie *et al.*, 2010; 2011). Other behavioral studies in insects have discovered melanic forms are strongly preferred in mate choice, further strengthening the notion that these pigments are strong indicators of quality (Seo *et al.*, 2008).

Many studies on pigmentation in insects have been performed on ladybird beetles. In particular the multicolored Asian lady beetle *Harmonia axyridis* is an attractive organism for research because of its highly variable red and black coloration and multiple color forms. In

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addition there is a strong genetic component to the pigmentation of *H. axyridis* which makes it a valuable organism for understanding how genes and the environment contribute to coloration (Wittkopp and Beldade, 2009; Brakefield and de Jong, 2011). *H. axyridis* is endemic to northeast Asia but has been well established throughout much of the U.S. following their introduction in the southern U.S. in the 1980s in an attempt to control aphid populations (Roy and Wajnberg, 2008). Color variation in *H. axyridis* can manifest itself in several different ways (Bezzerides *et al.*, 2007). There is variation in the fraction of the elytron palette simply covered by red versus black color. The portions of the elytra covered by red can vary both in terms of the hue and the intensity of the color. The degree of darkness of the black spots also varies considerably. Like many ladybird beetles, *H. axyridis* is defended by alkaloids present in the hemolymph of the animals and those defensive compounds can be bled reflexively through their joints when subjected to an attack. Previous research with this system demonstrated patterns present on the elytra are related to the overall defensive levels of the beetles (Bezzerides *et al.*, 2007). Interestingly, it was not the hue or degree of carotenoid concentration in the wings that was significantly related to defensive levels but simply the fraction of the elytron palette covered by red versus black. In short beetles with more of their wings covered by red had higher levels of chemical defense. This result was seen in both sexes, though the effect was stronger in females.

The focus of the study presented here is on the patterns present on the pronota of the beetles, whereas our previous work has focused on the elytra. In the pronota the patterns are made up entirely of melanin laid over a white background. As with the elytra, there is considerable intraspecific variation in the pronotal patterns. We hypothesize a strong relationship will exist between the degree of melanism on the elytra and that on the pronota. If supported this study would be the first to establish ladybird facial patterns as linked to the patterns of the elytra. This would be a significant finding because it would open up the possibility of there being information in the facial patterns of ladybirds, which could lead to new studies designed to investigate if those patterns are used in behaviors such as mate choice. In addition these studies aim to elucidate any significant sex differences in the degree of melanism on the pronota of *H. axyridis*.

METHODS

H. axyridis f. succinea ladybird beetles were collected from the wild in the fall of 2010 from a variety of populations in Rice Lake, WI (N 45.48, W 91.74), and frozen at -20°C upon collection. Beetles were sexed and the head, elytra, and pronota were dissected from 48 beetles (24 males and 24 females) and placed in individual Eppendorf tubes. The left elytron from each beetle was photographed and analyzed. Previous work had shown all pigment and color variables are significantly intercorrelated between the left and right elytra (Bezzerides *et al.*, 2007). Each elytron and pronotum was photographed individually under standardized lighting and background conditions using an Olympus C750 digital camera. For conversion from pixel count to area a 0.5 cm^2 red and black square from a Kodak Color Separation Guide strip was included in each photograph. For both the elytra and the pronota, the variables measured were the black spot brightness and the total fraction of the elytra and pronota covered by red and black. Brightness is a measure of the intensity and reflectance of

the dark spots on the elytra and pronota and was measured as described previously (Bezzerides *et al.*, 2007). Lower values indicate darker more intense black coloration.

Statistical analyses

In males two of the variables were nonnormally distributed. Those variables were the black brightness of the elytra and the fraction of the pronota covered in black. Both of those variables could be transformed to normal distributions following a square root transformation. In female beetles, without transformation, all analyzed variables were normally distributed. The data were analyzed with a backwards selection multiple regression generalized linear model (GLM) using the percent of the pronota as black as the dependent variable with the covariables of total elytra area (size), percent black of the elytra, and brightness of the elytra. This test was performed independently for both sexes. Another GLM test was performed using the brightness of the pronota as the dependent variable with the same set of covariables. In tests for sex differences, in cases where the distributions were normal and the variances equal, a *t*-test was used to assess differences. In cases where one or more of those assumptions was violated, the Wilcoxon rank sum test was used to test for differences.

RESULTS

Males

As with the elytra, the distribution of melanins on the pronota is a highly variable trait in *H. axyridis* (Fig. 1). The generalized linear model demonstrated the most significant predictor ($R^2 = 0.42$) of the percent of the pronota being covered in black in male beetles was the percent of the elytra covered by black (Fig. 2A, $P < 0.001$). That is, male individuals with a high fraction of their pronota covered in black tended to have a higher fraction of their elytra covered in black. When running the model focused on pronotal brightness as the dependent variable, the two strongest predictors ($R^2 = 0.41$) were the percent of the pronota covered by black ($P < 0.05$) and the total elytra size ($P < 0.05$).

Females

The strongest predictor ($R^2 = 0.38$) of the percent of the pronota covered in black in females was the brightness of the elytra ($P < 0.01$). Unlike in males, the percent black of the elytra was not strongly related to the percent black of the pronota (Fig. 2B). There was not a significant predictor of pronotal spot brightness in females.

Tests for sex differences

Females in the study had a greater fraction of their pronota covered in black than males ($t = 2.92$, $P < 0.01$, Fig. 3A). The melanic areas on the pronota of the females were more intense than those corresponding areas on males ($t = -2.33$, $P < 0.05$, Fig. 3B). There were not significant sex differences in terms of the fraction of the elytra covered in black or the brightness of the spots on the elytra (all $P > 0.51$).

DISCUSSION

These data show certain aspects of the patterns of pigment distribution on the pronota of multicolored Asian lady beetles are correlated with the patterns on their elytra. The correlations were found to differ between the sexes. In males the fraction of the elytra covered by black was a strong predictor of the fraction of the pronota covered in black. In addition males with darker, more intense spots on their pronota tended to be larger. Whether or not this latter trait is used by females in a mate choice context to judge the size of their potential suitors is an interesting area of future inquiry.

The relationship of pronotal patterns to elytral patterns in females was different than in males. In females the best predictor of the pronotal black/white ratio was the intensity of the spots on the elytra. Previous studies (Bezzerides *et al.*, 2007) showed that the intensity of elytral spots is strongly tied to the defensive levels of females. It is not known whether or not *H. axyridis* adorn their eggs with defensive compounds, as has been demonstrated in other chemically defended insects (Eisner *et al.*, 2000). It seems likely, given the vulnerability of their laid eggs and the strong degree of chemical defense they possess via harmonine and other alkaloids. If so, it would be beneficial for males to take mates with large quantities of alkaloids and they could potentially use female pronotal pigment coverage to discriminate between alkaloid poor and alkaloid-rich females.

The sex differences discovered in terms of both the intensity of the melanic areas on the pronota and their relationship to size suggest male and female ladybirds may be under different pressures in terms of their use of available compounds for pigment production. In the case of both the elytra and the pronota, females tend to have more of those surfaces covered in melanins and the black coloration is more intense than the comparable areas on males. The functional significance of these sex differences remains unknown. Given the relationships between degree of melanism, activity, and chemical defense, it seems likely that the sex differences in pigment distributions are driven by differences in size and energy constraints related to egg laying, egg-predation pressures, and sexual selection.

As with prior studies (Bezzerides *et al.*, 2007) these results suggest the health and vigor linked signals in this species may be those that are melanic in nature rather than carotenoid driven. This makes sense given that there is evidence that coccinellids are incapable of seeing into the red portion of the electromagnetic spectrum (Lin and Wu, 1998). Thus, in terms of assessing conspecifics in a mate-choice context, ladybird beetles would be limited to assessing the fraction of elytra or pronota covered in black/red and the intensity of the black spots. The results presented here indicate that both of those variables (fraction covered and intensity) may carry valuable information about the phenotypic quality of the beetle. Whether or not this result can be translated to other invertebrate species, as might be expected given the nature of pigment production in invertebrates, can only be assessed through more extensive testing of other taxa.

The fact there is potentially valuable information in the elytra and pronotal patterns of the beetles begs the question of whether or not that information is being used by either conspecifics or potential predators. Given the “face-to-face” nature of coccinellid courtship,

it seems more likely that pronotal patterns would be used to communicate information to conspecifics as opposed to elytron patterns. Elytron patterns seem the more likely of the two signals to indicate distastefulness to a predator with a “bird’s-eye-view”. Future studies will look at the role of elytral and pronotal patterns in dictating the outcome of both predation and mate choice trials.

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Figure 1.

All *Harmonia* have at least some melanin deposited on their pronotum but the amount and pattern of melanin distribution varies dramatically between individuals

Figure 2.
Relationship between the percent of the pronota covered in black and the percent of the elytra covered in black in male (A, $P < 0.001$) and female (B, $P > 0.5$) *H. axyridis*

Figure 3. Sex differences in the percent of the pronota covered by black (A, $P < 0.01$) and pronotum spot brightness (B, $P < 0.05$) in *H. axyridis* (note that bars indicate SEM and lower brightness values signify darker spots)