Bulletin of Insectology ***75*** *(1): 131-136, 2022*
ISSN 1721-8861 eISSN 2283-0332

Colour pattern plasticity in *Anthrenus isabellinus* (Coleoptera Dermestidae)

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Abstract

Twenty-three species belong to the Palaearctic *Anthrenus pimpinellae* complex. All *Anthrenus* spp. have intricate dorsal colour patterns. They can do this courtesy of the many small, coloured scales that coat their bodies, rather like Lepidoptera. All species within the complex are generally black/orange with an obvious trans-elytral wide band. One species, *A. isabellinus*, differs from most other species from the complex in that it displays a wide range of colour patterns, from the typical dark with white elytral fascia to almost pure white individuals. In the current study, we demonstrate that a continuous range of colour patterns exists within a population of *A. isabellinus* from Greece, negating the necessity to name variants as subspecies to account for the pattern variation. It is more likely that the pattern variation is an example of phenotypic plasticity. There is no evidence for sexual dimorphism in colour pattern variation. The range in colour pattern adopts a 'broken stick' pattern, which is discussed in relation to variation in numbers of individuals falling into different pattern categories, and the possibility that the broken stick appearance of the pattern range is an example of canalization. The white fascia across a dark background is consistent with a pattern that has evolved to disrupt the outline of the beetle (anti-predator). Most *A. isabellinus*, indeed most species within the *A. pimpinellae* complex, display this colour pattern so it is possible that canalization occurs to produce this colour pattern.

Key words: *Anthrenus pimpinellae* complex, Megatominae, colour variation, canalization.

Introduction

Anthrenus pimpinellae (F.) and *Anthrenus isabellinus* Kuster (Coleoptera Dermestidae) belong to the Palaearctic *A. pimpinellae* complex, a group of species bearing similar colour patterns (figure 1). Throughout the 19th and 20th centuries, our understanding of the taxonomy of this group of species was poorly developed. It was appreciated that there was a great deal of colour variation among individuals, but it was also believed that almost all individuals within the complex belonged to the same species: *A. pimpinellae* (Háva, 2021). To account for this degree of variation, several subspecies and variants were erected, such as *A. p. pimpinellae* and *A. p. isabellinus*. Beal (1998) was perhaps the first worker to begin questioning the validity of the taxonomy when noticing the great variation in male genital structure within the complex. It was not until the work of Kadej *et al*. (2007), followed by Kadej and Háva (2011) and Holloway (2019; 2020; 2021) that we have begun to appreciate just how many species might exist within the Palaearctic *A. pimpinellae* complex. The number of named species in the complex currently stands at 23.

A. p. isabellinus was declared a variant of *A. p. pimpinellae* (Schaum, 1862) and later a subspecies (Beal, 1998) by virtue of the striking difference in the dorsal colour patterns of the two taxa. Holloway *et al*. (2020) demonstrated that these two subspecies are different species, with *A. p. isabellinus* designated as *A. isabellinus*. Furthermore, *A. isabellinus* is conspecific with *Anthrenus dorsatus* Mulsant et Rey (Holloway *et al*., 2020). *A. dorsatus* is superficially similar to *A. pimpinellae* such that the two species have been consistently confused (Holloway *et al*., 2021). The consequence of the taxonomic work carried out by Holloway *et al*. (2020) was the establishment of a species, *A. isabellinus*, exhibiting a great deal of colour pattern variation. However, Holloway *et al*. (2020) suggested that *A. isabellinus* did not consist of a subspecies complex, instead proposing that the colour variation was most likely the consequence of phenotypic plasticity (Pigliucci, 2001). Phenotypic plasticity refers to the production of different phenotypes, in this case colour patterns, in response to different environmental conditions (Pigliucci, 2001). There are many examples of adult insect colour patterns that are influenced by conditions during the immature stages, often temperature affecting developmental period (Brakefield and Reitsma, 1991; Ottenheim *et al*., 1996; Marriott and Holloway, 1998). The complex colour patterns produced by the *A. pimpinellae* complex, in fact all *Anthrenus* spp., are made possible by the fact that *Anthrenus* are covered in small scales, similar to Lepidoptera (Peacock, 1993). Each individual scale can be white, orange or black. Holloway *et al*. (2020) essentially worked on two colour patterns to resolve the taxonomy but claimed that there was a range of patterns rather than discrete forms, hence plasticity rather than subspecies. Holloway *et al*. (2020) further stated that about 20% of *A. isabellinus* '…possess more whiteish scales…' but produced no further examination of the range of patterns possible.

The aim of the current study is to examine the range of colour patterns produced by *A. isabellinus* under field conditions and how the colour of the scales changes from one end of the pattern spectrum to the other. Data are further interrogated to consider whether there is sexual dimorphism in colour pattern (and morphology).

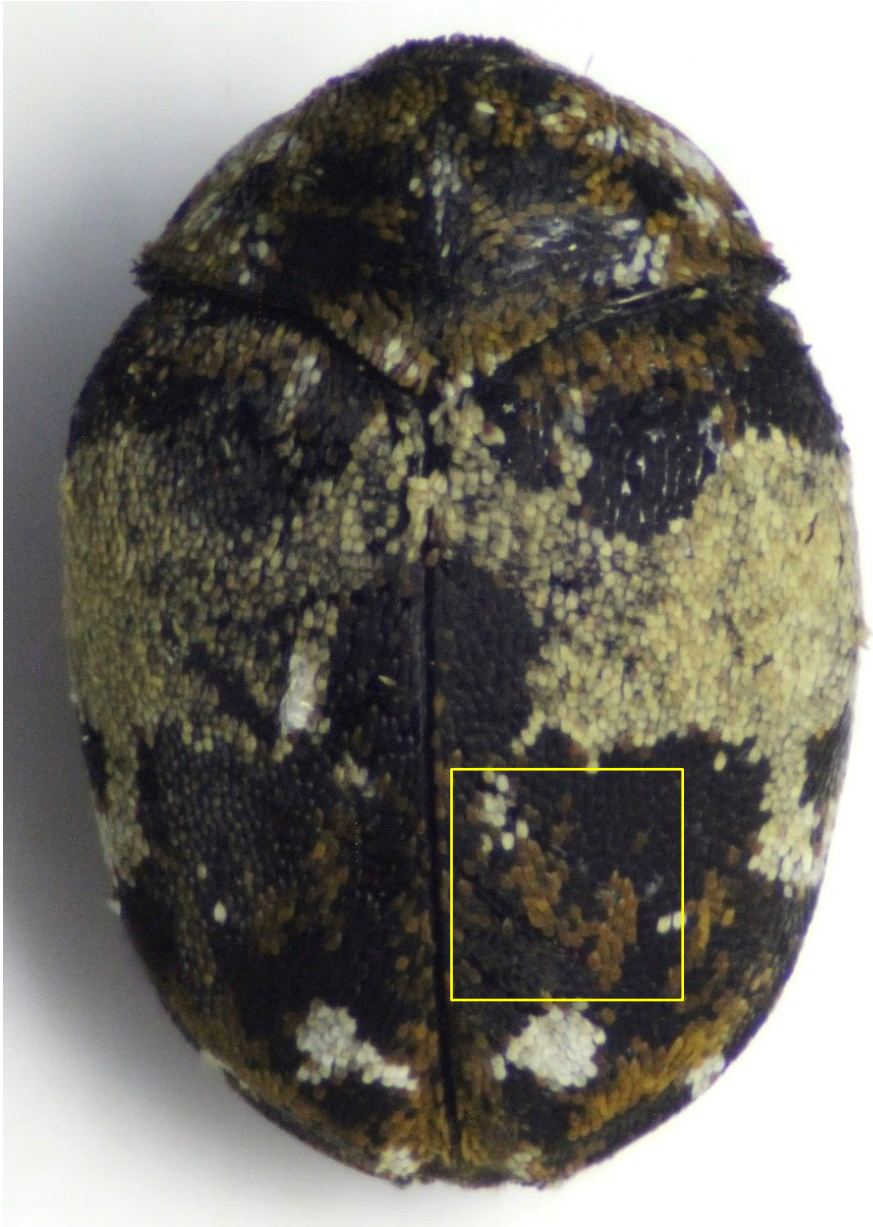

Figure 1. Example specimen of *A. isabellinus* showing placement of study square, positioned in contact with internal edge of right elytron, and in contact with but not including the top of the sub-apical white spot.

Materials and methods

Collection and imaging

345 specimens of *A. isabellinus* were collected from Thessaloniki, Greece, in May 2020. Insects were knocked from Apiaceae into plastic trays and then collected using an aspirator. Identification was confirmed by colour pattern and antennal structure (Holloway *et al*., 2019; 2020; Holloway and Bakaloudis, 2020). Images of whole insects were captured at ×20 magnification using a Canon EOS 1300D through a Brunel BMSL zoom stereo LED microscope and stacked using Helicon Focus 7-Pro focus stacking software. Morphometric measurements were taken using DsCap.Ink software. The following measurements (mm) were taken: i) body length (BL) (front edge of pronotum to the tip of elytra), ii) body width (BW) (maximum width across the elytra).

Colour analysis

The proportions of black, orange, and white scales on a specific area of one elytron of each specimen were determined using Image J (version 1.46). A standardized sample square measuring 0.2 × BL in each direction was taken from a colour variable section of the elytra, the central section between the trans-elytral white band and the white sub-apical spot (figure 1). This sample area represented the largest possible area that could be analysed without including scales of the white band or the sub-apical spot. The white scales in the band and the spot never show any variation; they are always white. The sample square was positioned so that it was in contact with the internal edge of the elytron and in contact with, but not including, the top of the sub-apical white spot (figure 1). Where possible the right elytron was used, and the left side only used if the right elytron contained patches devoid of scales.

Table 1. Mean upper and lower threshold values for three scale colours found on *A. isabellinus* specimens, based on the thresholds obtained via Image J where thresholds range from 0-255 for 20 randomly selected specimens.

Colour	Brightness upper limit	Brightness lower limit	Hue upper limit	Hue lower limit
Black (1)	0 ± 0.00	51 ± 2.87	0 ± 0.00	40 ± 0.00
Black (2)	0 ± 0.00	124 ± 6.16	41 ± 0.00	255 ± 0.00
Orange	52 ± 2.91	124 ± 6.16	0 ± 0.00	40 ± 0.00
White	125 ± 6.20	255 ± 0.00	0 ± 0.00	255 ± 0.00

The upper and lower limits of brightness and hue for each colour were set manually, via "colour threshold" in Image J, for 20 randomly selected specimens so that the software best measured the areas of each colour. Image J works to a scale where 0 = jet black and 255 = pure white. The default thresholding method was used. Images were not converted to 8-bit so that hue could be used alongside brightness. This allowed the software to better differentiate between orange and black scales, as black scales sometimes showed reflections that could be misinterpreted as orange. From the values obtained mean threshold values for each colour were determined (table 1). Using these mean thresholds, the proportion of the sample area taken up by the scales of each colour could be calculated. The proportion of black was calculated by the summation of two areas, black 1 and 2 (table 1).

Sexing

A random sample of 40 individuals were dissected to establish sex (Holloway *et al*., 2020). These definitively sexed individuals were used to look for sex-related differences in morphology and colour pattern.

Statistical analysis

All statistical analyses were carried out using Minitab version 19.1.1. For all statistical analyses, a significance level of $\alpha = 0.05$ was used. To compare sex related differences, two sample t-tests were carried out where samples were normally distributed or could be log-transformed to normality. Where normality could not be achieved a Mann-Whitney test was deployed. Correlation tests were deployed to examine the relationship between BW/BL and colour.

Results

Of the 345 specimens collected, 284 had at least one elytron in perfect condition and deemed usable. Forty individuals were randomly selected from the 284 perfect specimens and dissected to establish sex. Females (n = 20) were on average 9% larger (mean BL = 3.05 ± 0.26 mm - standard deviation) than males (n = 20, mean = 2.8 ± 0.22 mm) ($t_{38} = 3.28$, $p < 0.01$), but there was no difference between the sexes in the BW/BL ratio (0.72 ± 0.01, $t_{38} = 0.12$, ns - not significant). Proportion white and orange scales did not vary between the sexes (white - $\log_{10}$ transformed - $t_{38} = 0.75$, ns; orange - Mann-Whitney - W = 393, ns). There was no significant correlation between BW/BL and proportion white ($r = -0.035$, ns) or orange scales ($r = 0.198$, ns).

Colour patterns (in the sampled square) varied considerably between individuals, showing a continuous range of phenotypes from primarily black (figure 2a) to primarily white (figure 2e), although neither white, orange, nor black ever reached 100% coverage. As the colour patterns changed from typical (figure 2a) towards pale (figure 2e), increasing numbers of orange scales replaced the black scales, although the trans-elytral band was still clearly defined. In the paler individuals, the orange scales were in turn replaced by white until eventually the trans-elytral band could no longer be discerned (figure 2d and 2e).

Figure 3 shows individuals ranked based on the amount of white in the sample square (from least to most). Figure 2d shows an individual with about 20% white scales in the sample square and at this level the white fascia crossing the elytra begins to lose definition. Approximately 15% of the population had 20% or more white scales (figure 3) in the sample square increasing to 89% in the whitest individual. Figure 3 shows that 85% of the population had sufficient black (and orange) scales to retain the integrity of the white trans-elytral fascia.

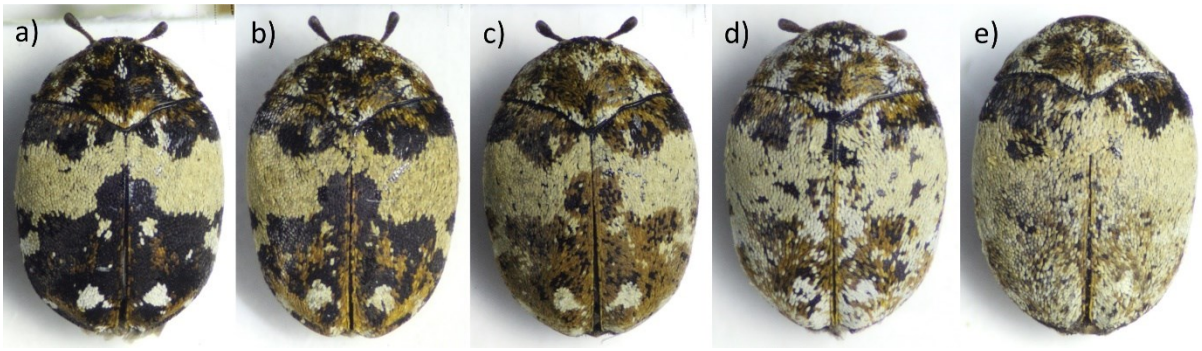

Figure 2. Examples of colour patterns along a spectrum defined by most black scales (**a**), through mostly orange scales (**c**), to mostly white scales (**e**) in the elytral study square (see methods).

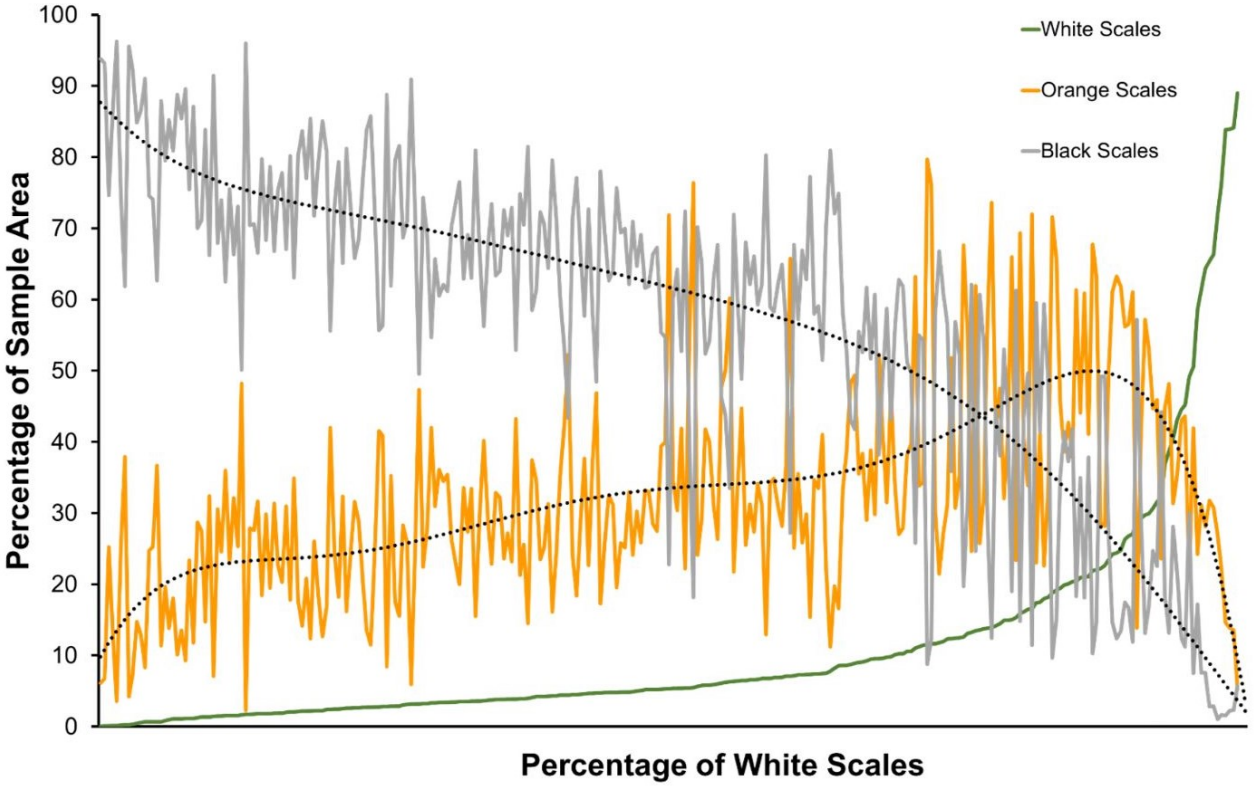

Figure 3. Proportions of white (green line), orange (orange line) and black scales (grey line) covering the study square on the sampled elytron of each individual (see methods) plotted on rank percentage white. Smoothed lines through percentage orange and percentage black scales are shown as dotted black lines.

Discussion

Holloway *et al*. (2020) argued that colour variation in *A. isabellinus* is an example of phenotypic plasticity negating any reason to erect subspecies or variants to account for the pattern variation. *A. isabellinus* is a single variable species distributed around the Mediterranean (Holloway *et al*., 2020) without any subspecific or discrete variant structuring. All possible colour patterns occur together (figure 2) facilitating free gene flow and this alone will ensure that subspecies cannot evolve. Whilst females are larger than males, there is no evidence for any sexual dimorphism for colour pattern. It is interesting to note that many subspecies have been proposed within the Dermestidae based on colour pattern variation, some with overlapping distributions (Háva, 2021). It is possible that at least some of these represent examples of colour pattern plasticity as described here.

The colour patterns of *A. isabellinus*, indeed of all species within the *A. pimpinellae* complex, consist of scales coloured white, or black, or a shade of orange. It is likely that the black pigmentation is eumelanin, and it is possible that the orange scales are pigmented with phaeomelanin (Barek *et al*., 2017). Melanins are common components of insect pigmentation (Peng *et al*., 2020) and are derived from dopamine common in the animal-based diet (keratins) of *Anthrenus* spp. (Barek *et al*., 2017). White scales most likely do not contain any melanins. The change in colour pattern from very dark (figure 2a) to almost totally white (figure 2e) could be achieved via a relatively simple mechanism. A parsimonious explanation for the change is that all scales contain both eu- and phaeomelanin but would appear black because the presence of black eumelanin smothers the expression of orange phaeomelanin. As eumelanin is withdrawn from scales, an individual would be more orange as an adult. After that, as phaeomelanin is withdrawn more scales would become white and eventually nearly all the dorsal surface of an adult would be covered in white scales. This hypothesis suggests that all coloured scales always contain phaeomelanin and only black scales carry an additional pigment. The modification of insect colour patterns in this manner has been found in the Syrphinae (Holloway unpublished data). In these flies all tergites are packed with yellow plant-derived pigments but some parts are also coated in a layer of black pigmentation. This produces the well-known black and yellow colour patterns of many Syrphinae hoverflies. The extent of black pigmentation deposited in adult hoverflies correlates with the length of the immature developmental period producing colour pattern plasticity in the adult (Marriott and Holloway, 1998). The colour pattern plasticity in this instance is adaptive (Ottenheim *et al*., 1999). The environmental cue that *A. isabellinus* is responding to is unknown but given that scales cannot change colour in the adult it is likely that the immature developmental conditions are involved.

Figure 3 shows the range of colour patterns found ranked on proportion white scales. It is clear that white individuals are less common than dark individuals.

Focusing on the white scale line, if figure 3 shows the result of typical phenotypic plasticity, a smooth, probably largely straight line might be expected. If the X-axis was the environmental gradient driving the plasticity, the line (reaction norm) might be straight, but because the darker, described here as 'typical', colour patterns are more common, a rank plot produces a 'broken stick' shaped line. An alternative explanation is physiological constraint to produce an adaptive colour pattern. This is referred to as canalisation (Hallgrimsson *et al*., 2019). A population might contain genetic variation capable of producing a wide range of colour patterns but only one pattern is adaptive. Perfect canalisation would produce the same colour pattern irrespective of genetic background. However, it is more likely that extreme combinations of genes cannot be canalised and under these circumstances a 'broken stick' reaction norm might be found with a 'break' at one or both ends of an otherwise flat reaction norm (Hallgrimsson *et al*., 2019). The consistent feature across species within the *A. pimpinellae* complex is the white, trans-elytral fascia. A dark colour with a white band across it could function as a disruptive colour pattern (Cott, 1940; Stevens and Merilaita, 2009), breaking up the outline of the insect especially when feeding on white flowers (e.g., Apiaceae) as they often do (Beal, 1998; Holloway and Bakaloudis, 2020). Another feature of effective disruptive colour patterns is that the boundary between contrasting elements is sharp, as is the case along the leading and trailing edges of the white fascia (Cott, 1940). Most individuals found displayed a clear white fascia. The fascia became less discernible as the proportion of white scales increased. Only 15% of individuals had 20% or more scales on the measured patch. The 'broken stick' appearance of the white scale line in figure 3 could be the result of colour pattern canalisation.

Conclusions

The study has demonstrated that the colour pattern variation in *A. isabellinus* is continuous with no need to erect subspecies to account for variation. Most adults display a white fascia across otherwise dark elytra, consistent with the operation of a disruptive colour pattern, and the colour patterns could be subject to canalisation to maintain the appearance of this colour pattern.

Acknowledgements

We are very grateful to three anonymous referees for their very constructive comments on the manuscript.

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Received August 13, 2021. Accepted February 28, 2022.