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ORIGINAL ARTICLE

Temporal and climatic variation in the colour forms of *Adalia bipunctata* and *Harmonia axyridis* (Coleoptera: Coccinellidae) populations in the United Kingdom*

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Abstract. Colour form polymorphism in *Adalia bipunctata* (Linnaeus, 1785) and *Harmonia axyridis* (Pallas, 1773) has been extensively studied in the past. Climate, season, and region are known to influence the colour form frequencies of these species, however, this effect is region specific, and the response of populations has changed over time. Here, 5862 photographic records from the UK Ladybird Survey from 2013–2017 were used to assess the geographic variation in colour form frequency (melanic versus non-melanic) of *A. bipunctata* and *H. axyridis* across the United Kingdom (UK) in relation to climate and season. Climate data from the UK Met Office were used to determine the effect of climate on melanic form. Seasonal variation in melanic form was observed in *H. axyridis* but not *A. bipunctata*. For *H. axyridis*, the probability of being melanic decreased in areas with higher annual sunlight hours. In *A. bipunctata*, there was an interaction between sunlight hours and rainfall. In areas with low rainfall, the probability of being melanic was negatively correlated with sunlight hours. Where rainfall was high, the probability of being melanic was positively correlated with sunlight hours. These findings highlight the role of climate in determining the proportion of colour forms in UK populations of *A. bipunctata* and *H. axyridis*. Furthermore, the results support the hypothesis that seasonal variation in melanism does not occur in *A. bipunctata* populations with a low overall proportion of melanics.

INTRODUCTION

The combined effects of climate change, habitat fragmentation, and biological invasions act as serious threats to insect biodiversity and can lead to biotic homogenisation (Tscharntke et al., 2002; Shaw et al., 2010; Knop, 2016). There are signs from multiple taxa that invasive non-native species can contribute to biotic homogenisation in certain ecosystems (Olden et al., 2006). This can be due to specialist species being outcompeted by generalists which can more readily adapt to environmental change (Warren et al., 2001; Ball-Damerow et al., 2014). The harlequin ladybird *Harmonia axyridis* (Pallas, 1773) is a near-globally distributed invasive alien beetle native to central and eastern Asia (Brown et al., 2011a; Roy et al., 2016). Its expansion began after being repeatedly introduced and used as a biocontrol agent throughout the twentieth century (McClure, 1987; Tedders & Schaefer, 1994; Brown et al., 2008), with populations establishing in North America from the late 1980s (Chapin & Brou, 1991). Populations of *H. axyridis* have now been established in five continents (Brown et al., 2011a) where they have been responsible for losses in the biodiversity of native coccinellids. Evidence of this has been seen in South America (Grez et al., 2016), North America (Brown & Miller, 1998), throughout Britain and mainland Europe (Roy et al., 2006; Adriaens et al., 2008).

The invasion success of *H. axyridis* is largely due to a range of physiological adaptations, typical of invasive non-native species, that allow it to colonise, disperse, and outcompete native species extremely effectively (Majerus et al., 2006). Competition with *H. axyridis* has been suggested as an explanation for the rapid decline of *Adalia bipunctata* (Linnaeus, 1758) throughout its native range in North America, Belgium, and Britain (Harmon et al., 2007; Roy et al., 2012). *Adalia bipunctata* is just one of the many coccinellids adversely impacted by the invasion of *H. axyridis*, however, it has been one of the most severely affected (Brown et al., 2011b).

Harmonia axyridis has four major colour forms, three of which are melanic, f. *conspicua*, f. *spectabilis*, and f. *axyridis* and one non-melanic, f. *succinea* (Dobzhansky, 1933; Tan & Li, 1934; Hosino, 1940; Komai et al., 1956; Gautier et al., 2018). In much of its natural range, me-

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lanic and non-melanic forms of *H. axyridis* exist together in populations in varying proportions depending on their geographic location (Komai et al., 1950; Komai & Chino, 1969; Seo et al., 2008). This includes colour form variation both between and within different regions. For example, in Japanese populations, the melanic forms f. *conspicua* and f. *spectabilis* increase in frequency from north to south (Komai et al., 1950; Komai & Chino, 1969). However, in Taiwan, the melanic form f. *conspicua* is almost entirely dominant (Komai & Chino, 1969), while in local populations in Korea, the non-melanic form f. *succinea* is dominant (Komai & Chino, 1969; Seo et al., 2008). This highly polymorphic nature of *H. axyridis* is beneficial for overwintering success through the seasonal variation of their colour form (Michie et al., 2010, 2011). Melanic forms are thought to be advantageous during cold periods as melanism enhances solar radiation absorbance, allowing organisms to heat up faster and be more active at low temperatures, but in the summer when solar radiation is persistently higher, this benefit is not as pronounced (Talleon et al., 2004; Michie et al., 2010). Seasonal change in the frequency of colour form within a population therefore allows a species to maximise its fitness in accordance with climate. In a study in the UK, Purse et al. (2015) found signs that the increased duration and intensity of sunlight may enhance the spread of the non-melanic colour forms of *H. axyridis*. This effect of climate has also been seen in the melanic forms of *H. axyridis*, which tend to be more prevalent at cold temperatures (Michie et al., 2011). This is further demonstrated by studies in China and Japan, where non-melanic colour forms of *H. axyridis* are more prevalent in the summer, while melanic forms are more successful in the winter (Osawa & Nishida, 1992; Su et al., 2009).

This process, known as thermal melanism, has also been used to explain variation in the occurrence of *A. bipunctata* melanic forms (Brakefield & Wilmer, 1985). *Adalia bipunctata* has three colour forms; f. *typica*, which consists of elytra with individual black spots on a red background (non-melanic form), alongside f. *sexpustulata*, and f. *quadrimaculata*, which both display predominantly dark elytra (melanic forms) (Lusis, 1932). Many studies have shown that the colour forms in local populations of *A. bipunctata* are highly variable in regions with an oceanic climate, including The Netherlands (Brakefield, 1984a, b, c), Norway (Bengtson & Hagen, 1975, 1977), northern Italy (Scali & Creed, 1975), coastal Russia (Zakharov & Sergievsky, 1978) and Britain (Creed, 1966, 1971a). Industrial pollution has been suggested as a factor influencing colour form variation in *A. bipunctata*, where melanic forms are more prevalent closer to industrial areas (Creed, 1966, 1971b, 1974; Brakefield & Lees, 1987). Alongside this, climate also influences colour form variability; previous studies in The Netherlands have shown that *A. bipunctata* colour forms vary geographically in relation to climate variables which influence the benefits of thermal melanism, including temperature, cloud cover, and wind speed (de Jong & Brakefield, 1998). This possibly explains why there is such high variability within and between regions. The colour forms of *A. bipunctata* vary seasonally in a contrasting manner to *H. axyridis*, as the melanic forms of *A. bipunctata* are selected against during the winter (Timofeeff-Ressovsky, 1940). It seems that this also varies by region, as selection for melanics during winter has been observed in *A. bipunctata* populations in The Netherlands (Brakefield, 1985). Regional differences are even further demonstrated in a range of studies which observe a lack of seasonal variation in colour form across different geographic areas (Ya, 1961; Honek, 1975; Bengtson & Hagen, 1975; Zakharov & Sergievski, 1980; Klausnitzer & Schummer, 1983). The studies on both of these species highlight that *H. axyridis* and *A. bipunctata* are capable of adapting to changes in climate by altering the frequency of melanism within a population, but the extent of colour form polymorphism varies for both species between different regions.

The UK Ladybird survey is part of the Centre for Ecology and Hydrology's Biological Records Centre (www.ladybird-survey.org). In this study, we build upon the work of Creed (1966, 1971a), Brakefield & Lees (1987) and Purse et al. (2015) using more recent datasets of photographic records from the UK Ladybird survey to identify the temporal and geographical variation of the melanic form frequency of *H. axyridis* and *A. bipunctata* ladybird populations throughout the UK in relation to year, climate, and season.

MATERIALS AND METHODS

Data collection

Photographic records of *A. bipunctata* and *H. axyridis* were extracted from the UK Ladybird Survey databases on 12 January 2021. As the recording scheme contains records from citizen scientists, each record must first go through verification by experts to confirm the species identification is correct. The dataset was filtered for only verified records of adult ladybirds from 01 September 2013 to 31 July 2018. This specific time period was chosen due to these years having large quantities of verified photo records ($n > 100$), whilst years outside of this time period had record numbers that were insufficient ($n < 15$) to provide a meaningful sample size.

The present study considers the periods 01 September to 31 December as the overwintering period, and records between 01 March and 31 July the following year as the spring period. Whilst the peak months for entering overwintering are October–November, and for becoming active post-winter are March–April, these periods have been extended as yearly record numbers in shorter time periods were insufficient ($n < 0$) to provide meaningful sample sizes for all species apart from *H. axyridis*. As August can be considered a peak emergence period for new generations of adults, records from August were not included to increase the likelihood that the same generation of adults from the overwintering period were being studied in the spring period. Records in the spring period act as an indication of the changes in the population structure during overwintering.

In this study, one year was considered to begin at the start of the overwintering period and finish at the end of the sampled spring period i.e. from 01 September to 31 July, giving a total of five years in the dataset. When referring to years, we refer to the year in which the overwintering period began. For *H. axyridis*, due to the large volume of records, 10% of the records in a given period were examined, giving a total of 4295 observations across all five

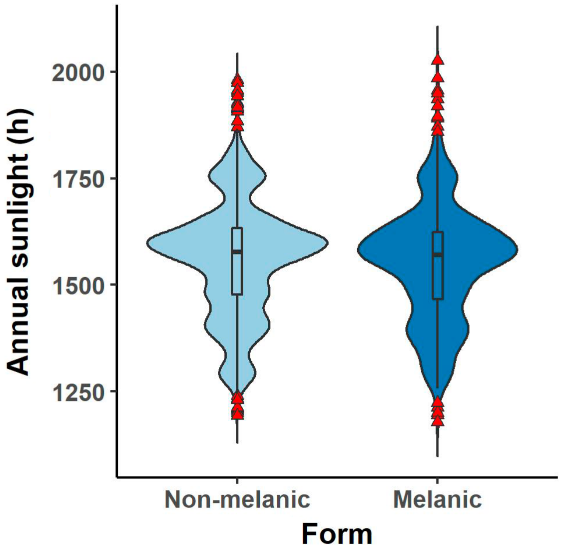

Fig. 1. Annual sunlight (h) in 1 km grid squares containing adult non-melanic and melanic *Harmonia axyridis* (n = 4295) from 2013–2017. Lower and higher boundaries of the square boxes indicate 25th and 75th percentiles, respectively. Whiskers above and below display the range and outliers are represented as red triangles. Median is marked with a black line in the box. Shaded areas illustrate the kernel probability density, indicating the proportion of *H. axyridis* found in grid squares exposed to that duration and intensity of sunlight.

years. For *A. bipunctata*, every record in each year was examined, giving a total of 1567 observations. Location, colour forms, and number of individuals were recorded. Variation in the proportion of melanic forms for *H. axyridis* and *A. bipunctata* between overwintering and spring periods was examined, with each year being treated as a replicate. The total yearly proportions of melanic forms were also compared between each year and species.

Ladybirds

When referring to colour forms, *H. axyridis* forms f. *spectabilis,* f. *conspicua* and f. *axyridis* are referred to as melanic, whilst f. *succinea* is referred to as non-melanic. For *A. bipunctata*, f. *typica* is referred to as non-melanic, and f. *sexpustulata* and f. *quadrimaculata* are referred to as melanic.

Climate variables

The impact of climate on the occurrence of melanic forms was assessed using the UK Met Office HadUK-Grid gridded and regional land surface climate observation datasets (Met Office, 2018). Data were extracted into a geographic information system from 1 km gridded annual observations of sunlight hours, average air temperature, rainfall, and average wind speed from 2013–2017. When creating the graphical heatmap, the 1 km gridded 30 year average for sunlight hours between 1981 and 2010 was used.

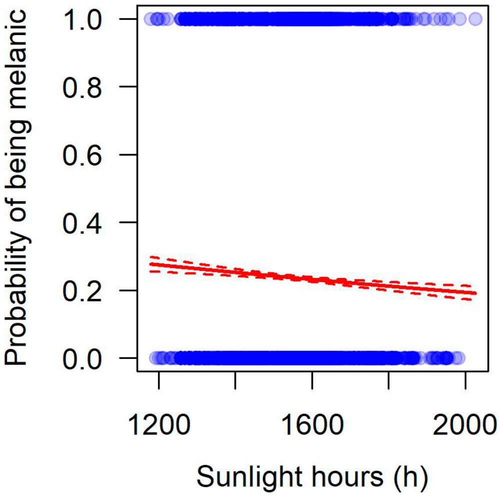

Fig. 2. Effect of annual sunlight (h) on the fitted probability of an observation containing the melanic forms of adult *Harmonia axyridis* (n = 4295) from 2013–2017 taken from the logistic regression (Table 1).

Statistical methods

All statistical analyses were conducted using R version 4.0.3 (R Core Team, 2021) and the "tidyverse" (Wickham et al., 2019), "betareg" (Cribari-Neto & Zeileis, 2010), "ggplot2" (Wickham, 2016), "sp" (Pebesma & Bivand, 2005), and "ggmap" (Kahle & Wickham, 2013) packages. The heatmap was created using ArcGIS pro. Z-tests were used when comparing the differences between proportions. Logistic regression including interaction terms between each variable was used to analyse whether climate predicts the occurrence of melanic forms. Model selection was computed using Akaike Information Criterion (AIC) for both species using backward stepwise regression. Prior to logistic regression, the linearity of the predictor variable and its logit was tested to verify no test assumptions were violated.

RESULTS

Climate, colour form, and distribution

For *H. axyridis*, only sunlight hours were significantly associated with the melanic forms, where the probability of being melanic decreased in areas with higher annual

Table 1. Logistic regression coefficients analysing the effect of annual sunlight (h) on the presence of melanic forms (1) and non-melanic forms (0) in 4295 records of adult *Harmonia axyridis* from 2013–2017. Significance is indicated by (*).

Predictor	Estimate	Standard error	95% CI	*P*	Chi-square
Intercept	–0.299	0.418	[–1.110, 0.528]	0.488	4.52
Sunlight	–0.0005699	0.0002680	[–0.001, -4.45×10^{-5}]	0.034*	

Table 2. Logistic regression coefficients analysing the effect of annual sunlight (h) and annual rainfall (mm) and their interaction on the presence of melanic forms (1) and non-melanic forms (0) in 1567 records of adult *Adalia bipunctata* from 2013–2017. Interaction is marked with (*).

Predictor	Estimate	Standard error	95% CI	*P*	Chi-square
Intercept	9.27	4.09	[1.460, 17.46]	0.023	5.2
Rainfall	–0.0097	4.28×10^{-3}	[–0.018, –0.002]	0.024	
Sunlight	–0.0072	2.58×10^{-3}	[–0.012, –0.002]	0.006	
Rainfall*sunlight	6.15×10^{-6}	2.72×10^{-6}	[9.62×10^{-7}, 1.16×10^{-5}]	0.024	

sunlight hours (Table 1, Figs 1, 2). This effect, whilst significant ($P = 0.034$), was small (Table 1, Fig. 2), and areas exposed to an increased duration and intensity of sunlight were often found to have a large number of the melanic forms (Fig. 3). In *A. bipunctata*, the probability of being melanic decreased as both rainfall and sunlight hours increased (Table 2, Fig. 4). However, a significant interaction between sunlight hours and rainfall was found. Where rainfall levels were low, the probability of being melanic decreased as sunlight hours increased. Where rainfall was high, this relationship reversed and the probability of being melanic increased with higher sunlight hours (Table 2, Fig. 5).

Melanic and non-melanic colour forms of *H. axyridis* were widely distributed throughout the UK. This was also true for the non-melanic forms of *A. bipunctata* (Fig. 6), however, other than their low level of occurrence, no apparent trends in the geographical distribution of the melanic form of *A. bipunctata* could be seen (Fig. 6).

Form variation between periods and species

On average throughout the five-year period, the proportion of the melanic forms were seen to be higher in spring periods than overwintering periods for *H. axyridis* (Z-test, $Z = 2.47$, df = 1, $p = 0.013$, Fig. 7), whereas no significant difference was seen between either period in *A. bipunctata* (Z-test, $p = 0.72$, Fig. 7). The non-melanic form was dominant, however, regardless of period, with the proportion of melanics never reaching above 40% in either species (Fig. 7).

The frequency of the melanic forms also varied among years for *H. axyridis*; 2013 and 2014 showed no significant

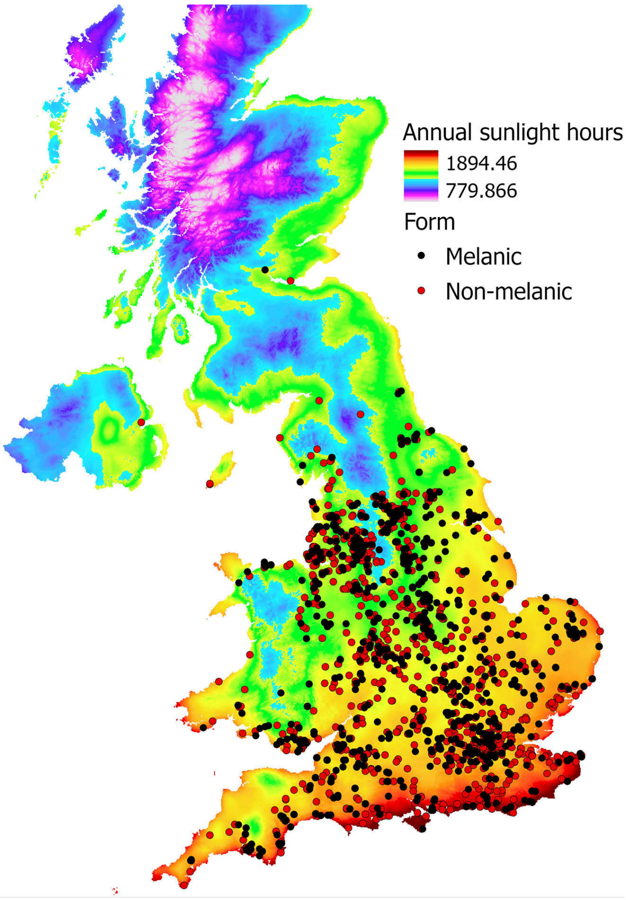

Fig. 3. Heatmap depicting the thirty-year average of yearly sunlight (h) (1981–2010) per 1 km in the United Kingdom. Points represent individual records of the melanic and non-melanic forms adult *Harmonia axyridis* (n = 4295) from 2013–2017.

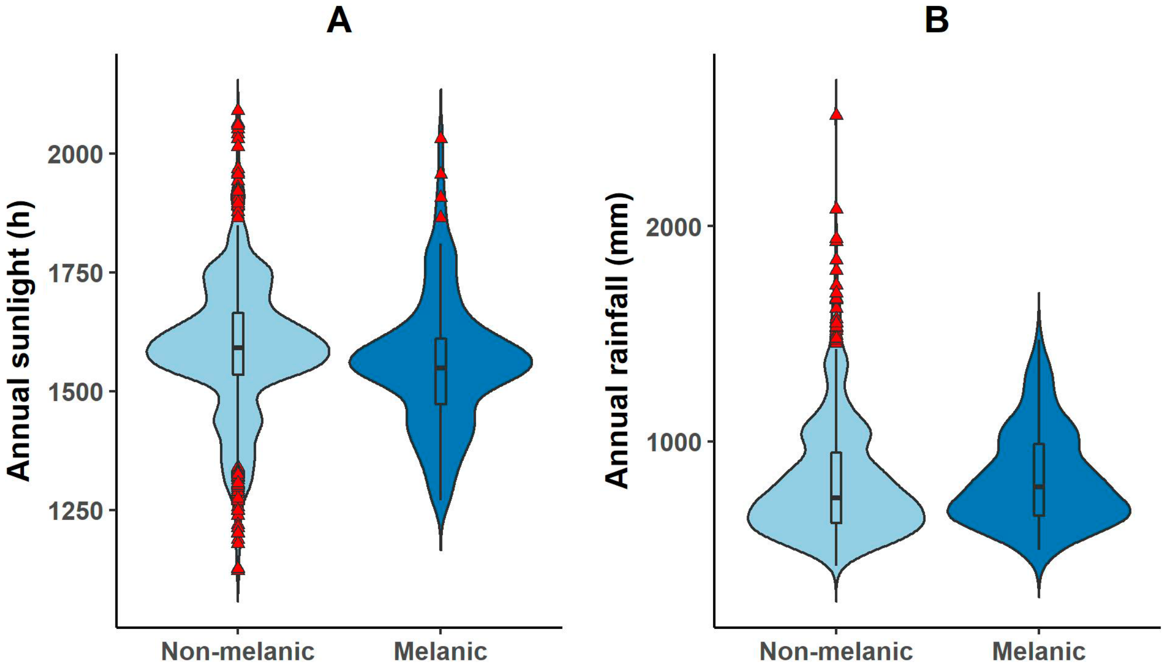

Fig. 4. (A) Annual sunlight (h) and (B) annual rainfall (mm) in 1 km grid squares containing adult non-melanic and melanic forms of *Adalia bipunctata* (n = 1567) from 2013–2017. Lower and higher boundaries of the square boxes indicate 25th and 75th percentiles, respectively. Whiskers above and below display the range and outliers are represented as red triangles. Median is marked with a black line in the box. Shaded areas illustrate the kernel probability density, indicating the proportion of *A. bipunctata* found in grid squares exposed to those levels of the variables.

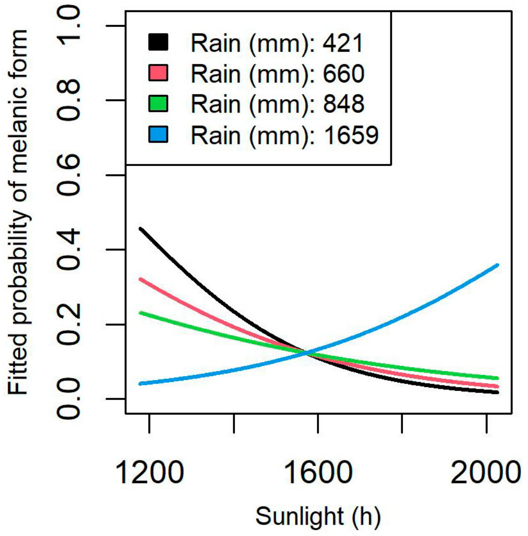

Fig. 5. Interaction effect between annual sunlight (h) and annual rainfall (mm) on the fitted probability of an observation containing the melanic form of adult *Adalia bipunctata* (n = 1567) from 2013–2017 taken from the logistic regression (Table 2). Rainfall has been split into tertiles at 0%, 33%, 66%, and 99%.

difference in the frequency of the melanic form to each other (Z-test, $p \geq 0.05$). However, from 2015 onwards each year had significantly lower proportions of the melanic forms than 2013 and 2014 (Z-test, $Z = 5.63$, df = 4, $p \leq 0.01$), but did not differ to each other (Z-test, $p \geq 0.05$; Fig. 8). No significant variation in the frequency of the melanic forms between years was seen for *A. bipunctata* (Z-test, $p \geq 0.1$).

DISCUSSION

Melanic polymorphism within ladybird populations has been extensively studied and its occurrence is now believed to be controlled by a mixture of both genetics and environmental influence (Honek, 1975; Osawa & Nishida, 1992; Honek et al., 2005; Lombaert et al., 2007; Michie et al., 2010, 2011; Purse et al., 2015). Research on UK populations of *H. axyridis* has shown that climatic factors, such as sunlight, may influence the distribution of certain colour forms (Purse et al., 2015). For UK populations of *A. bipunctata*, colour form polymorphism has been studied previously in relation to climate and industrialisation (Creed, 1966, 1971a, b; Muggleton et al., 1975; Brakefield & Lees, 1987), however, to our knowledge, there have been no studies on these UK populations in the last decade. Here, we have analysed the variation in the frequency of the melanic forms of *A. bipunctata* and *H. axyridis* populations in the UK in relation to the time of year and climate. This study provides a continued observation, using more recent data, of the geographic, climatic, and seasonal variation of *H. axyridis* and *A. bipunctata* colour forms seen previously

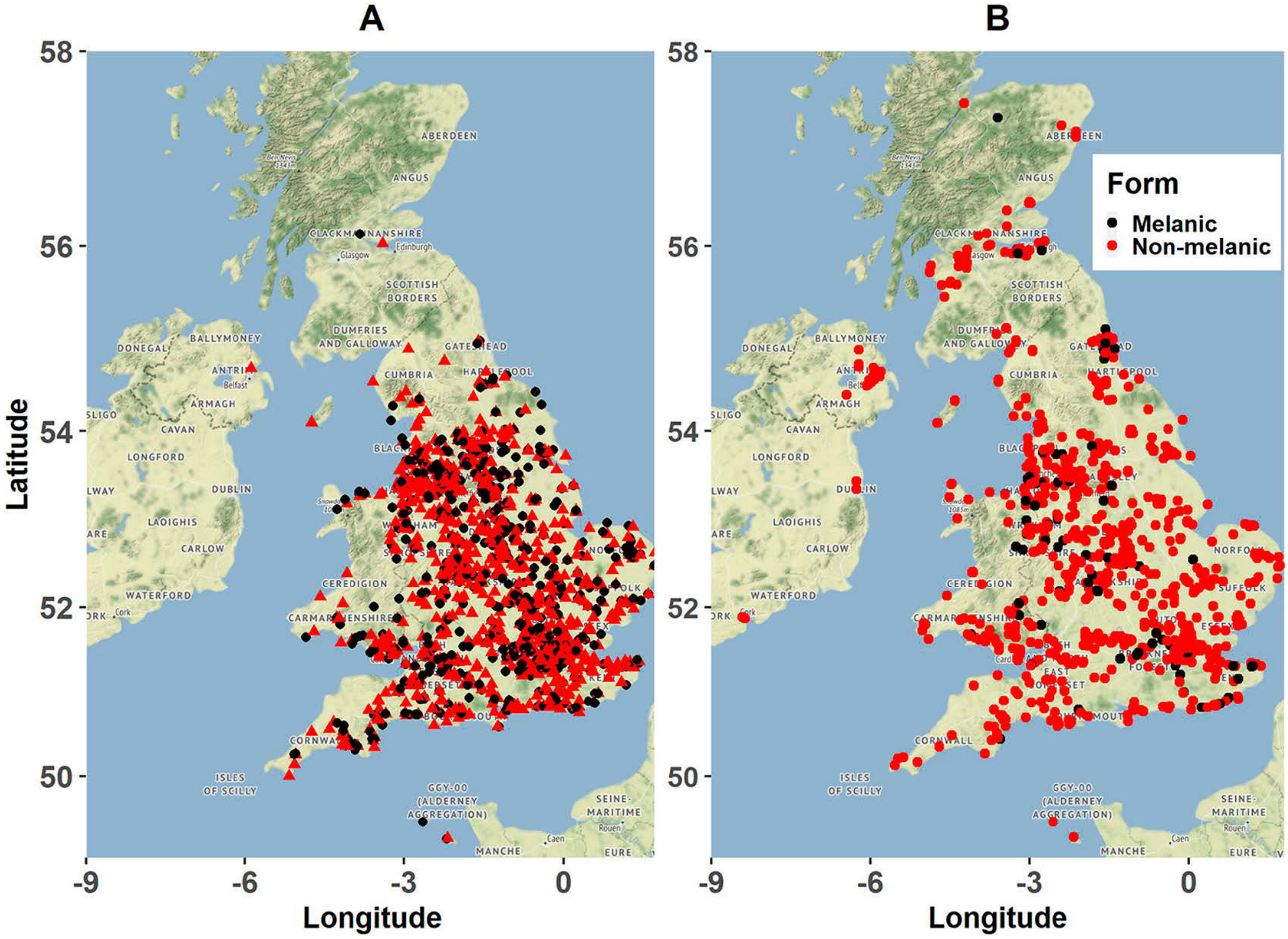

Fig. 6. Total distributions of (A) *Harmonia axyridis* (n = 4295) and (B) *Adalia bipunctata* (n = 1567) colour forms in the United Kingdom from 2013–2017.

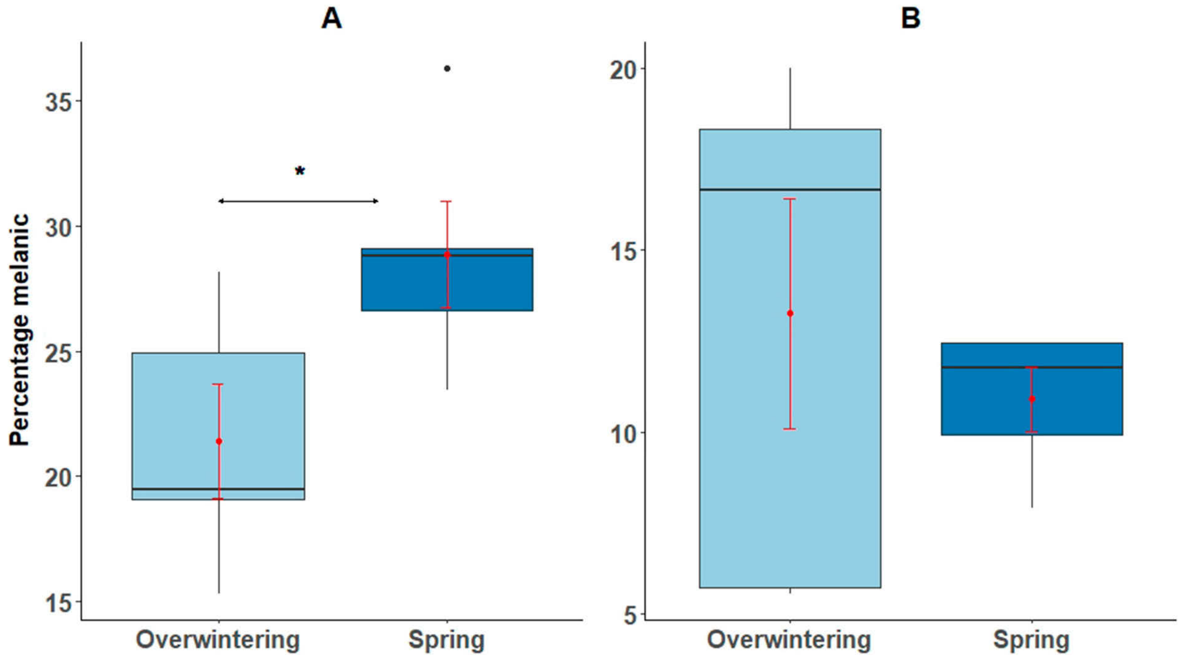

Fig. 7. Proportion of melanic forms during overwintering and spring periods for (A) adult *Harmonia axyridis* (n = 4295), (B) adult *Adalia bipunctata* (n = 1567). Significance is indicated by (*). Lower and higher boundaries of the boxes indicate the 25th and 75th percentiles, respectively. Whiskers above and below display the range. Median is marked with a black line within the box. Means are represented as points with standard error bars in red. Outliers are represented by points outside of the box.

in UK populations of these species (Creed, 1966, 1971a, b; Muggleton, 1975; Brakefield & Lees, 1987; Purse et al., 2015). These results highlight the important role that climate plays in determining colour form in both of these species and the decline in the frequency of melanism in the UK.

We found that in areas exposed to sunlight for longer periods of time, *H. axyridis* individuals were less likely to be melanic. This is explained by the increased duration and intensity of sunlight reducing the competitive advantage that melanics have over non-melanics by warming up faster (Soares et al., 2003). When sunlight is in excess it is no longer a limiting factor for fitness, meaning the cost of producing melanin (Talloen et al., 2004) becomes unfavourable. This supports the theory that melanism is selected against in the summer, when sunlight hours are longer, due to non-melanics benefitting more from increased sunlight (Michie et al., 2010; Purse et al., 2015). However, no significant effect of temperature on the frequency of the melanic forms was seen. This is in line with recent studies which found that the frequency of colour morphs in European populations of *H. axyridis* were not correlated with monthly or periodical changes in temperature during growing seasons (Honek et al., 2020). As we only selected for adult *H. axyridis*, this suggests that while temperature is important in determining the colour form during development (Michie et al., 2010, 2011), sunlight, rather than temperature, is more important when determining the frequency of the adult melanic forms. However, it must be noted that the melanic forms were also often found in areas where the duration and intensity of sunlight was very high (Fig. 3), as the effect of sunlight on predicting the melanic form was slight. This highlights the role of genetics in determining the colour form, contrary to the influence of climatic factors, a process which has been seen in some Japanese *H. axyridis* populations, where the distribution of the melanic forms have no relation to climate (Komai et al., 1950).

This effect was also true in *A. bipunctata*, however, the effect of sunlight on the melanic form frequency was dependent on an interaction with rainfall. We found that in areas with lower rainfall, the probability of being melanic was negatively correlated sunlight hours. In constrast,

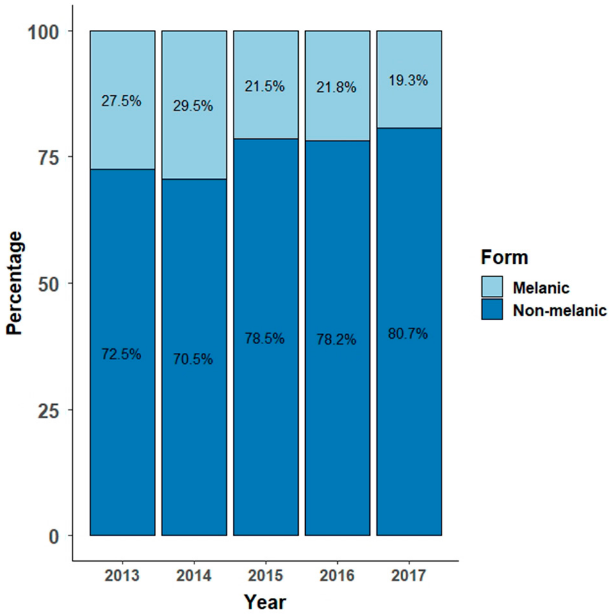

Fig. 8. Yearly percentage of the melanic and non-melanic forms in adult *Harmonia axyridis* (n = 4295) from 2013–2017.

where rainfall was high, the melanic form was positively correlated with sunlight hours. Cloud cover due to increased rainfall will lead to intermittent periods of sunlight exposure, therefore benefitting the melanic forms as they can more readily take advantage of these periods to warm up than the non-melanic forms. This has previously been suggested as an explanation for the clinal variation of *A. bipunctata* melanic forms in The Netherlands (de Jong & Brakefield, 1998). These results support the hypothesis that certain climatic variables are determining factors in predicting the melanic form in both of these species. However, while we saw evidence for this here, this may not be true for every population, as previously shown in Japanese populations of *H. axyridis* by Komai et al. (1950), the colour forms in ladybird populations sometimes do not respond to climate as expected. Honek et al. (2020) hypothesised that these geographic differences may be due to founder effects, such as the frequency of morphs in the original populations, or local complexes of Mullerian mimicry, but as of yet no studies have provided evidence to explain why this occurs. It would be interesting to see if similar trends occur in *A. bipunctata* populations from different continents.

Seasonal variation in the melanic forms of *H. axyridis* has been previously observed, in which melanism is selected for during winter, and selected against in summer (Osawa & Nishida, 1992; Su et al., 2009; Honek et al., 2020). However, this is area-specific, as in some populations no seasonal variation can be found (Kholin, 1990). In the present study, the proportion of the melanic forms of *H. axyridis* is higher post-overwintering in the spring period than it is at the beginning of the overwintering period. This suggests that selection for the melanic form during the overwintering period has taken place, and therefore, provides further evidence that UK populations of *H. axyridis* still undergo seasonal variation in colour form.

In *A. bipunctata*, no significant difference between overwintering and spring periods was seen, in contrast to studies suggesting that the colour form of this species undergoes seasonal selection (Timofeeff-Ressovsky, 1940; Brakefield, 1985). However, Brakefield (1985) took place in The Netherlands and Timofeeff-Ressovsky (1940) in Germany, suggesting the lack of a significant difference between periods in the present study may be due to variation in the response of colour forms to seasonal selection between different populations. Indeed, a lack of seasonal variation in the colour forms of *A. bipunctata* has been seen previously in populations from range of geographic areas across Europe (Ya, 1961; Bengtson & Hagen, 1975; Honek, 1975; Zakharov & Sergievsky, 1980; Klausnitzer & Schummer, 1983; Honek et al., 2005), including the UK (Majerus & Zakharov, 2000). Creed (1966) found only one local population in Birmingham in the UK which displayed seasonal variation. Honek et al., 2005 hypothesised that seasonal variation in the melanic forms of *A. bipunctata* may only occur when the proportion of melanics in a population is high. In the present study, the average proportion of melanics was very low (<15%) throughout. The lack of seasonal variation observed here supports this hypothesis and provides further evidence that UK populations of *A. bipunctata* lack seasonal variation in colour form. A possible underlying mechanism for this phenomenon may be explained by the climatic differences between countries. For example, The Netherlands tends to have colder winters in comparison to the UK (Brakefield, 1985; Met Office, 2021), and the populations studied in Brakefield (1985) withstood particularly cold winters. As cold temperatures promote the melanic forms of ladybirds (Michie et al., 2011), the milder winters in the UK potentially explain the lower overall proportion of melanics, leading to the lack of seasonal variation seen here.

This decline in the frequency of the melanic form of *A. bipunctata* in the UK has been documented previously in association with the level of air pollution (Brakefield & Lees, 1987), in which smoke levels act to reduce sunlight, favouring melanism. Therefore, as air pollution declines, so too does the melanic form. Climate change has also been suggested as an explanation for this phenomenon, as warmer weather is thought to favour low melanic form frequencies by reducing its competitive advantage of heating up faster (de Jong & Brakefield, 1998). The effect of climate change has been further demonstrated with continued surveys of this Dutch population of *A. bipunctata*; when sampled again in 2004, the once steep cline in melanic form frequency originally observed in Brakefield (1984b, c) had changed to a more uniform frequency of the melanic form of around 20% (Brakefield & de Jong, 2011). Similarly, in an area only 2 km away from the population of *A. bipunctata* in Potsdam, where the proportion of the melanic form was 25–75% (Timofeeff Ressovsky, 1940), the melanic form frequency was as low as 15.2% by 1973 (Creed, 1975), and only 5.0% in 1981 (Honek et al., 2005). These observations are reflected in the results of the present study, where we see the frequency of the melanic form of *A. bipunctata* range between 9 and 13% in total. This represents a decrease in the proportion of melanism in the UK since 1978 (Brakefield & Lees, 1987), where melanic form ranges between 13 and 36%, which is consistent with an increase in global temperature (Lenssen et al., 2019) and reduction in air pollution (Murrells et al., 2010) since the 1980s.

A decline in melanism was also seen in the frequency of *H. axyridis* melanic forms within the duration of this study; we found that the frequency of melanic forms decreased by almost 10% by the end of the five year period, with the average frequency of melanics never reaching above 30% in any given year. This result mirrors the trend seen in *A. bipunctata* and supports reports of a decrease in the frequency of the melanic forms in English populations of *H. axyridis* since its arrival in 2004 (Brown et al., 2008).

The methods in the present study, which used citizen science data from the UK Ladybird Survey, proved to be an effective method of monitoring colour form polymorphism in ladybirds. It was successful in identifying the same trends in colour form polymorphism found in a wide range of previous studies that actively sampled ladybirds (Creed, 1966; de Jong & Brakefield, 1998; Honek et al., 2020). This highlights the value of databases such as the UK Ladybird survey and iRecord for monitoring phenotypic changes in species in a changing climate.

This study provides evidence of the role of climate in determining the presence of the melanic forms of both *H. axyridis* and *A. bipunctata*, in particular, that sunlight is the most important factor determining the colour form in adults of these species. We found that the frequency of the melanic forms of these two species was very low, possibly due in part to the warming climate, continuing on from trends in overall melanic form decline seen previously. Furthermore, we show that *H. axyridis* exhibits seasonal selection for the melanic forms during winter and provide support for the theory proposed by Honek et al. (2005) suggesting that seasonal variation in colour form does not manifest in populations with low overall proportions of melanic forms in *A. bipunctata*. It is possible that the lack of seasonal variation may indicate a reduction in the fitness benefit of melanism during overwintering, resulting in the costs of melanism leading to selection against its occurrence. Continued observation of the trends in seasonal variation observed in this study for both species is therefore necessary. Alongside this, research should focus on whether the melanic form frequency continues to decline in these species as the climate warms further, and on identifying the mechanisms driving this decline in overwintering populations of *A. bipunctata*.

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