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SHORT COMMUNICATION

Declines in the frequency of melanic *Adalia bipunctata* (Coleoptera: Coccinellidae) in Northern UK populations

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Abstract. The two-spot ladybird, *Adalia bipunctata*, is polymorphic for elytral colour pattern. Whilst colour pattern polymorphism is complex, there are two predominant morphs: melanic and typical, which vary spatially in frequency. In the UK the melanic form has historically been most common in the industrialized North West of England. Evidence from industrial midland populations showed a decline in melanic frequency between 1960 and 1986 concordant with de-industrialization. We resampled the population of Merseyside in the North West of England, where the highest frequencies of melanic forms were historically observed, and compared our samples to data previously collected in 1962–1963. Data was consistent with a decline in frequency of melanics in the 60 year interval, but the melanic form was nevertheless still observed at around 30% frequency. We estimate the melanic allele has declined from 60–67% frequency to 13–18% frequency over the last 60 years, and that selection favouring the typical form has been of the order of 5–7% per generation. We note that selection against melanics in Wirral/Merseyside has been less strong than that observed in other Northern populations. We conclude that the decline in the melanic form is consistent with industrial thermal melanism as a primary factor driving melanism, but de-industrialization has not been sufficient to cause complete loss of the melanic allele in this location.

INTRODUCTION

Melanism – the possession of a dark colour phenotype underpinned by melanin deposition – is found widely across taxa (Majerus, 1998). Industrial melanism is particularly common in insects, of which the peppered moth is the classic model. Here, melanism is driven by reduced predation of dark forms camouflaged in sooty industrialized areas (Kettlewell, 1956). In UK populations, the two-spot ladybird *Adalia bipunctata* represents an unusual case of industrial melanism; it is widely considered to be related not to predation avoidance, but to the capacity of melanic forms to be active under lower incident solar radiation relative to the typical form. In this case, particulate pollution associated with industrialization drives reduced light levels that favour melanic forms, especially in Spring when irradiance is low and ladybird mating commences (Creed, 1974). This hypothesis is supported by field activity data indicating melanic morph activity earlier in the year and laboratory experimental data demonstrating a greater thermal excess of melanic *vs* typical forms (Brakefield, 1984; Brakefield & Willmer, 1985; Jong et al., 1996). However, there remains some scepticism that this form of industrial melanism, which involves the same mechanisms as that described in the "thermal melanism" hypothesis (Kettlewell, 1973), is universally relevant in this system (Majerus, 1998).

A key prediction of the industrial thermal melanism hypothesis is that the melanic form will decline in frequency following pollution control measures. In the UK, these were driven by the Clean Air Act 1956 that greatly reduced the burning of coal and other solid fuels in cities. The industrial melanism hypothesis was first examined in *A. bipunctata* in Birmingham, located in the industrial midlands of the UK. Consistent with a reduction in industrial melanism, a decline in frequency of melanic forms between 1960 and 1986 was observed, decreasing from a frequency of 30–55% (depending on location in the Birmingham area) to 10–25%; data that implies increasing selection in favour of the typical form (Brakefield & Lees, 1987). These data have been augmented by observations of Majerus (1994), noting a decline of melanics in Keele (Staffordshire, 29% melanics in 1980 vs 12% melanics in 1991), Sheffield (S. Yorkshire, 55% melanics in 1962–3 vs 7% melanics in 1990), and the Manchester area (91% in Altrincham 1962–3 vs 27% melanics in Manchester 1986). Studies in the Netherlands also support a decay of the historic clinal variation in melanic frequency, with populations now homogeneous for the frequency of melanism (Brakefield & de Jong, 2011).

Historically, the highest frequency of melanics in the UK was recorded in the Industrial North West of England and in Glasgow, Scotland. In Merseyside, 84% of *A. bipunctata* on the Wirral (south of the river Mersey) and 89% of *A. bipunctata* in Liverpool (north of the river Mersey) were of melanic phenotype in 1962–3 (Creed 1966). In this study, we examine the change in melanic frequency over the 60 year period in these two populations. To this end, we collected and scored the phenotype of *A. bipunctata* in Merseyside in March–September 2022, 2023 and 2024 and compared these data with records from the same localities in 1962–1963. We additionally confirmed the mode of inheritance of the two forms followed the expected model and estimated the frequency of the melanic allele for the two time periods. Finally, we calculated a standardized metric, the intensity of selection, for the Wirral and Liverpool populations, and compared this to the same calculation for other northern populations and Birmingham, to gain a broad view of the action of selection on the melanic phenotype in North England *Adalia bipunctata* populations.

MATERIAL AND METHODS

Adalia bipunctata (Linnaeus, 1758) were collected from overwintering sites (Anfield cemetery, window frames of houses), or from lime trees (*Tilia europaea*), common nettle (*Urtica dioica*) or dog roses (*Rosa canina*) in central Liverpool and on the Wirral during March–June 2022 and March–September 2023 (collection data including location sites: https://doi.org/10.6084/m9.figshare.26555704.v1). An additional collection was obtained between March–June 2024 from the Wirral. For the 2022–2023 collections, the majority of the collection were adults, but a limited number of pupae were also collected and allowed to eclose in the laboratory. Each ladybird was scored for colour form (melanic/typical; photographs of the two forms: https://doi.org/10.6084/m9.figshare.26553658.v1) and sex identified through the presence of flexure bands between tergites on the ventral abdomen of males and a notch in the ventral final abdominal tergite, following Randall et al. (1992). Whilst the majority of ladybirds were either melanic (quadrimaculata/sexpustulata forms), or the typical red with two black spots form, one annulata individual was noted that was not included in this study. For the Liverpool samples, heterogeneity in melanic morph frequency between 2022 and 2023 samples was tested using a Fisher exact test. For the Wirral, the three yearly samples (2022, 2023 and 2024) were likewise tested for heterogeneity of melanic frequency. Melanic frequency data were then combined across years for a single pool per population to compare against the historical 1962–1963 data of Creed et al. (1966), and heterogeneity between timepoints then tested using a Chi-squared contingency test.

To confirm the genetic basis of colour pattern, a selection of field collected individuals were allowed to oviposit, and progeny raised. Three melanic male progeny from typical females (liv2.1, liv2.3, liv2.5) – expected to be heterozygous – were then crossed to unmated typical females and the ratio of melanics:typicals tested against the 1 : 1 null model for melanism as a single locus dominant trait.

Allelic frequencies for the 1962–1963 and 2022–2024 populations were then estimated, with the frequency of the typical allele represented as the square root of the frequency of the typical form, under assumptions of Hardy-Weinburg equilibrium. The intensity of selection was calculated following Haldane (1924) equation 7, with $kn = u_n - u_0 + \ln(u_n / u_0)$ where k = strength of selection (positive values = selection in favour of the dominant allele, negative values = selection in favour of the recessive allele), n is the number of generations elapsed between samples (taking the estimate of Majerus (1994), of one generation/year in the UK), u_0 the relative frequency of the dominant allele to the recessive allele in 1962, and u_n the equivalent in 2022/3. This process was repeated for other populations across northern England for which melanic frequency data over time had been previously obtained but intensity of selection not calculated (contemporary data from Keele, Manchester and Sheffield from Majerus (1994)). In addition, we estimated the strength of selection for Birmingham likewise, using data from Brakefield & Lees (1987) Table 1, to allow direct comparison with our calculations. This calculation was based on start years and 1986 end point data.

Table 1. The frequency of melanic and typical *A. bipunctata* in Wirral and Liverpool populations. Data from the 1962–3 population is from Creed (1966).

Year	Population	Typical	Melanic	Proportion melanic
1962–3	Wirral	11	58	0.84
2022	Wirral	13	5	0.28
2023	Wirral	10	8	0.44
2024	Wirral	23	11	0.32
1962–3	Liverpool	3	25	0.89
2022	Liverpool	14	6	0.30
2023	Liverpool	26	7	0.21

RESULTS

Melanic *A. bipunctata* were observed in 2022–2024 in both Wirral and Liverpool populations but at lower frequencies than the 1962–3 samples (Table 1). There was no evidence of heterogeneity between the 2022 and 2023 collections in Liverpool (Fisher exact test for heterogeneity between year, $p = 0.52$) and no evidence for heterogeneity between 2022, 2023 and 2024 samples in the Wirral (Fisher exact test for heterogeneity between year, $p = 0.52$). The contemporary yearly samples were therefore pooled to create an overall sample for each population, and the frequency of melanics compared to the respective 1962–3 sample. This analysis supported heterogeneity between the historical and contemporary samples for both locations (Wirral: $p < 0.0001$; Liverpool: $p < 0.0001$).

Having confirmed the assumption that melanic was dominant to typical (for data, see https://doi.org/10.6084/m9.figshare.26240129.v1), we used the morph frequency data under the model of a single locus dominant trait (melanic > typical) to estimate allele frequencies, with the frequency of the typical allele *q* estimated as the square root of the proportion of typical individuals in the population, and the frequency of the melanic allele therefore as $1-q$. The estimated melanic allele frequency in the Wirral decreased from 0.60 to 0.19, and in Liverpool from 0.67 to 0.13, between 1962 and recent samples.

Examining previously recorded data on frequency changes of the melanic phenotype from other northern populations, the estimated melanic allele frequency in Sheffield declined from 0.33 to 0.04 between 1962 and 1990; that in Manchester from 0.70 to 0.15 between 1962 and 1986, and that in Keele from 0.16 to 0.06 between 1980 and 1991. For Birmingham, a midlands industrial city with good local records, melanic allele frequency declined from 0.58 to 0.14 between 1960–1962 and 1986 in Edgbaston, from 0.37 to 0.03 between 1961–1962 and 1986 in Maw Green, from 0.29 to 0.03 between 1961–1962 and 1986 in Hall Green, and from 0.24 to 0.05 between 1965–1966 and 1986 in Aston Church.

We then calculated Haldane's standardized intensity of selection *k* for these populations (Table 2). Selection against the melanic form was strong in all cases. The survival/reproductive success of typicals is estimated to exceed that of melanics by 5.1% on the Wirral and 7.4% in Liverpool. The estimated selection in favour of typicals is stronger in the other northern cities (Sheffield 11%, Keele 10.7%, Manchester 18.3%). Across the four Birmingham populations, the survival/reproductive success of typicals is estimated to exceed that of melanics by between 9.9% and 14.9%.

Table 2. Standardized intensity of selection calculation for the melanic allele in five northern English populations of *Adalia bipunctata* and four Birmingham populations following the method of Haldane (1924). u_0 = historic relative frequency of melanic allele; u_N = contemporary relative frequency of the melanic allele; N generations = number of generations between samples; k = standardized intensity of selection (k < 0 is selection against melanics).

Population	Period	u_0 Relative frequency of melanic allele in historic population	u_N Relative frequency of melanic allele in recent population	N generations	Standardized intensity of selection, *k*
Wirral	1962–2024	1.5	$u_{61} = 0.233$	61	–0.051
Liverpool	1962–2023	2	$u_{60} = 0.152$	60	–0.074
Sheffield	1962–1990	0.5	$u_{28} = 0.037$	28	–0.110
Keele	1980–1991	0.19	$u_{11} = 0.066$	11	–0.107
Manchester	1962–1986	2.33	$u_{24} = 0.171$	24	–0.183
Birmingham Edgbaston	1960–1986	1.38	$u_{25} = 0.156$	25	–0.136
Birmingham Maw Green	1961–1986	0.59	$u_{25} = 0.026$	25	–0.149
Birmingham Hall Green	1961–1986	0.408	$u_{25} = 0.035$	25	–0.113
Birmingham Aston Church	1965–1986	0.322	$u_{21} = 0.053$	21	–0.099

DISCUSSION

Industrial melanism is a classic example of 'evolution in action', with melanic forms rising and falling with the processes of industrialization and de-industrialization. In the UK, the *Adalia bipunctata* system represents a distinct case of industrial melanism, being a case of thermal melanism rather than differential predation by morph as found in Lepidoptera (Majerus, 1998). Within the industrialized zone of England, melanic frequencies were historically highest in North West England (Creed, 1966). We revisited these same North West England populations 60 years after a previous survey to examine the extent of decline in the melanic form in the post-industrial era. We recorded pronounced declines in the frequency of melanic morphs in Liverpool and Wirral. Using knowledge gained of the genetic basis of melanism, we also highlighted corresponding declines in the melanic allele frequency. Estimates indicate strong selection in action, with an individual with typical colour pattern producing on average 5.1% more surviving progeny per generation than a melanic individual during this period in the Wirral, and 7.4% more in Liverpool.

Revisiting previously collected data on melanic morph frequencies (Creed, 1966; Brakefield & Lees, 1987; Majerus, 1998) indicates selection against melanics was stronger in Birmingham, Manchester, Sheffield and Keele (individuals with typical colour pattern estimated to produce on average 10–18% more surviving progeny per generation than a melanic individual). Overall, these data paint a consistent picture of very strong selection against the melanic form across northern England but with lower magnitude of selection against melanics estimated in Liverpool and Wirral compared to other northern locations.

There are two hypotheses for the lower intensity of selection in Merseyside compared to other locations. First, selection against the melanic form from de-industrialization may have eased over recent time. The Merseyside data set continues for 30 years beyond that in the other locations, and one explanation for our data is that this end period may have been a more benign selective environment. Given the driver of melanic loss – declines in air pollution – has continued to decrease in recent time, this seems an unlikely explanation for the reduced intensity of selection. Further it is thought that climate warming – the other obvious change in the last 30 years – acts against the melanic form rather than in favour of it (Brakefield & de Jong, 2011), and would be expected to intensify selection. Thus, whilst reductions in selection intensity in recent time cannot be excluded, there is no clear environmental change that would drive this; indeed the likely changes work in opposition to easing of selection against melanics.

An alternate hypothesis – that we favour – is that there is some intrinsic aspect of selection operating in the Merseyside populations that maintains the melanic form in Merseyside (and not other places). Consideration of melanism in *Adalia bipunctata* has emphasised not only pollution as a driver of melanic variants, but also populations being near the coast. Majerus (1994) notes higher *A. bipunctata* melanic frequency in populations near the coast in Norway than inland populations and posits higher cloud cover near coasts as a potential environmental factor boosting melanic form fitness. The lower irradiance associated with clouds creates conditions that may favour melanics through both increased springtime activity under low irradiance (relative to typicals) and also melanic forms being less commonly damaged by thermal excess on otherwise bright summer days. These elements may explain the continued presence of melanic forms in the Merseyside populations.

Onward work should maintain monitoring of *A. bipunctata* morph frequency in UK coastal populations. A key prediction of the operation of both coastal and industrial thermal melanism is that the melanic form will not be lost or become very rare in these regions, as has happened in other ex-industrial non-coastal areas of the UK. Resampling the population in 10 years' time will permit this hypothesis to be tested.

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DATA. Collections sites, dates and sex of individuals, photographs of the two main forms, and crossing data can be found on Figshare: https://doi.org/10.6084/m9.figshare.26555704.v1, https://doi.org/10.6084/m9.figshare.26553658.v1, https://doi.org/10.6084/m9.figshare.26240129.v1