

ORIGINAL ARTICLE

A steep cline in ladybird melanism has decayed over 25 years: a genetic response to climate change?

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A cline in the frequency of melanic morphs of the two-spot ladybird, *Adalia bipunctata*, was first surveyed in 1980 along a transect extending inland from the coast in the Netherlands. At that time, the frequency of melanics increased over some 40 km from 10% near the coast to nearly 60% inland. Additional surveys made in 1991 and 1995 demonstrated some progressive change in cline shape. New samples from 1998 and 2004 confirm these dynamics, and show that over a period of about 50 generations for

the beetle, the cline had decayed rapidly to yield rather uniform frequencies of melanic morphs at around 20% along the whole transect by 2004. Climate data and evidence for thermal melanism in this species support our contention that these dynamics reflect a dramatic example of a rapid genetic response within populations to climate change and local selection.

Heredity (2011) **107**, 574–578; doi:10.1038/hdy.2011.49; published online 27 July 2011

Keywords: climate change; evolution; genetic response; selection coefficient; cline decay; melanism

Introduction

A variety of processes can enable organisms, including insects, to respond successfully to climate change (Stenseth *et al.*, 2002; Bradshaw and Holzapfel, 2006; Parmesan, 2006). These include habitat tracking, phenotypic plasticity and genetic adaptation or some combination thereof. Evidence for the first of these mechanisms is becoming comparatively commonplace. Thus, many species of butterfly on the northern and southern edges of their range are clearly responding with northern extensions in their range limits (Parmesan and Yohe, 2003; see also Thomas *et al.*, 2004; Hickling *et al.*, 2006), and species of moth and other insects are moving up altitudinal gradients (Chen *et al.*, 2009). The extent to which changes in phenotypic plasticity are (or will be) involved in the numerous reports of changes in phenology (Brakefield, 1987; Roy and Sparks, 2000; Amano *et al.*, 2010) is not clear but in some case studies, including the timing of egg hatching in the winter moth and of egg laying in the great tit, there is already evidence that strong selection can occur on the characteristics of the underlying norms of reaction (Visser and Holleman, 2001; van Asch and Visser, 2007). There are as yet few reports of genetic changes within populations linked to climate change, including in insects. The pitcher plant mosquito, *Wyeomyia smithii*, showed a genetic response to climate change, which involved changes in sensitivity to photoperiod (Brad-

shaw *et al.*, 2006). The change could be detected over a period as short as 5 years. On a wider geographic scale, changes in clines for the alcohol dehydrogenase polymorphism or in the frequencies of certain chromosome inversion polymorphisms have been detected in natural populations of species of *Drosophila* and linked to climate change (Umina *et al.*, 2005; Balanyá *et al.*, 2006).

Here, we report on the most recent data describing changes in a cline of melanism in the two-spot ladybird beetle, *Adalia bipunctata* L., along a transect that extends inland from the coast in the Netherlands. A steep cline when first surveyed in 1978–1981 (Brakefield, 1984a,b) showed some progressive decay up until 1995 (de Jong and Brakefield, 1998). This was correlated with an increase in hours of spring sunshine and in spring temperatures. These data, when considered together with a variety of field and experimental data in support of the involvement of thermal melanism in this species (Brakefield, 1984b; de Jong *et al.*, 1996), provided support for the change in the shape of the cline as reflecting a genetic response to climate change (de Jong and Brakefield, 1998). The documentation of further change in climate in the Netherlands since 1995 (see below) provided the impetus for us to continue monitoring the cline shape.

Materials and methods

Samples of *A. bipunctata* were collected in 1998 and in 2004 as adults or pupae from lime trees (*Tilia* spp.) and various shrubs and herbs from the same transect as that sampled in earlier years. Pupae were allowed to eclose in the laboratory, and then all samples scored for melanic morph (see Figure 1) in the same way as in previous studies (Brakefield, 1984a,b; de Jong and Brakefield,

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Received 17 August 2010; revised 11 January 2011; accepted 21 January 2011; published online 27 July 2011

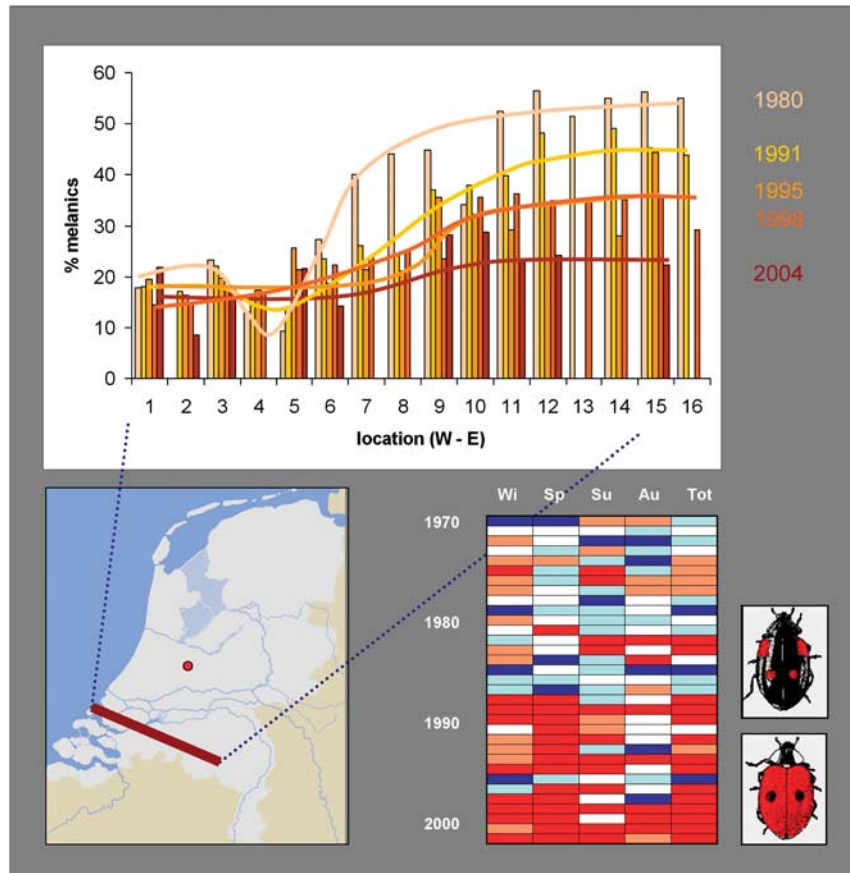


Figure 1 Changes over time in the proportion of the illustrated melanic and non-melanic morphs of the two-spot ladybird beetle along a transect of ca. 115 km in length in the Netherlands (bottom-left; source of map: <http://www.lesidee.nl/>). Samples were collected in each of the 5 years indicated at 16 more or less evenly spaced localities from west to east. Colouring of years matches the histograms for melanic frequency in the individual samples from each locality. Trend curves were fitted by eye with those for 1995 and 1998 overlapping inland. Localities are (1) Ouddorp; (2) Goedereede; (3) Stellendam; (4) Dirksland; (5) Middelhamnis; (6) Oude Tonge; (7) Achthuizen; (8) Ooltgensplaat; (9) Willemstad; (10) Klundert; (11) Zevenbergen; (12) Etten-Leur; (13) Oudenbosch; (14) Prinsenbeek; (15) Tilburg; (16) Waalre/Eindhoven. The smallest number of beetles in an individual sample was 35, with an overall mean of 818 (s.e. = 288 and median = 152). The panel on the bottom-right shows deviations in average temperature from a 'normal' season/year at De Bilt (red spot on map). From left to right, columns represent data for winter (Wi; December–February), spring (Sp; March–May), summer (Su; June–August), autumn (Au; September–November) and the overall year (Tot), respectively, and from top to bottom for different years beginning before the period of ladybird sampling. The colour of each block indicates the extent to which the average temperature in the particular season/year deviated from 'normal'; white = no deviation, blue = cooler than normal (dark blue more extreme than light blue), red = warmer than normal (dark red more extreme than light red). For further details and the source of this panel see: <http://www.knmi.nl/cms/content/71542/>.

1998). Some information on sample sizes is included in Figure 1. The full data are available on request from the authors.

The melanic polymorphism

The two-spot ladybird shows a number of genetically distinct colour morphs (Majerus, 1994). These morphs can be classified into two discrete classes, namely those that are predominantly black with red spots (melanics), and those that are red with black spots, usually one on each elytron (non-melanics; see Figure 1). A co-allelic series of alleles for melanism are genetically dominant to the recessive non-melanic, or *typica*, allele. The frequency of these morph classes varies geographically, and numerous examples of clines have been described in different countries (see Majerus, 1994). The geographical variation in morph frequencies in the Netherlands has been partly accounted for in field and laboratory studies by thermal melanism (de Jong *et al.*, 1996). This

hypothesis argues that the larger surface area of dorsal, black cuticle in the melanic morphs gives them a thermal advantage relative to non-melanics under conditions where activity is limited by the absorption of solar radiation (see de Jong *et al.*, 1996 and references therein). Based on the results from laboratory experiments measuring the temperature profiles and activity of beetles of each morph under differing conditions of radiation, heating and convective cooling, melanic beetles are expected to have a thermal advantage over non-melanic beetles in the field whenever: (1) ambient temperatures are low; (2) the amount of wind is low; (3) sunshine is intermittent; and/or (4) the radiation intensity is high (de Jong *et al.*, 1996). Thirty years ago, such conditions tended to occur in the east of the Netherlands away from the coast during the spring and early summer when ladybird beetles are emerging from hibernacula to feed on aphids and reproduce. Since that time, the climate inland at this time of the year has tended to become warmer with less cloud cover, and,

thus, more similar to that along the coast (see de Jong and Brakefield, 1998).

Results and discussion

We collected new samples of *A. bipunctata* in 1998 and in 2004 along the same transect. Unfortunately, invasion of this region by an exotic Asian ladybird, *Harmonia axyridis*, from around 2002 onwards had by 2004 already produced a clear negative impact on the numbers and distribution of the endemic ladybird fauna, including *A. bipunctata* (see chapters in Roy and Wajnberg, 2008). This invasion has progressed dramatically until it is no longer possible to re-survey the transect by obtaining samples of sufficient size for detailed analysis. The 10 samples obtained in 2004 were also generally smaller than in each of the earlier surveys (mean = 115 beetles; cf. Figure 1). In addition, we could not obtain any samples from five localities sampled in at least two of the previous surveys.

Figure 1 shows the results for 1998 and 2004, and compares these new data with the three previous surveys. A general linear model (GLM) analysis of the values for percentage melanics (Table 1; using ASIN-square root transformed yielding normally distributed data) with year as factor and distance from coast as a covariate gives a significant fit of year ($F=8.03$, $P<0.0001$) and distance ($F=10.08$, $P<0.0001$). Pairwise comparisons across years of the frequency of melanics in samples from each site (see Supplementary Table S1) show that at sites to the east of the transect, there are numerous examples of significant declines in melanism. In contrast near the coast, there are no declines and only a few significant increases at a single locality (Middelhamnis). Figure 2a illustrates this pattern by showing how the changes over the study period are much more dramatic inland reflecting decreasing melanism. Clearly what was originally in 1980 a steep cline with a change in melanic frequency from around 10% to nearly 60% over some 40 km had decayed progressively to a pattern in 2004 of rather uniform melanism with a melanic frequency of around 20%. There was significant heterogeneity among the samples obtained in 2004 (heterogeneity χ^2 -test = 25.86; $df=9$; $P<0.01$), but this is largely lost when the sample for the coastal site of Goedereede with only 8.6% melanics is excluded from analysis ($\chi^2=8.26$; $df=8$; P is NS; see Figure 2b). Assuming Hardy-Weinberg proportions, two alleles with the melanic allele dominant, a time span of 50 generations or two per year (Brakefield, 1984a,b), a decline in melanic frequency from 55 to 20%, and constant (weak) selection, a rough estimate of the selection coefficient (s) can be calculated (Hartl, 1980) for the melanic disadvantage over the area to the east of locality 10 (see Figure 1). This calculation yields a selection coefficient of 0.036 to account for the evolutionary dynamics of the cline shape inland in the Netherlands.

The climate trends that we described in 1998 have continued in to the first decade of this century (Figure 1 and see <http://www.cesar-database.nl>) with average temperatures, especially in the spring, increasing inland (see also Visser and Holleman, 2001). The invasion by *H. axyridis* began at the very end of the sampling period around 2002 and is unlikely to have contributed to the evolution in cline shape even in the final period of

Table 1 Numbers of melanic and non-melanic *Adalia bipunctata* in samples collected from the indicated locality in each of five surveys covering 1980 to 2004

Location	Distance to coast (km)	1980			1991			1995			1998			2004		
		# Melanic	# Typica	% Melanic	# Melanic	# Typica	% Melanic	# Melanic	# Typica	% Melanic	# Melanic	# Typica	% Melanic	# Melanic	# Typica	% Melanic
Ouddorp	5.6	19	88	17.8	25	114	18.0	25	104	19.4	22	130	14.5	18	65	21.7
Goedereede	8.0	25	83	23.2	33	112	17.0	14	71	16.5	14	82	14.6	13	139	8.6
Stellendam	11.6	57	383	13.0	25	117	22.0	19	78	19.6	26	119	17.9	17	82	17.2
Dirksland	19.0	770	7486	9.3	31	148	14.5	24	115	17.3	34	168	16.8	44	161	21.5
Middelhamnis	22.0	4158	11 059	27.3	54	175	13.8	76	221	25.6	43	158	21.4	5	30	14.3
Oude Tonge	28.6	20	30	40.0	39	110	26.2	19	70	21.4	21	68	23.6	49	124	28.3
Achthuizen	32.8	70	89	44.0	46	145	24.1	25	94	21.0	26	77	25.2	28	70	28.6
Ooltgensplaat	37.2	2565	3144	44.9	75	128	37.0	54	98	35.5	42	137	23.5	29	95	23.4
Willemstad	42.4	129	249	34.1	75	123	37.9	36	76	32.1	78	141	35.6	26	81	24.3
Klundert	50.0	1207	1139	51.5	75	113	39.9	225	545	29.2	102	179	36.3	16	56	22.2
Oudenbosch	50.3	2255	2054	52.3	93	100	48.2	80	152	34.5	59	110	34.9	37	66	35.9
Zevenbergen	55.4	2251	1744	56.4	78	81	49.1	34	87	28.1	87	161	35.1	33	80	29.2
Etten-Leur	60.4	151	124	54.9	89	107	45.4	100	126	44.3	37	66	35.9	16	56	22.2
Prinsenbeek	64.0	4546	3546	56.2	106	136	43.8	106	126	44.3	37	66	35.9	16	56	22.2
Tilburg	87.8	83	68	55.0	106	136	43.8	106	126	44.3	37	66	35.9	16	56	22.2
Waalre/Eindhoven	118.8	83	68	55.0	106	136	43.8	106	126	44.3	37	66	35.9	16	56	22.2

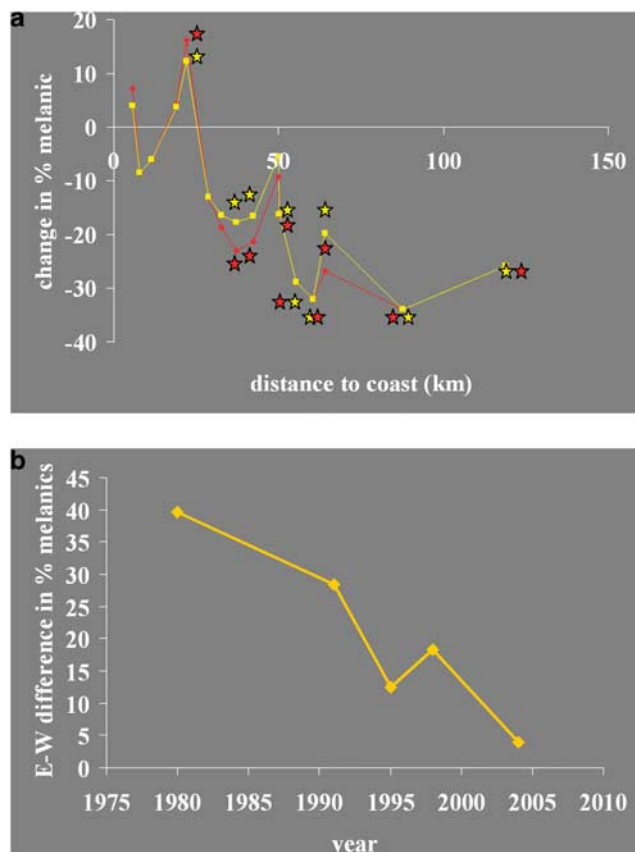


Figure 2 Pattern of change in melanic frequency over time along the study transect. (a) Plotted in red: the maximum difference in percentage melanics between any two samples collected at each locality over the study period; and in yellow: the comparable difference for the earliest and latest samples obtained for each locality. A negative sign indicates a decline in melanism over time, and a star indicates a significant change in frequency. (b) The difference in each sampling year between the median value for percentage melanics for the five most westerly localities near the coast and that for the five most easterly localities inland showing the approach to homogenization over the whole transect.

sampling. Thus, although no direct test is available, our data are more likely to be explained by a genetic response to climate change working via the process of thermal melanism than by any other factor.

A cline of melanism in the peppered moth between urban Liverpool and rural North Wales has decayed dramatically under strong selection in recent decades (Saccheri *et al.*, 2008 and references therein) as a region of high melanism in North-West England including Liverpool has almost disappeared (see also Majerus, 1998). This example has been explained at least in part by a change in visual predation as the effects of declining air pollution have affected the resting backgrounds of the moths in urban environments. Clines have not only been observed to decay rapidly but can also become established quickly, for example those involving chromosome inversions in colonizing populations of *Drosophila subobscura* presumably under the influence of local selection (Balanyá *et al.*, 2006). The present study adds to potential examples of how some organisms are likely to be responding to climate change through direct genetic responses within populations. It will be neces-

sary to understand such examples in detail to be able to assess properly the likely contribution of local adaptation and genetic responses in organisms to future climate change.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We dedicate this paper to the late Michael Majerus whose great enthusiasm for ladybirds and for melanism did so much to stimulate a broad interest in natural history and evolution. We are grateful to the Royal Dutch Meteorological Institute (KNMI) for access via their web sites to meteorological records. We also thank Suzanne Lommen and Kees Koops for help with collecting, and two anonymous reviewers for valuable comments on the manuscript. This project was supported for the most recent survey by the Technology Foundation STW, applied science division of NWO and the technology programme of the Dutch Ministry of Economic Affairs.

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Supplementary Information accompanies the paper on *Heredity* website (<http://www.nature.com/hdy>)