

Climate and change in clines for melanism in the two-spot ladybird, *Adalia bipunctata* (Coleoptera: Coccinellidae)

Peter W. de Jong and Paul M. Brakefield

Section of Evolutionary Biology, Institute of Evolutionary and Ecological Sciences, University of Leiden, PO Box 9516, 2300 RA Leiden, The Netherlands (brakefield@ruisfb.leidenuniv.nl)

The polymorphic two-spot ladybird, *Adalia bipunctata*, shows clinal variation in the frequency with which melanic and non-melanic morphs occur. Here we show that the clines in melanism in The Netherlands have changed over the past two decades: they have become shallower. This change has coincided with an increase in local ambient spring temperatures at most meteorological stations close to the sampling sites. The potential contribution of thermal melanism to the change in the shape of the clines is discussed.

Keywords: cline, melanism, *Adalia*

1. INTRODUCTION

The two-spot ladybird, *Adalia bipunctata*, shows colour polymorphism; a number of genetically distinct morphs exist (Majerus 1994), which can be classified broadly into those that are predominantly black with red spots (melanics), and those that are red with black spots (non-melanics). The frequency with which the various morphs occurs varies geographically, and has been described by clines (Benham *et al.* 1974; Muggleton *et al.* 1975; Brakefield 1984a). This clinal variation has been the subject of study in a number of countries, and several selection pressures have been proposed to influence the relative fitness of the colour morphs, and hence their relative frequency (for a review, see Majerus 1994).

One hypothesis proposed to explain the geographic variation of the frequency of the melanic alleles is thermal melanism. Several studies support this hypothesis, which argues that the larger area of black, melanized surface of the melanic morphs gives them a thermal advantage relative to the non-melanics under conditions where (reproductive) activity is limited by the absorption of solar radiation (see de Jong *et al.* (1996) and references therein). Our recent laboratory measurements of thermal budgets of two-spot ladybirds have identified the potential effects of several climatic variables on the higher-than-ambient temperatures in these beetles, and beetle activity (de Jong *et al.* 1996). The results, coupled with the application of a biophysical model (de Jong *et al.* 1996), showed that predicted temperature excesses were influenced differentially between melanic and non-melanic ladybirds by ambient temperature, radiation intensity, wind speed, and their interaction. Differences between the colour morphs arose through a difference in reflectance and transmittance of the elytra. Body temperatures, walking speed, and time needed to become active under a range of specified radiative regimes, ambient temperatures and wind speeds, were in qualitative agreement with, and

quantitatively closely corresponding to, the predictions from the model: melanic beetles generally had higher equilibrium temperatures and activity levels. An important factor reducing the difference in temperature excess and activity between melanics and non-melanics was wind, apparently because of a higher convective cooling in melanic beetles. Based on the results from this laboratory experiment, melanic ladybirds are expected to have a thermal advantage over non-melanic beetles in the field when (1) ambient temperatures are low, (2) the amount of wind is low, (3) sunshine is intermittent, and (4) the radiation intensity is high (de Jong *et al.* 1996).

Field studies have provided data supporting the hypothesis of thermal melanism (for a review, see Majerus 1994). For example, in The Netherlands, correlations were found between the relative frequency of melanics and the number of hours of sunshine in spring, and these correlations broke down outside this period (Brakefield 1984a). There was also evidence for a trend towards an earlier spring reproduction of melanic beetles (Brakefield 1984b, 1985). If thermal melanism indeed plays an important role in the field, and causes these correlations between melanic frequency and macroclimatic variables, similar correlations are expected on a temporal scale, i.e. any change in the relative frequency of the colour morphs with time is expected to be associated with a change in at least some climatic variables in the same period of the year.

This study provides long-term field data on morph frequencies of the two-spot ladybird in The Netherlands, as well as data on macroclimatic variables that are expected to be correlated with the morph frequencies.

2. MATERIALS AND METHODS

During 1978–1981, a large number of two-spot ladybird populations in The Netherlands were sampled, and clines in the frequency of melanic beetles were described (Brakefield 1984a).

In 1991, 1992 and 1995, a number of populations along the two steepest clines were resampled in a similar way; the positions of these populations are shown in figure 1*a*. Each transect, indicated by the stippled areas in figure 1*a*, was about 10 km wide (Brakefield 1984*a*). In each population, pupae and adult beetles were collected in the field, where they occurred mainly on *Rosa rugosa*, *Tilia* sp. and *Euonymus* sp. Pupae were taken to the laboratory, and morph frequencies scored upon emergence of the beetles. The sample size varied from 50–15 217. To test for the significance of changes in frequencies of melanic beetles for each population between the different collections, χ^2 tests were performed.

Climatic data were extracted from records of the Royal Dutch Meteorological Institute (KNMI). Here, it is relevant to mention that the climatic factors experienced by the beetles (i.e. the microclimate) determine their thermal budgets. If, however, microclimate and macroclimatic measurements are correlated, the correlations between thermal advantage and climatic variables as detailed in §1 are also expected when macroclimatic data are used. Data were analysed for the March–September period, because from autumn to spring the ladybirds overwinter and thermal melanism is unlikely to influence the fitness of the beetles through differential activity during this period (Brakefield 1985, but see §4). The climatic data were obtained from meteorological stations close to the field sites where ladybirds were sampled. We analysed the following climatic variables: (i) the average number of hours of sunshine per month; (ii) the average wind speed (m s^{-1}) for each month; (iii) the average daily maximum temperature ($^{\circ}\text{C}$); (iv) the average daily minimum temperature ($^{\circ}\text{C}$); and (v) the average daily temperature per month. (All measurements were standard meteorological observations.) Comparisons were made between meteorological stations for a number of consecutive years. Since the records were not always continuous for all stations, some missing values were excluded from the analyses. For the data on temperatures, a total number of eight meteorological stations were used (five main stations and three additional stations). The records for sunshine and wind were only available for the five main stations.

3. RESULTS

(a) *Morph frequencies*

The relative frequencies of melanic morphs along the two clines for the three collections are shown in figure 1*b,c*. A number of sites show significant changes in the relative frequency of melanic morphs, the pattern of which is shown in figure 1*d* (χ^2 tests with 1 d.f.; minimum sample size=50, usually ≥ 100 ; tests performed at significance level of 5%). Generally, both the north–south and the east–west clines have become shallower (figure 1*b,c*).

(b) *Climatic data*

There is a significant contribution of both year and site to monthly variation in the number of hours of sunshine (two-factor ANOVA performed for data over March–September for the 14 years (1981–1994) and five sites (figure 2); $p < 0.05$ in every case, usually $p < 0.001$). The level of sunshine in the spring is highest near the coast, and gradually declines with distance from the coast (figure 2). To determine whether this variation in sunshine showed any trend across years, linear regression analyses were performed. They showed no significant

relationships between hours of sunshine and year for 1981–1994 for any of the five sites (figure 2) or months (March–September: d.f.=10 or 11, since for some year–site combinations no data were available; $p > 0.05$ for each correlation). Additionally, we tested whether sunshine showed any evidence of a change since earlier decades by comparing the mean duration of sunshine for 1981–1990 (preceding the second collection of ladybirds) with the mean for 1951–1980 (preceding the first collection). Significant changes were only found for the month of June. Each site except Vlissingen showed a decrease in June sunshine. June, however, often comes after the main period of adult mating and early oviposition (Brakefield 1984*a*; Majerus 1994; de Jong 1997; P. W. de Jong, unpublished data).

Wind speed is highly variable across the five sites and 14 years (two-factor ANOVA for each month (March–September), $p < 0.001$ for each analysis); average wind speeds at the coast are relatively high, and they gradually decline with distance from the coast (figure 2). The only significant correlations between wind speed and year (1981–1994) were found for Eindhoven in April ($r = -0.56$, d.f.=13, $p < 0.05$), May ($r = -0.56$, d.f.=13, $p < 0.05$), and July ($r = -0.55$, d.f.=13, $p < 0.05$).

Average, minimum, and maximum temperatures per month (March–September) show significant variation over the years (1968–1994) and sites (eight sites, see figure 3; two-factor ANOVA for each month, $p < 0.01$ in each case, usually $p < 0.001$). Average daily maximum temperatures during the ladybirds' period of reproductive activity (April–May) tend to be higher inland than at the coast, while the reverse trend is observed for the mean daily minimum temperature (figure 3). Significant positive correlations were found between mean temperature and year (1968–1994) for most sites in April or May (table 1), but not outside this period. Some sites show non-significant trends ($p < 0.1$) for some months (table 1); only St Annaland did not show any trend or significant change with time. Figure 3 shows plots of the increase in temperature in April with time for these sites.

4. DISCUSSION

(a) *Morph frequencies*

Over the past two decades, for which data are available on morph frequencies of the two-spot ladybird in The Netherlands, the clines in relative frequency of melanics have changed significantly, and have become shallower. Most populations in the south-east of The Netherlands showed a significant decrease in relative frequency of melanics, while in the west of The Netherlands some populations showed a significant increase. Other studies in which the change in relative morph frequency was studied have generally provided evidence for a decrease in the frequency of melanics (e.g. Creed 1971; Bishop *et al.* 1978; Schummer 1983; Brakefield & Lees 1987), but data from the UK showed an increase in frequency of melanics in the south (M. E. N. Majerus, personal communication). Attempts to explain these changes in frequency of melanics include correlations with smoke levels (Creed 1971; Muggleton *et al.* 1975; Brakefield & Lees 1987), but their cause remains unclear (Majerus 1994).

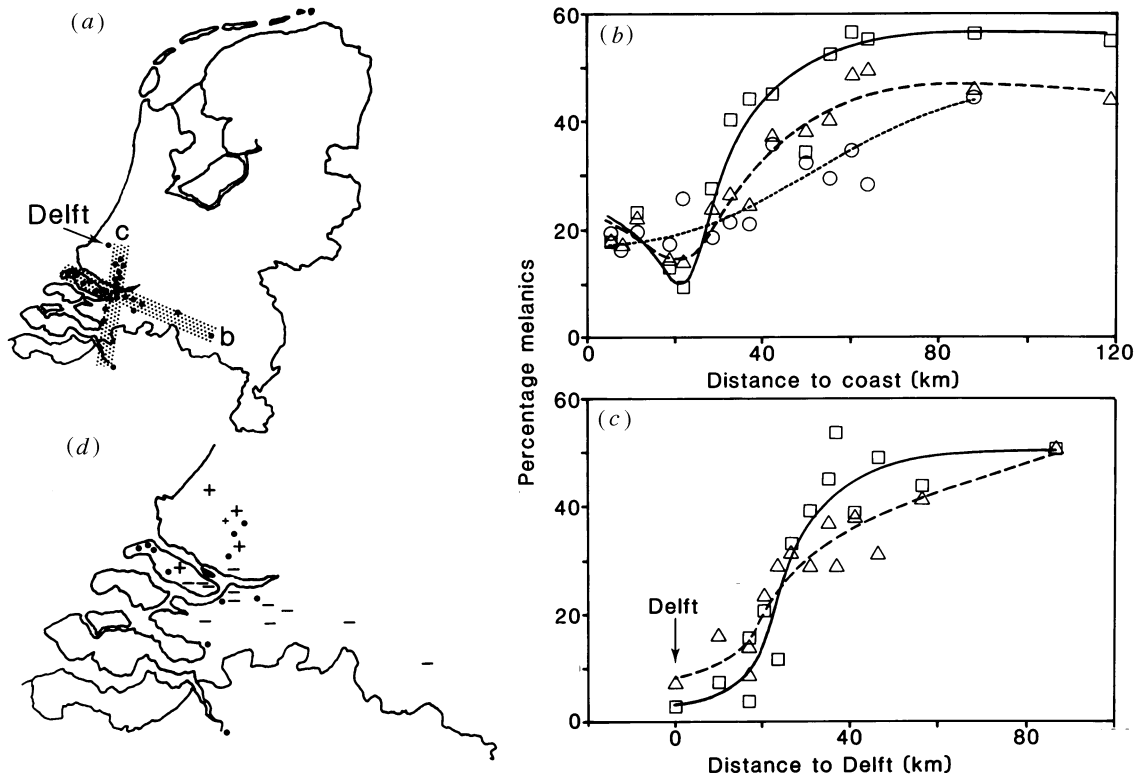


Figure 1. (a) Position of the two transects that were resampled in The Netherlands. Dots indicate collection sites along the transects. Delft is taken as a reference site to relate distances along the north-south cline to. (b) Clinal variation in the relative frequency of melanic two-spot ladybirds along the east-west transect and (c) the north-south transect for successive collections in The Netherlands. The collection from 1980 is described in Brakefield (1984a). (Some populations were sampled in the period of 1978–1981.) Squares indicate collections in 1980; triangles collections in 1991 (east-west cline) and 1992 (north-south cline); and circles collections in 1995. Curves are fitted by eye. (d) Geographic pattern of significant changes in the frequency of melanic beetles. A '+' indicates a significant increase, and a '-' indicates a significant decrease in the relative frequency of melanics. The small '+' is a site with an increase approaching significance, $p = 0.053$). Populations where there has been no change in the frequency of melanics are indicated with a dot.

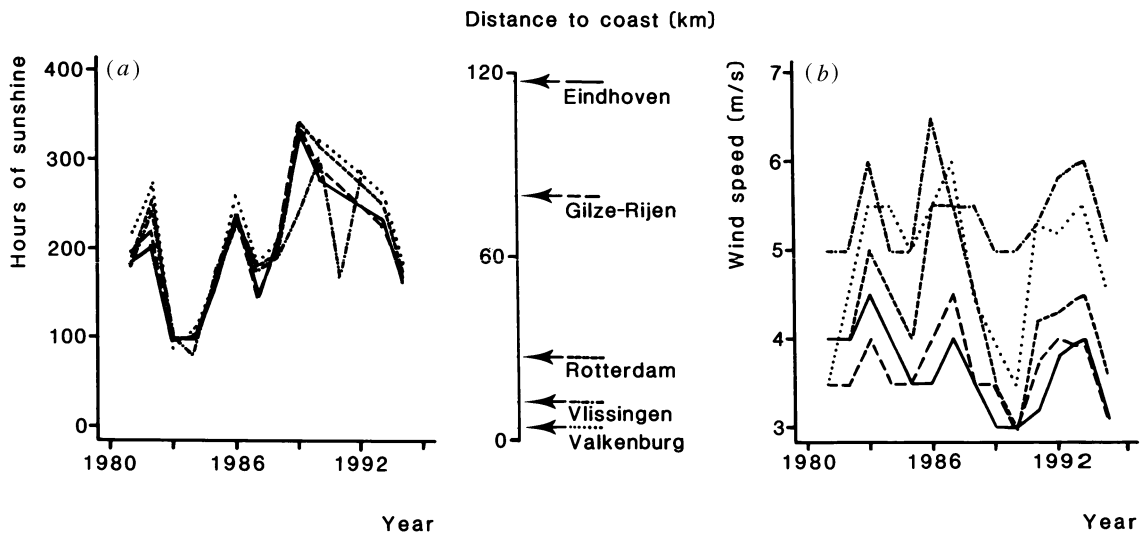


Figure 2. (a) The number of hours of sunshine in May through time for five meteorological stations close to the transects where the collections were made (see figure 3). The axis between figure 2a and 2b indicates the distance of the meteorological station to the coast. (b) The average wind speed (m s^{-1}) in May through time for the same meteorological stations.

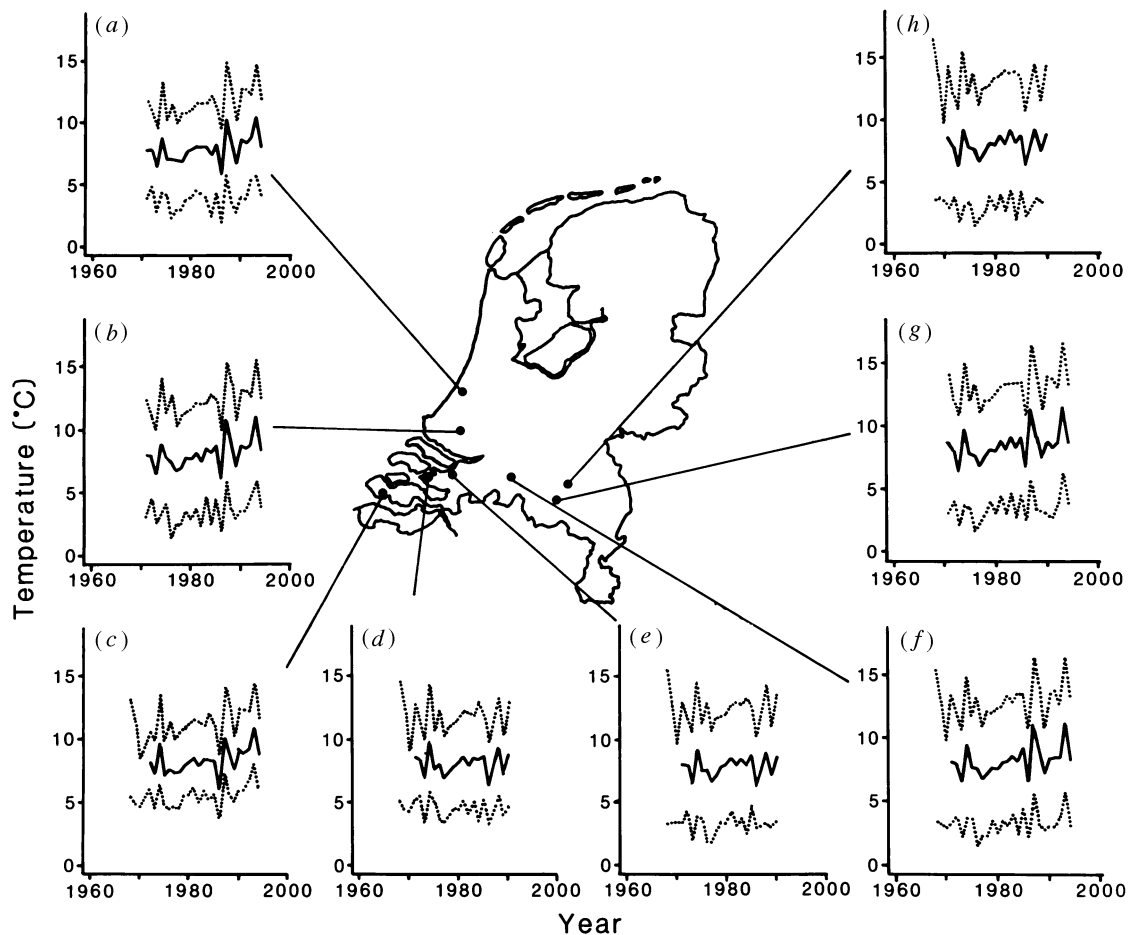


Figure 3. Average, minimum, and maximum daily temperatures in April through time at eight different meteorological stations close to the collection sites.

Table 1. *Correlations between mean temperature and year (1968–1994)*

(Only the trends which are significant or close to significance are given.)

site	month	r	d.f.	p
Eindhoven	April	0.40	23	=0.05
Valkenburg	April	0.45	22	<0.05
Gilze-Rijen	March	0.38	22	<0.1
	April	0.42	23	<0.05
Rotterdam	March	0.36	22	<0.1
	April	0.47	23	<0.05
Vlissingen	April	0.48	22	<0.05
	May	0.38	22	<0.1
	July	0.36	22	<0.1
Gemert	May	0.39	18	<0.1
Oudenbosch	May	0.44	18	<0.1

(b) *Correlation with climatic variables*

The variation in the key climatic variables, as identified through the laboratory studies (de Jong *et al.* 1996), is consistent with the shape of the ladybird clines. The high wind speeds along the coast are likely to reduce any potential thermal advantage to melanics. This factor has previously not been considered in the literature. The

lower minimum temperatures in the east are consistent with a relatively larger advantage to melanics when warming up early in the day. The fewer hours of sunshine in the east, due to higher cloud cover, would lead to an intermittent warming up which is expected to add to the advantage to melanics. These correlations between macro-climatic variables and the shape of the clines are in

agreement with those found by Brakefield (1984a). With respect to the factors considered here, however, the change in cline shape is correlated only with an increase in spring temperatures. Within the framework of the thermal melanism hypothesis, this correlation between change in cline shape and temperature can be interpreted as follows. An increase in temperature can lead to a reduction of the negative effect of wind on the fitness of melanics, which would be most pronounced in the west. Also, a general increase in temperature is expected to reduce the contribution of radiation to warming up of the ladybirds, and hence reduce the thermal difference between the morphs.

Although this association between changes in morph frequencies and ambient temperatures is consistent both with earlier interpretations of spatial correlations between climatic variables and ladybird clines in terms of thermal melanism, and the results from laboratory studies on the thermal biology of the two-spot ladybird, several other factors may influence spatial and temporal variation in two-spot ladybird morph frequencies. First, climatic factors may also influence the dynamics of the interactions between ladybirds and their prey, predators, and parasites. Second, recent field observations have corroborated earlier findings that cyclical selection may influence two-spot ladybird morph frequencies (Timofeeff-Ressovsky 1940; Brakefield 1985; de Jong 1997), indicating that net selective advantage may change direction over the seasons. The nature of the selection pressures involved needs to be understood before long-term changes in morph frequencies can be explained fully. Possible examples of these selection pressures include winter mortality (Brakefield 1985) and sexual selection (e.g. Muggleton 1979; Majerus *et al.* 1982; Kearns *et al.* 1990, 1992).

However, in addition to both recent laboratory studies (de Jong *et al.* 1996) and field observations (de Jong 1997), this study provides further evidence for the operation of thermal melanism in the two-spot ladybird. If the change in the clines is indeed at least partly due to an increase in ambient temperature, this study provides an example in an insect species of a possible direct influence of increasing ambient temperatures, consistent with, and in the same order of magnitude as, the expectations from an enhanced greenhouse effect (Gordon 1994; Kerr 1995; Beebee 1995; MacCracken 1995; Anonymous 1995). A next step in the analysis will be to deploy portable thermal imaging equipment and measure microclimate in the field, in order to produce a direct link at the individual beetle level between macroclimatic data, microclimate, body temperature of ladybirds, their activity, and their reproductive success.

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