Differential winter mortality and seasonal selection in the polymorphic ladybird Adalia bipunctata (L) in the Netherlands

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Seasonal selection acting on the melanic polymorphism in the two-spot ladybird Adalia bipunctata was investigated in The Netherlands. An increase in melanic frequency over the spring-summer reproductive period was quantified. The selective advantage gained by melanics averaged 9%, but significant heterogeneity occurred between populations. Adult hibernation behaviour is described. The beetles when outdoors show a highly clumped distribution both between and within trees. The distribution of the morph classes between aggregations is random. Survivorship in a hibernating cohort (initial n = 1898) on a grid of 70 lime trees near Utrecht was monitored by making three counts over the winter of 1981-1982. Intense selection favouring each melanic morph occurred during December and January. The relative fitness of non-melanics was 0.55 (melanics = 1). The discovery of dead beetles in late January (about 5°_{0} of total losses) and the absence of spatially density-dependent mortality were consistent with a climatic stress rather than selective predation. The period of selection was associated with very cold temperatures averaging up to 4°C below normal and an overall mortality of nearly 75%. There was no change in morph frequency, near normal temperatures and a lower mortality from February to early April. Examination of groups of nearby trees in late January strongly suggested that similar differential mortality had occurred except on some willows. This difference was probably due to the more protected hibernation sites available on these trees. Samples of hibernating cohorts at three other sites showed no evidence of differential mortality. Laboratory experiments with hibernating beetles found no difference in survivorship or rate of weight loss between starved non-melanics and melanics in temperature regimes with and without periods of adult activity. It is concluded that the intense winter selection on the study limes is probably exceptional. Examination of changes in morph frequency through the annual cycle suggests that at some sites the selection favouring melanics during reproduction is counterbalanced by selection against melanics in late summer or early autumn. The results are discussed in relation to mathematical models of cyclical selection and to other field studies including that of Timoféeff-Ressovsky (1940), who found large decreases in melanic frequency during hibernation in Berlin.

KEY WORDS:—Coccinellidae – Adalia bipunctata – melanism – polymorphism – seasonal selection – cyclical selection – reproduction – selective advantage – hibernation – differential mortality.

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INTRODUCTION

Timoféeff-Ressovsky (1940) found that the frequency of the melanic forms of the two-spot ladybird Adalia bipunctata declined substantially over the period of winter hibernation in Berlin (see also Timoféeff-Ressovsky & Svirezhev, 1966). His study provides one of the most striking examples of the operation of seasonal selection. Such cyclical patterns of selection are of considerable interest because theoretical work has shown that they can maintain polymorphism or at least delay the time to fixation (e.g. Haldane & Jayakar, 1963; Hedrick, 1974; Hoekstra, 1975). However, the conditions necessary for protected polymorphism are usually very restricted but are much less so when gene frequencies are intermediate and selection coefficients large. These conditions prevailed in the population studied by Timoféeff-Ressovsky.

The polymorphism in A. bipunctata is controlled by a number of alleles at a single gene locus with melanics dominant to non-melanics (Lus, 1928, 1932). Timoféeff-Ressovsky (1940) sampled a population at the beginning and end of the winter at hibernacula in crevices between the stone blocks of a ruined church. He obtained large samples in each of the 12 consecutive years, 1929–1940, with a single gap in spring, 1932. The consistent decrease in melanic frequency during hibernation averaged about 20%. In each of three years Timoféeff-Ressovsky collected all the A. bipunctata from an area of wall prior to the end of hibernation. Comparison of living and dead beetles demonstrated that the change in melanic frequency was due to differential mortality. Some other workers have obtained considerably fewer data for hibernating populations of A. bipunctata, which have not indicated a difference in winter survivorship between the morph classes (Bengtson & Hagen, 1975; Takharov & Sergievsky, 1980).

My study of A. bipunctata in The Netherlands was designed to examine the dynamics of the polymorphism in more detail than previous workers (Brakefield, 1984a, b, c). Comparisons of mating and non-mating A. bipunctata at sites along clines have shown that melanics gain a mating advantage in post-hibernation populations (Brakefield, 1984c). Analysis of frequency data for sites where large numbers of mating animals were found strongly suggested that this mating advantage resulted in an increase in melanic frequency in the next generation. More extensive data sets are analysed in this study which confirm that such an increase occurs over the reproductive period in The Netherlands. The survivorship of cohorts of hibernating A. bipunctata is then examined to determine whether differential winter mortality counterbalances this increase. Finally, the changes in melanic frequency which occur through the whole annual cycle of the species are followed.

MATERIALS AND METHODS

Sampling in the summer

The study sites were on four transects which traversed an area between a region of low, and one of high melanic frequency. Details of the sites, methods of sampling, geographical variation of melanism and population biology are given in Brakefield (1984a, b). Sequences of samples from each summer generation were obtained from a number of sites with differing frequencies. Their analysis examines the combined data for the two melanic morphs, quadrimaculata and sexpustulata, and those for the non-melanic typica.

Sampling in the winter

The main study site for hibernating A. bipunctata was Utrecht E. (site 11, Brakefield, 1984a) in the central region of The Netherlands. This is a recreation area of roads, grass and planted trees just outside the city boundary. In the winter of 1981–1982 three separate counts of hibernating beetles were made on grid L_1 of 70 lime trees (*Tilia* sp.) planted in two, or sometimes three, rows (Fig. 1A). The trees were about 40 years old. Since beetles were rarely completely hidden (Fig. 1B & C) a thorough search of the bole and main branches of each tree revealed most, if not all, of them. The beetles were not disturbed. During the mid-winter visit collections of all beetles were made from surrounding groups of trees.

A similar series of counts were made of a smaller cohort on a grid of 48 poplar trees (*Populus abeele*) at the nearby site of De Uithof (no. 12). Some samples of hibernating beetles were collected from an eight-storey building and surrounding walls and trees at this site.

Laboratory studies of survivorship

Some hibernating A. bipunctata were used to investigate survivorship of the morph classes in the laboratory. Beetles were assigned at random to cohorts of at least 90 individuals of each morph class. They were weighed and placed in stoppered 9 ml glass vials. Survivorship and loss of weight (in two cohorts) were then examined at regular intervals. Cohorts of each morph class were kept without feeding in three controlled environment rooms.

RESULTS

Selection during summer reproduction

The combined frequency data for 26 post-hibernation adult populations and for their offspring collected as pupae or adults are given in the Appendix. In some years and at some sites the later samples include members of a second summer generation (Brakefield, 1984a). Some of the samples of adults collected after emergence from pupae began, included surviving adults of the parental generation. This source of error is excluded when the 'second' generation samples are made up of pupae only. Figure 2 shows that there is a trend of increasing melanic frequency over the reproductive period. The changes in



Figure 1. A, Lime trees on grid L₁ at Utrecht E in December 1981 (looking NE); B and C, examples of hibernating aggregations of polymorphic Adalia bipunctata on the limes; D, a group of beetles on Salix alba exposed after removal of bark.

melanic frequency are quite small, averaging 3.2% for all data. The magnitudes of these changes are not strictly comparable since overall melanic frequencies ranged from 3 to 58% (see Appendix). Figure 2 includes frequency distributions of estimates of the selective advantage gained by melanics over the reproductive period. For all data this advantage averages 9%. The data presented in Fig. 2 involve the factors: site (S), generation (G) and frequency (F). The statistical significance of the changes in melanic frequency is



Figure 2. Frequency distributions for the change in frequency of melanic *Adalia bipunctata*, and the selective advantage of melanics (non-melanics = 1) over the period from post-hibernation adults to their progeny collected either as A pupae or B pupae and adults, at individual sites. Shaded histograms show sites for which sequential samples were obtained in each generation. Mean $\pm 95\%$ C.L. are shown for each total distribution.

examined using a 3-way G test (Sokal & Rohlf, 1981) to analyse: (a) the full data set; and (b) those data for sites at which sequential samples were obtained in each generation and including only pupae in generation 2. These analyses show that the change in melanic frequency between generations is significant but that it is not independent of site ((a) $G_{(S)GF} = 145.2$, df = 26, P < 0.001; $G_{SGF} = 47.21$, df = 25, P < 0.01 and (b) $G_{(S)GF} = 56.30$, df = 12, P < 0.001; $G_{SGF} = 30.40$, df = 11, P < 0.01). Thus there are differences between sites in the selective advantage gained by melanics over the reproductive period. Nevertheless, the increase in melanic frequency over this period is consistent with the occurrence of seasonal selection in The Netherlands similar to that in Berlin (Timoféeff-Ressovsky, 1940) but involving considerably smaller changes in frequency.

Hibernation behaviour

Adult Adalia bipunctata in The Netherlands hibernate from late October or early November until April or early May. In many built-up urban areas they usually hibernate inside buildings. At De Uithof where modern multistorey buildings are surrounded by large planted areas, some beetles enter the buildings while others remain on trees or are found in crevices on the concrete blocks of the buildings' walls. In villages and smaller towns a similar diversity of hibernation sites occurs. In more rural areas with few buildings most beetles hibernate on trees; particularly species of lime (*Tilia*), willow (*Salix*), poplar (*Populus*) and plane (*Platanus*), but also others including hawthorn (*Crataegus*), ash (*Fraxinus*) and oak (*Quercus*).

Adalia bipunctata hibernating on young lime trees (Fig. 1A) are usually more or less exposed in small crevices or cavities on the bole or beneath large branches (Fig. 1B and C). They are found from about 60 cm up to several metres above ground on all sides of the trees. On species of tree with fissured bark (e.g. *Salix alba*, Fig. 1D) or with bark scales (e.g. *Platanus X hispanica*) the beetles are hidden under bark.

Small aggregations of beetles are found at hibernacula on trees (Fig. 1B to D). The largest group on the study limes at Utrecht E. on 2 December 1981 was of 59 individuals. Nearly 20% (n = 351) of all beetles on that date were in groups of at least 15. However, about 10% (n = 196) were isolated with no neighbour within 5 cm. Some observations made by C. Lane (Rothschild, 1962) on *A. bipunctata* hibernating indoors suggested that melanics might tend to aggregate together. Figure 3 shows that there is no evidence for such behaviour



Figure 3. Comparison by group size of frequency distributions of aggregations of hibernating Adalia bipunctata with differing numbers of melanics. Group size is given above each histogram. Solid histograms show observed frequencies and broken ones expected frequencies calculated for single beetles from overall melanic frequency and for groups from a binomial distribution (where necessary expecteds are combined for higher numbers of melanics). Data are for grid L_1 of lime trees at Utrecht E. on the dates indicated for all group sizes with at least ten aggregations.

on the study limes since, for hibernating groups of the same size, the distribution of melanics between groups is random (for each comparison by chi-square, P > 0.05).

The coccinellids Adalia decempunctata (L.), Exochomus quadripustulatus (L.) and Synharmonia conglobata (L.) were sometimes found within aggregations of A. bipunctata. E. quadripustulatus was abundant on ash trees at De Uithof.

The spring dispersal from hibernacula to areas of shrubs where feeding and reproduction begins (Brakefield, 1984a) takes place over several weeks. Observations on the decline in numbers of A. bipunctata (initial total = 634) at hibernacula on young trees and supporting stakes with ties were made at three sites (nos 12, 23 and 25) in April to May 1982. For example, at site 25 there were 121 beetles on 3 April (none on nearby shrubs), 61 on 23 April, 54 on 10 May and none on 13 May (many on shrubs). At each site most of the dispersal probably occurred during relatively warm spells before 23 April and 13 May. However, some factor other than temperature (e.g. photoperiod, see Hodek, 1973) appears to be involved in initiating dispersal since there was no emigration on warm days in late March. There was no evidence at any of the sites that the timing of dispersal differed between the morph classes (heterogeneity chi-square tests, P > 0.1). Movement to hibernacula in the autumn was not closely monitored (one marked melanic released on lime trees at De Uithof on 15 September 1980 was found on a concrete wall about 75 m away on 8 April 1981, see Brakefield, 1984a).

Selection during winter hibernation

Survivorship of a cohort: Table 1 gives the frequency data for the three winter counts of A. bipunctata on the study limes at Utrecht E. There was a highly significant increase in the proportion of each melanic morph between early December and late January. During this period nearly 75% of all beetles died. The following period of similar length extending nearly to the commencement of dispersal from hibernacula was associated with much lower mortality (33%)and no further change in morph frequency.

The number of beetles per tree in December varied widely (range = 0-76, mean = 27.1) with an extremely clumped distribution $(s^2/\bar{x} = 15.51)$. Because of this variability and the presence of many trees with few or no beetles, the effects of selection are analysed further by dividing the linear grid of trees into eight

Jate	Number of non-melanics	Number o quad.	f melanics ^a sexp.	Total (n)	% melanic	Chi-sq. $(df = 2)$
?-3 Dec. 1981	1581	224	93	1898	16.70	06 20***
?6 Jan. 1982	362	89	42	493	26.57	20.39+++
April 1982	237	47	36	320	25.94	2.60

Table 1. Changes in the frequency of the non-melanic and melanic morphs of Adalia bipunctata in a hibernating cohort on grid L, of limes at Utrecht E

, quad. = quadrimaculata; sexp. = sexpustulata. **P < 0.001.



Figure 4. Analysis of counts of *Adalia bipunctata* made on 2-3 December 1981 (O) and on 26 January 1982 (\odot) in sections of grid L₁ of lime trees at Utrecht E. A, frequency of melanics on each data; B, relative fitness of non-melanics (melanics = 1) over the study period; and C, percentage mortality over the study period. Vertical ranges show standard errors and arrows indicate mean values.

sections of roughly equal length and of six to ten trees. Figure 4 shows that the change in melanic frequency between early December and late January occurred in each of these sections. Application of a 3-way G test shows that this change was significant ($G_g = 23.17$, P < 0.01) and independent of selection (3-way $G_7 = 4.34$, P > 0.1). The mean relative fitness (\pm S.E.) of non-melanics is 0.554 ± 0.052 (melanics = 1) which is equal to the overall mean.

The mortality of A. bipunctata over the first period was considerably higher in three sections of trees at one end of the grid (overall $\chi_1^2 = 141.1$). There is no evidence of a relationship between mortality and intensity of selection for the eight sections (Fig. 4; r = +0.28, P > 0.1). A comparison of melanic frequency between the two groups of sections with differing mortality showed no significant differences before or after selection ($\chi_1^2 = 2.71$ and 0.01 respectively).

Although A. bipunctata is distasteful and warningly-coloured some predation by birds occurs (refs in Muggleton, 1978; Brakefield, 1984a). Furthermore, Betts (1955) showed by gut analysis that the closely related A. decempunctata can form a substantial component of the winter diet of great tits Parus major in oak woodland. Titmice and other insectivorous birds were present at Utrecht E. I have no direct data on the involvement of bird predators in the differential mortality in the hibernating cohort of A. bipunctata. However, there is no relationship between the number of beetles on a tree in early December and the percentage mortality up to late January (minimum five beetles and arcsin transformation: b = -0.014, $F_{(1,61)} = 0.02$, P > 0.1). This argues against a major rôle of predation by birds since they would be expected to act in a density-dependent manner. Thus, studies of predation by titmice have found strong spatially density-dependent mortality of overwintering larvae of the moths *Ernarmonia conicolana* in pine cones (Gibb, 1958), and *Cydia pomonella* under the bark of apple logs placed within trees (Solomon & Glen, 1979; and see other refs therein). The range in density and mortality in Solomon and Glen's experiments was similar to that at Utrecht E.

The presence of some dead A. bipunctata lodged in cavities on trees within the study area in late January was consistent with mortality associated with a climatic stress. The 59 dead beetles on the study limes represented less than 5% of those disappearing since early December. The total dead beetles (n = 185) showed no difference in melanic frequency to the counts of all living beetles made in December or January ($\chi_1^2 = 0.47$ and 1.59 respectively). A sample collected on 11 March 1982 inside a building at De Uithof similarly showed no differences between living and dead beetles ($\chi_1^2 = 0.01$, with n = 366 and 244 respectively), although in this case there was also no evidence of a change in melanic frequency over the winter.

Lusis (1961) suggested that greater changes of body temperature in melanics than non-melanics, as recently demonstrated by Brakefield & Willmer (1984) for *A. bipunctata* under illumination, is involved in winter selection on the polymorphism. Differential activity is one expected consequence of the differing thermal properties. Some slow locomotory activity was observed on the study limes on sunny winter days. This will lead to turnover in the hibernating aggregation in addition to losses due to death. If differential activity occurs, changes in the distribution of melanics between aggregations of different sizes may be expected (for example, an accumulation of melanics as isolated individuals or within small groups). Figure 5 shows no indication of such changes. There is no significant heterogeneity in melanic frequency between the size classes shown in Fig. 5 (Dec.: $\chi_{14}^2 = 9.87$; Jan.: $\chi_7^2 = 3.35$).

Winter climate: Some climatic data for the period of hibernation are summarized in Table 2 for De Bilt 4 km north-east of Utrecht E. Extreme cold conditions occurred in December 1981 and January 1982 with temperatures averaging up to 4°C below normal. Temperatures of below -12° C were recorded in each of these months. Continuous periods of days with a frost occurred from 7–28 December and 5–24 January. Thus the period of intense selection and high mortality of A. bipunctata at Utrecht E. coincided with a time of extreme cold. In other months, including February and March 1982, when no selection and lower mortality occurred, temperatures were similar to normal.

Survivorship of laboratory cohorts: Details of the conditions used in the laboratory experiments are given in Fig. 6. The three regimes represent one in which no beetle activity occurred (A), one in which periods of days with activity alternated with those without (B), and one with constantly high temperatures and high illumination during the hours of light (C). The survivorship curves of the cohorts of non-melanic and melanic A. bipunctata were closely similar in each regime (Fig. 6). Furthermore, the loss of body weight in cohorts A and B was



Figure 5. Frequency of melanic *Adalia bipunctata* in combined counts for hibernating aggregations of different sizes on grid L_1 of lime trees at Utrecht E. on the dates indicated. Vertical ranges show standard errors.

similar for each morph class (see Fig. 6). Comparisons of the initial weights of beetles dying up to the last date when mortality was < 25% (see Fig. 6) with those surviving, show that smaller beetles tend to die earlier (A: $t_{183} = 8.46$; B: $t_{182} = 6.88$, with P < 0.001). The percentage of initial weight lost over this period by surviving beetles decreases with size, although the relationship is only significant for cohort B (A: b = -0.439, $F_{1,139} = 4.02$, $P \cong 0.1$; B: b = -0.445, $F_{1,149} = 7.30$, P < 0.05). A general increase in mortality rate with higher temperatures is evident in Fig. 6.

Survivorship in other natural cohorts: The spatial variation in melanic frequency in late January on surrounding trees to the main grid of study limes (L_1) at Utrecht E. is shown in Fig. 7. The three areas of limes have homogeneous frequencies ($\chi_2^2 = 3.06$, overall = 24.9% mel.). However, those for all the areas of trees are heterogeneous ($\chi_9^2 = 22.84$, P < 0.01). Examination of Fig. 7 suggests

Table 2. The winter climate at De Bilt. Mean monthly figures for daily maximum and minimum temperature and relative humidity are given for 1981–1982 together with differences to the 30-year means for 1951–1980 in parentheses. The number of days with maximum and minimum temperatures below 0°C are included

	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
Maximum °C	11.9 (-2.4)	9.8 (+1.1)	1.7 (-4.0)	4.3 (-0.1)	6.1 (+0.7)	9.6 (+0.8)	12.6 (+0.1)
Minimum °C	4.8 (-1.6)	3.2 (+0.3)	-3.4 (-3.9)	-2.4 (-1.7)	-0.5 (+0.2)	1.2 + 0.1)	2.6 (-0.7)
Days with max. < 0°C	0 (0)	0 (-1)	10 (+7)	7 (+3)	(+3)	$\begin{pmatrix} 0 \\ (-1) \end{pmatrix}$	0 (0)
Days with min. < 0°C	2 (0)	8 (0)	23 (+10)	21 (+6)	16 (+1)	11 (-3)	$\frac{3}{(-2)}$
R.H. %	87 (+1)	85 (-3)	93 (+4)	87 (-2)	82 (-4)	80 (-1)	74 (-4)



Figure 6. Survivorship of cohorts of non-melanic (\Box) and melanic (\blacksquare) Adalia bipunctata in controlled environment rooms. A, 4°C with 12:12 h light: dark; B, 2 days at 18°C: 5 d at 4°C, 12 L: 12 D; C, 16 h L at 25°C and 60% R.H.: 8 h D at 16°C and 90% R.H. Initial fresh weights in mg (mean $\pm 95\%$ c.L.) and those on the latest dates when mortality was < 25% (see arrows) are shown for non-melanics (above) and melanics in cohorts A and B. Note that the time axes are not directly comparable.

that there is a lower frequency on each of four large willows, *Salix alba*, within a copse of trees with few or no coccinellids. These four trees give homogeneous frequencies ($\chi_3^2 = 0.14$), but the combined sample is different from those for all other areas ($\chi_1^2 = 16.64$, P < 0.001) and for the three other willows which are isolated and alongside a canal ($\chi_1^2 = 11.69$, P < 0.001, see Fig. 7). When the



Figure 7. Sketch map of the study area at Utrecht E. showing the frequency of melanic Adalia bipunctata with sample sizes, for different habitats on 20-27 January 1982. L, grid of lime trees; M, group of mixed trees including ash and oak; W/A, group of willow and ash; W_o, isolated willows and W_i, four willows within other trees with no hibernacula (\bullet).

four willows are excluded the remaining areas give homogenous samples $(\chi_8^2 = 5.35, P > 0.1)$. The melanic frequency on the four willows is 13.3%, which is similar to that on the main grid of limes in early December before selection had occurred $(\chi_1^2 = 2.02, P < 0.1)$, see Table 1). Thus the selection which favoured melanics on the main grid of limes apparently operated on hibernating cohorts of *A. bipunctata* on other trees in the study area but not on willows within a copse. Salix alba has highly fissured bark with shallow ridges forming an enclosed network within which the beetles hibernate. They can only be collected following removal of bark (Fig. 1D). This suggests that selection did not operate because of a more protected microclimate resulting from the nature of the hibernacula and from the more sheltered position of the particular trees.

Counts of the cohort of A. bipunctata on the grid of poplars near De Uithof were made on 3 December 1981 and on 1 February and 1 April 1982. The mortality in the consecutive periods was similar to, although somewhat less intense than on, the study limes at Utrecht E. (49.5 and 22.1%). There was no heterogeneity in melanic frequency between counts ($\chi_2^2 = 0.09$; with consecutive melanic frequencies of 24.27\% (n = 206), 25.00% (104) and 25.93% (81)). Thus although this cohort was hibernating in similarly exposed positions on trees close to the limes at the main study site, there is no corresponding differential mortality.

Table 3 includes some details of counts of *A. bipunctata* made on trees at Middelharnis and Oude-Tonge near the coast in early January and early April. At neither site was there a change in melanic frequency.

Overall seasonal changes in melanic frequency

Table 3 gives the melanic frequency in different periods of an annual lifecycle for those sites where samples of hibernating A. bipunctata were obtained. An increase in frequency over the reproductive period is evident at three or four of the six sites. The samples from the three sites to the left of Table 3 give some indication that this increase is counterbalanced by selection against melanics before hibernation begins. There is a significant heterogeneity (P < 0.01) in melanic frequency between periods at each of these sites. The data for later hibernation at Utrecht E. are likely to be biased since they only cover the cohort exhibiting strong differential winter mortality and not the whole population. The two coastal sites with low melanic frequencies show no seasonal changes. At De Uithof there was apparently no counterbalancing selection against melanics following the reproductive period.

DISCUSSION

Analysis of mating data strongly suggests that the increase in melanic frequency over the reproductive period in The Netherlands (Fig. 2) is a consequence of a mating advantage gained by melanics (Brakefield, 1984c). A similar but much larger increase presumably occurs in Berlin to counterbalance the higher winter mortality of melanics (Timoféeff-Ressovsky, 1940 and see Lusis, 1961). There are apparently three annual generations in Berlin as against one or two in The Netherlands (Timoféeff-Ressovsky & Svirezhev, 1966;

Table 3. Changes in the frequency of melanic (mel) Adalia bipunctata over the annual cycle at six sites in The Netherlands. Data are combined samples of post-hibernation adults (generation 1) and their offspring collected in the summer (generation 2) and of hibernating adults collected outdoors in mid- (December to early January) and late (end February to early April) winter. Significant values of chi-square are indicated for comparisons of consecutive periods

Period	Utrecht E.		Oude-Tonge		Zevenb	ergen W.	Del	Uithof	Middelharnis		Delft ^a	
	n	% mel	n	% mel	n	% mel	n	% mel	n	% mel	n	$% \mathcal{O}_{0}^{\prime \prime $
1981:				_								
Spring gen. 1	261	16.09	3433	24.00	3337	45.43	325	19.69	3650	9.15°	906	2.98
Summer gen. 2	1279	21.27	2603	29.47	1001	50.85	2849	25.41	979	9.40 [*]	1488	2.90
Mid-winter	1898	16.70	221	23.98 †	141	44.68	206	24.27	390	10.00	96	4.17
1982:												
Late winter	320	25.94	121	32.23	_		577	22.18	164	12.80	_	
Spring gen. 1			1452	27.41	1122	43.05	2293	24.25			234	1.71

"Sequence for 1980-1981. "Samples for 1980.

P < 0.01; *P < 0.001; †P approaches 0.05.

Brakefield, 1984a). Samples collected at different times in the summer period of adult activity by other workers in various regions of Europe have provided little evidence of changes in melanic frequency (Meissner, 1907a, b, 1909, 1910; Creed, 1966, 1975; Lusis, 1973; Bengtson & Hagen, 1975; Honek, 1975; Muggleton, 1978; Zakharov & Sergievsky, 1980; Klausnitzer & Schummer, 1983). However, many samples are small and comparisons often involve single sampling occasions or grouped data for 'early' and 'late' periods which cover only part of the whole reproductive period. The occurrence of overlapping generations and of spatial and temporal heterogeneity in morph frequency within generations (Brakefield, 1984a, b) means that care is necessary in interpreting such data.

My observations in The Netherlands indicate a similar diversity of hibernation sites to those found in Britain (Benham & Muggleton, 1978). The aggregative behaviour is characteristic of the species (see Hodek, 1973).

This study shows that differences in survivorship between non-melanic and melanic A. bipunctata may occur in hibernating cohorts in The Netherlands, but that they are probably exceptional. The only cohorts which exhibited such a difference were associated with exposed hibernacula at a single study site and experienced particularly harsh climatic conditions. The subpopulation with differential mortality showed an increase in melanic frequency from about 17 to 27% over the mid-winter period of powerful selection and heavy mortality. This selective advantage contrasts with the intense winter selection against melanics in Berlin observed by Timoféeff-Ressovsky (1940). Some samples of hibernating A. bipunctata obtained by other workers have provided no examples of differential winter mortality (Zakharov & Sergievsky, 1980; see also Bengtson & Hagen, 1975; Honek, 1975). Thus, although differences in winter survivorship of the morphs may occur in some populations of A. bipunctata, there is no indication that they are a general phenomenon. Evidence of changes in the frequency of melanic forms during hibernation has been obtained in two other species of coccinellids (Tan, 1948; Parry & Peddie, 1981).

The winter climate in Berlin is usually colder than in The Netherlands (Table 4). However, the difference was considerably less during the mid-winter period of selection at Utrecht E. (cf. Table 2). Thus general differences in climate are unlikely to account for the difference in direction of selection at Utrecht E. and Berlin or for the absence of selection in other cohorts in The Netherlands.

Hibernating coccinellids, including A. bipunctata, characteristically have high levels of stored fat and glycogen, inactive ovaries and reduced respiration rates. They can have very low supercooling points which may change with time (see,

	Materr	aximum da 1perature (aily °C)	M	inimum da operature (iily °C)	Relative humidity (%)			
	Dec.	Jan.	Feb.	Dec.	Jan.	Feb.	Dec.	Jan.	Feb.	
De Bilt Berlin	5.4 3.1	4.3 1.7	5.3 2.9	0.6 - 1.4	-0.8 -3.5	-0.8 -3.1	88 88	86 84	83 82	

Table 4. Comparison of the mid-winter climates at De Bilt and Berlin. Figures are 30-year means for 1931-1960

e.g. Hariri, 1966; Hodek, 1973; Parry, 1980). Factors other than temperature and energy reserves are likely to influence overwintering survivorship in *A. bipunctata*. One such factor is moisture in the air, since insects freeze internally at higher temperatures when their surfaces are wet than when they are dry (Salt, 1936). The extreme period of cold in December 1981 in The Netherlands coincided with relative humidities which were higher than normal in the study area or Berlin (Tables 2 and 4). This may then have contributed to the climatic stress experienced by *A. bipunctata* at some exposed sites. Further laboratory experiments on winter survivorship using very low temperatures would be valuable. However, the significance of the results would be difficult to interpret without more understanding of the microclimates at hibernacula.

The data collected for some sites in The Netherlands (Table 3) suggest that the increase in melanic frequency over the reproductive period is counterbalanced by selection against melanics before hibernation commences. More data sets which include large samples taken in early hibernation are necessary to establish how general this pattern of selection is. Brakefield & Willmer (1984) found that the temperature excess over ambient air reached by larger melanic A. bipunctata under illumination similar to bright sunlight can be > 10°C (compared to 7°C for non-melanics). Thus a possible causal mechanism for the selection against melanics is the occurrence of greater heat stress in conditions of prolonged sunshine and very high ambient temperatures. Unfortunately the late summer and early autumn period of the life-cycle is the least well understood (see Brakefield, 1984a). Field observations in The Netherlands suggest that the species becomes less active and more widely dispersed during this period. They may tend to aestivate in diapause (see Iperti, 1966).

The relevance of mathematical models of cyclical selection (e.g. Haldane & Jayakar, 1963; Hedrick, 1974; Hoekstra, 1975) to the dynamics of melanism in A. bipunctata in populations in The Netherlands is unclear, since a second annual generation probably does not always occur and, in such cases, the period of selection in opposing directions must operate within single generations (see Goux, 1978; Pasteur, 1977). In regions with more than one annual generation adults from each one probably overwinter and are subject to opposing selection pressures when these occur. The moderate nature of the selection coefficients involved in seasonal selection in The Netherlands (Fig. 2) and their variability between populations (see also Brakefield, 1984c) suggests that in any case they are unlikely to provide a unitary explanation for the polymorphism. Seasonal selection may, however, contribute in some regions to maintaining the polymorphism by interacting with other factors such as a balance between migration and selection or frequency-dependent effects introduced by forms of sexual selection or differential timing of reproduction (see Brakefield, 1984b; O'Donald et al., 1984).

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APPENDIX

Numbers of non-melanic (n-m) and melanic (mel) Adalia bipunctata in the combined samples for each generation in the summer period of reproductive activity for sites in The Netherlands. The twelve sites at which sequential samples were obtained in each generation are given above (see text for details)

					Generation 2								
	Site		Genera	tion 1	թսբ	oac	adu	ılts	Overall				
no.	name	Year	n-m	mel	n-m	mel	n-m	mel	⁰ _₀ mel				
23	Middelharnis	1979	901	76	689	58	569	60	8.24				
4	Delft	1980	879	27	1052	31	394	11	2.88				
10	Utrecht C.	1980	902	223	1786	357	966	249	18.49				
12	De Uithof	1980	1367	407	373	148	1814	727	26.51				
23	Middelharnis	1980	3316	334	521	47	366	45	9.20				
28	Willemstad	1980	823	605	566	446	434	398	44.28				
32	Zevenbergen W.	1980	1265	1028	249	266	1619	1603	48.04				
33	Zevenbergen E.	1980	502	454	418	450	514	696	52.74				
38	Tilburg	1980	1184	1522	385	553	461	736	58.07				
25	Oude-Tonge	1981	2609	824	1727	722	109	45	26.36				
32	Zevenbergen W.	1981	1821	1516	461	474	31	35	46.68				
33	Zevenbergen E.	1981	169	187	297	310	45	57	52.02				
4	Delft	1978	229	3	60	2	26	1	1.87				
10	Utrecht C.	1978	191	38	1469	287	470	77	15.88				
4	Delft	1979	61	1	215	8	53	4	3.80				
7	Harmelen	1979	47	9	118	26	_		17.50				
10	Utrecht C.	1979	245	62	377	89	106	32	20.09				
64	Zierekzee	1979	217	32	78	14			13.49				
25	Oude-Tonge	1980	326	68	1133	503	3750	1483	28.28				
31	Oudenbosch	1980	770	798	38	46	121	133	51.26				
52	Antwerp	1980	257	225	383	380	432	506	50.89				
10	Utrecht C.	1981	109	25	4472	1048	_		18.98				
11	Utrecht E.	1981	219	42	1007	272			20.39				
12	De Uithof	1981	261	64	2125	724	_		24.83				
28	Willemstad	1981	230	156	787	719	130	81	45.46				
38	Tilburg	1981	984	1137	45	56	8	12	53.75				