

ECOLOGICAL STUDIES ON THE POLYMORPHIC LADYBIRD *ADALIA BIPUNCTATA* IN THE NETHERLANDS. I. POPULATION BIOLOGY AND GEOGRAPHICAL VARIATION OF MELANISM

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SUMMARY

(1) Samples of the polymorphic two-spot ladybird *Adalia bipunctata* were collected at seventy-five sites in the Netherlands and northern Belgium. Most sites were on four transects up to 120 km long. Sequential sampling at thirteen sites was used to examine basic population biology.

(2) Shrubs, especially *Rosa rugosa* and *Crataegus* spp., provide feeding and mating habitats in late April and May following hibernation. Some oviposition may also occur. Adults disperse from mid-May to trees, particularly *Tilia* spp., which are the principal habitats for egg laying in many populations. At some sites in some years a substantial second, late summer or autumn generation occurs. Reproduction probably tends to occur earlier inland than on the coast. There are differences in timing between years.

(3) Frequencies of melanics are 1–15% in the north-west and >50% inland in the south-east. Steep clines occur over part of the transition between these regions, possibly due to a partial barrier to gene flow. Frequency changes were probably more marked for the *quadrimaculata* than the *sexpustulata* melanic morph.

(4) Among the correlations between melanic frequency and climatic variables are negative ones with an index of oceanity, relative humidity and length of sunshine. The last is consistent with thermal melanism. The interpretation of the relationships with environmental variables is discussed.

INTRODUCTION

The two-spot ladybird beetle *Adalia bipunctata* (L.) is polymorphic for several non-melanic and melanic forms. These are controlled by a number of alleles at a single gene locus with the melanic morphs being dominant to non-melanics (Lus 1928, 1932). A number of studies have examined geographical variation in morph frequency. Several workers have carried out regression analysis of the relationships between climatic variables and the frequency of melanics (Lees, Creed & Duckett 1973; Creed 1975; Muggleton, Lonsdale & Benham 1975; Scali & Creed 1975; Bengtson & Hagen 1977). These studies have led to the development of several theories to account for the variation (see review by Muggleton 1978).

This paper describes the results of a survey of morph frequency in the Netherlands and

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northern Belgium. The relationships between the frequency data and some climatic variables are examined. The work on polymorphism in *A. bipunctata* has been conducted without an extensive ecological understanding of the species. This study therefore examines the basic population biology in the Netherlands. The observations were made in conjunction with a comparison of the adult movement and reproductive activity of melanics and non-melanics (Brakefield 1984a,b).

METHODS

Study area

In the Netherlands *A. bipunctata* is an abundant species found in most urban areas. Figure 1 shows the distribution of study sites. Some were scattered through the Netherlands and northern Belgium. Most sites were, however, located along four transects in central and southern Holland: A and B running approximately eastwards from the coast and C and D, bisecting these two from north to south. The transects were 90 or 120 km long.

The samples

Samples of *A. bipunctata* were collected from each site between 1978 and 1982. In the Netherlands only three morphs of *A. bipunctata* are abundant; all others together comprising less than 1% of a population. The three morphs are the non-melanic red *typica* and the melanic *quadrimaculata* and *sempustulata* with four and six red spots respectively.

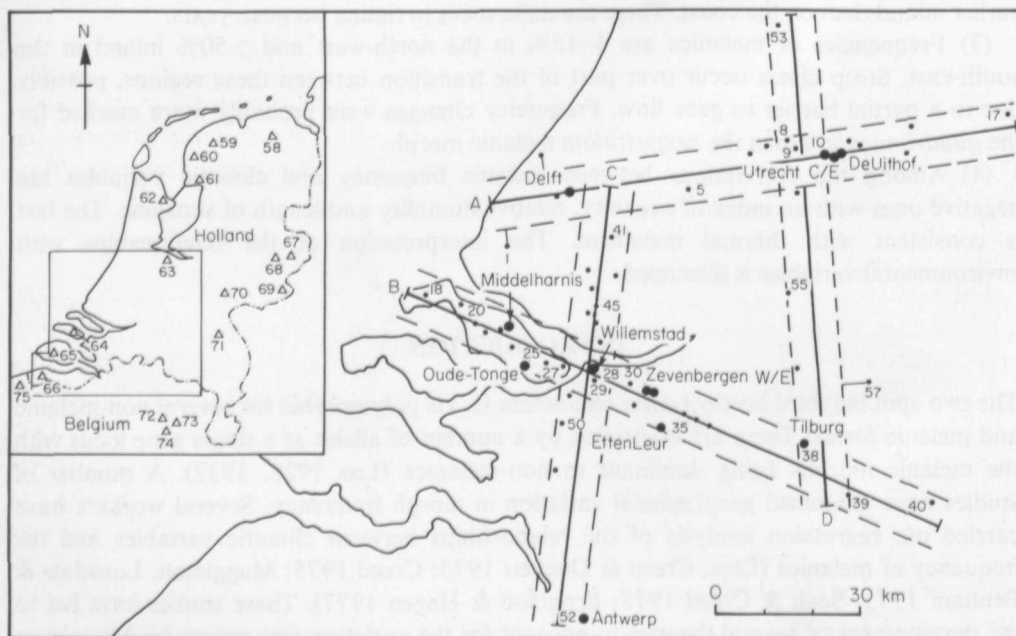


FIG. 1. Map of the study sites: (Δ), general sites; (\bullet), sites on four transects A-D; (---), ± 5 km limits for transect width (transect sites outside these limits are connected by lines). Names refer to larger circles (\bullet) and indicate sites from which series of samples of pupae were obtained. Sites are numbered individually or in sequence in intervals of five. All sites names are given in Appendix 1.

Thirteen sites from the transects were selected for further study in 1980 and 1981 (Fig. 1). Sequential sampling of adults and of prepupae and pupae was carried out at each site, usually in a number of different habitats. Samples of adults from shrub habitats where a high sampling intensity was possible represent counts rather than collections. During sampling of a habitat the same area was searched systematically and in a manner that was consistent from one occasion to another. Adults emerging from pupae in the laboratory were scored following complete development of elytral phenotype. Tests of random sampling of pupae within a habitat with respect to morph class were made. Eleven comparisons of morph frequency in subsamples collected from: (i) different areas of a habitat; (ii) trunk and leaves of trees; (iii) upper and lower leaf surfaces or (iv) leaves with single or grouped pupae, showed no differences ($P > 0.25$ for each test).

Laboratory dissection showed that sex differentiation was unreliable in the field. The sexual dimorphism in size was quantified for some populations by weight analysis of the members of mating pairs following drying to constant weight at 60 °C. Some estimates of sex ratio in successive samples from one site were obtained by comparison of the frequency distribution of weight in non-mating adults with that in the mating males and females. For each weight class the proportion of each sex in the non-mating beetles is estimated as equivalent to the proportion in the mating sample. Dissection of one of these samples of non-mating beetles ($n = 140$) showed no difference between the actual and estimated proportions ($G = 0.97$, d.f. = 1, $P > 0.1$). The weight data are also analysed to examine temporal changes in reproductive condition.

RESULTS

Population biology

Hibernation

Adults hibernate from October or early November to April. Hibernation occurs mainly on trees at rural sites but in buildings in urban areas. Behaviour during hibernation will be described in detail elsewhere. Migration both to and from hibernacula continues over several weeks.

First generation

The data for number of pupae collected on separate dates in different habitats at a site are used to examine the pattern of pupation. In 1980 the pattern at several sites indicates a parallel sequence of breeding habitats on plants supporting the aphid prey of *A. bipunctata* (Fig. 2a). Early recruitment in May and June occurred on shrubs, particularly hawthorn *Crataegus* spp. and the ornamental and extensively planted *Rosa rugosa*. Later recruitment in June and July was on trees, principally lime *Tilia* spp. The same pattern is evident in the post-hibernation adult populations (see Brakefield 1984a, Fig. 5). Early adults are found on shrubs where mating occurs at high frequencies of up to 44%. Oviposition and subsequent recruitment on the shrubs may or may not occur. Dispersal to *Tilia* begins following budburst in mid- or late May. Figure 2b shows that in 1981 pupation on *Tilia* was earlier and that higher densities of pupae occurred. At most sites there was little evidence of earlier recruitment on shrubs although such habitats did provide adult mating sites.

Male *A. bipunctata* are smaller than females (Table 1). There are no differences in dry weight between morphs or between populations. The weight analysis of samples of post-hibernation adults from Tilburg yields evidence of an overall excess of males on *Rosa*

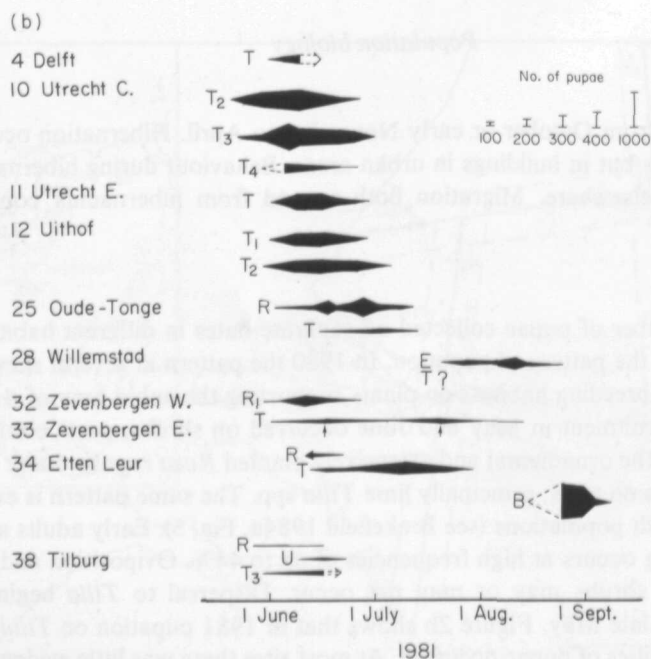
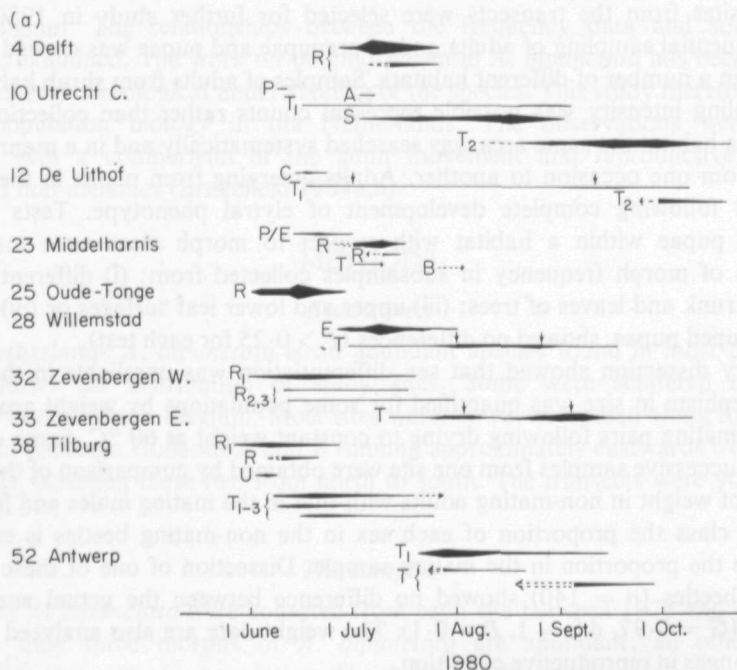


FIG. 2. Temporal changes in the size of sequential samples of pupae of *Adalia bipunctata* at the sites indicated. Plant habitats: A = *Acer campestre* L., B = *Betula* spp.; C = *Crataegus* spp.; E = *Sambucus nigra* L.; P = *Prunus* spp.; R = *Rosa rugosa* Thunb., S = *Salix* spp.; T = *Tilia* spp.; U = *Urtica dioica* L. (not shown: *Euonymus europaeus* L., *Acer pseudoplatanus* L.). Arrows indicate two peaks in numbers.

TABLE 1. Dry weight (mean \pm 95% C.L.) of the non-melanic *typica* (typ) and melanic *quadrifasciata* (m4) and *sempustulata* (m6) morphs of male and female *Adalia bipunctata* collected in copula in 1980. Samples are those of post-hibernation adults from the sites indicated

no.	Site name	n pairs	Males				Females			
			typ	m4	m6	all	typ	m4	m6	all
38	Tilburg	222	4.209 \pm 0.130	4.129 \pm 0.125	4.290 \pm 0.279	4.185 \pm 0.087	6.042 \pm 0.218	5.872 \pm 0.222	5.963 \pm 0.433	5.954 \pm 0.148
31	Oudenbosch	180	4.212 \pm 0.151	4.188 \pm 0.150	4.002 \pm 0.277	4.165 \pm 0.101	5.891 \pm 0.229	6.156 \pm 0.241	6.147 \pm 0.600	6.042 \pm 0.163
12	De Uithof	119	4.129 \pm 0.120	4.337 \pm 0.246	4.486 \pm 0.320	4.180 \pm 0.107	5.846 \pm 0.238	5.714 \pm 0.484	6.318 \pm 0.633	5.880 \pm 0.206

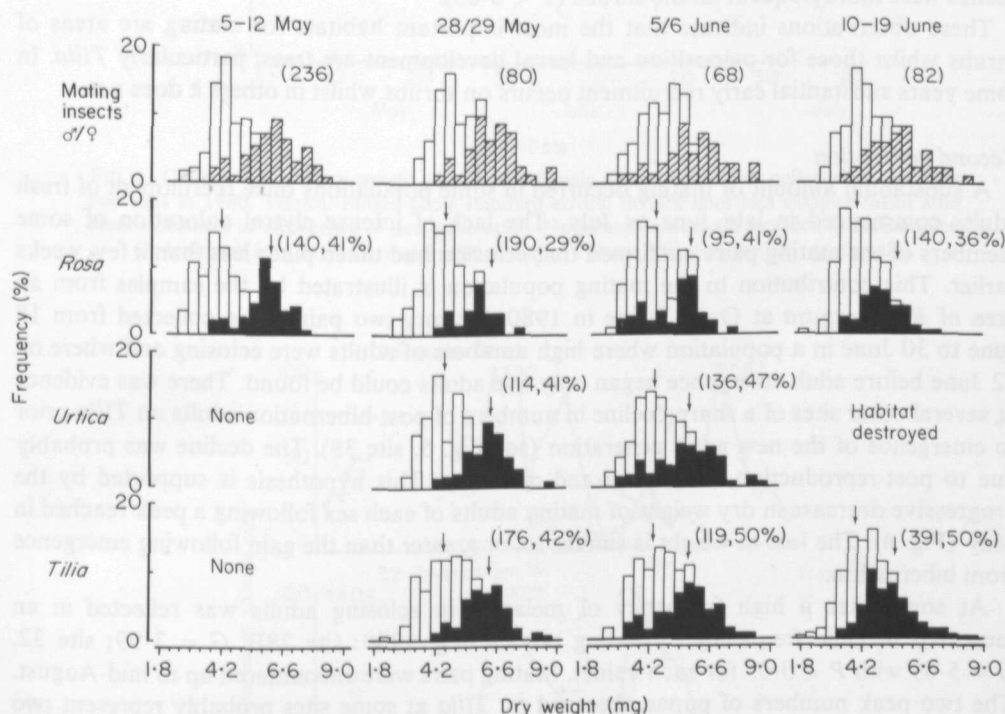


FIG. 3. Frequency of dry weight in samples of *Adalia bipunctata* from Tilburg (site 38) collected in consecutive periods in 1980 on different plant species. The top row shows distributions for males (unhatched) and females (hatched) in all mating pairs. The lower rows are those for total samples from each plant species (arrows show means for each sex in all mating insects). The distributions for mating insects are used to estimate the relative proportion of each sex in each total sample for the same period as indicated by shading. The sample size and estimated % frequency of females is indicated for each sample.

and a more even sex ratio on *Tilia* (Fig. 3; combined non-mating samples: estimates of % females = 36% and 48%, respectively with $G = 19.57$, $P < 0.001$). The status of the samples from *Urtica dioica* is uncertain although the initial movement to *Urtica* is probably synchronous with that to *Tilia* (Fig. 3). A similar difference is indicated from a comparison of weight distribution in samples from areas of *Rosa* and *Tilia* about 100 m apart collected at Antwerp on 22 May 1980 soon after bud burst on the trees (mean dry weight in mg (n) for *Rosa* and *Tilia* respectively = 4.80 (220) and 5.68 (139);

Mann-Whitney $U = 19\ 163.5$, $P < 0.001$). A bimodal frequency distribution on *Rosa* contrasted with *Tilia* where only a high mode was evident. Such differences suggest a stronger tendency for movement by females from the shrubs to trees than by males.

At each of the sites for which comparisons can be made there is evidence that over the mating period up to commencement of emergence from pupae, mating occurs at a substantially higher frequency on shrubs than on *Tilia* trees (site 12, 1980: $G = 95.6$; site 38; 1980, $G = 16.74$ and 1981, $G = 41.3$ with 1 d.f. and $P < 0.001$ for each value). The overall frequencies of mating beetles for these sites were 23.5% and 10.4% on shrubs and trees, respectively. In four of the five comparisons of the first sample from *Tilia* with that collected on the same date from nearby shrubs (see Brakefield 1984a, Table 6) mating beetles were more frequent on the shrubs ($P < 0.05$).

These observations indicate that the most important habitats for mating are areas of shrubs whilst those for oviposition and larval development are trees, particularly *Tilia*. In some years substantial early recruitment occurs on shrubs whilst in others it does not.

Second generation

A substantial amount of mating occurred in some populations once recruitment of fresh adults commenced in late June or July. The lack of intense elytral coloration of some members of the mating pairs confirmed that eclosion had taken place less than a few weeks earlier. This contribution to the mating population is illustrated by the samples from an area of *Rosa rugosa* at Oude-Tonge in 1980. Seventy-two pairs were collected from 18 June to 30 June in a population where high numbers of adults were eclosing and where on 12 June before adult emergence began only five adults could be found. There was evidence at several other sites of a sharp decline in numbers of post-hibernation adults on *Tilia* prior to emergence of the new adult generation (see Fig. 5, site 38). The decline was probably due to post-reproduction senescence and mortality. This hypothesis is supported by the progressive decrease in dry weight of mating adults of each sex following a peak reached in May (Fig. 4). The loss in weight is similar to, or greater than the gain following emergence from hibernation.

At some sites a high frequency of melanics in eclosing adults was reflected in an increased melanic frequency in mating beetles (e.g. 1980: site 28E, $G = 3.99$; site 32, $G = 5.69$ with $P < 0.05$ for each value). Mating pairs were encountered up to mid-August. The two peak numbers of pupae observed on *Tilia* at some sites probably represent two essentially separate generations (Fig. 2). A late second generation was sometimes found in Autumn on birch *Betula* spp. (Fig. 2, e.g. site 34, 1981) and on *Tilia* where no early breeding had occurred (e.g. site 10, 1980). The second generation is almost entirely restricted to trees. Although second generation recruitment can be substantial it is not clear how general it is in relation to differences between sites and years and to what extent a long oviposition period by a surviving cohort of the overwintered generation contributes to it.

Variability between sites

A closer examination of differences in the biology of *A. bipunctata* at three similar areas of *Rosa rugosa* is possible from Fig. 5. The areas are at sites on transect C at increasing distances from the coast (Oude-Tonge, 28.5 km; Zevenbergen W., 54 km; Tilburg, 88 km). Figure 5 shows that in 1981 maximum adult density on *Rosa* was earliest at the most inland site, Tilburg. Dispersal to *Tilia* also occurred earlier than nearer the coast. At Oude-Tonge nearest the coast there is evidence of two adult peaks, the second probably

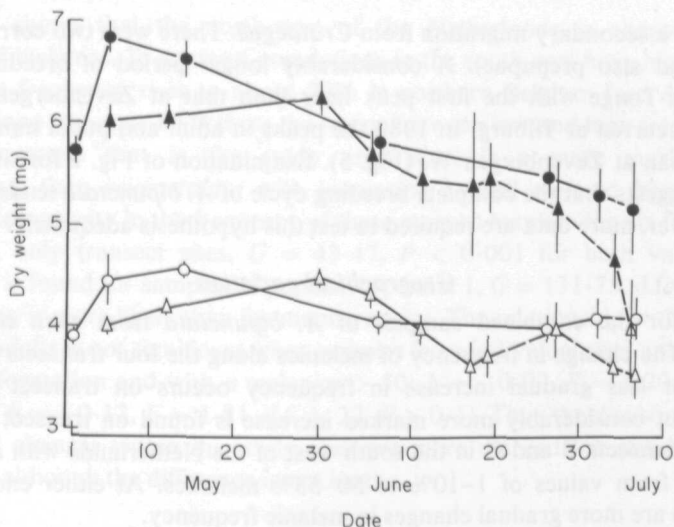


FIG. 4. Change in mean dry weight of male and female *Adalia bipunctata* collected in copula at two sites in 1980. Vertical ranges show standard errors. Broken lines join samples taken after commencement of emergence of new generation. No teneral insects are included in the samples. Oudenbosch (site 31): (○), males; (●), females. Tilburg (38): (△), males; (▲), females.

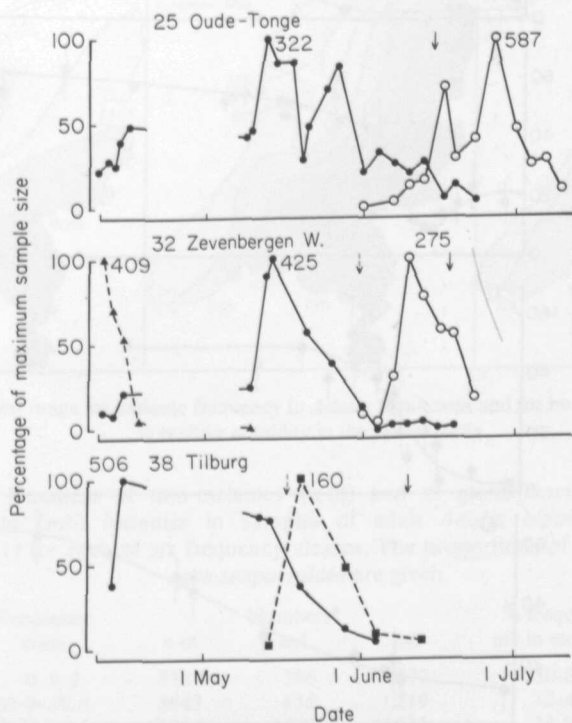


FIG. 5. Change in counts of adults (●), and samples of pupae (○) of *Adalia bipunctata* from single habitats of *Rosa rugosa* at the sites indicated in 1981. Data for adults from adjacent habitats of *Crataegus monogyna* Jacq. (▲) and *Tilia europaea* L. (■) are also shown. Breaks in the plots indicate a period of no adult activity. Figures give maximum sample sizes. Solid arrows show peaks for pupal samples in 1980 and broken arrows those for adult counts.

resulting from a secondary migration from *Crataegus*. There were two corresponding peaks for pupae (and also prepupae). A considerably longer period of breeding occurred on *Rosa* at Oude-Tonge with the first peak later than that at Zevenbergen W. Negligible recruitment occurred at Tilburg. In 1980 the peaks in adult and pupal numbers at Tilburg were earlier than at Zevenbergen W. (Fig. 5). Examination of Fig. 2 for sites 23–38 along transect C suggests that the complete breeding cycle of *A. bipunctata* tends to occur earlier inland. However, more data are required to test this hypothesis adequately.

Geographical variation

The data for the combined samples of *A. bipunctata* from each site are given in Appendix 1. The change in frequency of melanics along the four transects is shown in Fig. 6. A more or less gradual increase in frequency occurs on transect A. A similarly progressive but considerably more marked increase is found on transect D. Steep clines occur along transects B and C in the south-west of the Netherlands with an increase over some 20 km from values of 1–10% to 50–55% melanics. At either end of these steep portions there are more gradual changes in melanic frequency.

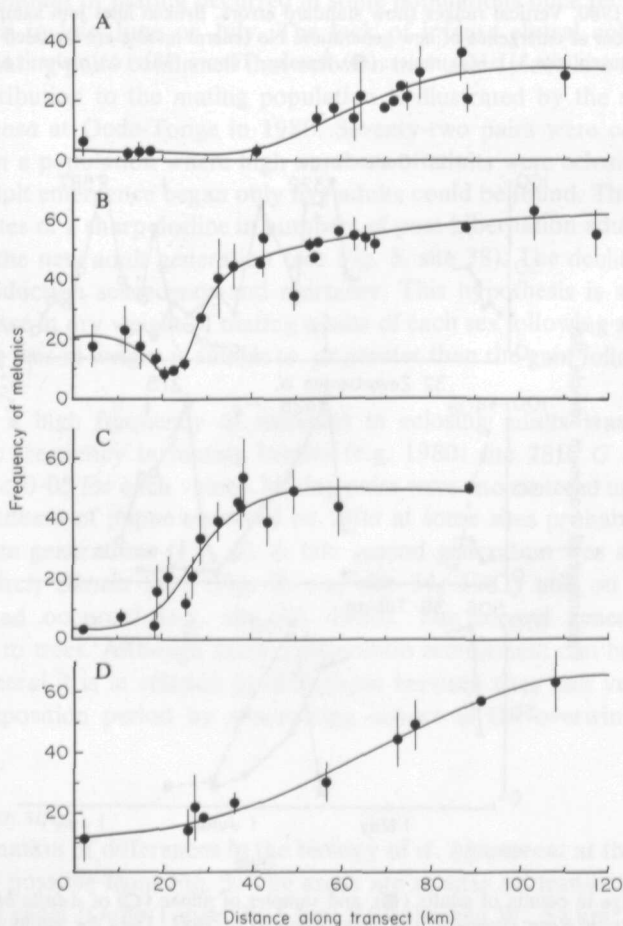


FIG. 6. Clines in the frequency of melanic *Adalia bipunctata* on the four study transects A–D. Vertical ranges show 95% C.L. Curves are fitted by eye.

Figure 7a shows that the north-west of the Netherlands is characterized by low frequencies of melanics. In contrast populations in the south-east have higher than 50% of melanics. The frequency rises to about 70% in northern Belgium (see Appendix 1). The steep clines along transects B-D cross the region between low and high frequencies.

Table 2 suggests that in the study area there is a more marked increase in *quadrimaculata* than *sexpustulata* with increasing overall melanic frequency. There is significant heterogeneity in the frequency of these morphs between the six frequency classes ($G = 44.96$; only transect sites, $G = 43.47$, $P < 0.001$ for both values). A similar heterogeneity is found for samples of pupae (Appendix 1, $G = 131.75$, d.f. = 4, $P < 0.001$) but mis-scoring is more likely than for mature adults. The relationship with overall melanic frequency in adults is not significant when samples from individual sites are analysed (using angular transformation and with n melanics > 50 ; $b = -0.09$, $F = 1.20$, d.f. 1, 24; only transect sites, $b = -0.12$, $F = 1.81$, d.f. 1, 22, $P > 0.1$). Thus there is some evidence that the frequency changes within the study area are more marked for *quadrimaculata* than *sexpustulata*, although the difference is not large.

Climatic correlations

There is a negative relationship between the hours of spring sunshine and the frequency of melanics (Fig. 7a,b). The relationship tends to break down outside the months April to

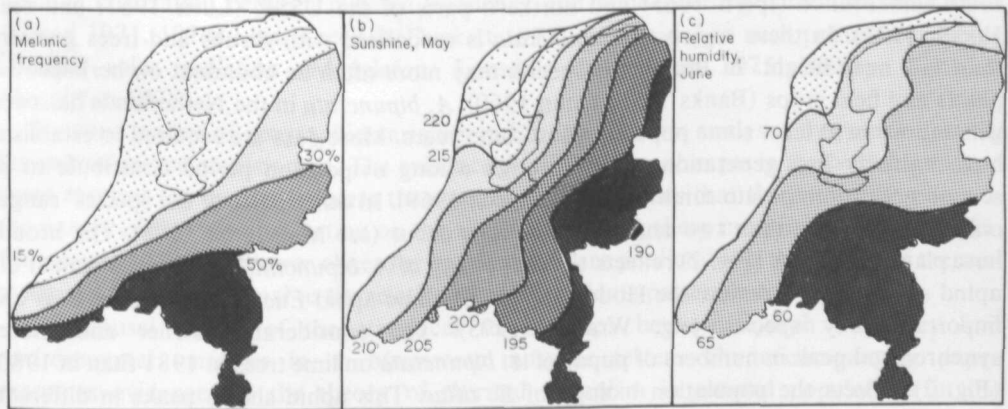


FIG. 7. Contour maps for melanic frequency in *Adalia bipunctata* and for hours of sunshine and % relative humidity in the Netherlands.

TABLE 2. Numbers of non-melanics (n-m) and of *quadrimaculata* (m4) and *sexpustulata* (m6) melanics in samples of adult *Adalia bipunctata* (data in Appendix 1) for each of six frequency classes. The proportions of melanics which are *sexpustulata* are given

Frequency class	Numbers*			% frequency m6 in melanics
	n-m	m4	m6	
0-9.9	7167	396	177	30.89
10.0-19.9	3843	455	219	32.49
20.0-29.9	11714	2492	1012	28.88
30.0-39.9	838	307	126	29.10
40.0-49.9	5189	3143	1011	24.34
50.0-59.9	5229	4397	1471	25.07

* Figures for $> 60.0\%$ melanics are 32, 39 and 16 respectively.

June and particularly in winter (cf. climatic maps in K.N.M.I. 1972). The correlation between the average annual hours of sunshine (1951–1980) and melanic frequency for twenty-one study sites which can be matched to meteorological stations is -0.45 ($P < 0.05$). This is somewhat lower than, but in the same direction as, the corresponding correlations shown in Britain (Muggleton, Lonsdale & Benham 1975). It is consistent with the theory of thermal melanism which predicts that melanic beetles are favoured in conditions of low sunshine because of a more efficient absorption of solar radiation (Lusis 1961; Benham, Lonsdale & Muggleton 1974; Muggleton, Lonsdale & Benham 1975; Brakefield & Willmer 1984). There are, however, stronger relationships with other climatic variables in the study area. The negative correlation with relative humidity is illustrated in Fig. 7a and c. This relationship is also strongest in the months April–June. The correlation of average annual mean relative humidity and melanic frequency is -0.75 (d.f. = 12, $P < 0.01$). The highest value of -0.90 (d.f. = 12, $P < 0.001$) is found with the index of oceanity examined by Bengston & Hagen (1977). These observations and the existence of significant correlations between climatic variables emphasizes the caution necessary in interpreting such data (see Bishop, Cook & Muggleton 1975).

DISCUSSION

The sequence of breeding habitats for *A. bipunctata* in the study area is similar to those in south-east France (Iperti 1965) and northern parts of the USSR (Lusis 1961; and see Hodek 1973). In these regions *A. bipunctata* is associated with shrubs and trees greater than 1–2 m in height. In Britain the species may more often be abundant on herbaceous plants and field crops (Banks 1955; Dunn 1960). *A. bipunctata* in the Netherlands has two generations in at least some populations in some years. More data are required to establish how regularly first generation adults through a long oviposition period contribute to a second generation in late summer (see Ellingsen 1969). In other parts of the species' range only one, or more than two annual generations occur (see Muggleton 1978). The broad host plant utilization (Fig. 2) reflects the polyphagy of *A. bipunctata* and the movement of aphid species (for a review see Hodek 1973). The lime aphid *Eucallipterus tiliae* L. is an important prey species (e.g. Wratten 1973). The considerably earlier and more synchronized peak in numbers of pupae of *A. bipunctata* on lime trees in 1981 than in 1980 (Fig. 2) reflects the population biology of *E. tiliae*. This aphid shows peaks in different years in June or in August–September. The regulation of this variation is dependent on the number of fundatrices hatching from overwintered eggs and on spring temperatures (Dixon 1971).

The steepest parts of the clines in the Netherlands (Fig. 6) occur in areas where populations are restricted to villages and are rather small and where rural environments include few suitable breeding habitats. Changes in melanic frequency were considerably more gradual in regions with large urban sites. This is consistent with Endler's (1977) theoretical prediction that clines will become steepened or stepped in the vicinity of a partial barrier to gene flow. The steep changes occur to one side of a wide (c. 1.75 km) waterway and not across it (Figs. 1 & 6). This may, however, represent no more of a barrier than a similar stretch of rural environment (see Brakefield 1984a). The greater width of the cline along transect D, which is further inland (Figs 1 & 6), probably reflects a more gradual change in selection coefficients. There are areas of this transect where a similar distribution of habitats occurs to those characterized by steep changes in transects B and C. It is possible, however, that no such area is within the critical range of the point at

which net genic selection changes over from favouring non-melanics to melanics (see Endler 1977).

The negative correlation between melanic frequency and length of sunshine in the Netherlands is similar to those found in Britain (Muggleton, Lonsdale & Benham 1975; see also Creed 1975). Bengtson & Hagen (1977) show a negative correlation with annual number of clear days in Norway (sunshine itself was not analysed). The stronger relationship in April–June in the Netherlands is consistent with an influence of thermal melanism since this period is that of peak mating and oviposition activity and is when adults most commonly occur on low growing shrubs exposed to direct solar radiation rather than shaded among trees. Muggleton, Lonsdale & Benham (1975) found higher correlations in the early spring and autumn months when ambient temperatures are lower. The strongest relationship found by Bengtson & Hagen (1977) was a positive one between melanic frequency and an index of oceanity (see also Lusi 1961; Hodek 1973). This contrasts with the negative correlation in the Netherlands although the range in the index falls below, and is only about one-tenth of that in Norway. It is possible that the significant rise in melanic frequency in the coastal strip of transect B (Fig. 6) is related to a local positive influence of maritime climate. Other workers have described correlations with temperature variables (e.g. Scali & Creed 1975; Creed 1975). There are no mechanistic explanations of the relationships with climatic variables other than length of sunshine. The positive correlation between melanic frequency and atmospheric pollution in some areas (Creed 1971, 1974; Lees, Creed & Duckett 1973) may be due to such pollution reducing incident solar radiation (Muggleton, Lonsdale & Benham 1975; Bishop, Cook & Muggleton 1978).

Previous studies of geographic variation in Europe have not analysed frequency data for the individual melanic morphs. The evidence that frequency changes in the Netherlands are more marked for *quadrimaculata* than *sexpustulata* suggests that there are differences in the nature of the selection acting on the alleles controlling these morphs (see Creed 1971; Muggleton 1978). Shallower clines for *sexpustulata* are predictable if the effects of thermal melanism are proportional to the relative extent of melanic pattern in the morphs.

The causal nature of the statistical relationships between morph frequency and environmental variables in *A. bipunctata* must be tested. The accompanying paper examines evidence for the theory of thermal melanism in natural populations in the Netherlands.

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APPENDIX 1

Frequency data for combined samples of *Adalia bipunctata* from the sites indicated (see Fig. 1). Frequencies for individual morphs are given for samples for which morph determination was effective and those for morph classes where it was not. Overall sample sizes and % frequency melanics are given. n-m, non-melanic/*typica*; mel, melanic; m4, *quadrimaculata*; m6, *sexpustulata*

no.	Site name	Adults generation 1*		Adults generation 2*		Pupae 1978 + 1979		Pupae 1980 + 1981		Total <i>n</i>	% mel
		n-m	m6	n-m	mel	n-m	mel	n-m	m6		
1	Brielle	27	0	22	3	43	2	8	0	107	6.54
2	Den Haag	281	3	0	0	0	0	0	0	288	2.43
3	Den Hoorn	102	3	0	0	0	0	0	0	105	2.86
4	Delft	1283	21	547	19	275	10	1284	19	3493	2.98
5	Gouda	141	1	0	0	110	6	0	0	259	3.09
6	Woerden	120	14	8	3	101	17	0	0	267	14.23
7	Harmelen	49	7	0	0	129	29	0	0	218	18.35
8	Vleuten	15	4	0	0	58	7	0	0	85	14.12
9	De Meern	40	4	0	0	0	0	0	0	51	21.57
10	Utrecht C.	2093	289	1124	291	2057	415	6202	857	13993	17.99
11	Utrecht E.	3455	620	0	0	0	0	1007	152	5634	20.80
12	De Uithof	2895	598	1458	580	40	11	2498	571	9219	25.25
13	De Bilt	9	1	0	0	217	61	0	0	288	21.53
14	Zeist	16	6	1	0	55	23	0	0	101	29.70
15	Maarn	58	31	5	0	7	1	0	0	102	36.27
16	Amersfoort	14	2	74	25	166	38	0	0	321	20.87
17	Apeldoorn	103	28	0	0	0	0	0	0	143	27.97
18	Ouddorp	88	12	7	0	0	0	0	0	107	17.76
19	Stellendam	83	15	10	0	0	0	0	0	108	23.15
20	Melissant	62	5	8	0	0	0	0	0	75	17.33
21	Dirksland	383	38	19	0	0	0	0	0	440	12.95
22	Sommelsdijk	361	20	12	0	0	0	0	0	393	8.14
23	Middelharnis	4833	343	1079	148	976	85	598	33	8256	9.33
24	Nieuwe-Tonge	464	37	232	42	24	6	0	0	816	11.76
25	Oude-Tonge	4238	1019	3912	1543	49	15	2860	838	15217	27.32
26	Achthuizen	14	8	5	0	10	5	6	1	50	40.00
27	Ooltgensplaat	43	33	14	7	19	11	13	5	159	44.02
28	Willemsstad	1053	569	618	538	53	45	1420	978	5709	44.93
29	Helwijk	26	18	0	0	0	0	0	0	56	53.57
30	Klundert	249	80	0	0	0	0	0	0	378	34.13
31	Oudenbosch	980	750	121	133	0	0	38	33	2346	51.46
32	Zevenbergen W.	3803	2344	1650	1638	0	0	713	549	11633	47.00
33	Zevenbergen E.	550	381	130	911	43	38	738	571	4309	52.33
34	Etten Leur	85	102	29	0	0	0	1659	557	3995	56.35
35	Prinsenbeek	57	51	64	74	3	3	0	0	275	54.91

APPENDIX 1

no.	Site name	Adults generation 1*		m6	Adults generation 2*		Pupae 1978 + 1979		Pupae 1980 + 1981		Total n	% mel
		n-m	m4		n-m	mel	n-m	mel	n-m	m4		
36	Breda W.	236	185	90	0	0	0	0	0	0	511	53.82
37	Breda C.	324	237	102	29	35	88	103	11	5	938	51.81
38	Tilburg	2638	2458	706	482	773	0	0	426	446	8092	56.18
39	Westelbeers	32	39	16	0	0	0	0	0	0	87	63.22
40	Eindhoven	68	54	29	0	0	0	0	0	0	151	54.97
41	Rotterdam	88	4	2	65	2	163	17	0	0	341	7.33
42	Rhoon	51	1	1	0	0	0	0	0	0	53	3.77
43	Barendrecht	49	6	3	0	0	0	0	0	0	58	15.52
44	Heinenoord	99	19	7	0	0	0	0	0	0	125	20.80
45	Mijnsherenland	243	21	11	0	0	0	0	0	0	275	11.64
46	Westmaas	96	15	10	0	0	0	0	0	0	121	20.66
47	Klaaswaal	83	34	7	0	0	0	0	0	0	124	33.06
48	Numansdorp	73	28	16	2	0	51	37	0	0	207	39.13
49	Dinteloord	95	41	19	0	0	0	0	0	0	155	38.71
50	Steenbergen	90	67	19	0	0	0	0	0	0	176	48.86
51	Bergen op Zoom	58	26	19	0	0	0	0	0	0	103	43.69
52	Antwerp	265	161	72	432	506	0	0	497	310	2417	50.60
53	Amsterdam S.	60	5	2	0	0	0	0	0	0	67	10.45
54	IJsselstein	205	34	19	10	3	10	5	53	12	359	22.56
55	Gorinchem	121	42	10	0	0	0	0	0	0	173	30.06
56	Waalwijk	58	36	10	0	0	0	0	0	0	104	44.23
57	'S-Hertogenbosch	70	60	7	0	0	0	0	0	0	137	48.91
58	Groningen	0	0	0	68	10†	0	0	0	0	78	12.82
59	Leeuwarden	0	0	0	100	2†	0	0	0	0	102	1.96
60	Nijland	0	0	0	50	6†	0	0	0	0	56	10.71
61	Oudemirdum	0	0	0	81	9	317	14	0	0	421	5.46
62	Medemblik	0	0	0	64	3†	0	0	0	0	67	4.48
63	Naarden	0	0	0	80	16†	0	0	0	0	96	16.67
64	Zierikzee	217	17	15	0	8	78	14	0	0	341	13.49
65	Middelburg	0	0	0	26	8	28	8	0	0	70	22.86
66	Oostburg	0	0	0	8	13	0	0	30	25	89	57.30
67	Hengelo	160	41	12	0	0	0	0	0	0	213	24.88
68	Markelo	49	13	10	0	0	0	0	0	0	72	31.94
69	Winterswijk	110	38	10	0	0	0	0	0	0	158	30.38
70	Arnhem	301	90	29	0	0	0	0	0	0	420	28.33
71	Cuyk	0	0	0	43	75†	0	0	0	0	118	63.56
72	Diest	0	0	0	0	0	0	0	8	30	56	85.71
73	Hasselt	0	0	0	50	133†	0	0	0	0	183	72.68
74	Sint-Truiden	0	0	0	18	39†	0	0	0	0	57	68.42
75	Brugge	0	0	0	39	77†	6	10	0	0	132	65.91

* generation 1 = mature adults (November-June); generation 2 = samples include recently emerged individuals (June-October) or melanics were not sub-divided.
 † Data at least in part for 1969 or 1973 ♀-♂ kindly provided by F. R. Crees