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A decline of melanism in the peppered moth *Biston betularia* in The Netherlands

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Melanic forms of the peppered moth *Biston betularia* were well established in The Netherlands by the end of the 19th century, indeed the first records of the black *carbonaria* form in 1867 are only about 20 years later than in England. Analysis of extensive sampling data collected by B. J. Lempke for a period of several years beginning in 1969 shows that *carbonaria* was at a frequency of about 60 to 70% in most of the country where epiphyte communities on trees were reduced due to the effects of air pollution. The pale *typica* and the three intermediate *insularia* forms were each at similar, low frequencies. Only in the extreme north and south-east of The Netherlands where epiphyte floras were richer was *carbonaria* at a lower frequency of less than 40%. Samples collected from seven localities in 1988 show that *carbonaria* has dramatically declined to a frequency of less than 10%. In contrast to England, the fully black form is being replaced not only by *typica* but also by the darkest of the *insularia* phenotypes. The decline in melanism coincides with a period of decreasing levels of sulphur dioxide and of increasing species diversity of lichens on trees.

KEY WORDS:—*Biston betularia* – peppered moth – industrial melanism – evolution – polymorphism – natural selection – air pollution – epiphytes.

CONTENTS

INTRODUCTION

Studies of industrial melanism have gained new impetus in recent years with the discovery of further evolutionary change occurring in the form of declines in the frequency of melanic forms of the peppered moth *Biston betularia* and the two-spot ladybird beetle *Adalia bipunctata* in some urban environments in Britain (Clarke, Mani & Wynne, 1985; Cook, Mani & Varley, 1986; Howlett & Majerus, 1987; Creed, 1971; Brakefield & Lees, 1987). Such phenomena provide excellent opportunities for experimental analyses of natural selection involving both visual and non-visual effects of the major genes controlling the melanic

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polymorphisms. Insights from such studies will be critical to improving our rather rudimentary understanding of this classic example of the spread of adaptive phenotypes under the influence of natural selection (see Brakefield, 1987; Mani, 1990). Although both the moth and ladybird appear to be responding to decreases in air pollution, there are clearly important differences in the underlying mechanisms (see Brakefield & Lees, 1987; Brakefield, 1987; Liebert & Brakefield, 1987).

The present study provides a basic description of a recent decline in the frequency of the black *carbonaria* form of *B. betularia* in The Netherlands. Although this phenomenon tends to parallel the changes in Britain there are at the same time some interesting differences in detail. Unfortunately, unlike the monitoring studies of *B. betularia* at Caldy near Liverpool (Clarke *et al.*, 1985) and of *A. bipunctata* in Birmingham (Creed, 1971; Brakefield & Lees, 1987), data points are only available for the present-day and for some 15 to 20 generations (years) earlier.

SURVEYS OF *BISTON BETULARIA* IN THE NETHERLANDS

The 19th century

The first recorded observation of a black peppered moth in England was made in 1848 in Manchester (see Cook, 1981). The first record from The Netherlands is not much later (see Lempke, 1970). A mating pair of black moths was found on an elm tree at Breda in Noord-Brabant in 1867 (Heylaerts, 1870). The pairing is depicted in a plate by S. van Vollenhoven. The discovery of a mating pair suggests that even by 1867 a dominant *carbonaria* allele was present at a substantial frequency in certain populations. In any case inspection of material in the Zoological Museum of Amsterdam shows that by the end of the 19th century melanic forms of *B. betularia* were well established in the greater part of The Netherlands. These melanic forms included not only *carbonaria* but each of the three main phenotypes recognized within the class of intermediate *insularia* melanics. These phenotypes are all determined by a series of alleles at the *carbonaria* locus. Lempke (1970) states that the earliest specimens of the four forms (see below) in the Zoological Museum of Amsterdam are dated 1871, 1895, 1888 and 1884 (in order of increasing melanization).

1969 to 1973

B. J. Lempke working in the 1960s recognized the value of a survey of *B. betularia* throughout The Netherlands. Entomologists were enlisted to send samples obtained in light traps to him for scoring and synthesis. Moths were scored after consultation with H. B. D. Kettlewell and study of Kettlewell's collection. Lempke distinguished five phenotypes: fully-black *carbonaria*; light *typica*; dark *insularia* (I_3), very dark with very light, or light specking; medium *insularia* (I_2) with substantial speckling; pale *insularia* (I_1), more black scaling than *typica*, especially on the body (see also Lempke, 1970). The sampling began in 1969 with collections at some localities extending over more than one year and at others, not commencing until a later year. Nearly all the samples date from the period 1969 to 1973. A subset of the data for 1969 are included in Kettlewell

TABLE 1. Percentage frequency of the five major non-melanic and melanic phenotypes of the peppered moth *Biston betularia* in pooled samples obtained in ten Dutch Provinces by B.J. Lempke from 1969 to 1973 (see text). Total sample sizes are also given

Province	Percentage frequency					Total sample size
	typica	*insul. 1*	*insul. 2*	*insul. 3*	*carbonaria*	
Friesland	19.1	16.3	16.3	14.7	33.6	194
Drenthe	16.2	20.9	17.4	4.7	40.7	86
Overijssel	13.9	9.6	9.3	8.4	58.8	330
Noord-Holland	12.5	10.1	8.8	6.4	62.3	377
Gelderland	7.0	8.5	17.4	7.9	59.1	328
Utrecht	9.0	10.2	13.3	9.8	57.8	256
Zuid-Holland	4.3	7.8	9.9	7.3	70.7	232
Noord-Brabant	3.7	3.0	10.4	10.8	72.1	297
Zeeland	12.1	18.2	22.7	9.1	37.9	66
Limburg	2.3	4.5	10.8	9.6	72.9	1706

(1973: table 6.1). Lempke most generously made the complete data set available for the present analysis.

A total of 4486 male *B. betularia* were obtained from 81 localities. However, 32 sites yielded less than ten moths and only 27 samples were of 40 or more moths. The samples provide a fairly thorough coverage of the country, only the extreme north (the Province of Groningen) being unrepresented. Preliminary examination of the data indicated broad regions of similar morph frequencies. Chi-square (χ^2) heterogeneity tests were applied to the frequency data for the localities grouped by Province or by contiguous groups of Provinces. The tests provided similar results when the phenotypes were grouped either by *typica* and all melanics or by *typica:insularia:carbonaria*. The larger samples from most of the individual Provinces are homogeneous ($P>0.05$). This included Limburg in the south-east, the Province with the highest frequencies of *carbonaria* and the only one with more than two or three separate samples ($\chi^2=48.58$, d.f.$=36$ and ten sites, $P>0.05$). The data pooled by Province are given in Table 1. Each of the five phenotypes was collected throughout the country. The *typica* form and each of the three *insularia* phenotypes were of roughly similar abundance. The geographical variability in the frequency of *carbonaria* is illustrated in Fig. 1 together with an indication of the pattern of χ^2 homogeneity across Provinces. Most of The Netherlands was characterized by high frequencies of *carbonaria* of about 60 or 70%. However, Friesland and Drenthe in the north, and Zeeland in the extreme south-west had lower frequencies of around 40%.

1988

Large samples of *B. betularia* were obtained in May to July 1988 at several sites around Leiden and Rotterdam in the Province of Zuid-Holland by the use of assembly traps or the release of held virgin females onto trees (see Liebert & Brakefield, 1987). In addition, smaller samples were obtained from central Amsterdam and Sint Nicolaasga in Friesland. The moths were scored in a manner as close as possible to that employed by Lempke and Kettlewell following inspection of many moths together with B. J. Lempke and T. G.

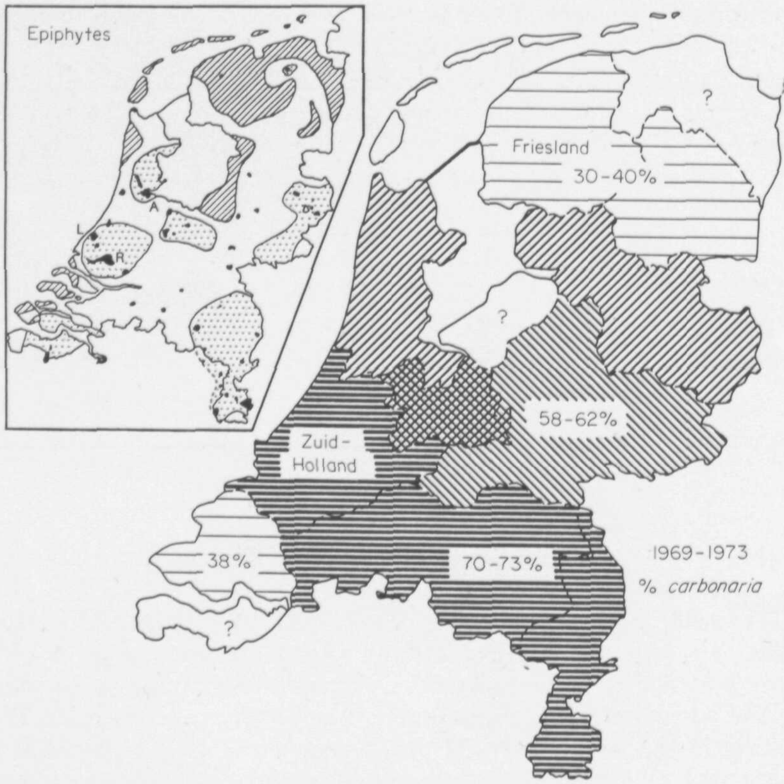

Figure 1. Map of The Netherlands showing the frequency of the *carbonaria* form of *Biston betularia* in the period 1969 to 1973 (+). Broken lines show the borders between the Dutch Provinces, two of which are named. Shading indicates the pattern of homogeneity in morph-frequencies across Provinces (see text; data of B. J. Lempke). No samples were collected in the areas indicated by question marks. The inset map shows the epiphytic vegetation in the 1960s with "deserts" *dotted*; transitional zone in *white* with poor to locally subnormal vegetation; normal or luxuriant areas *hatched*. Larger towns and main industrial areas are in black. Three cities near which samples were obtained in 1988 are indicated: A, Amsterdam; L, Leiden and R, Rotterdam (after Barkman, 1969).

TABLE 2. Numbers of the five major non-melanic and melanic phenotypes of the peppered moth *Biston betularia* in samples obtained from the indicated localities in 1988. The first three localities are in the vicinity of Leiden and the next two are just to the west of Rotterdam (see Fig. 1)

Province/ locality	Numbers					Total
	typica	*insul. 1*	*insul. 2*	*insul. 3*	*carbonaria*	
Zuid-Holland:						
Leiden	138	23	31	116	32	340
Warmond	51	4	6	31	5	97
Voorschoten	53	10	8	17	10	98
Hoogvliet	33	14	15	41	4	107
Schiedam N.	56	19	17	54	5	151
Noord-Holland:						
Amsterdam	18	2	8	6	3	37
Friesland:						
Sint Nicolaasga	39	2	8	17	0	66

Liebert. It should, however, be recognized that the phenotypes intergrade and thus there is inevitably some subjectivity in assigning certain individuals to particular phenotypes. This is probably especially so for the boundaries between *typica* and *insularia 1* and between *insularia 3* and *carbonaria* (see also Lees & Creed, 1977; Clarke, 1979). My own experience is that the body colouration is critical

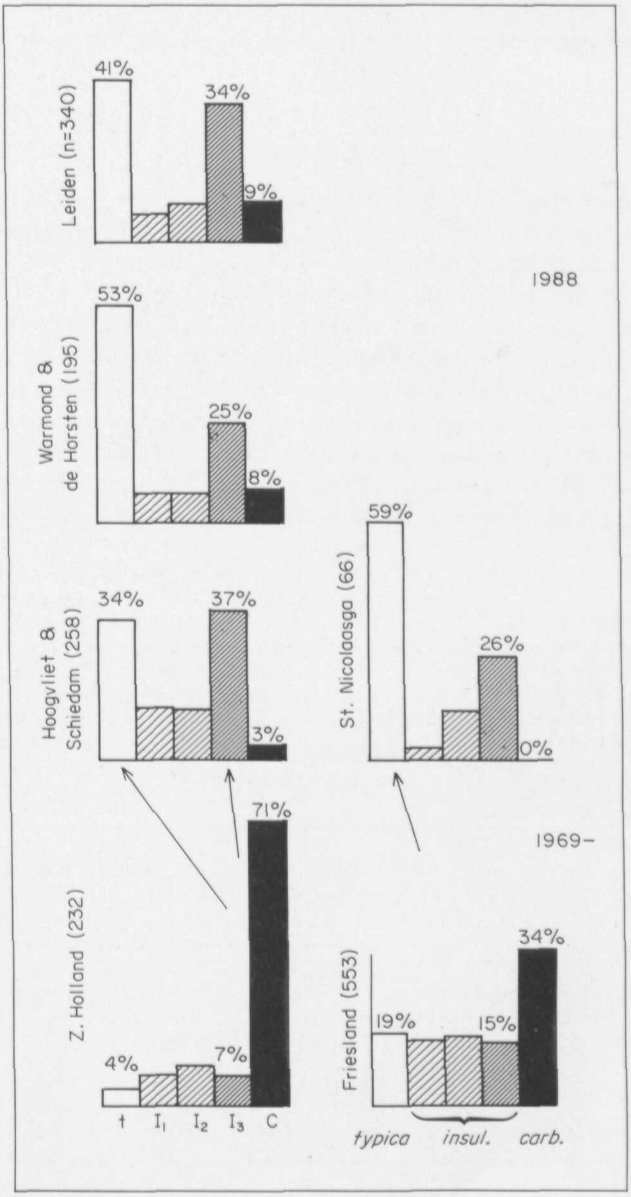

Figure 2. Comparison of morph frequencies in 1969–1973 (lower-most histograms) with those of 1988. The earlier samples are those pooled for each of two Dutch Provinces (see Fig. 1). The later samples from the same Provinces are either for individual sites or pooled for neighbouring localities (see Table 2). Percentage frequencies are indicated for the three morphs showing substantial changes in frequency.

in the former case, and the presence or absence of a more or less complete ring of light white speckling on the upper hindwing, in the latter.

The frequency data for the samples collected in 1988 are given in Table 2. Figure 2 illustrates the dramatic changes which have occurred in less than 20 generations. In the area of Zuid-Holland and Noord-Holland the frequency of *carbonaria* has declined from about 70% to less than 10%. Apparently it is being replaced not only by *typica* but also by the darkest form of *insularia*. The two paler forms of *insularia* have not changed substantially in frequency. There has also been a dramatic decline in *carbonaria* in Friesland but from initially lower levels.

DISCUSSION

The inset diagram in Fig. 1 shows that there was some spatial correspondence in the late 1960s between melanism in *B. betularia* in The Netherlands and the growth of epiphytic lichen communities on trees (Barkman, 1969; see also map for 1972 given by de Wit, 1983). The only two regions with low frequencies (<40%) of *carbonaria* were those in the north and extreme south-west with comparatively luxuriant lichen communities. The rest of the country which includes the major industrial conurbations was characterized by high frequencies of *carbonaria* of around 60 or 70% and by impoverished epiphyte growth (Fig. 1) and high levels of air pollution, especially sulphur dioxide (reports of the Dutch Rijksinstituut v. Volksgezondheid). Thus during this period *B. betularia* seems to have displayed classic industrial melanism with a correlation between air

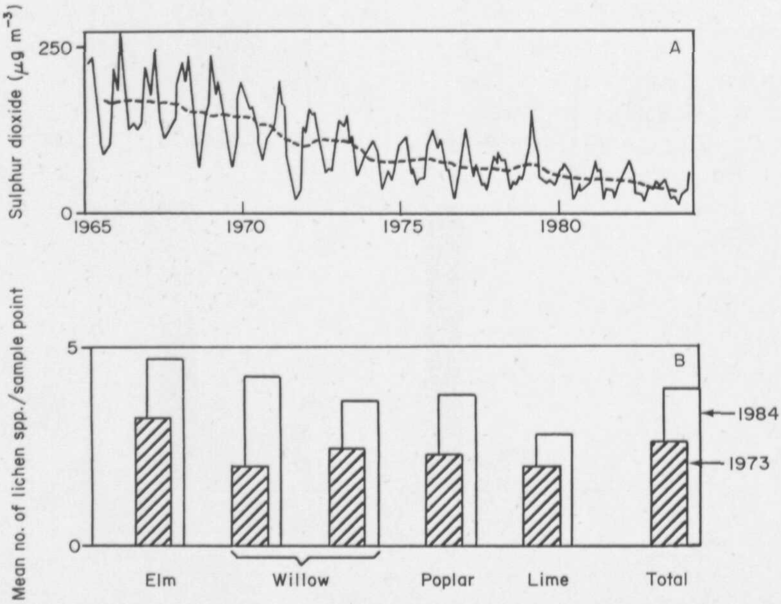

Figure 3. A, Average concentration of sulphur dioxide recorded monthly at Schiedam in Zuid-Holland from 1965 to 1983; broken line shows running annual mean. B, Mean number of species of epiphytic lichens recorded at numerous sites in Zuid-Holland on different tree species in the years 1973 and 1984 (both parts redrawn from Bakker *et al.*, 1987).

pollution and melanic frequencies. However, the apparently early establishment of the melanism in The Netherlands is noteworthy since during the mid- to late nineteenth century, industrialization was much less intensive and more localized than in northern England.

The frequency of *carbonaria* has declined quite dramatically in the last 20 or so years. This appears to parallel the change in many populations in northern England and which is best documented at Caldy near Liverpool (Clarke *et al.*, 1985) where *carbonaria* has declined rapidly from about 90% to 40% since 1970. Cook *et al.* (1986) show that this change is consistent with a more or less constant selective disadvantage to *carbonaria* of about 12% compared to the earlier period. However, at Caldy *carbonaria* is being replaced largely by *typica; insularia* increasing from below 1% up to only about 4%, with each of the three classes being represented (Mani, 1990; C. A. Clarke, personal communication). This suggests that a more complex change in the relative fitnesses of the various genotypes is occurring in The Netherlands where both *typica* and (darker) *insularia* are increasing at the expense of *carbonaria*.

Models of the decline in *carbonaria* at Caldy have shown that it is closely correlated with a reduction of sulphur dioxide in the locality (Mani, 1990). Fig 3A shows a typical data set illustrating the progressive decline in levels of sulphur dioxide which has occurred since the early 1970s in the Province of Zuid-Holland and elsewhere in The Netherlands. Although sulphur dioxide is not the only significant factor influencing changes in the epiphytic lichen communities in The Netherlands (de Wit, 1983) there is clearly a correlated increase in lichen species diversity on a variety of tree species in Zuid-Holland (Fig. 3B). Small colonies of foliose lichens now occur on the trunks and upper surface of branches in trees in cities in this area. This may be an important factor, together with more general changes in bark colouration, influencing relative crypsis in *Biston* moths (see discussion in Liebert & Brakefield, 1987).

Mani (1990) in a modelling approach to examining changes in fitness in the population at Caldy assumes that *insularia* suffers less loss of fitness with reduction in air pollution than *carbonaria*. The frequency data from The Netherlands suggest that the darker *insularia* phenotypes may indeed experience less loss of fitness there and, moreover, that they may have similar fitness to the *typica* form. Future experimental analyses of moth crypsis will investigate the possibility that changes in the available resting backgrounds for *B. betularia* in trees in central Holland have led to a switch from a bias towards those giving *carbonaria* the closest background-matching to a mixture of some favouring darker *insularia* and some, *typica*.

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