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Polymorphisms for Melanism

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THE RESPONSE OF TWO SPECIES OF MOTHS TO INDUSTRIALIZATION IN NORTHWEST ENGLAND

I. POLYMORPHISMS FOR MELANISM

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[Plate 1, pullouts 1 and 2, microfiche]

Industrial melanism was brought to the public gaze as a textbook example of rapid evolution by the surveys and experimental studies of Dr H. B. D. Kettlewell. The late Professor P. M. Sheppard, F.R.S., recognized the importance of a detailed study of melanism in an area where there is a steep cline in gene frequency, and initiated the present work. It is a pleasure to record our indebtedness to these two men and to dedicate the following contributions to them.

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Northwest England is one of the oldest industrial regions in the world and has suffered intense air pollution since early in the nineteenth century. Soot has blackened trees and walls in the area and sulphur dioxide has killed most of the lichens that were originally present. Night-flying moths, in particular the Peppered Moth *Biston betularia*, rest on such surfaces by day and are sought by birds as food. In 1848 a black, industrial melanic form of *B. betularia* was discovered near Manchester and by 1900 had almost completely replaced the light-coloured typical form. Kettlewell showed that this was because the *carbonaria* melanic was better camouflaged than typical when it rested on trees and walls affected by air pollution. In the last 25 years clean air policies have markedly reduced smoke and sulphur dioxide pollution in the region.

A polymorphism with visually distinct forms of *B. betularia* exists in northwest England and adjacent areas of Wales. These forms are determined by a series of multiple alleles, with the darkest phenotypes (*carbonaria*) dominant to the lighter (*insularia*) while typical is recessive. *Carbonaria* is by far the commonest melanic in the northwest and is most abundant in the populations of *B. betularia* in Greater Manchester and Merseyside and is relatively rare in northern Wales. Data for the frequency of *carbonaria*, *insularia* and typical from 158 sites, between Leeds in the east to beyond Bangor in the west, are tabulated. The date of collection and the map reference of the sampling site are given to facilitate prospective sampling as the environment continues to improve. Some evidence suggests that the increase in the frequency of typical moths at Caldy, Merseyside and in Central Manchester may be due to reductions in air pollution.

Gonodontis bidentata is another geometrid moth with a melanic (*nigra*) dominant to the non-melanic. The intensity of pigmentation of the non-melanic varies and specimens from northwest England are darker than those from the south of the country. The species is secretive and in the study area does not rest on exposed surfaces. Nevertheless *nigra* shows much local variation in frequency; it is most common in urban districts and least in the intervening rural areas. (In this way it is unlike *B. betularia* where *carbonaria* remains at over 85 % frequency throughout.) Superimposed on this pattern of local differentiation is a tendency of *nigra* to increase in frequency from west to east. In Liverpool the highest frequency attained is 45 % whereas in Manchester it reaches 80 %. Data from 112 sites are also tabulated. There is no evidence of a fall in the frequency of *nigra* associated with a decline in air pollution. Some results are given which suggest that adults emerge at different times in different parts of the survey area.

The two spot ladybird *Adalia bipunctata* also shows increases in the proportion of melanics in populations that inhabit the inner urban areas. The advantage conferred by melanism is not completely understood since the species is warningly coloured and distasteful. Melanics may be at an advantage in the presence of certain pollutants or they may be favoured by the lower amounts of sunshine received by areas suffering air pollution. In the ladybird there has been a spectacular decline in the frequency of melanics in Merseyside between the late 1960s and 1976. (Data from 40 samples are given.) This rapid response would be expected if the environment is acting directly on the species rather than through changes in the background on which the species rests as is the case in *B. betularia*.

The maintenance of the polymorphism for melanism in *B. betularia* is discussed. Three possibilities are examined in relation to the evidence available from the study area as well as from the rest of the United Kingdom: (1) heterozygous advantage is important irrespective of selective predation by birds, (2) there is a balance between the disadvantage of the melanic homozygote and the forces of selective predation, and (3) predation is the sole important selective agent while migration of animals from different environments occurs to produce polymorphism. At present the available evidence is insufficient to distinguish between these possibilities, the existence of heterozygous advantage, assumed to be of primary importance by other authors, has yet to be established.

1. Introduction

Industrialization of northwest England was well under way by the end of the eighteenth century and the process relied on locally available coal as a source of power. In the early 1800s the consumption of coal by industry and by a continuously increasing human population, which used it for heating and cooking, led to a sudden rise in atmospheric pollution. Although the harmful effect of this pollution on health, property and the environment had long been recognised (an early work on the subject, by John Evelyn, was published in 1661) the emissions of smoke and combustion gases from coal burning remained at a high level throughout the region until the mid-twentieth century. The last quarter of a century, however, has seen the introduction of legislation rigorously controlling the use of coal and this has greatly reduced the amount of smoke in the atmosphere although the use of fuel oil as a substitute for coal has resulted in a less spectacular reduction of sulphur dioxide. In 1946 Manchester was the first local authority to obtain powers to enforce 'smokeless zones,' although Coventry established the first such zone in 1951 (Anon. 1974). Manchester's first smoke control area which covered 104 acres (42.1 ha) was established the following year (Richards 1965). The national act to establish smoke control areas (The Clean Air Act) was passed in 1956. Since that time control of atmospheric pollution has become the rule in the northwest of England, and there has been a striking improvement in weather, visibility, public health and the survival and growth of plants. The situation in the Greater Manchester area is reviewed by Wood, Lee, Luker & Saunders (1974).

The period of high atmospheric pollution brought about a natural experiment in population genetics. While numerous plant and animal species were extinguished locally by the extreme effects of urbanization, others underwent genetic adaptation to fit them for the new conditions. The increase in frequency of dark coloured morphs in several species of moths (called industrial melanism) is the classical example of a rapid genetic response to a man-made change. Its significance in relation to the study of evolution was recognized at an early date (for example, by the Evolution Committee of the Royal Society in 1900). Investigations were made into the possible agents causing the response, and particular attention was paid to the Peppered Moth, *Biston betularia* (L.). This period is reviewed by Kettlewell (1973 ch. 5). A discussion of the position in continental Europe is given by Cleve (1970), who draws on earlier work by Ule (1925*a*, *b*). Nevertheless, despite the interest, the details of the increase in frequency and dispersal of melanic genes which were recorded are sketchy and anecdotal (see Kettlewell 1973). In his study of *B. betularia* in Manchester, H. S. Leigh (1908, 1911) recognized the importance of survey data and statistical samples. In his note published in 1908 he asks entomologists to supply him with details of the exact numbers of melanic and non-melanic types in samples together with the localities from which they were taken, and he did in fact collate quantitative data for the area. Unfortunately, his survey does not appear to have been published and we can find no record of it either at the University of Manchester or in the records of the Royal Society, from which he received financial support. The picture is even less complete with respect to other melanic species.

The conditions which favoured industrial melanism have now been reversed. The region remains an industrial one with relatively high levels of some atmospheric emissions such as exhaust gases from road traffic, but with a radically reduced smoke level and a lesser but substantial drop in sulphur dioxide (Wood *et al.* 1974). The appearance of the cities has greatly changed: the buildings have been cleaned or replaced and more trees and shrubs are present. There is already evidence that the frequencies of melanic morphs have reached a turning point (Clarke & Sheppard 1966; Cook, Askew & Bishop 1970; Creed 1971 *b*; Lees & Creed 1975; Whittle, Clarke, Sheppard & Bishop 1976) and a progressive decline may be expected in the future. It is the primary purpose of this paper to present a detailed picture of the distribution of morph frequencies in two species of moth, *Biston betularia* and the Scalloped Hazel, *Gonodontis bidentata* Clerck, at the time of the downturn in frequency. The present survey should be of value for comparison in subsequent investigations of the frequency of the melanic forms of the two species. It forms part of a general study of industrial melanism in the area by the present authors in association with a number of others (Askew, Cook & Bishop 1971; Benham, Lonsdale & Muggleton 1974; Bishop 1972; Bishop & Cook 1975; Bishop, Cook & Muggleton 1976; Bishop Cook, Muggleton & Seaward 1975; Bishop & Harper 1970; Boardman, Askew & Cook 1974; Cook, Askew & Bishop 1970; Cook & Wood 1976; Muggleton, Lonsdale & Benham 1975; Whittle, *et al.* 1976). In the discussion we review the evidence for selective agents changing morph frequencies and maintaining polymorphisms.

2. Materials and methods

(*a*) *Natural history*

The survey shows the frequencies of the principal melanic morphs of the Peppered Moth, *Biston betularia*, and the Scalloped Hazel moth, *Gonodontis bidentata*. Both species belong to the family Geometridae, and were chosen because they are abundant, relatively easy to catch and because the melanic forms are distinct and segregate in a simple genetic manner. Both are univoltine and have flying periods lasting five to ten weeks. In northwest England and north Wales adult *B. betularia* occur from mid-May to mid-August but are most abundant from early June to late July, a period of seven weeks (Bishop 1972). *Gonodontis bidentata* is found from mid-May until late June with the majority of moths flying, in any one area, during a period of three weeks (Bishop & Harper 1970). There are frequently differences in the peak flying time of this species and Scalloped Hazel moths on Merseyside emerge about two weeks before those in Greater Manchester (see §4). There are also differences in the peak flying times from year to year in the same locality.

Adults of both species are extremely difficult to find in the wild. *Biston betularia* has been observed resting on tree trunks and walls during the day by a number of observers. When it is allowed to emerge from its pupal case in a cage an individual *B. betularia* is likely to rest on an exposed surface. *Gonodontis bidentata*, on the other hand, is secretive and may hide under leaves and in crevices in the wild. The one specimen that we have observed in its natural resting place was in a crevice, and this species also retreats into cracks when allowed to emerge in a cage (Bishop *et al.* 1975). Characteristically *G. bidentata* will move to the corners of assembling traps while *B. betularia* remains out on the gauze surfaces. In the artificial habitats inside our cages *G. bidentata* remained near the ground whereas *B. betularia* climbed to a considerable height. Observations made in the field while releasing moths during capture, recapture experiments were consistent with this pattern of behaviour. Kettlewell (1973), on the other hand, states that at Cannock, Staffs., *G. bidentata* rests on the surface of pine tree trunks, so that resting behaviour of this moth may not always be so secretive. Kettlewell (1973) demonstrated that morphs of *B. betularia* settled on backgrounds they most resembled. Kettlewell & Conn (1977) have recently shown that *nigra* and non-melanic *G. bidentata* show a marked preference for resting on twigs of their appropriate colour. The difference in behaviour, as measured by χ^2, is far less marked when the two morphs are allowed to chose a resting place on tree trunks.

Adult *B. betularia* emerge from their pupae in the late afternoon and early evening and fly and move to assembling traps from dusk (21h 00 G.M.T.) onwards. Hitchen, Hitchen, Jackson & Meers (1969) observed that the species was attracted to light throughout the night at Woodchester Park, Gloucs. *Gonodontis bidentata* emerge rather later in the evening (18h 00–22h 00 G.M.T.) and at Aigburth in south Liverpool (site 87) do not assemble in appreciable numbers before 23h 00 G.M.T. (table 3). One male was observed, however, flying and feeding from lilac flowers at about 21h 15.

Copulation in *B. betularia* is prolonged. Mating may take place on the evening a female emerges and the pair frequently does not separate until late the following afternoon. In spite of this, if the moths are separated as early as 07h 00 on the morning after copulation commences fully fertile eggs are produced (Bishop 1972). The female produces many (*ca.* 2000) small, pale-green eggs. These eggs are ovoid, less than 1 mm long and the female uses her elongated ovipositor to insert them into crevices in bark. *Gonodontis bidentata* mates for a short time (1–2 h) and produces a relatively small number (100–300) of large (2–3 mm long) pale-green eggs. The eggs are cemented in groups onto flat surfaces and turn from green to purple in about three days if they are fertile.

Newly hatched larvae of *B. betularia* are small and may hang from silk threads; at this stage there is a possibility of wind-blown dispersal. *Gonodontis bidentata* larvae are larger, start feeding immediately on emergence from the egg and are not likely to disperse in this way. Unlike some of the noctuids, females of the species in this family appear to fly rarely, those that are caught usually being virgins (Clarke & Sheppard 1963).

The larvae of both species feed on the foliage of a wide variety of deciduous trees. *G. bidentata* also feeds on privet (*Ligustrum ovalifolium* Hassk.) and in towns the larvae are sometimes so abundant that they will defoliate a section of hedge several metres in length. The caterpillars of *B. betularia* can be either bright green or chocolate and some individuals are mottled with both colours. In our study area the larvae of *G. bidentata* were always black and showed a spectacular resemblance to dead privet twigs. Kettlewell (1973 pl. 4.2) illustrates a larva from Scotland showing an excellent resemblance to a lichened twig. We have found this form in southwest England. The larvae of *B. betularia* may feed throughout the day, whereas those of *G. bidentata* feed only at night. In consequence the larval life-history of the latter species is prolonged and pupation occurs in late October or November. *B. betularia* pupates naked in the soil, whereas *G. bidentata* pupates in the leaf litter on the soil surface or in debris hanging above the ground, spinning a delicate silken cocoon that incorporates leaves and twigs from the vicinity of the site. The majority of *G. bidentata* pupae we have collected from the bottom of privet hedges in Manchester and Nottingham have been attacked by the fungus *Paecilomyces farinosus* (Dickson ex Fr.) Brown & Smith and others have produced two hymenopteran species *Meteorus ? deceptor* (Wesmael) (Braconidae) and *Dusona* sp. (Ichneumonidae).

(*b*) *Trapping*

Trapping was carried out with light traps and assembling traps. The light traps were adaptations of the Robinson type (Heath 1970; Robinson & Robinson 1950; Southwood 1966) lit by 125 watt mercury vapour discharge lamps with pearl glass covers (Ecko MB/U) and run from mains power supplies. This type of lamp, emitting light in both the ultraviolet and the visible range, has been shown to be very effective as an attractant for moths (Heath 1970; Mikkola 1972). The assembling traps utilized the sex pheromones of one to four virgin females contained in a mesh compartment in the centre of an oblong box with funnel-shaped entrances at each end. At a few sites more than one assembling trap was used. *G. bidentata* is less often caught in mercury vapour light traps than *B. betularia* and is taken more frequently in assembling traps. Whenever possible the traps were emptied every day and the moths either removed or marked and released. In some cases both light and assembling traps were used together at the same site.

There is no reason to believe that the morph frequency of a sample is affected by trap type. In the long sequence of captures of *B. betularia* by both methods made by Clarke & Sheppard at Caldy (see table 1 (appendix) and Whittle *et al.* 1976) the frequency of the *carbonaria* melanic in the two sets of samples may be compared. In only one case does the difference approach significance (for 1966, $\chi^2_1 = 3.59$) and a test for a difference carried out by Whittle *et al.* provides $\chi^2_{11} = 5.98$, which is to be expected by chance. The samples from assembling traps consist only of males, as do, with very few exceptions, the light trap samples. Some of the results for *G. bidentata* are for samples of larvae collected just before pupation in September and October. These are identified in table 2 (see appendix 1).

The main series of data was collected between 1971 and 1975, but for completeness we also include earlier figures for the area taken from published sources. The complete series are given in tables 1 and 2, listed from east to west. The Ordnance Survey six-figure grid reference for each site is given, with the name of the town or district and in some cases the street at which the trap was situated. The position of sites as numbered in table 1 is shown in figure 2. The survey would not have been possible without the generous cooperation of many individuals and local authorities who allowed us to use their gardens and parks; to these people we are extremely grateful. A social effect of the urban environment made itself felt during the course of the work; in the poorer districts of the cities sampling was often impossible owing to damage to the traps by vandals.

We are grateful to Professor Sir Cyril Clarke and Professor P. M. Sheppard for permitting us to include data on *B. betularia* for several sites at the west of the transect. These include the long series from Caldy and Meols, sites 108 and 101, which are treated more fully elsewhere (Whittle *et al.* 1976). Dr S. L. Sutton has kindly allowed us to use results for both species collected by him in Leeds (sites 1 and 2, both tables).

(*c*) *Scoring of morphs*

If one could select from a large number of specimens of *B. betularia* taken from several localities in different parts of Britain it would be possible to assemble a series of moths showing a complete spectrum of variation from almost completely black (the *carbonaria* morph) to the very pale non-melanic or typical form illustrated by plate 9.1 of Kettlewell (1973). However, series of moths from one site or local region show marked discontinuities between phenotypes and we have distinguished three classes: *carbonaria*, *insularia* and typical.

The black form *carbonaria* has a very distinct phenotype controlled by a gene dominant in its effect. However, *carbonaria* does show variation: some individuals that we have seen, particularly from the Wirral area of Merseyside and from north Wales, have areas of white on the anterior surfaces of the hindwing. These patches of pale pigment would normally be hidden by the black forewing while the animal was at rest. Several individuals have been collected in the Wirral that resemble 'ancient *carbonaria*' illustrated in pl. 7.1 of Kettlewell (1973). One such male (very similar to 3 in Kettlewell's plate) was mated to a heterozygous female of 'modern' *carbonaria* phenotype. Unfortunately only a few offspring were reared and only three males retained for examination, two being *carbonaria* and one typical. This confirms that the original male was a heterozygote. It is therefore possible that the class that we have designated *carbonaria* is genetically heterogeneous. The genetics of the so-called 'ancient *carbonaria*' form requires investigation.

Table 3. Assembly times of *Gonodontis bidentata* at Aigburth, south Liverpool

(Traps were inspected at half-hour intervals until 23 h 00, they were examined at 08 h 00 the following morning. Dusk was at approximately 20 h 30 G.M.T.)

	number assembled	
date of observation	before 23h 00	after 23h 00
16–17 May 1974	1 (at 22h 15)	21
17–18 May 1974	0	14
18–19 May 1974	0	4
total	1	39

Table 4. Results of crosses of *B. betularia* involving the *insularia* form

parents		progeny			
male	female	*ins*^{5}	*ins*^{4}	*ins*^{2}	typical
1. *ins*^{5}/typ	× *ins*^{4}/typ	30	17	0	13
2. *ins*^{4}/typ	× *ins*^{5}/typ	21	10	0	8
3. *ins*^{4}(wild)	× *ins*^{5}/typ	10	7	0	9
4. *ins*^{5}(wild)	× *ins*^{4}/typ	86		0	24
5. *ins*^{4}/typ	× typical	0	12	0	12
6. typical	× *ins*^{4}/typ	0	11	8	8

The name *insularia* is used for phenotypes intermediate between *carbonaria* and typical, which vary considerably in appearance. They are controlled by alleles at the same locus as *carbonaria*, recessive to it but dominant to typical. Two *insularia* alleles are known (Clarke & Sheppard 1964; Lees 1968), and there are probably several with progressively darker expression. At Woodchester Park, Gloucs., where the frequency of *insularia* is high, at least two categories are present (Askew *et al.* 1971). The darkest is similar to *carbonaria* but has a small amount of white flecking on fore and hind wings (like the individual referred to as *ins*^{5} in pl. 9.1 of Kettlewell 1973, or the insect in pl. 1c, top right, of Lees & Creed 1975), while the paler form is intermediate between it and the typical (Kettlewell's *ins*^{3}). The genetics of *insularia* has recently been discussed by Lees & Creed (1977) and the phenotypes by Steward (1977).

Among our reared material, obtained from crosses of moths bred by Mr A. G. Liebert, are some progenies which have a bearing on the genetics of *insularia* (table 4). These involve dark and medium *insularia* individuals originally taken from the wild in Somerset and Gloucestershire respectively. The dark ones resemble *ins*^{5}, while the medium ones are like *ins*^{4}. Animals scored as typicals are frequently more strongly flecked than the one so labelled in Kettlewell's pl. 9.1 and resemble *ins*^{1}. Pale *insularia* individuals like *ins*^{3} are, however, definitely distinct from the typical class.

In table 4 the parents of known genotype are derived from crosses of *ins*^{5} to typical or *ins*^{4} to typical and are heterozygous for the *insularia* and typical alleles. Broods 1 and 2 suggest a dominance series of *ins*^{5} to *ins*^{4} to typical. The *ins*^{4} parent of brood 4 is very dark; if it is a misscored *ins*^{5} then the cross is showing segregation of *ins*^{5} and typical, and does not differ from a 3:1 ratio. There is a range of phenotypes in the non-typical class, varying from *ins*^{5} to *ins*^{4}. Brood 6 indicates segregation of typical and very pale *insularia* (*ins*^{2}). The typical allele of the female is from the same stock as that of the female in brood 1, so that the other parent may have been misscored. There are therefore some crosses showing clear segregation of *insularia* into separate classes, but two cases where some of the insects cannot be classified with confidence. Kettlewell (1965*a*) has previously shown that the genetic background may modify the expression of the melanic morphs.

Where the frequency of *carbonaria* is high in northwest England almost all the *insularia* which are present are of the very dark type. In north Wales, however, the *insularia* are pale in appearance (Bishop 1972). Only one category is given in table 1 (see appendix 1), the frequency being low throughout the transect.

Typical may also be a heterogeneous class. Those that we collected in areas where the frequency of *carbonaria* was low (less than 10 %) resembled that depicted by Kettlewell, pl. 9.1. Individuals collected to the east of this had more melanin and appeared to be intermediate between the pale specimens and the typical example of the north American species *Biston cognataria* illustrated in the same plate. Bailey, Clarke, Sheppard & Whittle (1973) occasionally found difficulty in distinguishing pale *insularia* from typical in samples from the Isle of Man.

Two phenotypic classes were distinguished in *G. bidentata*. The dark brown, almost black morph *nigra* is dominant and there is a polymorphism for this morph and the lighter non-melanic form throughout northwest England and Yorkshire. It is also found in the Midlands at Cannock (Kettlewell 1973) but its place is taken in Birmingham by a dark form under multifactorial control (Kettlewell 1973). Further to the south, e.g. at Wytham Wood, Berks. and Woodchester Park, Gloucs., the typicals are very pale and *nigra* is absent, but *nigra* occurs again in London (Kettlewell 1973; A. G. Liebert, personal communication). In the northwest the non-*nigra* individuals are darker than in southern England and sometimes approach *nigra* in phenotypic appearance. Dark typical and *nigra* individuals are, however, easily distinguished; the latter have uniform dark brown wings which contrast with pale beige hairs on the thorax, while dark typicals have the wings and thorax of the same general hue but the typical black markings on the wings are plainly visible (figure 1, plate 1). We were unable to separate the typicals into distinct categories, so that table 2 is divided into two classes, *nigra* and typical.

(*d*) *Local atmospheric pollution*

In the Warren Spring Laboratory publications the northwest industrial belt is divided into Merseyside, Central, and Manchester sub-regions. Of these, Manchester has the highest levels of smoke and sulphur dioxide while the Central area around Warrington has the lowest (Warren Spring Laboratory 1972). Merseyside and the Central area are very similar in sulphur dioxide concentration. The differences between the sub-regions are associated with the effectiveness of dispersion, Manchester, lying beneath the Pennines, having the lowest dispersion rate and the Central area of low lying and wind swept ground the highest. This picture is backed

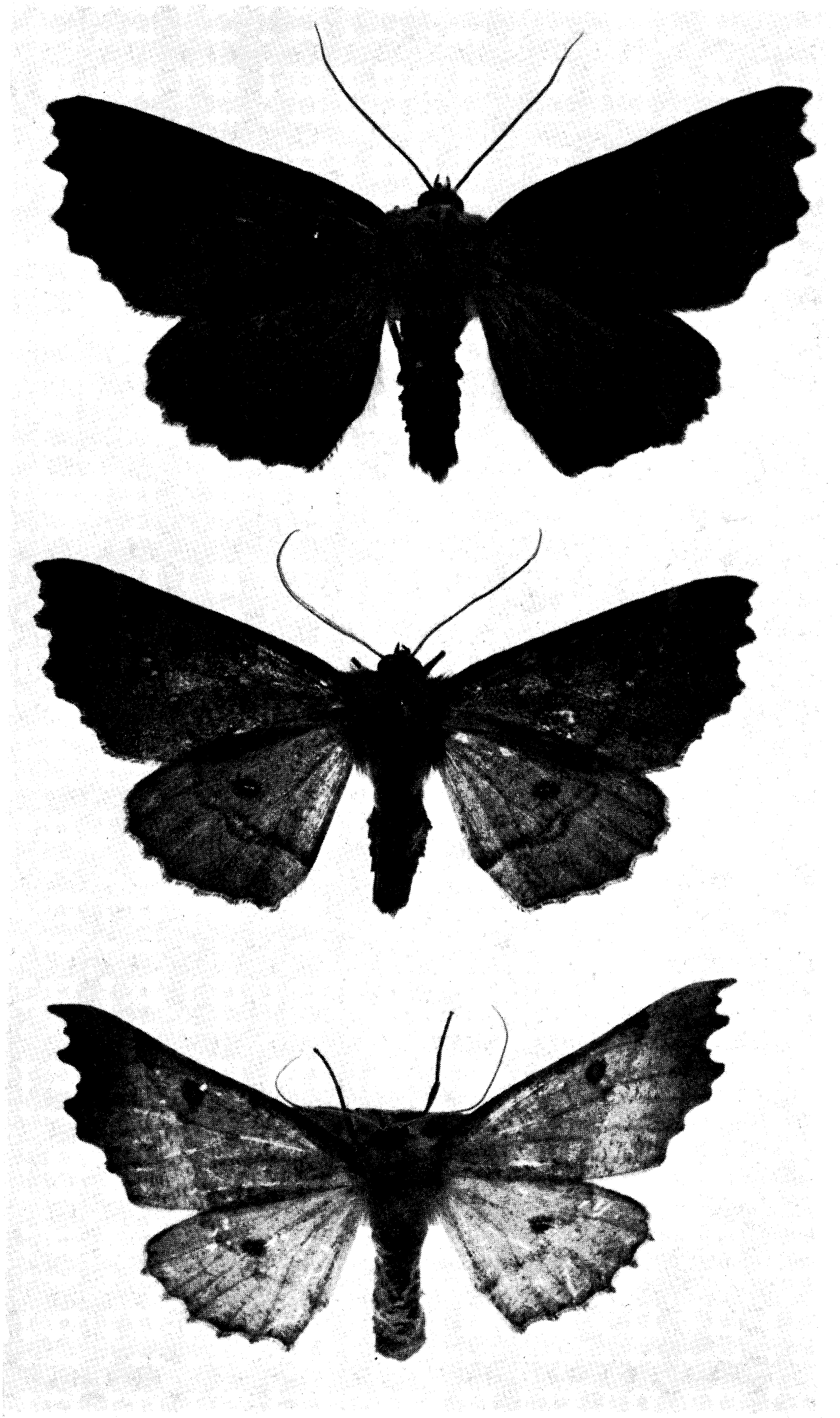


FIGURE 1. *Gonodontis bidentata.* Top, the melanic form *nigra*; centre, a dark non-melanic individual from the study area in northwest England; bottom, a pale non-melanic of southern English type.

(*Facing p.* 496)

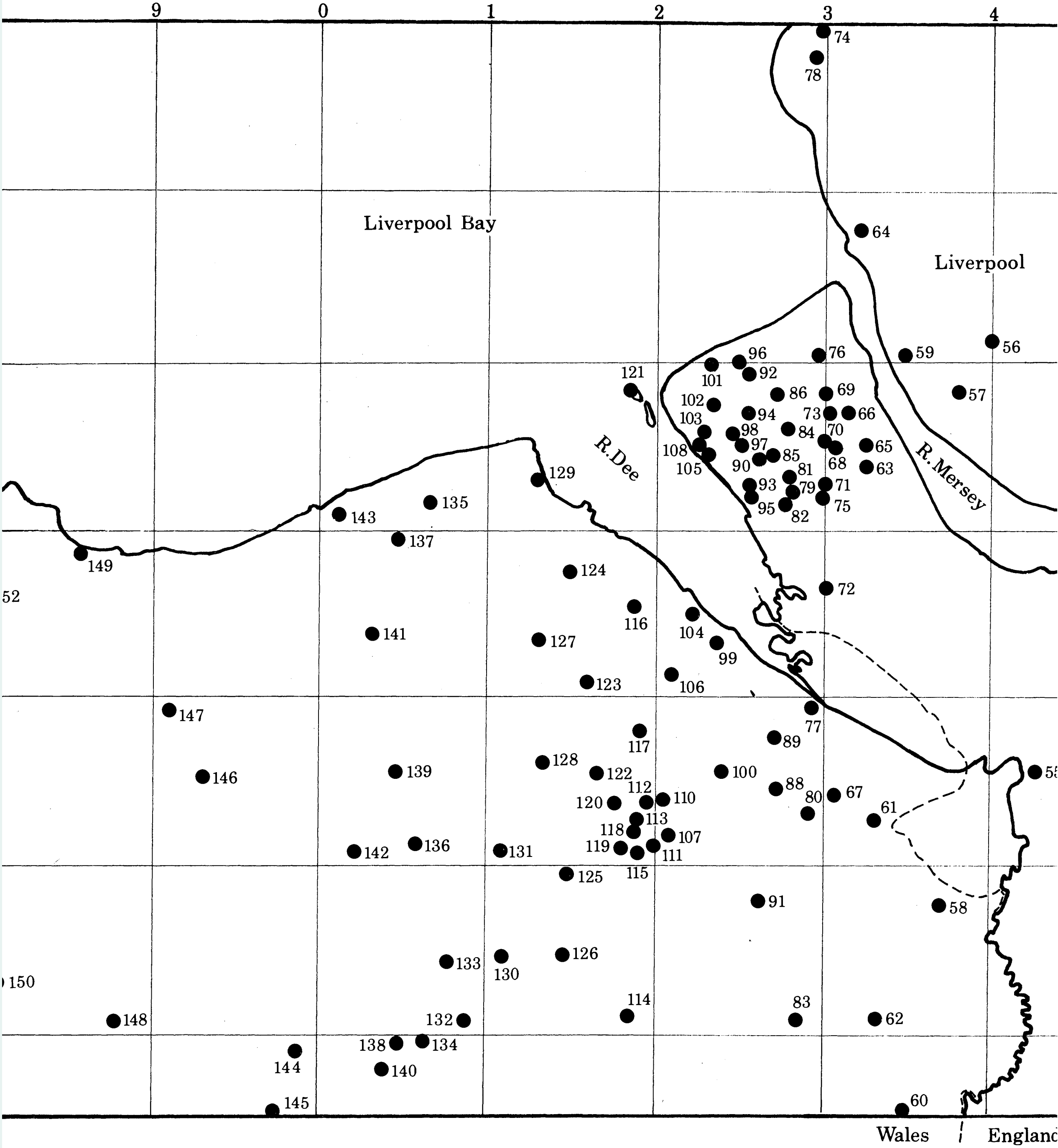
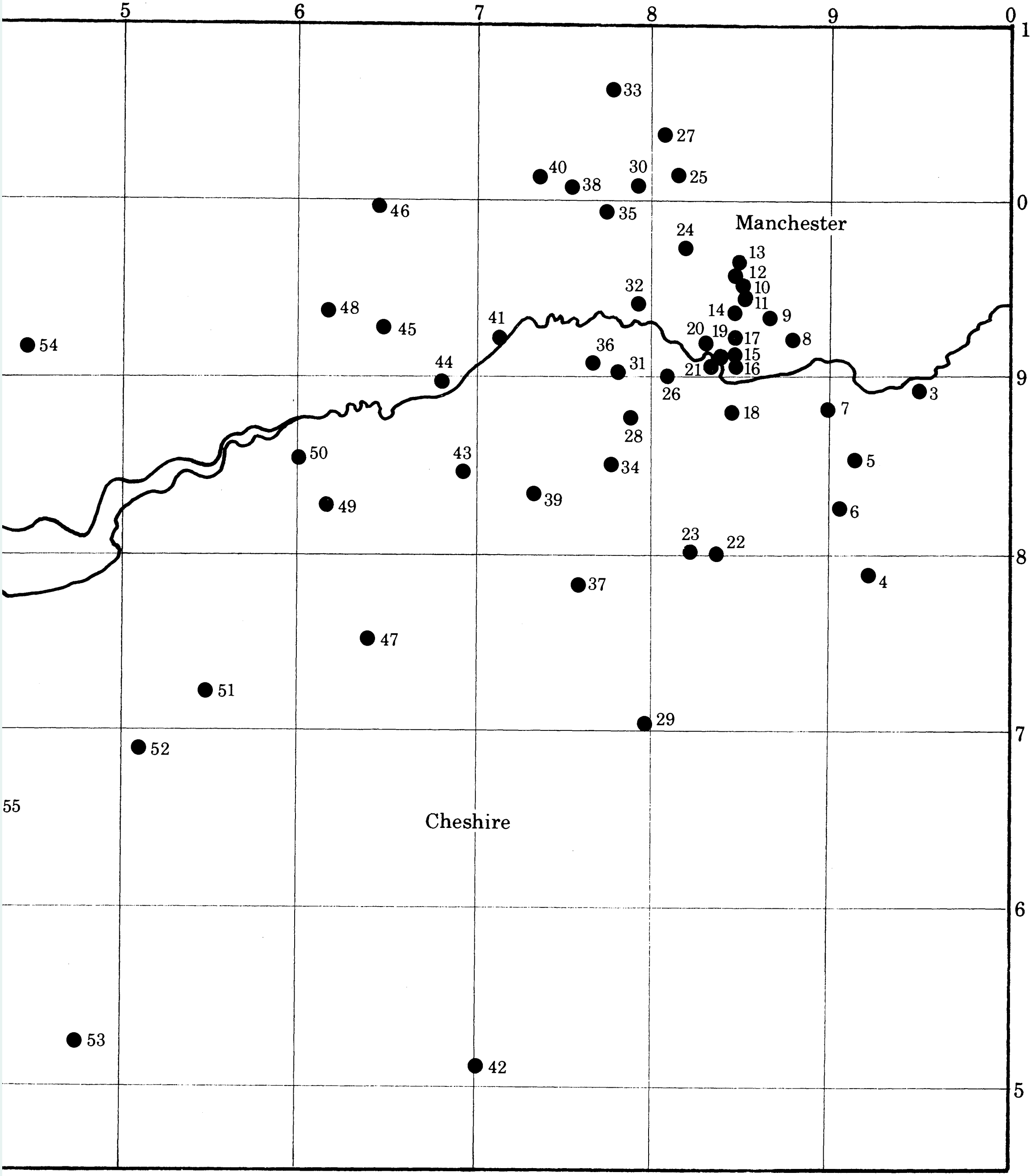


FIGURE 2. Map showing outline of the study area. The posit
Survey 10 km grid. Trapping sites for *Biston betularia* a



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sition of the coast is shown and the Ordnance are indicated, numbered as in table 1.

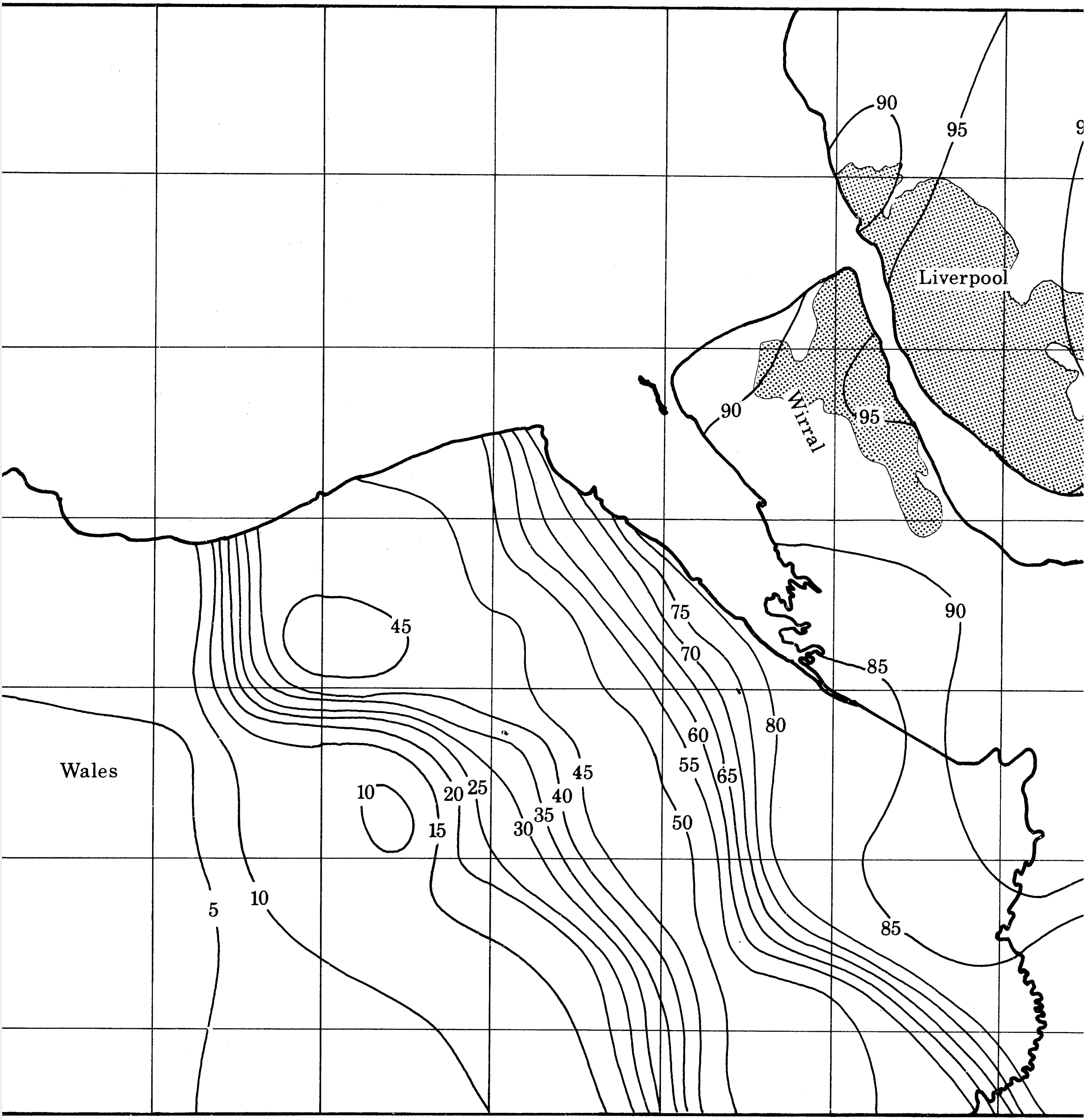
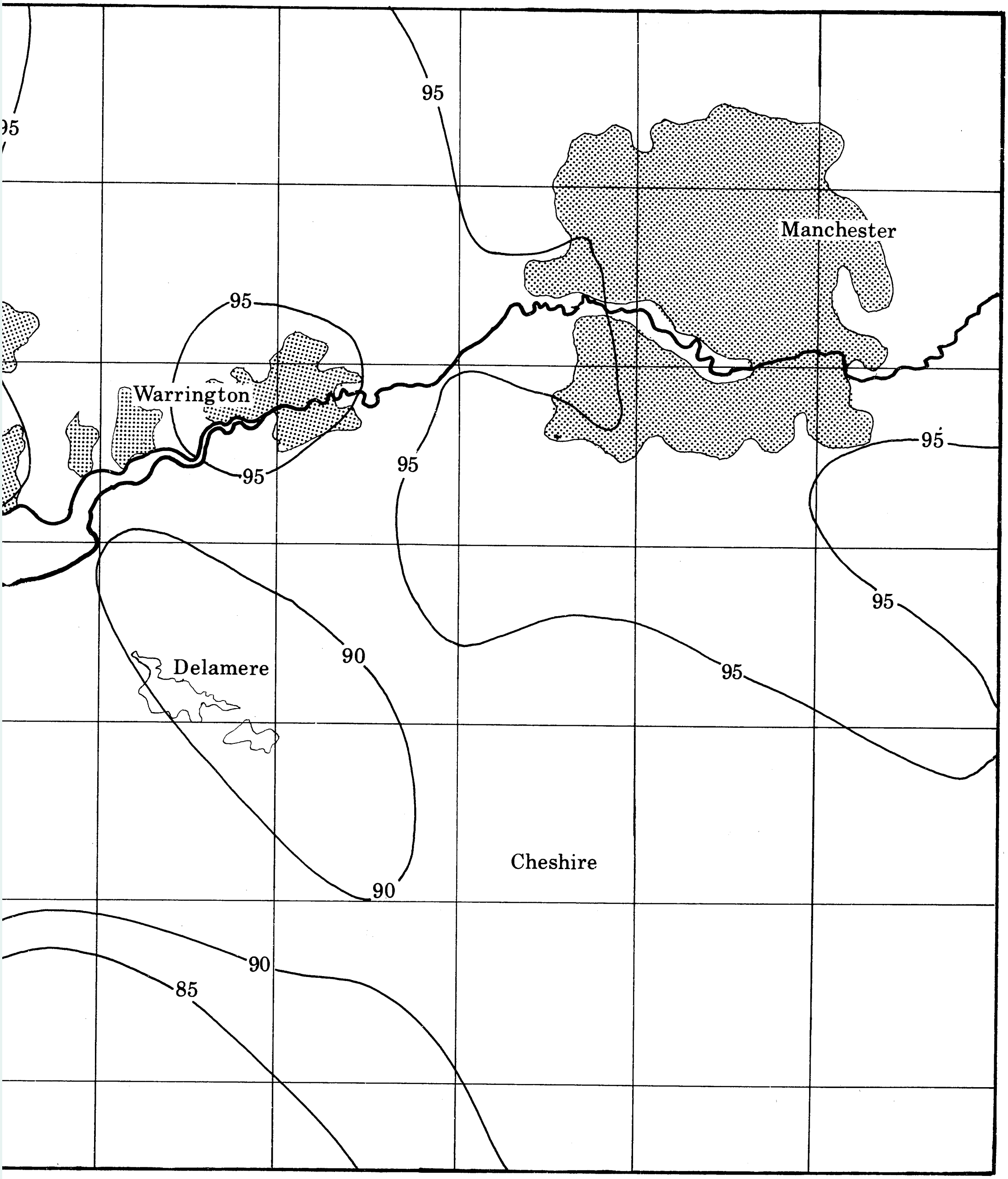


Figure 3. Map showing contours of frequency of the melanic m
are produced by interpolation between estimates for the sa
the site frequency by its variance. The Ordnance Survey 1



orph *carbonaria* in *Biston betularia*. The contours mpling sites listed in table 1, after weighting of 0 km grid is indicated.

up by the records for 1973–4 shown in table 5 (see appendix 1). The linear regression of annual concentration on distance east for smoke is significant ($P < 0.01$). For sulphur dioxide the much more irregular annual values, some of them inflated by high summer averages do not show a significant increase to the east, but the Central sub-region appears to have fewer very high records than the other two sub-regions.

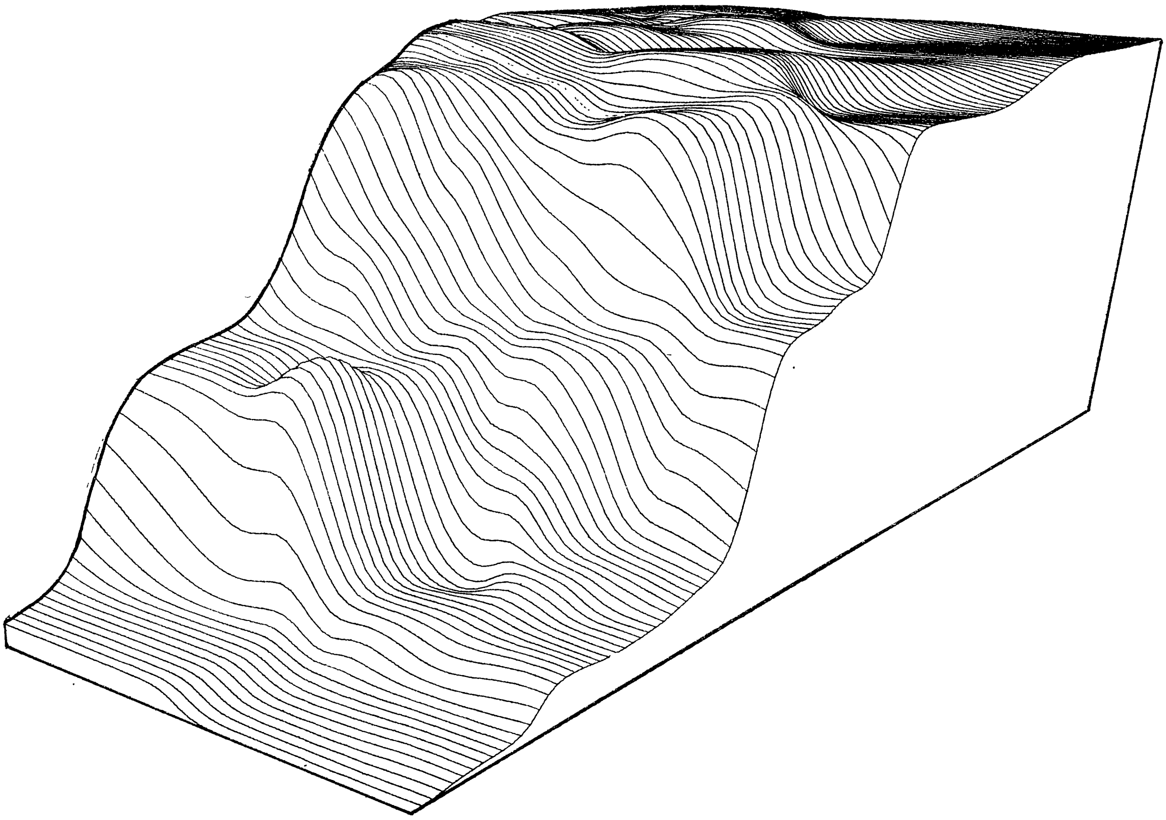


Figure 4. Three dimensional representation of the morph frequency data for *carbonaria*. The surface is viewed from the south-west. The axes at the base of the figure run west to east and south to north, the height indicates morph frequency. The viewing angle is 30° from the horizontal.

3. Graphical display of results

The tabulated data are shown graphically in figures 3 to 6. For this purpose the sites were located to the nearest 1 km^2. Unless there is significant change in frequency the results for separate years have been combined at each site and the frequency was then weighted by dividing it by its variance so as to adjust for variation in sample size. If the frequency was 1 or 0, then in order to introduce a reasonable weighting it was assumed that one allele of the rare type was present when calculating the binomial variance. The weighted results for each trapping site were then used to calculate interpolated values for each corner of a rectangular 1 km grid. The program to do so is based on an algorithm by Adams (1970), and was written for the purpose by Bishop and Dr R. J. White at the University of Liverpool. The calculated grid has then been used as the data input for programs to produce contour maps (figures 3 and 5) and three-dimensional perspective veiws of morph frequency (figures 4 and 6). The programs used here are CONTOUR, written originally at the University of Manchester Institute of Science

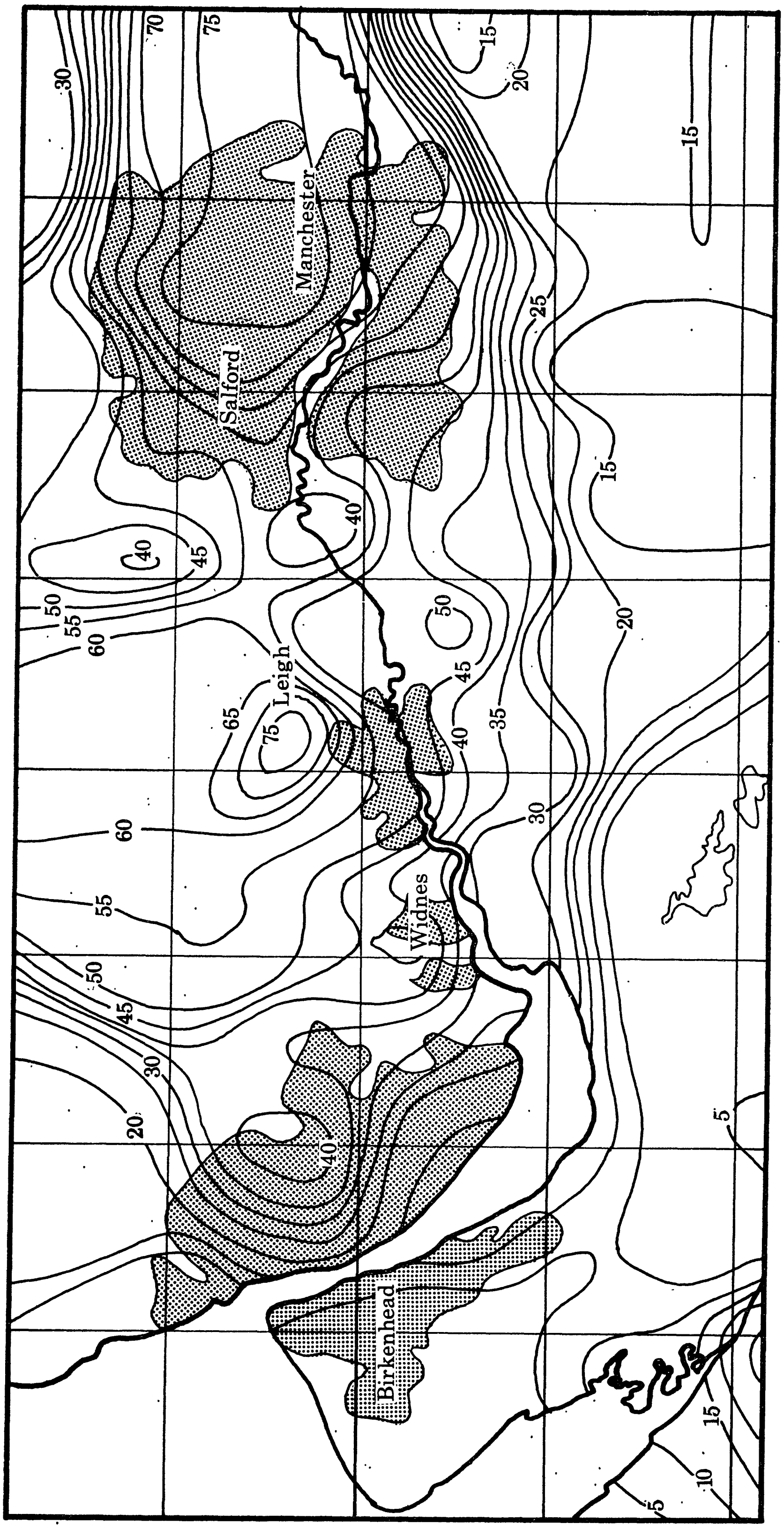


FIGURE 5. Contours of frequency of the *nigra* morph of *Gonodontis bidentata*. The map is prepared like figure 1, by using data shown in table 2. The area covered is smaller than that of figure 1.

and Technology, and SYMVU, originating from the Laboratory for Computer Graphics at Harvard University and modified at the Edinburgh University Regional Computing Centre. Both of these are available at the University of Manchester Regional Computing Centre, where they have received further extensive modification. In this way we have produced maps which are reasonable objective estimates of the more or less continuous pattern of morph frequency represented by our sample sites. The three-dimensional views in Bishop & Cook (1975) were produced using the program SUDS from the University of Liverpool Computer Laboratory.

The surface produced for *B. betularia* consists of a plateau of high melanic frequency over the industrial area and extending southwards to the rural farmland of the Cheshire plain. It falls slowly from over 95% in the Manchester region in the east to around 90% on the Wirral peninsula, but the move from urban and industrial to rural parts of the area produces no perceptible change. There is a sharp decline in frequency beginning at the high land to the west of the Welsh border, where the frequency falls from 85% to 10% over a distance of about 25 km. Along the north Welsh coast the melanic frequency declines at a somewhat lower rate than it does inland. The picture is essentially a simple one, in which a plateau of high frequency to the east is connected to one of low frequency to the southwest by a transition area of limited extent. At neither end are the populations monomorphic.

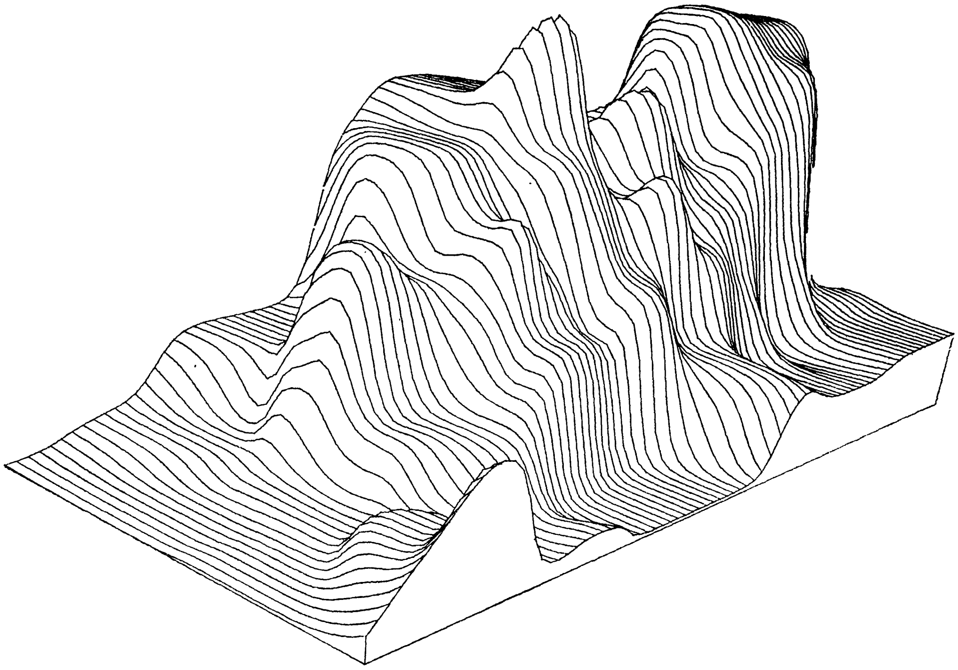


FIGURE 6. Three dimensional representation of morph frequency of *nigra* in *Gonodontis bidentata* viewed from the southwest.

Gonodontis bidentata exhibits local differentiation on a much smaller scale. There is a peak of around 80% melanic frequency in Manchester and others at Warrington, Widnes and Liverpool along the main industrial spine, each separated by lower frequencies. These peaks decline from east to west, so that Liverpool has no more than half the frequency of Manchester. The River Mersey appears to constitute a southern boundary to this ridge of high melanic frequency; there is a sharp drop from Liverpool to Birkenhead at its mouth and from those parts of Manchester north of the valley to the suburbs south of it. In the southern area frequencies drop from around 20 % in the east to about 5 % in the west, with a local peak at the industrial area at the head of the Dee estuary.

4. Change in frequency with time

The history of industrial melanism suggests that a reduction in atmospheric pollution is likely to result in a drop in the frequency of melanic forms although for *B. betularia*, at least, the change is expected to be slow. There are two reasons for this. The main selective advantage of the *carbonaria* morph in polluted areas is its crypsis on the lichen-free and blackened tree trunks which are the daytime resting sites of this moth. In the past high levels of sulphur dioxide in the air have prevented lichen growth in urban and industrial areas. The reduction of sulphur dioxide levels allows the reappearance of lichens and other tree trunk epiphytes and their presence on the trunks reduces the cryptic advantage of *carbonaria*. As all these epiphytes are slow growing there will inevitably be a time lag between the reduction of sulphur dioxide levels and the reappearance of sufficient epiphyte growth to reduce the crypsis of *carbonaria*. An additional factor is that reduction of sulphur dioxide levels has not yet been achieved throughout the area (Bishop *et al.* 1975; Cook & Wood 1976). However, even if the selective value of typicals compared to *carbonaria* has changed substantially the sigmoid pattern of response of gene frequency to selection means that there will be only a small initial decrease in an area like the study transect where the *carbonaria* morph frequency is high.

Nevertheless, individual cases of a drop in melanic frequency have been recorded. In a series of collections starting in 1959 Clarke & Sheppard (1966) found a significant decline in *carbonaria* at Caldy (site 108). The series up to 1975 is shown in table 1, and the drop in frequency continues throughout the period. These results are considered fully by Whittle *et al.* (1976), who also consider the effect of selective predation by bats. In Manchester Cook *et al.* (1970) obtained significantly more typicals in 1966–9 than were present in an equivalent survey made between 1952 and 1964 (Kettlewell 1965*a*, 1973).

More recently, Lees & Creed (1975) examined the data for the whole of England, either by comparing Kettlewell's 1952–6 data with later samples, or where data for 1952–6 are lacking, by dividing the subsequent results into two halves. They calculated χ^2 from the 2×2 contingency tables so produced and treated the signed square root as a normal deviate when combining results from different samples. It is evident that northeast of a line roughly from north Wales to London, including the industrial Mersey area and Birmingham and also rural East Anglia, the frequency of *carbonaria* has decreased (combined $\chi^2_1 = 41.5$), while in southwesterly parts of the country there has been a net increase in *carbonaria* (χ^2_1 from combined data $= 24.9$ $P < 0.001$).

The results in table 1 have been examined in the same way with a view to detecting a change in frequency of *carbonaria* compared to non-*carbonaria*. Since we do not know exactly when the drop is likely to begin all the sites represented by more than one record have been examined even though in some cases the comparison is between a record in the 1950s and one in the 1960s while in others it is between two successive years in the early 1970s. When more than two samples are available the data have been divided into approximately equal halves. These procedures allow a total of 35 comparisons to be made. Taken individually, Hale (site 34) shows a significant increase in *carbonaria*, Caldy, Wirral and Bodynys (sites 108, 109 and 131) show significant declines, while for the rest the change is non-significant. It is in the downward direction at 22 sites, upwards at the other 13. The overall χ^2 measuring the decline in frequency at the 35 sites is 4.29 ($P < 0.05$, 1 d.f.). The same calculation used to compare non-typicals (i.e. *carbonaria* plus *insularia*) to typicals indicated a non-significant decline in the non-typical frequency ($\chi_1^2 = 3.28$).

The test of all available data therefore shows only slight evidence of a response of morph frequency to change in the environment. The test is not very accurate when expected numbers in some classes are small, as in many of the samples included, but this is likely to lead to an overestimate of the significance of the change rather than an underestimate. On the other hand, change is not expected at all sites at the same time, but will vary in an unknown way; in addition, the early and late results are closely spaced. If sites have been included where the data span a period before the change has begun they will reduce the significance of the trend. At Delamere, comparison of the 1952–6 figures with those for 1961–2 shows a non-significant decline in *carbonaria* measured by a χ^2 of 2.70; comparison of the 1961 result with 1962 indicates a significant increase ($\chi^2 = 16.93$). Thus, although there is good evidence that *carbonaria* is decreasing in frequency in some places, much the best results for the area being those of Clarke and Sheppard from Caldy, a general decrease over the whole region has yet to be established.

A similar result is obtained with *G. bidentata*. Twenty-six sites were examined and combining the data from them we find a net tendency for *nigra* to decrease, but again this is not significant, the overall χ^2 value being 1.87 ($P < 0.2$). A significant decline in frequency of *nigra* was found at only two sites. A χ^2 value of 24.34 is obtained when the two samples recorded from Delamere by Kettlewell (1973) are compared with our 1974 sample (sites 67 and 66 in table 2), and there is also a significant drop at Newsham (site 91, $\chi_1^2 = 5.25$) between a larval sample and an adult one. The results from both these sites, however, must be treated with caution. Delamere is in an area where very dark typical insects are found, so that there is a possibility that the early sample with the high frequency of melanics was scored differently from ours, and not only are larval samples collected under different conditions from adult ones but there is also the possibility of differential survival of the larvae of different morphs. If Delamere and the larval samples are excluded the χ^2 value measuring the trend is reduced to 1.36, so that again the net trend is not significant.

For comparison with the results of the moth surveys we also present some data on melanism in the Two-spot Ladybird, *Adalia bipunctata*. This coccinellid beetle is polymorphic for the non-melanic red form with black spots and a number of genetically controlled melanic morphs. The melanics are at high frequency in industrial parts of Britain and at a low frequency in rural south England. Creed (1971 *a*, 1975) has surveyed the distribution of morph frequencies in Britain and Europe and concluded that the high frequencies of melanics in the British Isles are a direct result of industrial pollution. He has also shown (1971 *b*) that the frequency declined in Birmingham from 1960 to 1969, after smoke control areas had been introduced. The cause of the melanism has been questioned by Muggleton *et al.* (1975, cf. Creed 1975), who argue that the amount of sunlight is the most important environmental agent and that the correlation with industrial areas most probably comes about via the effect of smoke on the number of sunshine hours. Table 6 shows data collected after 1971 and mostly in 1976 for our study area.

There are relatively high frequencies of melanics (70–90 %) in south Lancashire and north Cheshire from Manchester to Liverpool. Chester and Delamere to the south and Billinge to the north of the main industrial transect show rather lower frequencies of 40 to 50 %, and there is a sharp drop on the Wirral to 20 to 40 % from the high frequencies of urban Birkenhead and Liverpool. At the northeastern extreme melanic frequencies in Leeds, an industrial conurbation, are low. The three dimensional representation of these data for the same area as for *G. bidentata* is shown in figure 7.

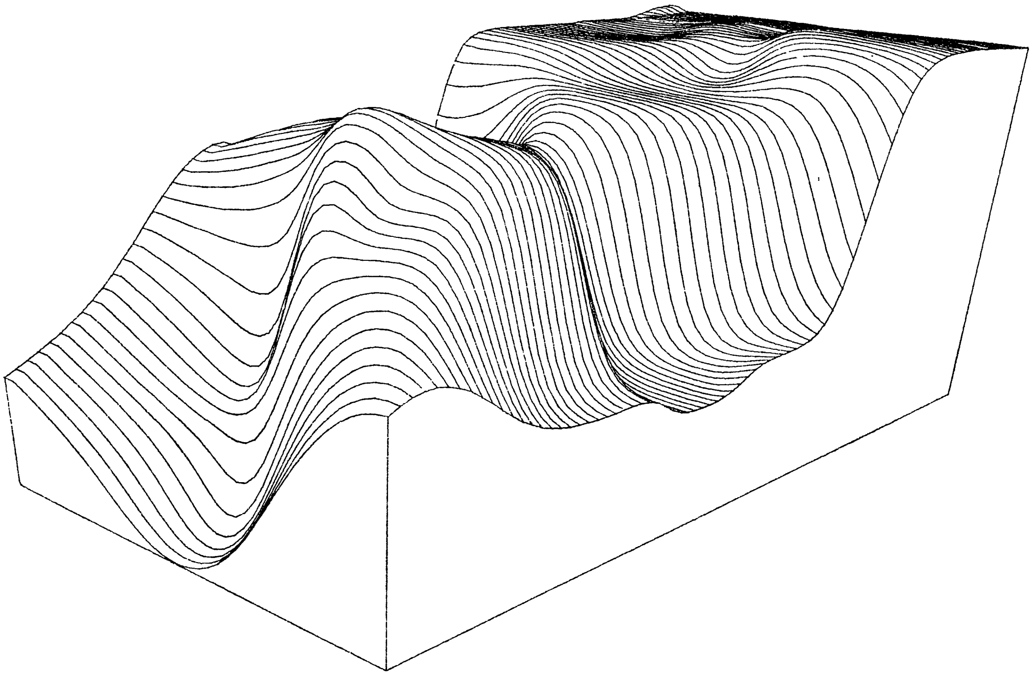


Figure 7. Frequency of melanics in *Adalia bipunctata*. The area and viewing direction are the same as for figure 6. Since the surface is based on fewer data, however, a greater degree of smoothing has been used to give a general indication of the geographical trend in frequency.

Where these records can be compared with earlier ones for the area listed by Creed (1971 *a*) there is almost always a decline in melanic frequency. Eight pairs of samples (site nos. 2, 5, 11, 16, 24, 32, 35, 40 in table 6 (see appendix 1)) have been compared by the method used above, to produce a χ^2 value of 116.5 ($P < 0.001$). A drop is much more evident than in either of the species of moths. This fact is consistent with the belief that the environment has some direct effect (for example, one or more of those mentioned) and does not act via longer term agencies such as change in the appearance of the background resulting from changed epiphyte cover.

5. Date of emergence in *G. bidentata*

The steep clines in the melanic frequency of *G. bidentata* compared to *B. betularia* have been explained in terms of low migration of the adults (Bishop & Cook 1975). In the course of the work, however, we have found considerable differences between localities in emergence time of the adults, which would themselves limit gene flow even if the migration rate is higher than supposed. There also appears to be variation in time of emergence of the two morphs. Bishop & Harper (1970) examined their data from two localities in the Liverpool area and found no evidence of differential emergence. However, some of the later samples do indicate a differential, the evidence for which is presented here.

Daily emergence was recorded at five sites in 1973 and 1974. All were situated in or adjacent to areas of parkland with mixed deciduous woods. The number of moths caught varies from day to day as a result of change in weather conditions and varying attraction of the females, so that for ease of interpretation the results are presented as accumulated daily totals (figure 8) or grouped into periods of a few days (tables 7 and 8).

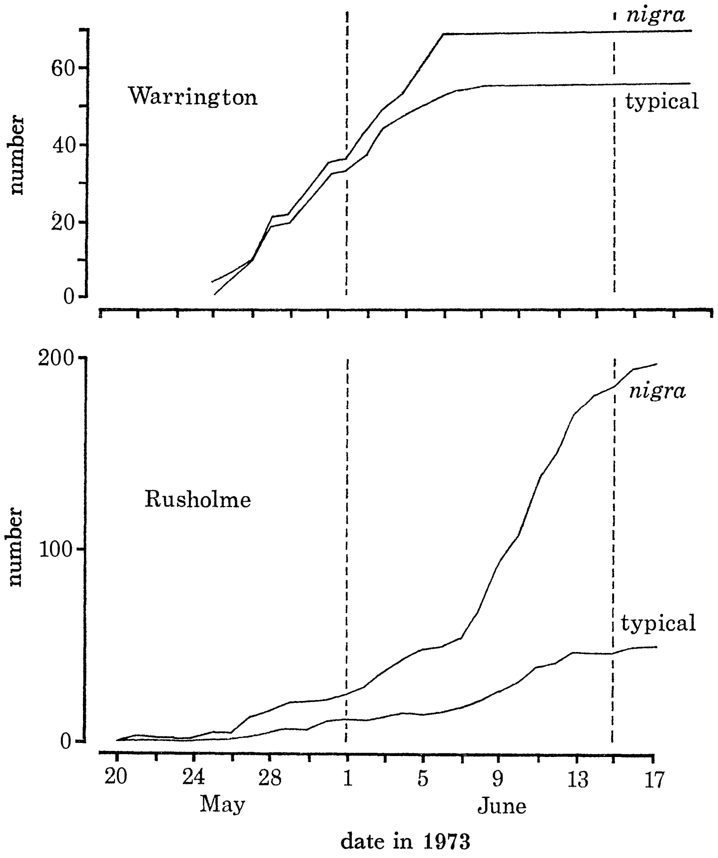


Figure 8. Samples of *Gonodontis bidentata* from Warrington (site 62) and Rusholme (site 18) in 1973. The curves show accumulated daily totals of insects caught for the first time. At Warrington 50% of the total was taken by 1 June, at Rusholme this fraction was not reached until 10 days later. Both sites show a later emergence of *nigra* than of non-melanics.

The 1973 data in figure 8 indicate that at Warrington the moths emerged considerably earlier than those at Rusholme. In 1974 (tables 7 and 8) the moths at Rusholme were later than at Wythenshawe, which in turn had a later emergence than at Aigburth. The difference in flight time of about two weeks between Aigburth and Rusholme is of the same order as the difference occurring between seasons in the Large Blue butterfly *Maculina arion* L. during various periods in the last hundred years, which can be correlated with marked climatic changes (Muggleton 1974). The major difference appears to occur between Wythenshawe and Rusholme, a distance of 7 km, and to explain this in terms of local climate would require a steep climatic gradient. We have no evidence of such a gradient; moreover, the mean temperature in cities is known to be somewhat higher than in the surrounding countryside (Chandler 1962), so that if it were environmental the order of emergence at these sites should be reversed. At Didsbury, which lies between the other two sites there appear to be two periods of emergence: an earlier one corresponding with that at Wythenshawe and a later one which may be equated with the latter part of the emergence at Rusholme. This later part is made up mostly of *nigra*.

The sites with the greater melanic frequency have the later emergence. It is therefore possible that melanics emerge later than typicals so that the differences between sites are genetic in origin. This can be seen by examining the tables. At both Wythenshawe and Didsbury melanic frequency increases towards the end of the season, while at Aigburth and Rusholme the numbers in the rare morph class are so small as to make any difference impossible to detect.

The emergence of typicals at Didsbury occurred with a single mode in numbers, whereas the emergence of *nigra* took place in two phases. The *nigra* gene is visually dominant and it is tempting to suggest that one of the genotypes appears with the typicals while the other makes up the late-emerging component.

G. bidentata is not the only moth for which an increase in melanic frequency during the season has been proposed. Kettlewell (1973) reports that C. J. Cadbury observed an increase from 1.4 % to 8.7 % in the frequency of the melanic morph *nigricata* of *Cleora repandata* L. over seven days in a rural situation in Scotland. This suggests that the melanics were emerging later, although the data could also be explained by a greater survival of melanics compared to typicals. That explanation does not appear to fit our data as we found an abrupt change in frequency unlike the steady increase seen by Cadbury. Poitout (1973), examining recessive melanics in laboratory stocks of the moth *Spodoptera exigua* Hubn., found that individuals of the melanic genotype had a longer total development than those of the non-melanic genotypes. Thus the melanic individuals tended to emerge from the pupae later than the non-melanics. Kettlewell (1973) found some evidence of differential larval growth in laboratory-reared

TABLE 7. *G. BIDENTATA*

(Daily results grouped into six periods for three sites in 1974. The periods were chosen to give maximum information. For each site the first two columns give the numbers of *nigra* and typical caught and the third is the fraction of the total sample emerging in that period.)

	Aigburth			Wythenshawe			Rusholme		
	nigra	typ	percentage of total	*nigra*	typ	percentage of total	*nigra*	typ	percentage of total
before 19.5.74	5	63	15.4	26	31	8.8	0	0	0.0
19–27.5	23	167	43.1	96	115	32.4	18	5	14.7
28.5–5.6	19	126	32.9	101	131	35.6	26	9	22.4
6–14.6	5	32	8.4	61	47	16.6	31	8	25.0
15–23.6	0	1	0.2	22	20	6.5	35	9	28.2
after 23.6	0	0	0.0	1	0	0.2	12	3	9.6

TABLE 8. *G. BIDENTATA*

(Grouped daily results for the Didsbury site (site 21). The layout is as for table 7 but because collecting started later different periods have been used.)

	nigra	typ	percentage of total
15–23.5.74	28	18	15.0
24–31.5	63	27	29.4
1.6–8.6	54	38	30.1
9–16.6	7	7	4.6
17–23.6	52	12	20.9

B. betularia, the larvae which pupated first producing a higher proportion of typicals than those pupating later. If the present example can be substantiated, however, it is the first time that a difference between the two genotypes of a dominant melanic, which could readily affect their relative fitness, has been detected in the wild. Differential emergence between genotypes will result in non-random mating and modify the proportions of the genotypes in the succeeding generation.

6. Discussion

(*a*) *The comparison between the two species*

In this paper we have presented the results of surveys of morph frequencies in *B. betularia* and *G. bidentata* in an area of the British Isles including both polluted industrial and unpolluted rural localities. Experiments on relative survival of the morphs and population parameters are discussed in the accompanying paper, and throw some light on the pattern of morph frequency. The two species present some sharp contrasts. Estimates of fitness in *B. betularia* show a good correspondence with morph frequency. At sites where the environment is unpolluted the typical form has a substantial (50–100%) advantage over *carbonaria*, while in polluted areas typical is at a disadvantage. Agreement between the capture-recapture experiments and those with dead insects indicates that this is due to differential survival resulting from selective predation. Previous studies are in agreement with ours, with the exception of the results of Lees and Creed for East Anglia, which may indicate a different pattern of selection there. The component of fitness measured favours one morph in some regions and the other in others, but populations everywhere are polymorphic. A unitary theory accounting for the polymorphism therefore has to include heterozygote advantage resulting from a balance of selective forces other than those measured, or frequency-dependent selection, or extensive migration from place to place, or some combination of these. In *G. bidentata* the higher fitness values of non-melanics are again recorded at sites where the frequency of non-melanics is higher. The trend is not significant, however, and is seen largely because the estimates from Rusholme, where the non-melanic frequency is very low for the species, are significantly lower than unity. The morph frequency in this species changes over smaller distances than that in *B. betularia* so that if the source of selection measured by the experiments was determining gene frequency and the amount of movement was similar we should expect a steeper regression in *G. bidentata*, not a shallower one.

In fact, migration appears to be very much less in *G. bidentata* than in *B. betularia*. For the latter species the results of Bishop (1972) (discussed by May, Endler & McMurtrie (1975)) and others considered here and in Bishop, Cook & Muggleton (1977) agree in suggesting a root mean squared distance moved per generation of around 2.5 km. In contrast to *B. betularia*, evidence for movement of *G. bidentata* from one trap site to another is almost completely lacking. This fact, together with the sharp changes in gene frequency and the absence of moths at some sites close to ones where samples are large, indicates a much lower distance moved, perhaps no more than one tenth of that in *B. betularia*.

There is an even greater difference in density between the two species. In *B. betularia* the total seasonal population per square km is probably usually in the hundreds (estimates of about 400 on the Wirral and 1200 at Loggerheads have been obtained), while in *G. bidentata* it may be in tens of thousands (three estimates gave 40000 at Wythenshawe, 400000 at Aigburth and 150000 at Cressington), although the distribution is patchy. We therefore have a picture of one species (*B. betularia*) existing at low densities with high mobility and large variation in size and direction of visual selection from place to place, while the other (*G. bidentata*) is much more dense, more static, more patchy in distribution and subject to less variation in visual selection. In many areas an emerging adult *B. betularia* has a high probability of dying in a locality with different selection from that where it emerged, while in *G. bidentata* this probability is small.

(*b*) *Maintenance of polymorphism in* B. betularia

The *carbonaria* morph has been at a high frequency in northern England for about one hundred generations, but in no area are the populations monomorphic for *carbonaria*. Polymorphism is also the rule in areas of Europe where *carbonaria* occurs (Kettlewell 1973; Cleve 1970), and it is also found in most other species of moth with melanic morphs (Lees 1971; Kettlewell 1973; Bishop *et al.* 1976). Although the phenomenon of industrial melanism is well known, the mechanisms involved in maintaining the polymorphisms are imperfectly understood.

In *B. betularia* it is known that *carbonaria* is favoured by strong visual selection in industrial areas and selected against in unpolluted areas, the selective agent being predation by a variety of bird species (Kettlewell 1956, 1958, 1973; Clarke & Sheppard 1966; Bishop 1972; Lees & Creed 1975; Bishop *et al.* 1977). It is not clear how or to what extent other, non-visual components of fitness interact with selective predation to maintain a polymorphism. Apart from moths a number of other insect species exhibit industrial melanism and of these the most studied is the aposematic ladybird *Adalia bipunctata* which behaves as an industrial melanic in some industrial and urban areas. For this species it seems that physiological differences related to the toxic properties of pollutants (Creed 1971), to differences in the thermal response of the morphs (Lusis 1961; Muggleton *et al.* 1975), to a seasonal difference in viability between the morphs (Timofeyev-Ressovsky & Svirezhev 1965) or to a combination of these (Creed 1975) are the prime factors involved, with selective predation perhaps playing a minor rôle in certain localities.

In B. betularia there is a certain amount of circumstantial evidence for non-visual fitness differences, although it is still not clear exactly what part they may play in maintaining the polymorphism. The main question is the extent to which they contribute to fitness differences between genotypes rather than morphs. Visual selection results in a disadvantage to the unfavoured morph in polluted industrial areas or in unpolluted rural ones of around 10 to 60 %. A non-visual fitness difference will make a material contribution to maintaining polymorphism or to changing gene frequency if it is of the same order. It will have a negligible effect if it is much smaller, for example between mutation rate and about 1 %. It is therefore essential to establish whether the non-visual fitness effects operate within the higher or the lower range. In order to focus the issue, three possible situations may be considered.

Heterozygote advantage exists irrespective of selective predation.

For non-visual reasons the heterozygote is substantially fitter than both the typical and the *carbonaria* homozygote. Visual selection then acts upon the system to suppress the melanics in unpolluted areas while permitting them to reach high frequencies in polluted ones.

Selective predation and homozygous melanic disadvantage are balanced.

The *carbonaria* homozygote is substantially less fit than the heterozygote for some physiological or metabolic reason. This difference is balanced by selection against typicals in industrial areas, so that polymorphism results there although not where typicals are at an advantage.

Both of these systems could operate if migration rates were very low.

Selective predation is the sole important selective agent.

The non-visual fitnesses of the three genotypes are more or less equal (within about 1 %) and the pattern of morph frequencies is due to variation in visual selection from place to place. The selection may be frequency-dependent in effect or balanced by migration to result in polymorphism.

(i) *Heterozygote advantage exists irrespective of selective predation*

The first of these possibilities is one which implies a highly evolved gene. Either the melanic mutant, when it first arose, exhibited fortuitous heterosis, an unlikely event, or the heterosis has developed by modification of dominance of expression. Ford (1971, and in earlier books and papers) has argued the case for evolution of gene expression most powerfully. In the present example the time scale seems short if we are to allow adjustment of expression since the Industrial Revolution, but it may be supposed that *carbonaria* was at one time favoured, that it developed heterozygote advantage during the favourable period and was then kept down to mutation frequency during the intervening period until industrialization provided backgrounds on which melanic insects again became cryptic and advantageous. This possibility has been discussed in relation to the general rise in frequency of melanic morphs in moth species (Ford 1937; Kettlewell 1973). Kettlewell (1973) supposed the advantage to have existed in the past (*ca.* 10000 years B.P.) when the prevalence of pine trees provided contrasting backgrounds against different elements of which typicals and melanics were conspicuous. If this argument is correct then the northern pine forests of Europe should favour melanics at the present time and yet *carbonaria* reaches high frequencies only in southernmost Sweden and eastern Denmark (i.e. outside the main coniferous belt) and decreases rapidly northwards (Douwes cited in Kettlewell 1973).

The theory that *carbonaria* exhibits heterozygote advantage in the absence of visual selection has been discussed in detail by Lees & Creed (1975). They point out that *carbonaria* is at a high frequency in rural East Anglia (up to 80 %), that they have indirect evidence of non-visual fitness differences favouring *carbonaria* over typical, and that certain improvements can be made to the fit of a theoretical cline to Bishop's (1972) data for north Wales if a large reduction of fitness of the typical is assumed compared to the fitness of the typical/*carbonaria* heterozygote. They suggest selection against typicals of 30 % and against *carbonaria* homozygotes of 10 %, compared to the heterozygote.

The situation in East Anglia described by Lees & Creed (1975) appears unlike that in any other regions where estimates of selection have been made, in that high frequencies of *carbonaria* are not accompanied by a visual selective advantage of the melanic. It is generally agreed that the frequency of *carbonaria* in most parts of the country is related to the condition of the tree trunks on which the moths rest. Measurements in East Anglia indicate that the tree surfaces are pale; data collected by Lees & Creed would be associated with lower melanic frequencies in other parts of the country (Creed, Lees & Duckett 1973). However, lichen cover in East Anglia is also low. Epiphyte cover may be used to estimate sulphur dioxide level, and Creed *et al.* found that their biological method of estimation based on a number of parameters related to the condition of tree trunks gives consistently higher estimates than would be expected from a rural area. Similar results have been obtained in surveys which used only lichens as indicators (Hawksworth & Rose 1970; Gilbert 1974). Direct measurements of atmospheric sulphur dioxide have been made in the region at Framlingham and Sibton (Warren Spring Laboratory 1973).

They show levels which are about half those estimated by Creed *et al.* (1973) from the appearance of the trees. It seems probable, therefore, that in East Anglia some factor other than sulphur dioxide is reducing the epiphyte flora of the tree trunks. This is an area of intensive agriculture and the absence of lichens may be at least partly due to agricultural sprays drifting in the wind (Hawksworth, Coppins & Rose 1974). We do not know how much paleness *per se*, as compared to the variety of epiphyte flora, contributes to the relative fitness of the morphs of *B. betularia*. Creed *et al.* (1973) have assessed the relative importance of different factors by multiple regression analysis, but we have already emphasized the weakness of this approach (Bishop *et al.* 1975). It remains possible that *carbonaria* is in fact better protected than appears at first sight.

The question then is whether the frequency data are consistent with the results of predation experiments in East Anglia. The experiments of Lees & Creed (1975) were not so rigorous as those of other workers (Clarke & Sheppard 1966; Bishop 1972; Bishop *et al.* 1977), but even if the selection against *carbonaria* is established the degree of selection measured in 1973 is not necessarily an indicator of conditions in the area when high frequencies of *carbonaria* first arose. The high frequency could in part be due to a cryptic advantage of the morph at some time in the history of melanism in the area.

Lees & Creed also re-examined the data for the north Wales cline discussed by Bishop (1972) and included with additional data in this paper. The difficulty here is that given the estimated selective values along the length of the cline, simulations of the expected change in gene frequency do not fit the observed change exactly when simple assumptions are used (Bishop 1972; Bishop *et al.* 1977). The *carbonaria* morph remains at a high frequency further into rural north Wales than is predicted, and there is a tail of frequencies of 5–10 % *carbonaria* to the southwest and 5–10 % typical to the northeast which is not explained (figure 3). Lees & Creed found that by invoking a 30 % disadvantage to typicals in addition to the observed change in visual selection they could make the two curves coincide around the point of inflexion at 50 %. This figure is in agreement with the one needed for East Anglia (provided further assumptions about fitness and movement are made), but there are arguments against accepting it for north Wales. First, the measured visual selection is based on insects glued to tree trunks, and using the estimates for a series of points the simulation was generated assuming that the regression of estimated selection on distance is linear: both these procedures leave room for accidental variation in fit. Secondly, and most importantly, the long tail of the cline stretching south westwards as far as Bangor where frequencies of up to 5 % melanic occur cannot be explained, because both *carbonaria* genotypes are there at a disadvantage. If their presence is due to migration then additional fitness differences are probably unnecessary to account for the distribution. We therefore regard the supposition of a large non-visual fitness difference between heterozygotes and typical homozygotes as unproven and, in the northwest region, unlikely. More generally, if this kind of differential were usual it is surprising that local pockets of high melanic frequency are not more common throughout the range of the species. With the possible exception of East Anglia, *carbonaria* is known only from areas of high atmospheric pollution. The northern birch forests of Europe should favour melanics as the ancient British pine forests were supposed to have done, yet *carbonaria* occurs there only in insignificant numbers (Douwes, Peterson & Vestergren 1973; K. Mikkola, personal communication).

(ii) *Selective predation and homozygous melanic disadvantage are balanced*

Heterozygote advantage may also arise as a result of a balance of visual selection against typicals and non-visual selection against the melanic homozygote. This is intrinsically more likely, implying as it does that in the unpolluted environment the mutant is on average deleterious and shows intermediate dominance in fitness. The case for this system has been advocated by Clarke & Sheppard (1966). The assumptions would produce a pattern similar to that observed even in the complete absence of migration.

There are four sets of evidence in favour of a lower fitness in one of the homozygotes than in heterozygotes. First, Haldane (1956) argued that the observed frequency of 98 % *carbonaria* in Manchester may be a stable one, so that if selection against non-*carbonaria* was at the level of 50 % the fitness of *carbonaria* homozygotes would be about 8 % less than that of heterozygotes. Secondly, at several sites Kettlewell (1958) estimated the selective advantage of melanic over typical, assuming dominance of fitness, at a period early in the rise of melanism when the frequency was low, and compared it with a later estimate when the frequency is high. There are three localities for which two such estimates can be made and in each case the selective advantage of the melanic is greater in the early period. As Clarke & Sheppard (1966) point out, this result would be expected if there was heterozygote advantage, since the increase in *carbonaria* homozygotes with increasing frequency would tend to depress the estimate obtained by the method used.

Thirdly, Clarke & Sheppard observed a significant decrease in frequency of *carbonaria* at Caldy (Wirral) from 1959 to the present but most noticeable in the data published in their 1966 paper. This was at the time when smoke control legislation was introduced. The change was consistent with an advantage to the typicals of 23 % (if there is no heterosis), whereas experimental evidence from the area suggested a 20 % disadvantage. It is therefore possible that the change in frequency was brought about by a small relaxation in selection against typicals resulting in a decrease in melanics because of the selection against *carbonaria* homozygotes.

Fourthly, Kettlewell (1958) has bred backcrosses of *carbonaria* × typical and compared the resulting ratios of progeny with backcrosses bred at the turn of the century. These crosses of Birmingham moths produced a significant deficiency of typicals while the material from 1900–5 resulted in an excess of typicals. The difference between the two sets was significant, which could suggest that the fitness of the heterozygotes compared to typicals has increased during the intervening time. This is the kind of result to be expected if the genes are evolving a state of balance.

All these lines of evidence may be questioned. Kettlewell also crossed *carbonaria* individuals to typicals from Devon and obtained no distortion of segregation. This might indicate that the Devon stock, from which the melanic form is absent has not become adjusted like the Birmingham one. However, backcrosses made by Clarke & Sheppard (quoted by Kettlewell 1973) using insects from Lancashire and Cheshire where the adjustment should have occurred also failed to show a distorted ratio, and additional early crosses from 1892 and 1910 showed a slight excess of *carbonaria* and equal frequencies respectively. The only exception is Kettlewell's Birmingham data. If it is excluded there is no significant difference between the early and late breeding results. Ford (1940) carried out experiments on the related moth *Cleora repandata* L. which showed the melanics to occur in excess in backcrosses where the progeny were subject to starvation, but not in those given sufficient food. The present evidence for *B. betularia* may indicate the same effect; it does not demonstrate variation in fitness from place to place or from the beginning to the middle of the century. It cannot be concluded from this evidence alone that a general adjustment has taken place.

When Haldane made his calculation he specifically excluded the possibility of migration between areas of different frequency, on the basis of the then existing information. The result only stands if there is no migration. It may also be noted that the sample in which there was 98 % *carbonaria* came from Didsbury, a suburb of Manchester which has since shown a rather higher frequency of carbonaria than other parts of the conurbation (see table 1). Although numbers of the rarer morphs are small there is now a suggestion of heterogeneity between different parts of Greater Manchester within 5–10 km of each other. Also, in the data considered by Haldane the rare morph was *insularia*, not typical, so that unlike in the other examples the estimate concerns the relative fitness of *carbonaria* homozygotes and *carbonaria/insularia* heterozygotes.

The estimates made by Kettlewell are of necessity based on very incomplete early data. The only one for which two of three frequencies involved come from known samples is from Delamere, Cheshire. In this case the melanic morph was first seen in 1860. Assuming dominance of fitness, Kettlewell gives an estimated selective advantage for the period from then to the turn of the century or 18 %, but the figure is only 5 % for the change from then to the 1950s. The other samples, which agree with this one in direction, are based on considerably less adequate data.

In Kettlewell's (1973) table and text there are altogether 28 estimates of selective advantage for parts of the period from the late 19th century to 1967. Although they refer to widely differing lengths of time, we should expect on average that if there is heterozygote advantage, the estimated selective advantage of melanics would decline with increase in the frequency finally achieved. Overall, the data do not show this to be so convincingly. There are three very high estimates associated with low frequencies, 45 % at Marlborough, where the frequency had reached 9 %, 52 % in London, where the frequency had reached 37 % (in a sample of 27) and 60 % at King's Lynn, where the *carbonaria* form had become 'prevalent' (which Kettlewell takes to be 50 %). Apart from these examples, two of which hardly rest on sufficient data, the trend is in the reverse direction, from 2–10 % estimated selective advantage when the frequency is low to 2–25 % when it is high.

The best documented study is that at Caldy on the Wirral peninsula discussed by Clarke & Sheppard (1966) and Whittle *et al.* (1976). Even here, however, another interpretation is possible if large migration rates are assumed. The site lies on the north side of the Dee estuary. The drop observed from 1959 to 1965 was from about 93 % *carbonaria* to about 90 % over six years, with a sharp decline in the middle of the period. This locality is surrounded by others with high melanic frequency; 93 % to the S.E. at Heswall, 87 % nearby at Caldy, Thorswood, and 87 % to the N.W. at Meols. Values of 86 and 88 % occur at sites nearby at Heswall Hills and Pensby. The area is one where frequencies vary over a range of up to 10 % between sites a few km from each other, and migration takes place on the Wirral between sites as close as these (Bishop 1972). Twelve kilometres away on the other side of the estuary, frequencies are as low as 55–65 %. Clarke & Sheppard's estimate is based on evidence for high selective predation on typicals in the area of about 20 % and on the assumption of low migration.

Formal representation of the interaction of selection and migration leads to equations requiring many (often unguessable) parameters such as rate of movement, net direction of movement, gene frequencies at each point, population sizes, etc. However, if we accept an estimate of 20 % selection against typicals and a stable morph frequency of 93.5 % before the drop some rough calculations may be made to give an idea of the migration rate and gene frequency of the migrants. These two variables interact, but figures between 10 % immigrants per generation with 88 % melanic morph frequency, and 3 % immigrants with 67 % melanic frequency will ensure that the frequency at Caldy remains unchanged despite 20 % selection *in situ* against typicals. Now if we assume 4 % immigration, a new mean level of 91.5 % *carbonaria* will be achieved if the disadvantage of typicals is reduced from 20 to 15 %: the reversal to a 23 % advantage envisaged by Clarke & Sheppard in the absence of heterosis and migration is not required. Of course, for the new level to be reached rapidly a larger drop is necessary. Against this, the frequency of *carbonaria* in the immigrants may reasonably be assumed to be declining at the same time, thus speeding the process of change. The rate at which the change takes place also affects Clarke & Sheppard's estimate. If the drop occurred in one generation from 1962 to 1963 the homozygous disadvantage would have to be 24 %.

Whittle *et al.*, in their discussion, make some further observations. First, if migration was lowering the frequency at Caldy then the frequency of melanics among captured females should be higher than among males, since they move very much less far. This is the case, although the sample is too small for the difference to be significant. Secondly, they have for several years sampled the moths of Hilbre Island, a small island in the Dee estuary which does not have the vegetation to support the larvae, so that the local catch consists of immigrants. Hilbre Island (site 121) is much closer to the Wirral than to the Welsh side of the estuary, and has a melanic frequency lying between those of the two sides. A simple calculation using the small amount of data available suggests that about 24 % of the insects arrive from the more distant Welsh coast (8 km away). They also point out that the frequency in a sample from Meols, on the north coast of the Wirral is 87 %, compared to 91 % at a similar date at Caldy on the west coast on the Wirral. If selection is identical at the two sites, migration from Wales sufficient to account for the Meols frequency would depress the Caldy frequency even further than it has done. However, as pointed out above, there is no reason to suppose that selection is identical at the two sites, nor that a particular migration rate at one point implies an identical one somewhere else. If migration is affecting the frequency some irregularities are to be expected given the sharp disparity in frequency between the two coasts of the Dee. By contrast, at the Manchester end of the cline where selective pressures appear to be similar over a much larger area, there is greater consistency in morph frequency from site to site.

In the foregoing argument we do not dispute the fact that the available evidence may indicate heterosis. In the light of the evidence on movement, however, another interpretation is possible, namely the third model proposed earlier (p. 507) and discussed below, that polymorphism results from a balance of directional selection and migration.

(iii) *Predation the sole important selective agent*

At present, the evidence is insufficient to reject this interpretation. Recently, various aspects of the interaction of selection and spatial separation, either in a mosaic environment or along a cline, have received theoretical attention in connection with the general problem of maintaining polymorphisms (for example, among others, Endler 1973; Slatkin 1973; Strobeck 1974; Felsenstein 1975; Christiansen & Feldman 1975; Nagylaki 1976; Karlin & Richter-Dyn 1976; reviewed by Felsenstein 1976). These studies have shown how readily polymorphism may be generated. May *et al.* (1975) and Endler (1977) have summarized the conclusions of many of these papers concerning clines. What is known of the ecology of *B. betularia* suggests that it is an insect of low density and high mobility. The density estimated for the Wirral by Bishop (1972) is about 10/km^2 per night. This indicates a total seasonal emergence of somewhere around 250 insects per km^2. The root mean squared distance moved per season is estimated from release and recapture studies as around 2.5 km (Bishop 1972; Bishop *et al.* 1977; May *et al.* 1975). During the increase in melanism in northern Germany the wave of advance of melanics appears to have moved about 400 km in twenty years, or 20 km per season (Cleve 1970). This figure is consistent with the estimate of 2.5 km per season for the root mean square since the gene was strongly advantageous and dispersal curves are known usually to be leptocurtic. A large fraction of insects at any locality may therefore be immigrants from some km away, and this is a factor which cannot be ignored when attempting to account for a particular observed pattern of morph frequency. Given these parameters for movement and the experimental results for spatial change in selective value, May *et al.* (1975) showed that the shape of the cline is very close to their theoretical prediction. We therefore feel that the existence of heterosis in *B. betularia* has not yet been established. The resolution of this problem must await experimental work on possible fitness differences and ecological field studies on a scale which has not yet been applied to the species.

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APPENDIX 1: MICROFICHE

The microfiche contained in this issue includes the following tables referred to in the text of this paper.

TABLE 1. NUMBER OF *CARBONARIA*, *INSULARIA* AND TYPICALS CAUGHT IN SAMPLES OF *BISTON BETULARIA*

(This includes all known data from northwest England and north Wales. Sample data from 158 sites (figure 2) between Leeds in the east and Abersoch in west Wales are presented. Six figure grid references from the 1:50000 Ordnance Survey are usually given. The frequency of the *carbonaria* melanic is summarized in figures 3 and 4.)

TABLE 2. NUMBER OF *NIGRA* AND NON-MELANICS CAUGHT IN SAMPLES OF *GONODONTIS BIDENTATA*

(This includes data from 111 sites in northwest England and north Wales. Grid references are given. The frequency of the *nigra* melanic is summarized in figures 5 and 6.)

TABLE 5. RECORDED LEVELS OF SMOKE AND SULPHUR DIOXIDE FOR 1973–4 FROM WARREN SPRING LABORATORY SURVEY

TABLE 6. NUMBER OF MELANIC AND NON-MELANIC INDIVIDUALS IN SAMPLES OF THE LADYBIRD BEETLE *ADALIA BIPUNCTATA*

(This includes data from 40 sites in northwest England. Grid references are given. The frequency of the melanic is summarized in figure 7.)

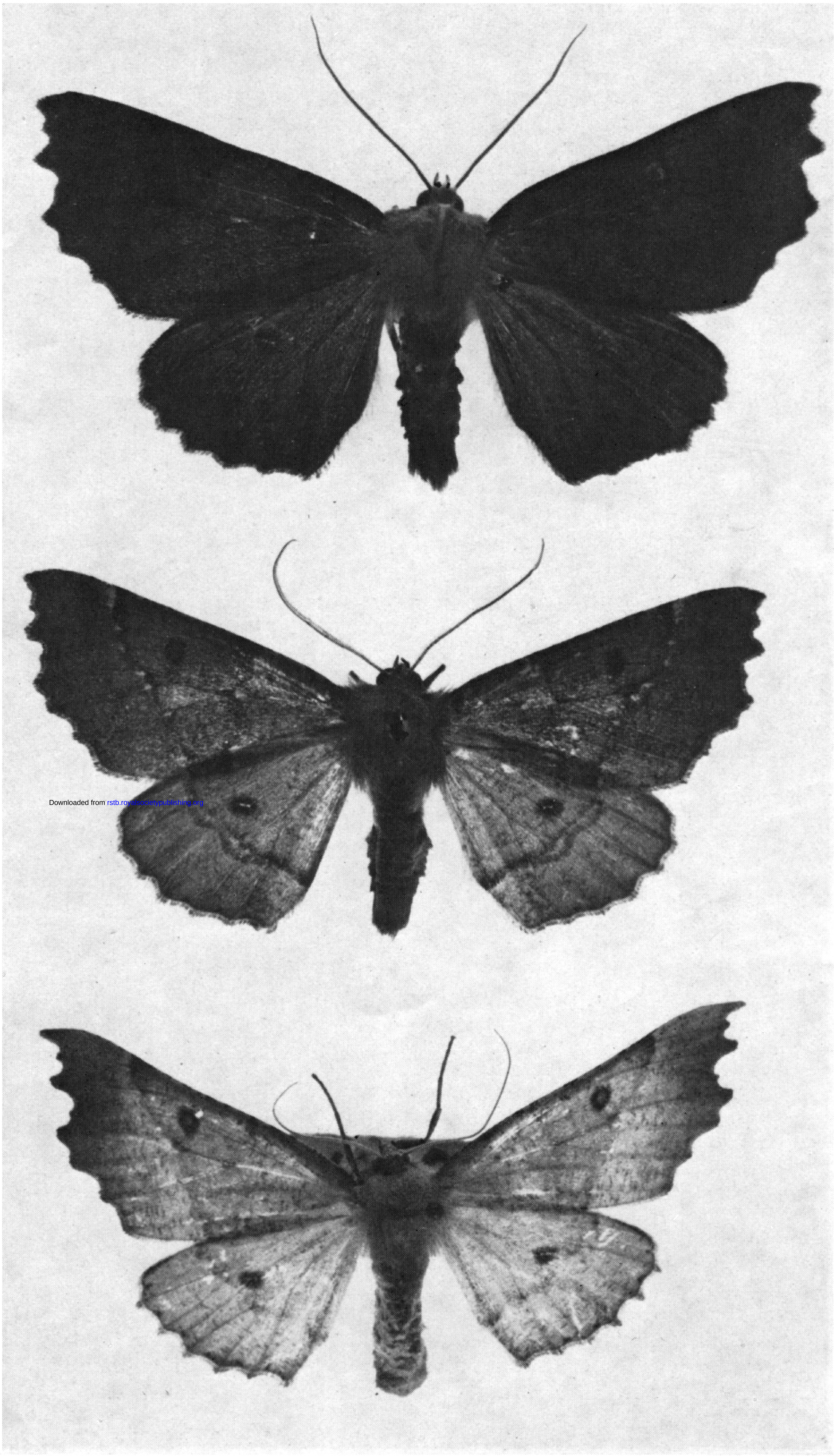

FIGURE 1. *Gonodontis bidentata*. Top, the melanic form *nigra*; centre, a dark non-melanic individual from the study area in northwest England; bottom, a pale non-melanic of southern English type.

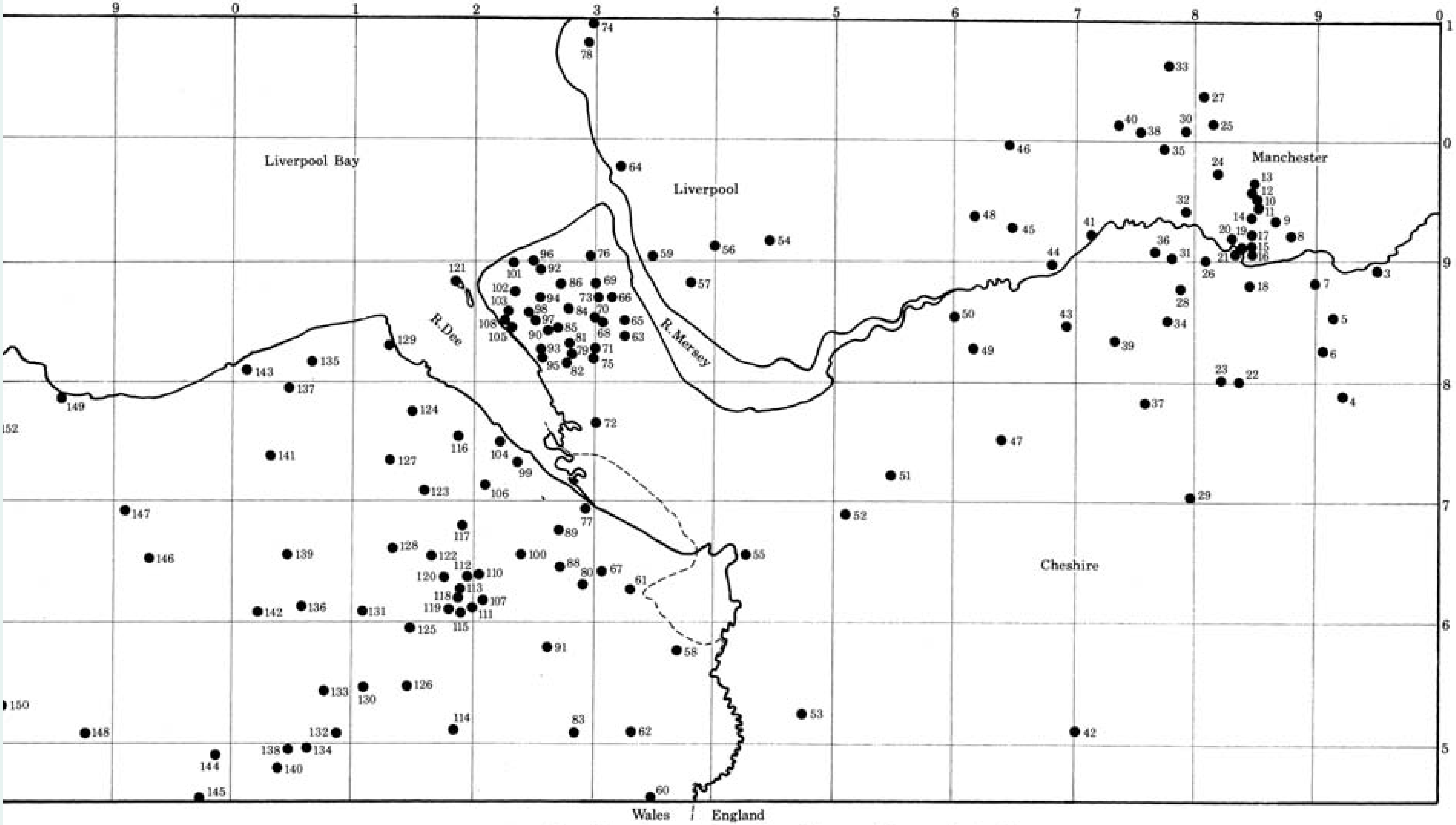

Figure 2. Map showing outline of the study area. The position of the coast is shown and the Ordnance Survey 10 km grid. Trapping sites for *Biston betularia* are indicated, numbered as in table 1.

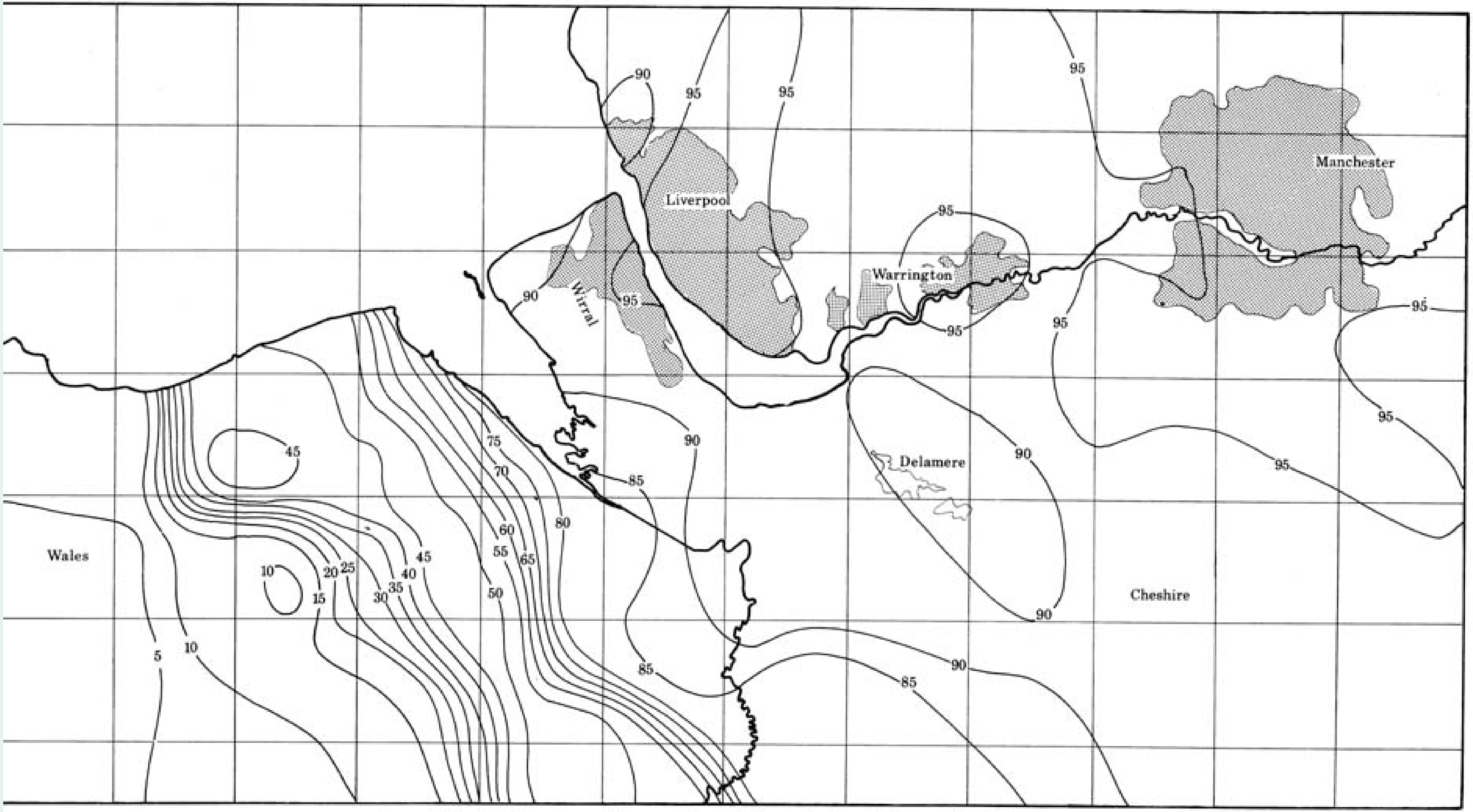

FIGURE 3. Map showing contours of frequency of the melanic morph *carbonaria* in *Biston betularia*. The contours are produced by interpolation between estimates for the sampling sites listed in table 1, after weighting of the site frequency by its variance. The Ordnance Survey 10 km grid is indicated.