A SURVEY OF THE FREQUENCIES OF *BISTON BETULARIA* (L.) (LEP.) AND ITS MELANIC FORMS IN GREAT BRITAIN

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1. FOREWORD

Biston betularia, the Peppered Moth (previously placed in the genera *Amphidasys* and *Pachys*), is one of about 70 species of Macrolepidoptera occurring in this country which are at present in the process of changing their populations from light to dark. This is referred to as Industrial Melanism. In nearly every instance it results in the substitution of a complex pattern by a dark or black coloration. Industrial melanism is found, in fact, only among those species which depend for their survival by day on their efficiency in harmonising with the specialised background on which they rest, such as lichened trunks, boughs, rocks or wood. It is not found in those species which exhibit other types of protective coloration. The black mutant is nearly always inherited as a simple Mendelian dominant. In compiling a survey, such as the one about to be described, it was necessary to choose a species which had the following attributes :—it must be widespread, forming fairly continuous populations both in industrial and rural areas ; it must indulge in considerable periods of flight to encourage gene-flow ; and be easy to capture at mercury vapour traps or by other methods. *B. betularia* has all these qualifications. Accordingly, in 1952 I was fortunate enough to recruit a team of about 80 entomologists (which has since risen to over 150) living in different parts of the country, and to them I owe my sincere thanks for more than 20,000 records of this species which they have sent me in the last four years.

2. THE PHENOTYPES

Typical *B. betularia* is whitish overlaid with innumerable thin black lines and dots. At rest on lichened boughs it is often almost invisible. The first melanic appeared about 1848 in Manchester (Barrett, 1901), and is referable to as f. *carbonaria* Jordan (syn. *doubledayaria* Millière).

Another melanic form is known as *insularia* Th. Mieg. It is not as extreme as *carbonaria* and is variable. Occasionally it is difficult to differentiate from the *typica* form, and still less frequently from *carbonaria*. These two melanics are not allelomorphic (Kettlewell, 1953). Hitherto there has been no evidence of linkage. There is, however, a distinct possibility that f. *insularia* may be controlled by a series of alleles. The three forms are figured in a previous paper (Kettlewell, 1956*a*). *Carbonaria* is unifactorial and dominant to the *typica* form, and *insularia*, which is also dominant or semi-dominant to the type, cannot be recognised phenotypically in the presence of *carbonaria*.

3. PREVIOUS INVESTIGATIONS

The following facts have been recorded by me and have a direct bearing on the distribution of the three phenotypes in Britain :—

(*a*) " To the human eye, *carbonaria* proved much better concealed on the lichen-free tree trunks, blackened by pollution near Birmingham, but this advantage was reversed in favour of the pale form in unpolluted country. *Insularia* possesses an intermediate advantage in both places " (Kettlewell, 1956*a*).

(*b*) Mark-release-recapture experiments showed that in the industrial area of Birmingham we recaptured more than twice as many f. *carbonaria* as *typica* in a total release of 584 of these two forms. In contrast, a release of 969 individuals into an unpolluted and heavily lichened wood in Dorset resulted in twice as many of the *typica* form being recaptured as of the f. *carbonaria*. In both experiments, the recaptures were usually made within 24 hours of the release.

(*c*) This was shown to be due to bird predation. Six species of birds were recorded as taking part in this and filmed as they captured resting moths whilst under observation (by Dr Niko Tinbergen). In Birmingham, Redstarts, *Phoenicurus phoenicurus* L., were seen to take 58 *betularia* which had been released in a 1 : 1 ratio, melanic : typical ; 43 were *typica* and 15 f. *carbonaria*. On the other hand, in the unpolluted wood of Dean End, Dorset, when both forms were released in equality, 5 species of birds were observed to eat 190 *betularia*, of which 164 were f. *carbonaria* and 26 f. *typica*. These findings provided evidence for stating that " the difference in cryptic coloration alone could be responsible for the rapid spread of the Industrial Melanics "

(*d*) Nevertheless, we have provided certain basic evidence that there exist both physiological and behaviour differences between the melanic and typical forms (Kettlewell, 1956*b*).

4. EARLY HISTORY OF MELANIC SPREAD

Prior to the middle of the last century, there is every reason to believe that melanic forms were maintained in the population solely by recurrent mutation. For the next fifty years these melanics were much sought after by collectors, so that it is reasonable to suppose that their capture would be recorded assiduously when they first made their appearance. Table 1 gives a list of the earliest records

TABLE 1

Earliest records of f. carbonaria *in Britain. Approximate selective advantages (S.A.) have been estimated for a period of 100 years on the assumption that the local frequency of f.* carbonaria *was not greater than 1 per cent. when first recorded*

County Locality (recorder)	1st *carbonaria* recorded	1900-06 survey (when known)	S.A.	Locality (recorder)	1952-56 frequency sample size	S.A.	Locality (recorder)	S.A. over total period
BERKSHIRE (Barrett)	1885	...	...	...	11% (63)	...	Newbury (Saundby)	3%
CAMBRIDGESHIRE Cambridge (Farren)	1892	" now seen every year "	...	(Farren)	93% (88)	...	Cambridge (H.B.D.K.)	14%
Ely (Cross)	1895	...	...	...	...	...	...	15%
CHESHIRE Chester (Barrett)	Delamere 1860	83% sample 180	18%	Chester (Arkle)	94% (124)	5%	Chester (G. Smith)	10%
ESSEX Colchester (Harwood)	1892	...	...	...	86% (822)	11%	Bradwell (Dewick)	11%
Dovercourt (Mathew)	1902	...	...	...	"	14%	"	14%
GLOUCESTERSHIRE	...	" black not observed "	...	Stroud (Prideaux)	16% (185)	>6%	Nailsworth (Demuth)	>6%
HAMPSHIRE New Forest (Barrett)	1897	...	...	...	10% (324)	4%	Eastleigh (Goater)	4%
IRELAND Castle Bellingham (Thornhill)	1894	...	...	...	7% (58)	3%	Belfast district (Wright)	3%
ISLE OF MAN (Cassall)	1904 2 sps.	...	...	...	13% (69)	6%	Santon (Hedges)	6%
KENT	...	" black not observed "	...	Farnborough (Christy)	73% (224)	>12%	E. Malling (Groves)	>12%
LANCASHIRE Manchester (Edleston)	1848	" black prevalent. Type occurs "	...	Manchester (Clutton and Tait)	98% (350)	...	Manchester (Michaelis)	15%
LINCOLNSHIRE (Barrett)	*c.* 1860	" both light and black "	...	(Fowler)	91% (158)	...	Louth (H.B.D.K.)	9%
LONDON DISTRICT Woodford (Mira and Bacot)	1897	37% (sample 27)	52%	Woodford (Main and Harrison)	90% (327)	8%	Whetstone (Lovell)	14%

S.A. = Selective advantage of f. *carbonaria* as calculated from Haldane's (1924) Table.

TABLE 1 (*continued*)

County Locality (recorder)	1st *carbonaria* recorded	1900-06 survey (when known)	S.A.	Locality (recorder)	1952-56 frequency sample size	S.A.	Locality (recorder)	S.A. over total period
MONMOUTH Newport (Wheeler)	...	" 1870 two forms about equal "	...	...	...	...	...	...
NORFOLK Kings Lynn (Atmore and Baker)	1892	" Prevalent " (=50%)	>60%	(Atmore and Baker)	77% (137)	<3%	Fritton (H.B.D.K.)	10%
STAFFORDSHIRE Cannock Chase (Barrett)	1878	" all black " (=≥80%)	>25%	(Frere)	90% (42)	3%	Cannock Chase (Richardson)	11%
SUFFOLK Ipswich (Morley and Pyett)	1894	...	...	...	75% (56)	10%	Lowestoft (Burton)	10%
SURREY Croydon (Gower)	1906	" black not observed "	...	Reigate (Meyrick)	77%(1615)	>13%	Woking (C. de Worms and Trundell)	>13%
SUSSEX	...	" black not observed "	...	(Hewitt)	79% (1347) 16% (383) 26% (106)	>13% >6% >7%	Ottershaw (Bretherton) Hastings (Astbury) Eastbourne (Ellison)	>13% >6% >7%
WARWICKSHIRE Birmingham	...	" 50% black "	...	(Barrett)	87% (1611)	5%	Birmingham (H.B.D.K.)	5%
WESTMORLAND Kendal and Windermere (Moss)	1870	" black commoner than type " (= 50%)	14%	(Barrett)	49% (47)	...	Kendal (Birkett)	...
WILTSHIRE Marlborough (Prentice)	1951	" black not observed "	...	Marlborough (Davis)	9% (460)	>45%	Marlborough College	>45%
YORKSHIRE Bradford (Butterfield)	scarce 1876	" black now prevalent " (= >50%)	>10%	(Butterfield)	96% (508)	<10%	Bradford (Briggs)	10%
Huddersfield (Porritt)	1861	" now only black " (= ≥80%)	≥15%	(Porritt and Morley)	99% (409)	...	Sheffield (H.B.D.K.)	17%

S.A. = Selective advantage of f. *carbonaria* as calculated from Haldane's (1924) Table.

of f. *carbonaria* from different localities, and it has been assumed that at these dates *carbonaria* would not be at a local frequency higher than say 1 per cent. In 1900, The Evolution Committee of the Royal Society began an investigation into the problem of industrial melanism. It is unfortunate that approximate percentages only are given (Doncaster, 1906), but by this date scientific opinion had evidently become conscious of the biological importance of the rapid spread of the melanic forms. Only on two occasions, in fact, are there actual figures for random samples. The same unfortunate shortcomings are found in the later investigations of Mera (1925) and Adkin (1925). In fact nowhere are the figures given which would have helped so much in the present investigation.

Certain information can, however, be deduced from these earlier records. It appears that f. *carbonaria* was taken for the first time in many widely separated places between 1848 and 1900. Whether this represents migration from one centre, or numerous discrete mutations, will be discussed later. Secondly, very shortly after the initial captures this melanic form increased rapidly in a brief space of time. Thirdly, until the end of the century, *carbonaria* was unknown in southern England. At this time it had a midland and eastern county distribution and was not taken in the London area till 1897. Fourthly, when these earliest records are considered with our recent data, the selective advantages (p. 64) for the *carbonaria* form can, with a reasonable degree of accuracy, be assessed for each locality over the period involved. The ability to do this in one of the most transient polymorphisms ever known, makes these earliest records most valuable.

5. PRESENT SURVEY

In view of the inadequate nature of the data on the frequencies of the melanic forms at any one period in the past, I decided in 1952 to obtain as much data on *betularia* as quickly as possible, with the object of providing figures for future reference. At the same time, a great deal of information has been collected, some of which is capable of being analysed at present.

Table 2 gives a list of frequencies of the three forms of *betularia* from many areas of Great Britain. Furthermore, the last column gives the estimated true frequency of *insularia* in the non-*carbonaria* fraction of the population (assuming no linkage), *carbonaria* being epistatic to this form. It will be appreciated that the higher the frequency of *carbonaria*, the greater the error in assessing the *insularia* frequency, and that even in large random samples from industrial areas the data are often inadequate.

TABLE 2

Phenotype frequencies of Biston betularia *and its two melanics, f.* carbonaria *and f.* insularia, *from 83 centres in Britain (1952-56)*

Locality	Observer	Per cent. T	Per cent. C	Per cent. I	Total	Estimated frequency per cent. I
BEDFORDSHIRE						
Woburn	S.H.K.	4·76	95·23	0	21	0
Leighton Buzzard	J.F.R.	15·43	82·28	2·29	175	12·90
BERKSHIRE						
Newbury	R.S.	79·37	11·11	9·52	63	10·71
CAMBRIDGESHIRE						
Cambridge	H.B.D.K., B.O.C.G.	4·54	92·95	3·41	88	42·85
CHESHIRE						
Chester	S.G.S.	5·65	93·55	0·81	124	12·50
Delamere	W.E.A.	6·68	90·86	2·46	569	26·92
Heswall	D.E.H.	6·82	93·18	0	44	0
Wirrall	A.C.	1·56	98·44	0	192	0
CUMBERLAND						
Penrith	W.F.D.	60·71	26·79	12·50	56	17·07
DERBYSHIRE						
Chesterfield	J.H.J.	0·59	99·41	0	170	0
DEVON and CORNWALL						
Torquay	F.H.L.	100	0	0	100	0
Ashburton, Bude, Plymouth, Tavistock, Tiverton	S.T.S., A.H., F.W.J., D.J.W., F.H.L.	100	0	0	500	0
DORSET						
Dean End Wood	H.B.D.K.	93·64	0·87	5·49	346	5·54
Broadmayne	V.W.P.	97·02	0	2·98	168	2·98
ESSEX						
Bradwell on Sea	A.J.D.	6·69	86·25	6·57	818	49·54
Westcliffe on Sea	H.C.H.	5·95	85·55	8·5	353	58·82
GLOUCESTERSHIRE						
Hardwicke	R.D.	38·89	23·61	37·5	72	49·09
Nailsworth	A.R.	48·65	15·68	35·67	185	42·31
HAMPSHIRE						
Bournemouth	F.M.B.C.	60·00	30·00	10·00	40	14·29
Chandler's Ford	B.G.	82·1	10·49	7·41	324	8·27
Borden	D.W.	65·43	20·29	14·28	3095	17·92
Fordingbridge	P.J.B.	86·84	5·26	7·89	38	8·33
Winchester	R.S.M.W.	84·85	15·15	...	33	0
Fleet	A.W.R.	47·31	46·03	6·66	315	12·35
HEREFORDSHIRE						
Malvern	R.K.J.	46·30	27·80	25·90	54	35·90
HERTFORDSHIRE						
Bishops Stortford	C.C.	4·76	89·18	6·06	231	56·01
Rothamsted	C.B.W.	11·08	83·69	5·23	325	32·08
Tring	L.G.	16·89	74·30	9·81	214	38·18

TABLE 2 (*continued*)

Locality	Observer	Per cent. T	Per cent. C	Per cent. I	Total	Estimated frequency per cent. I
IRELAND						
Belfast district .	W.S.W.	93·10	6·90	...	58	0
Dublin district .	E.S.A.B.	96·30	3·70	...	27	0
ISLE OF MAN						
Santon . .	A.H.	50·70	13·11	36·20	69	41·67
ISLE OF WIGHT						
Freshwater . .	K.E.B.	...	...	...	50	...
KENT						
Bromley . .	M.G.M.	9·26	88·89	1·85	54	16·66
Folkestone . .	A.M.M.	46·43	42·86	10·71	140	19·75
East Malling .	D.E.	...	...	...	...	...
Maidstone . .	J.R.G.	21·43	73·21	5·36	224	20·00
LANCASHIRE						
Formby . .	N.G.L.	5·32	94·68	...	94	0
Southport . .	K.L.G.	10·00	90·00	...	120	0
Manchester . .		0	98·00	2·00	350	100
LEICESTERSHIRE						
Market Harborough	H.A.B.	11·83	84·95	3·32	93	21·43
LINCOLNSHIRE						
Grimsby . .	G.A.T.J.	7·41	88·89	3·75	81	33·35
Louth . . .	H.B.D.K.	6·33	91·13	2·53	158	28·57
LONDON AREA						
Whetstone . .	R.L.	5·81	90·21	3·98	327	40·63
MIDDLESEX						
Pinner . . .	W.E.M.	9·14	84·95	5·91	558	39·28
NORFOLK						
Cromer . .	J.B.	20·93	67·44	11·63	43	35·71
Fritton . .	H.B.D.K.	14·60	77·37	8·03	137	35·48
NORTHANTS						
Wellingborough .	P.J.G.	14·24	79·88	5·90	288	29·31
OXFORDSHIRE						
Oxford district .	H.B.D.K. P.M.S. G.V.	41·00	34·03	24·97	717	37·84
Steeple Barton .	H.B.D.K.	49·13	34·26	16·61	289	25·26
SCOTLAND						
Glasgow . .	H.B.D.K.	10·33	89·66	...	29	...
Kinloch Rannoch .	R.L.	100	...	...	285	...
Newtonmore .	G.H.	100	...	...	100	...
SOMERSET						
Portishead . .	J.A.B.	50·00	20·00	30·00	10	37·50
SUFFOLK						
Beccles . .	E.T.G.					
Lowestoft . .	J.B.	17·86	75·00	7·14	56	28·57

TABLE 2 (*continued*)

Locality	Observer	Per cent. T	Per cent. C	Per cent. I	Total	Estimated frequency per cent. I
SURREY						
Cobham	J.B.P.	17·98	76·86	5·17	484	22·32
Cranleigh	H.B.D.K.	50·00	39·30	10·70	28	17·65
Ottershaw	R.F.B.	13·73	79·93	6·34	1435	31·60
Woking	C.deW. E.T.	16·84	76·66	6·88	1615	25·88
SUSSEX						
Brighton	R.W.D.	41·18	52·94	5·88	17	12·50
Eastbourne	R.E.E.	40·57	26·42	33·02	106	44·87
East Grinstead	M.G.	66·70	53·30	...	21	...
Hastings	C.F.A.	74·15	15·67	10·18	383	12·07
Petworth	P.D.	79·66	11·86	8·47	59	9·61
NORTH WALES						
Dolgelley	T.T.	95·37	2·78	1·85	108	1·90
Llandudno	J.A.T.	100	...	...	100	0
HEREFORD						
Ross on Wye	J.E.K.	39·29	21·43	39·29	28	50
WARWICKSHIRE						
Birmingham	H.B.D.K.	8·94	87·09	3·97	1611	30·77
Cannock Chase	R.P.D. A.R.	7·5	90·00	2·50	40	25·00
Tysoe	T.T.	22·22	66·67	11·11	18	33·33
Wyre Forest	H.B.D.K.	31·34	64·18	4·48	67	12·50
WESTMORLAND						
Kendal	N.L.B.	40·43	48·94	10·64	47	20·83
WILTSHIRE						
Warminster	R.J.	83·33	9·26	7·41	54	8·17
Marlborough Col.	C.R.P. R.W.C.V. S.L.S.	79·78	8·91	11·30	460	12·41
YORKSHIRE						
Aysgarth	H.B.D.K.	4·00	94·00	2·00	100	33·33
Bradford	J.B.	1·97	95·67	2·36	508	54·54
Grassington	G.deC.F.	...	100	...	60	...
Sheffield	H.B.D.K.	0·5	99·00	0·50	409	50·00

Throughout this paper the letters T, C, I stand for *Biston betularia* f. *typica*, f. *carbonaria* and f. *insularia* respectively.

A distribution map (fig. 1) of the frequencies of the three forms shows :

(*a*) A correlation between the frequency of the melanic forms and the industrial areas of Britain.

(*b*) A high frequency of *carbonaria* throughout eastern England from north to south, though far removed from industrial centres. This is, in my opinion, the indirect effect of long continued smoke fall-out carried by the prevailing south-westerly winds from central England.

(*c*) Western Britain, with the exception of Cheshire, Lancashire and Westmorland (Kendal) is virtually melanic free.

(*d*) Northern Scotland has no melanic *betularia* but in the Glasgow district f. *carbonaria* is about 90 per cent.

(*e*) In Ireland, *carbonaria* has been recorded, first in 1894 from the Belfast district and aso Dublin, but is of rare occurrence. It has been found nowhere else.

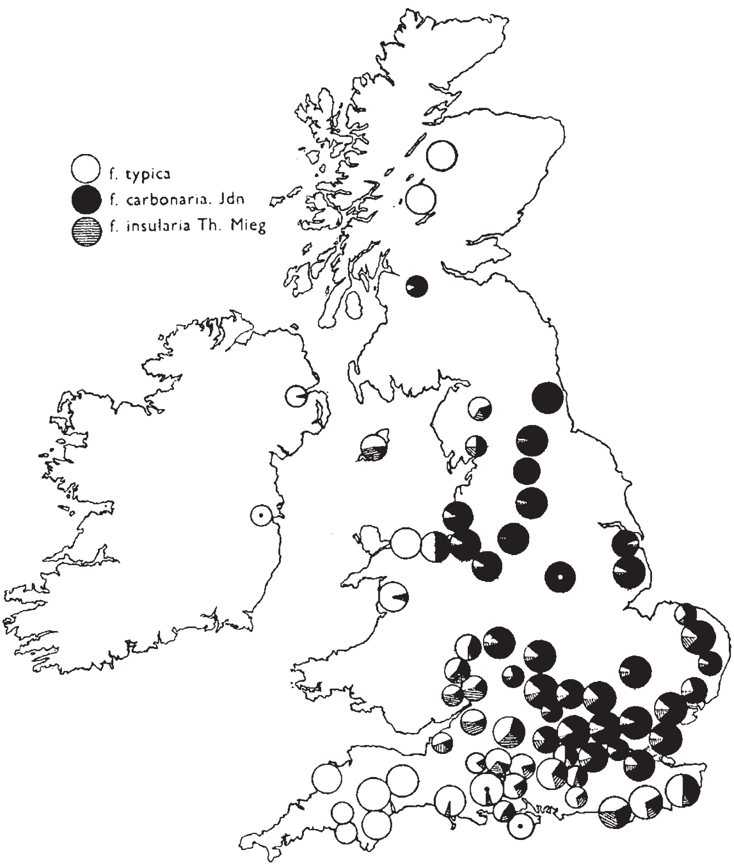


FIG. 1.—A frequency map of *Biston betularia* and its two melanics, f. *carbonaria* and f. *insularia* (1952-56), comprising more than 20,000 records from 83 centres in Britain.

(*f*) The map shows multiple clines running out from the higher *carbonaria* frequencies in the centre of England. There may be a rapid decline from east to west as, for instance, from Cheshire to North Wales, or from Birmingham to Wyre Forest, or they may be gradual as always found in an easterly direction.

(*g*) The highest frequency of f. *insularia* phenotype is, for the most part, found in the Gloucester and Severn Valley district of England.

6. ANALYSIS OF F. *CARBONARIA* FREQUENCIES

(*a*) *Evidence of mutation rate*

If one reviews the first county records, it is indeed difficult to resist the conclusion that the new mutant has radiated outwards from an original centre of mutation near Manchester. There is no doubt that following the original capture in 1848, the nearest counties to Lancashire were the next to record its appearance ; Cheshire in 1860, Yorkshire in 1861, Staffordshire in 1878, and Westmorland in 1870. The London district, on the other hand, and southern England did not report a *carbonaria* until about twenty years later [1897). It is significant also that the eastern counties of Norfolk, Suffolk and Cambridge all recorded their first *carbonaria* practically simultaneously between 1892 and 1895.

All this is consistent with the spread of a successful gene with no ecological barriers, from its centre of origin. Furthermore, on the Continent, the same spread was taking place, being recorded by A. Hofmann from Hanover in 1884, the Netherlands and Thuringia in 1888 " and in the next few years in various parts of the Rhine Valley, indeed he [Hofmann] thinks that its progress was up the Rhine " (Barrett, 1901).

In a short series of evening releases undertaken in open country near Louth, Lincs., in 1956 I attempted to find the distance which *betularia* flies per night. Using different releasing points, different markings, and several collecting centres of known distance apart, I was able to show that of 93 releases, 6 travelled 1½ miles, and 9 over half a mile within 48 hours. At Fritton in Suffolk, of 78 releases over three occasions, 16 were recaptured three-quarters of a mile distant, 12 within 24 hours. From this, having regard to the random flight of the releases, it can be accepted that *betularia* frequently flies a mile per night, probably much farther. Nevertheless, dispersal cannot be accepted as the actual cause of the widespread distribution of f. *carbonaria*. There is considerable evidence that recurrent mutation, at a fairly high rate, also takes place. *Carbonaria* has constantly appeared in isolated centres separated from others by usually impassable barriers. Thus it was taken in Dublin about 1950, near Belfast in 1894, the Isle of Man in 1904, and in 1956 at Torquay, each locality being separated by fifty miles or more from the nearest possible contacts. It is, in fact, likely that the *carbonaria* allele has a high mutation rate. This is in contrast with certain melanics of other species, such as f. *nigra* of *Ectropis consonaria*. In this country, only on two occasions has the melanic mutation successfully maintained itself, first near Maid tone in Kent, and secondly near Stroud, Gloucestershire, where a sample of 121 recorded by me in 1956 were 43 per cent. of the melanic form. It would appear that this melanic mutation has not yet taken place in an industrial centre in this country. It has, however, in Germany where it has become an industrial melanic.

(*b*) *Period of adjustment*

Haldane (1924) has pointed out that if in Manchester in 1848 the *betularia* population was 99 per cent. the *typica* form, and if by 1898 it was 99 per cent. f. *carbonaria*, this represents an approximate 30 per cent. advantage of the black form over the light. Magnitudes, only rather less than this, are borne out by table 1.

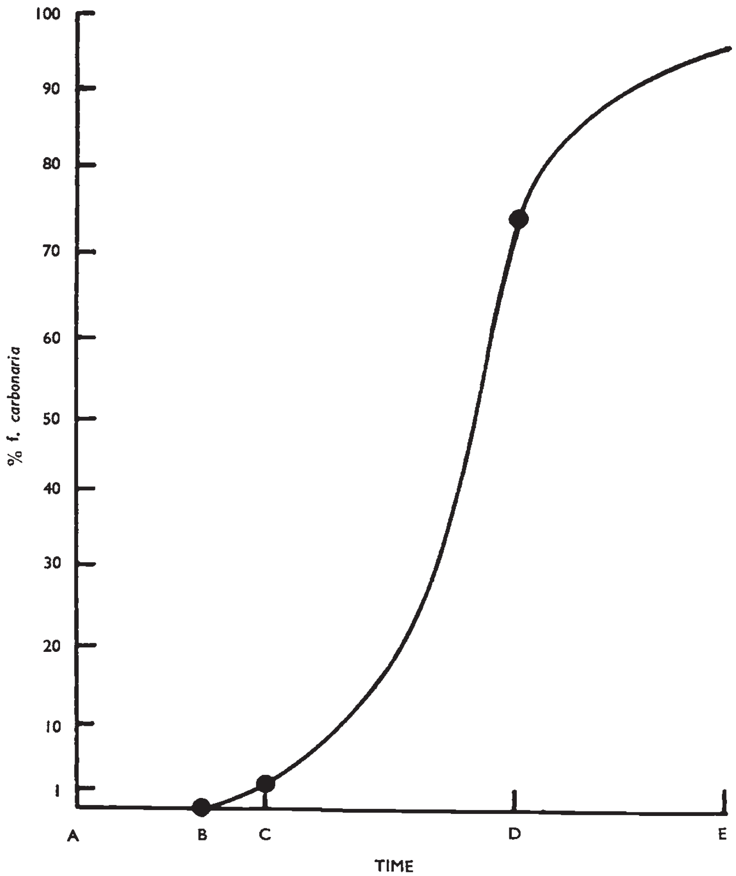


FIG. 2.—Diagram showing rate of increase of a melanic (dominant) mutant with a mutation rate of one in a million, assuming a constant advantage of the heterozygote throughout (which in practice will not occur), with a 30 per cent. selective advantage over its typical form.

It is now necessary for us to consider, therefore, the time lag between the original mutation and its arrival at the 1 per cent. level. At frequencies below this, it is unlikely to have been recognised in the population because of the poorer techniques in sampling used at that date. Let us examine an hypothetical case and assume that in a local population of the order of a million, a single melanic mutation has occurred. With a 30 per cent. advantage, it would take about 29 years to reach the 0·1 per cent. level, and 38 years to 1 per cent. Fig. 2 is a diagram showing the type of spread expected from a new mutant from the time of its origin until it has reached a state of balanced polymorphism or, alternatively, has eliminated its allele from the population. Present data are in agreement with this type of curve. The period under present consideration is represented by BC on the abscissa, and if, in fact, there was only one mutation in Manchester, and by 1848 *carbonaria* has reached 1 per cent. of the population, we can say that this original mutation took place in about 1810, which is approximately fifty years after the commencement of the Industrial Revolution.

During this initial period (BC), the black mutants, though having great cryptic advantage, nevertheless would find themselves in a gene-complex entirely fitted for the specialised pattern of the *typica* form. It is of interest that many of the earliest examples of f. *carbonaria* which I have obtained by searching innumerable old collections, are different from the majority of heterozygous *carbonaria* found to-day. Plate I shows two rows of probable heterozygous *carbonaria*, the right-hand one of which is comprised of insects, all of which were caught in the last century. They bear no resemblance to f. *insularia*. In contrast, the left-hand column shows five modern heterozygotes, and it will be noted that they differ considerably from those collected seventy years previously. Many of the earliest specimens show white markings on all the wings in both sexes greatly in excess of what are generally found to-day. The present-day heterozygote only occasionally shows minor degrees of white markings on the wings. On the other hand, the majority of *carbonaria* have white dots around the head and at the base of the wings. In the Sheffield area and, to a lesser degree, in other centres (Chester for example), these spots are also disappearing in present-day specimens, leaving a completely black insect. This must be brought about by modifying genes through the effect of natural selection. It provides evidence that during this period the gene-complex was adjusting itself to attain its greatest advantage. Furthermore, it suggests that in the early days f. *carbonaria* had not full dominance. We are at present attempting to verify this by out-crossing *carbonaria* into West Country stock whose complex has had no experience of this mutant.

Of even more interest is the situation I have recently found in regard to physiological advantages and disadvantages. It is accepted among lepidopterists that nowadays an excess of melanics occur in backcross broods. Table 3*b* gives a list of my own results of backcross *betularia* broods fed on unwashed Oxford Sallow (*Salix* sp.) which corroborates this fact. It will be seen that there is a significant excess of melanics. In contrast to this, Doncaster (1906) records figures which deviate in the opposite direction for backcross broods occurring between 1900 and 1906 (table 3*a*). It is probable that these also were fed on unwashed foliage. Both sets of data are homogeneous but are significantly different. (The $\chi^2_{(1)}$ is 13·27.) It appears, therefore, that in its early phases, the melanic mutant was not at a physiological advantage to the *typica*, and that only after a considerable period of adjustment did it become so. It is possible then that certain major modifying genes, previously adapted for keeping the complicated typical pattern in check, freed from cryptic responsibilities, were now

TABLE 3

A comparison between early and recent backcross broods of Biston betularia *segregating for* typica *and* carbonaria *showing a deficiency of the two forms in opposite directions*

(*a*) Earliest broods recorded 1900-1905				(*b*) Recent broods 1953-1956				
T	C	Total	Breeder	T	C	Total	Breeder	Brood no.
123	109	232	Bacot	14	22	36	H.B.D.K.	B/2/52
57	47	104	Main and Harrison	7	10	17	H.B.D.K.	19/53
18	11	29	Fletcher	1	5	6	H.B.D.K.	30/53
57	50	107	Harrison	28	30	58	H.B.D.K.	15/54
				1	2	3	H.B.D.K.	19/54
				14	39	53	H.B.D.K.	11/54
255	217	472	C = 46·6%	65	108	173	C = 62·4%	

(*c*) 2 × 2 Table			
T	C	Total	Year
255	217	472	1900-1906
65	108	173	1953-1956
320	325	645	

(*a*) $\chi^2_{(1)} = 2{\cdot}90$; P 0·089

(*b*) $\chi^2_{(1)} = 10{\cdot}20$; P 0·0014

(*c*) $\chi^2_{(1)} = 13{\cdot}27$; P 0·0003

able to exploit themselves in bringing about the best physiological advantage. This period then (BC) must be regarded as a time during which the gene-complex was adjusting itself to the new mutant. The disappearance of a complicated pattern may make it easier for alleles, previously adjusted to this end, to contribute more in other directions.

(c) *Period of rapid spread*

In theory, when once adjusted, the new mutant is free to spread, provided the selective pressure is maintained. As in the earlier periods, because backcross matings will leave a higher percentage of melanic offspring, this increase will proceed with great rapidity up to a time when 50 per cent. of the population is of the *carbonaria* form, and thereafter it will tend to slow down at a speed directly proportionate to the increasing *carbonaria* frequency when the number of heterozygotes in the population will commence to drop as pointed out by Fisher (1937). If we review the progress of *carbonaria* frequencies in those few localities (table 4) where it stands between 1 and 40 per cent. for the short time they have been under observation, there are, indeed, indications of rapid spread. In each, it will be seen that the *carbonaria* frequency has risen significantly. It is, however, possible that the very high selective advantage, apparent in the Marlborough figures, may be due to the initial sample being inadequate. This period of rapid spread is shown on the graph (fig. 2) as CD during which, under certain conditions, it is probable that f. *carbonaria* can change from being 10 per cent. of the local population to 70 per cent. within a period of ten to fifteen years, which reflects accordingly a minimum selective advantage of from 35 to 23 per cent.

(d) Period of slow elimination of f. typica *or alternatively a balanced polymorphism*

The data in table 2 show that in every industrial area f. *carbonaria* is now at least 85 per cent., but in no large sample is the value 100 per cent. Even after one hundred years, Manchester and Sheffield still have 1-2 per cent. of non-*carbonaria* forms, and Lincolnshire 9 per cent. Furthermore, the only actual sample figures available are for Chester, which show that f. *carbonaria* rose in the last fifty years from 83 to 94 per cent. only, giving a selective advantage of 5 per cent., after having achieved the earlier frequency from 1 per cent. in forty-five years (= selective advantage 18 per cent.). All the available evidence goes to show that f. *typica* continues in the population however great are the apparent selective pressures against it. This period is referred to as DE in the diagram (fig. 2), and may represent many hundreds of years, depending on unknown variables. It is probable that by now, after experiencing one hundred generations, the new mutant will have succeeded in achieving a gene-complex suitable for its optimum expression, and that by this time the heterozygote will be at an advantage to both the homozygotes. This will have been brought about by Natural Selection. Modifying genes, disadvantageous in the homozygous state, but advantageous as heterozygotes, will have become more closely linked to the new mutant and one would probably find a figure below the expected 3 : 1 ratio as the result of heterozygous pairings in the wild, because of the elimination of the homozygous *carbonaria*. This will inevitably be followed by a balanced polymorphism.

As a corollary of this, it can be anticipated that the rare f. *typica*, being eliminated on nearly every occasion because of its conspicuousness, and now finding itself in a gene-complex no longer adapted to it but to the melanic, should, in theory, diverge from its previous fixed pattern. I have, therefore, recently commenced to collect all the f. *typica* from districts where it occurs at a very low frequency. With the small numbers at present available, and the difficulty in analysing the degree of divergence, all that can be stated is that considerable variation does occur. Whether this is more than is found in samples from districts where f. *carbonaria* is absent, I am so far unable to tell.

Conclusions on carbonaria *frequencies*

The autocatalytic or sigmoid curve, suggested by Haldane (1924) as representing the likely course of spread of f. *carbonaria*, has been fully borne out. The present investigation shows selective advantages for this mutant of a slightly lower magnitude than that suggested by him, and this, no doubt, reflects more accurate data for present-day frequencies than were available to him. Alternatively, an apparent lower selective advantage would be brought about if a stable polymorphism was taking place in populations with a high frequency of f. *carbonaria*.

7. ANALYSIS OF F. *INSULARIA* FREQUENCIES

The early history of f. *insularia* is unsatisfactory in every respect. It is generally agreed that it appeared about the same time as f. *carbonaria*, but in certain districts, particularly in the south, before it. Doncaster (1906) tried to differentiate "two distinct forms", a light and a dark, and he may in fact be correct. Furthermore, the earlier breeders were confused on finding that backcross *carbonaria* × *typica* broods, on some occasions, "produced intermediates" (= *insularia*), whilst on others Mendelian segregation took place (Doncaster, 1906).

Insularia occurred in Ireland in 1894, in Scotland (Kincardine) some time prior to 1906, and was found at the end of the last century in most central industrial areas. It was recorded on the Continent (Belgium) in 1886. All observers agree that it is an industrial melanic, and that it gets rarer following the upsurge of *carbonaria*.

It is not surprising that f. *insularia*, being intermediate in appearance between f. *typica* and f. *carbonaria*, is found most frequently at the present time on the periphery of industrial areas, outside centres with a high frequency of *carbonaria*, in whose presence it is impossible to detect phenotypically. Background scoring efficiency (Kettlewell, 1955), for the few releases undertaken by me, give *insularia* a position intermediate between f. *typica* and f. *carbonaria* in both industrial and rural environments. It is, in fact, admirably suited for resting on boughs covered with Pleurococcus and not lichens. Nevertheless, it must be pointed out that, in contrast with *carbonaria*, the phenotype frequency of *insularia* has never been found higher than 40 per cent.

Insularia has the great drawback, previously referred to, that it varies from those individuals which are indistinguishable from f. *typica* to those which are as dark as f. *carbonaria*. The frequency figures, unlike those for f. *carbonaria*, are, therefore, subject to personal error.

To estimate this variability, four observers, all living within three miles of each other, and each rated as a most reliable lepidopterist, have supplied independent data over the last few years. It is clear from the table that the estimates of *carbonaria* percentages do not differ very markedly. Comparing the four values by use of a 2×4 contingency table does, in fact, disclose some heterogeneity, since $\chi^2_{(3)} = 7{\cdot}88$, and the 5 per cent. point is 7·81. Although this is formally significant, it does not contradict the view that variations between observers are probably small in respect of *carbonaria* classifications, since the total sample size of 3534 is large enough to detect very small differences.

Percentage phenotypes					
T	C	I	Total	Name	I. estimated frequency
18 per cent.	77 per cent.	5 per cent.	484	J.B.P.	22·32
14 ,,	80 ,,	6 ,,	1435	R.F.B.	31·60
18 ,,	78 ,,	4 ,,	1191	C.deW.	18·80
15 ,,	74 ,,	11 ,,	424	E.T.	40·54

To make a similar test for *insularia*, we have examined the 2×4 contingency table based on the *insularia* and *typica* figures. (This is because the *carbonaria* class is largely uninfluenced by the possibility of misclassification, as explained above.) We find $\chi^2_{(3)} = 23{\cdot}64$, for which $P < 0{\cdot}001$. Thus a very appreciable degree of heterogeneity is revealed in spite of the much smaller sample (777) now used, and this is reflected proportionately in the estimated frequencies of the *typica* and *insularia* forms which for this district vary, with comparable methods of collecting, between 18 and 40 per cent.

Nevertheless, in spite of identification difficulties, f. *insularia* offers many points of considerable interest. As pointed out by Haldane (personal communication), a triangular graph of the three phenotypes (fig. 3) shows that high values of f. *insularia* are associated with *carbonaria* frequencies of from 10 to 30 per cent. Districts showing this, for the most part, are centred around the Severn Watershed and Gloucestershire which, without doubt, receive constant pollution fall-out from Bristol and South Wales. Also the Isle of Man, which is subject to occasional but definite smoke drift from the industrial areas of Lancashire, when the wind is in an easterly direction, has a high *insularia* frequency of 36 per cent. In those areas where *carbonaria* is common, the frequency of visible *insularia* falls, and the random sample, therefore, becomes less accurate, varying in direct proportion to an increased *carbonaria* frequency.

Unfortunately earlier records of f. *insularia* are, for the most part, lost, and what I have been able to extract is largely circumstantial.

For example, the Giles Collection of Lepidoptera in the Folkestone Museum was amassed between the years 1880 and 1890. It was a local collection and in it is a series of 18 *betularia*, 12 *typica* and 6 *insularia*. They are by appearance not bred, in fact many of both forms are worn, and it is likely that this is a small random sample, and reflects correctly a frequency of about 33 per cent. *insularia* at this date. *Carbonaria* did not appear in Folkestone until a very much later date, about 1927 (Morley, personal communication). As will be seen from table 2, the present *insularia* frequency is about 11 per cent., with *carbonaria* standing at 42 per cent., which gives an estimated

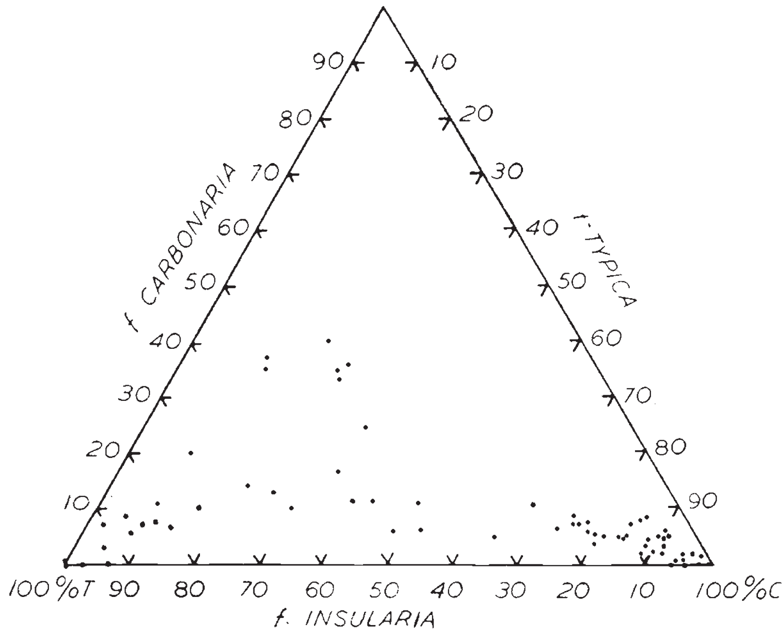


FIG. 3.—Graph showing frequencies of the three phenotypes of *Biston betularia* in Britain.

insularia frequency, on the assumption of there being no linkage, of 17 per cent. It has, in fact, dropped 16 per cent. in thirty years, during which time the frequency of *carbonaria* has risen from approximately 1 to 42 per cent. The true frequency of f. *typica* would, therefore, have increased during this period from 66 to 83 per cent. If this is true, f. *carbonaria* has expanded at the expense of f. *insularia* and not f. *typica* and it suggests that *insularia*, in the presence of heterozygous *carbonaria*, may interact in a disadvantageous manner.

At the upper end of the *carbonaria* frequency, there appear to be only one set of early figures available. Arkle (1901) took a sample of 180 *betularia* at electric light in Chester, and this can be compared with a sample of 124 taken during 1956 by S. Gordon Smith. In a period of over fifty years, the estimated frequency of *insularia* has dropped by only 7·5 per cent. in the population in spite of its cryptic disadvantage. Furthermore, if one includes the combined figures for Cheshire, the *insularia* frequency is still 22·76 per cent.

Percentage phenotypes					
Year	T	C	I	Total	I Estimated frequency
1901	13 per cent.	83 per cent.	3 per cent.	180	20 per cent.
1956	6 ,,	93 ,,	0·81 ,,	124	12·5 ,,

The *insularia* records obtained in the last few years are a good deal more helpful. Nowhere have we so far obtained a phenotype frequency at a level higher than 40 per cent. This can be interpreted in any one of several ways. Either on every occasion f. *carbonaria* has swept

TABLE 4

Frequency of f. carbonaria *recorded in the last four years for districts having a low* carbonaria *frequency*

Locality (observer)	Year	Per cent. T	Per cent. C	Per cent. I	Total	Estimated *insularia* frequency
Oxford . . (P. Sheppard)	1952 1954 1956	45·65 42·50 34·82	28·26 34·20 39·70	26·09 23·50 25·40	46 219 224	36·37 35·45 42·22
Wiltshire . . (Marlborough College)	1951 } 1952 } 1955 1956	76·67 86·30 76·38	1·66 7·19 11·81	21·67 6·48 11·81	60 139 254	22·03 6·98 13·39
Glos. Nailsworth. (A. Richardson)	1952 1956	43·14 50·75	11·76 17·16	45·10 32·09	51 134	51·11 38·74
Hants, Bordon . (D. Wright) .	1952 1953 1954 1955	59·66 64·44 69·39 66·08	21·33 19·53 18·38 22·02	19·01 16·03 12·23 11·90	647 599 941 908	24·16 19·92 14·90 15·25

This gives the following selective advantages for f. *carbonaria* : Oxford 12·2 per cent., Wiltshire 44·6 per cent., Gloucestershire 12 per cent., Hampshire 2 per cent. (approx.).

through the population, an increase in pollution having taken place, thereby favouring this more extreme mutant and enabling it to overtake *insularia* ; or, alternatively, homozygous *insularia* are at a considerable disadvantage to the heterozygotes. A third explanation could be that *insularia* has partial linkage to *carbonaria*, so that fewer *insularia* phenotypes appear in the population when the *carbonaria* mutant becomes common. Table 4 does, in fact, suggest, with the exception of Oxford, that *carbonaria* increases at the expense of *insularia* and not of f. *typica*.

If we assume that there is no linkage, we are able to graph the estimated frequency of *insularia* in any population, in which it may be to a great extent concealed by *carbonaria*. The higher the *carbonaria* frequency, however, the greater the error because of the paucity of the *insularia* sampled. Fig. 4 shows the result of graphing all estimated frequencies shown in table 2 in which the total sampled was larger than fifty individuals. The data suggest the following deductions :—

(1) When f. *carbonaria* and f. *insularia* simultaneously enter a population consisting of f. *typica* only, f. *insularia* will rise to about

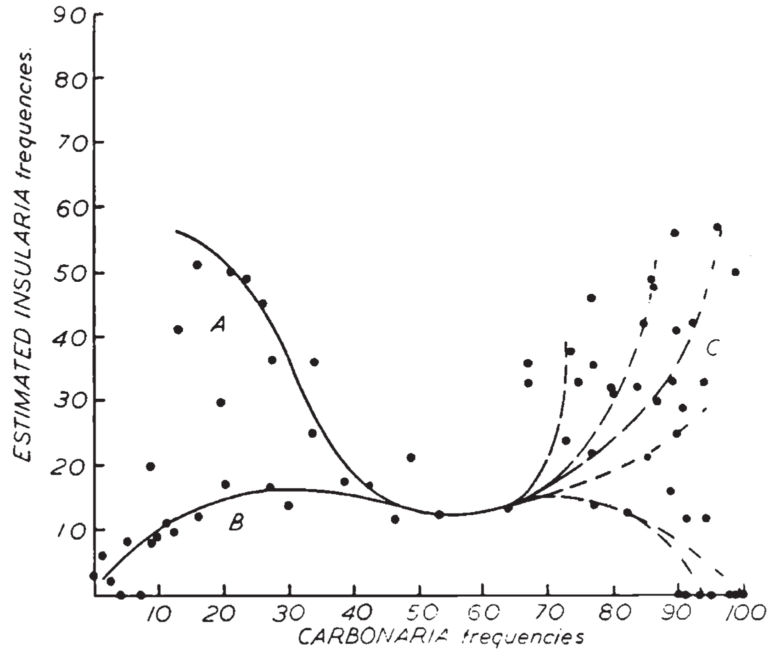


Fig. 4.—Graph showing frequencies of *insularia* for the majority of districts in Britain (plotted against *carbonaria* frequencies).

15 per cent., but no higher as long as the *carbonaria* frequency remains under 10 per cent. (District B).

(2) When f. *carbonaria* enters a population which has a high frequency of f. *insularia* (40 per cent.), it is likely that this will drop to this same level of 15 per cent. (District A).

(3) *Carbonaria* frequencies of between 40 and 65 per cent. are associated with the lowest gene frequencies of *insularia*, and this coincides with the most rapid spread of f. *carbonaria* (fig. 2).

(4) Thereafter, a rising *carbonaria* frequency is associated with *insularia* frequencies varying from high to low (District C). It is of interest then to find that each of the three main groupings, designated with a letter, correspond geographically with the different areas of Britain in the majority of recordings. Thus group A, with a high

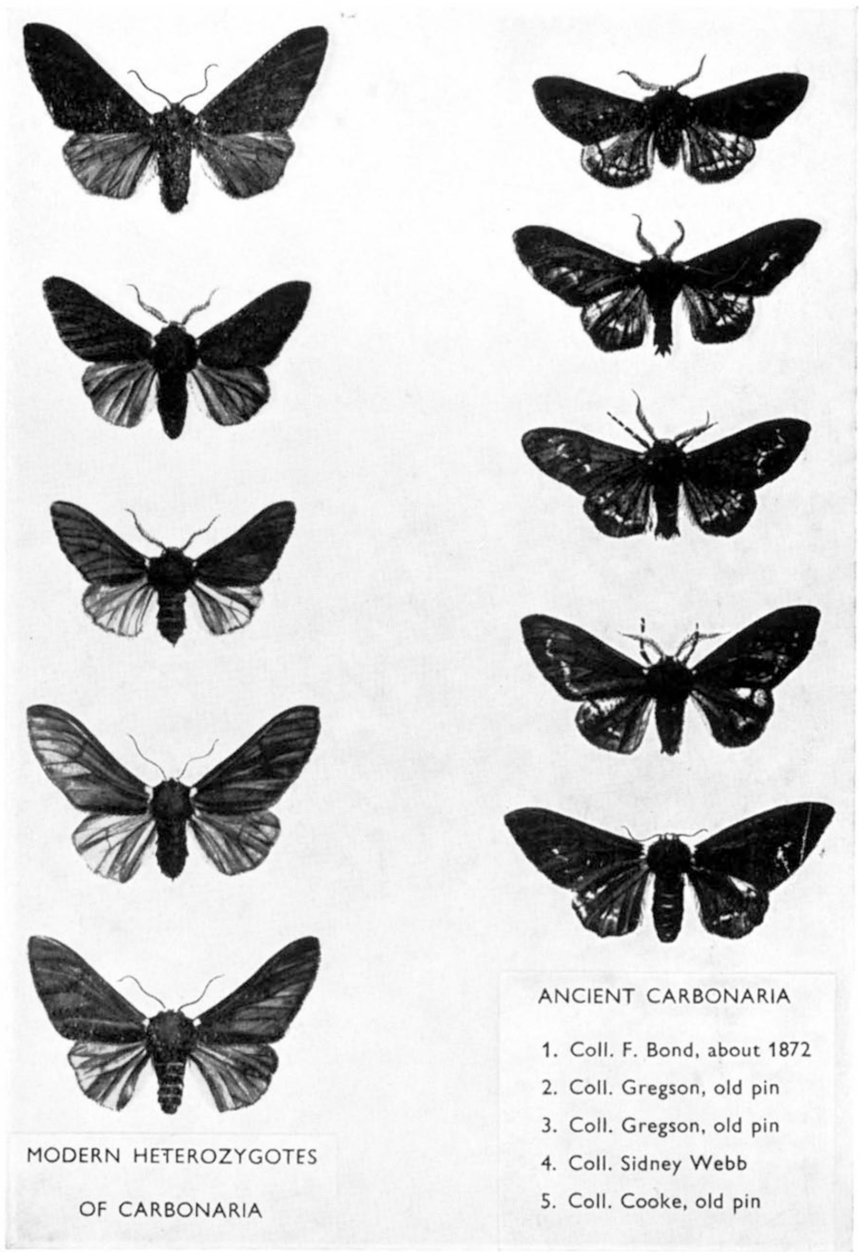
frequency of *insularia* is largely constituted by the Severn Valley, Gloucestershire and Oxfordshire ; group B the counties of Berkshire, Wiltshire, Hampshire and Dorset ; while group C represents the whole of the eastern half of England from Yorkshire to London, an area which corresponds with the earlier map of *carbonaria* as drawn up by Adkin (1925).

For the purpose of analysis, it is convenient to divide the graph (fig. 4) into two portions : below 50 per cent. *carbonaria* frequency and above.

Below a 50 per cent. *carbonaria* frequency, it is evident that no matter at what level the *insularia* has been maintained in the population, the entry of *carbonaria* into it will canalise the *insularia* for a period of time at an approximate 15 per cent. level. It is possible on the other hand that this may represent an equilibrium frequency in which f. *insularia*, in the presence of prolonged but slight pollution fall-out, is capable of adjusting itself in the population at this level in a state of balanced polymorphism. It is a more likely explanation, however, that it is in a transient state, in which *carbonaria*, the more extreme melanic, is replacing a less suitable one on the presence of ever-increasing air pollution. It could, however, be a further indication that *insularia*, in the presence of *heterozygous carbonaria*, is at a disadvantage.

Above a 50 per cent. *carbonaria* frequency (fig. 4), the estimated *insularia* values vary from 0 to 65 per cent., the majority being high except in populations practically entirely overrun by f. *carbonaria*, where the *insularia* samples are always inadequate. This can be brought about by one of two alternative ways. The initial cryptic advantage of *insularia*, being now largely lost in the *carbonaria* phenotype, it is likely that heterozygous *insularia* have some definite physiological advantage in the presence of *homozygous carbonaria*. Alternatively, it could be argued that over levels of 65 per cent. *carbonaria* frequencies, the *insularia* cryptic advantage is largely lost, and *insularia* would find itself floating in a *carbonaria* population, and subject to drift. This could constitute a new type of circumstance, allowing drift, but in my opinion it is most unlikely that *insularia* has a completely neutral physiological value apart from its initial cryptic advantage. Furthermore, if homozygous *insularia* are eliminated as seems possible, drift cannot be considered as an explanation.

Standard statistical analysis shows a highly significant degree of heterogeneity ($\chi^2_{(38)} = 99{\cdot}86$) in the proportions of f. *insularia* in populations with over 65 per cent. *carbonaria*, and it is evident that f. *insularia* continues to maintain itself within a *carbonaria* population even after losing its cryptic advantages and, in fact, may even expand. On the other hand, slow elimination is indicated by the subsequent history of the only recorded early frequency known to me, which indicates that in Chester the estimated frequency of *insularia* has dropped 7·5 per cent. in 55 years, but the sample for this assumption is inadequate. It must be remembered that at the beginning of this



Plate

Ancient and modern heterozygotes of *Biston betularia* f. *carbonaria*. The former were extracted from old collections made in the last century and have many white markings. Specimens like these are but rarely found to-day.

(Photograph by John Haywood from painting by Christine Court, Oxford).

period 3 per cent. of the population were still appearing as f. *insularia* which would, on most occasions, be eliminated because of their cryptic disadvantage in heavily polluted districts. Only future records will be able to settle what really happens, and their foundation is now laid in this paper. At the same time, it has served to point out that the natural history of the T-C-I complex in this rapidly changing countryside is likely to raise problems of unusual interest.

8. SUMMARY

1. The early history of *Biston betularia* and its two melanic forms in Britain, *carbonaria* and *insularia*, has been recorded. Selective advantages have been estimated for the period of over 100 years since the commencement of industrial melanism, and shown to be similar to those suggested by Haldane (1924) on the ground of theory, but slightly smaller.

2. The present survey includes over 20,000 records of *betularia* from many districts of Britain which have been made by over 150 observers. A table of these is given, and a frequency map has been prepared from them. The data show correlation between the industrial centres and high frequency of f. *carbonaria*, the more extreme melanic. *Carbonaria* is also abundant throughout the eastern half of England ; this is probably the result of the indirect effects of smoke-drift following the prevailing south-westerly wind. The south-western parts of England and the whole of northern Scotland are free of melanics.

3. The rate of spread of *carbonaria* follows a sigmoid curve. The original light form is maintained at a very low frequency in all industrial populations. This may eventually lead to a balanced polymorphism if the heterozygous *carbonaria* are at an advantage to either homozygote.

4. The highest phenotype frequencies of *insularia* are found in populations having 10 to 30 per cent. *carbonaria*, and these are for the most part centred around the Severn Watershed, Gloucestershire and Oxfordshire. *Insularia* phenotype never occurs higher than 40 per cent., and this may be because the homozygote is at a disadvantage.

5. A graph, showing the estimated frequencies of *insularia*, has been prepared. It has been shown that *carbonaria*, when between 20 and 50 per cent. of the population, expands at the expense of *insularia* but not of f. *typica*.

6. In populations of more than 65 per cent. *carbonaria*, the *insularia* gene frequency may be either high or low, in spite of ever increasing loss of its earlier cryptic advantages.

7. This paper is intended to be a foundation for future records which may disclose the true behaviour of two dominant genes both rapidly and simultaneously spreading through a population.

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