PRESIDENTIAL ADDRESS

BUTTERFLIES AND MOTHS IN SCIENTIFIC RESEARCH

BY E. B. FORD, F.R.S.,
All Souls College, Oxford

The feature which has attracted generations of naturalists to the study of butterflies and moths is the one which fits these insects so well for certain types of scientific research: that is to say, their beautiful colour-patterns which make it possible to analyse their variation with exceptional ease and efficiency. This brief survey of such work will be devoted to aspects of it that are not yet widely known. Foreign species will be excluded; indeed it seems appropriate to restrict the examples in such a way that they can be drawn entirely from forms to be found in or near Somerset. No account therefore, can be given of the remarkable investigations now being carried out on butterfly mimicry, using African and North American material.

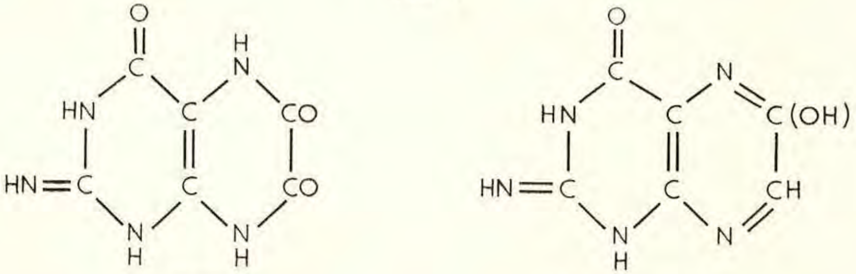
In order to study any large assemblage, whether of words, butterflies or finds of pottery in an excavation, it is necessary to classify it, and to do so hierarchically. There are many different ways in which the Lepidoptera could be classified but the established one is based upon relationship, so that it demonstrates one particularly important aspect of reality: that presented by evolution. Evidently, therefore, if successfully carried out, the insects placed within each group, whether large or small, will be more closely related to one another by actual descent than they are to any other organisms upon earth. Thus if the arrangement be represented diagrammatically, the result should produce a genealogical tree.

It may well be asked whether such an object has been achieved: if indeed the various subdivisions of butterflies and moths are anything more than human fabrications, in no way reflecting affinity. Clearly a test could be applied to this matter. Some qualities should be studied which were not used in devising the accepted system; should they, nevertheless, prove to be closely correlated with it, we should have independent evidence of its validity.

It was not difficult to pick upon such features: the chemistry of the wing-pigments provided them. Not only had these been ignored in assessing relationship but they were themselves largely unknown and their nature had first to be determined. Space will not allow of more than two examples illustrating the way in which the distribution of these substances has been applied to classification.

The white and yellow pigments of the Lepidoptera are of a number of chemically distinct types. One of them is derived from uric acid, a waste product of the body of butterflies as of men, built up to a slightly larger molecule. When obtained in this way, the white colouring matter, "leucopterin", has one more oxygen atom than the yellow, "xanthopterin" (Figure 1).

FIG. 1



LEUCOPTERIN
(white)

XANTHOPTERIN
(yellow)

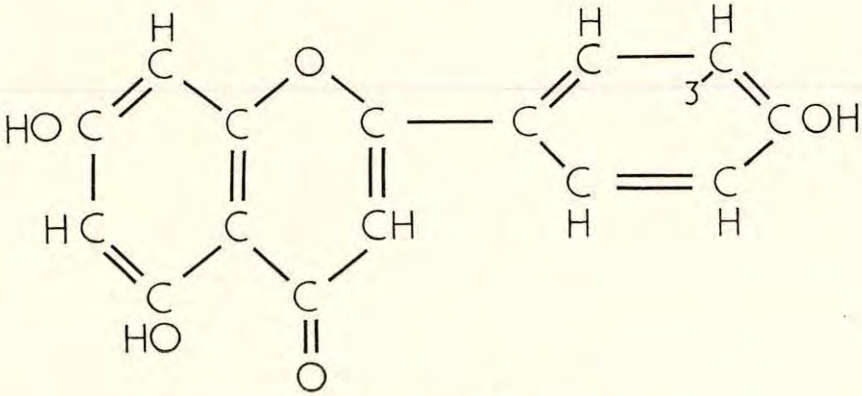
These uric acid pigments can be detected by a "murexide" test, a simple form of which, using chlorine, is available (Ford, 1947). Their occurrence proves to be restricted to a single family, the Pieridae, comprising 67 genera and several hundred species. In these they are always present and they have never been found in any other butterflies and moths; a fact entirely unknown until 1947, long after these insects had been classified. Clearly, therefore, the Pieridae (which include the ordinary Cabbage Whites and other White Butterflies, the Brimstone and the Clouded Yellows) are correctly grouped together since their association has been attested on independent evidence.

It will be desirable to take a second instance within the same family so as to determine the application of this type of argument at a different hierarchical level. The Wood White, *Leptidea sinapis*, L., is a very local butterfly found in a few woods in Somerset and in other parts of Southern England and Ireland. It is widespread in Europe, where the closely related *L. duponcheli* Stgr. also occurs, while a third very similar species, *L. gigantea* Leech, is known from

China. This little genus of three White Butterflies is an anomalous one. Detailed consideration of their structure led to the surprising conclusion that they belong to the subfamily Dismorphiinae, otherwise known only from Central and South America.

So strange an ascription needs to be justified. Since the Dismorphiinae belong to the Pieridae, they possess uric acid pigments as just described. But in some, though not all, of the species white and yellow flavones are present in addition. These are of a very different nature, being non-nitrogenous (Figure 2); moreover, they are purely plant products. Animals, apparently, can never manufacture them but are sometimes adapted to obtain them from their food and utilize them. They can be extracted with ethyl acetate giving, when filtered, a colourless solution. This turns yellow on shaking with sodium carbonate if flavones be present. They can also be detected by means of ammonia with which they form unstable yellow compounds, as can easily be seen on fuming the Marbled White, *Melanargia galathea* L., with that gas. This is a common butterfly of the Somerset downs and roadsides; it is, however, not a member of the Pieridae but belongs to a very different family, the Satyridae.

FIG. 2



APIGENIN, a white flavone

Flavones are unknown in any of the Pieridae except the Dismorphiinae. It is therefore a matter of great interest to find them throughout the genus *Leptidea* including, of course, the Wood White

(Ford, 1941). Their presence provides entirely independent confirmation for the view that this little butterfly of the Somerset woods belongs, with its two foreign relatives, to a group otherwise confined to Central and South America.

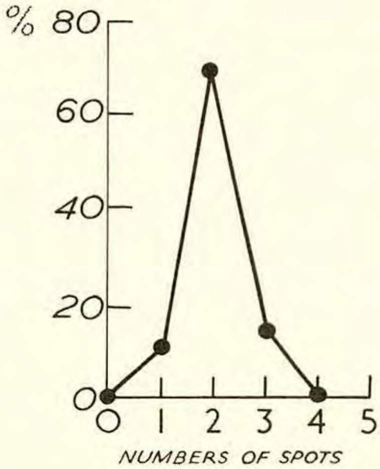
It will be realized, therefore, that tests are available which confirm on independent evidence that the accepted classification of the Lepidoptera correctly reflects relationship. Consequently it is reasonable to enquire how the different groups have evolved. A hundred years after the publication of *The Origin of Species*, and after sixty years of Genetics, there can surely be no need to stress that they do so, in general, by the interplay of three agencies: natural selection operating upon heritable variability in populations subdivided by some form of isolation. The variation must, of course, be of the Mendelian type; that is to say, controlled by a system which ensures both extreme heritable diversity and extreme heritable stability. The need for isolation was admitted by Darwin and has been recognized by all biologists subsequently.

It is now so well known that the actual process of evolution has been detected, studied in the field and reproduced in laboratory experiments, that it is unnecessary to develop that aspect of the subject here. Yet the work of the last twenty years or so has at least in one respect altered fundamentally our views upon it. For the magnitude of the selective forces ordinarily operating in nature had previously been greatly underestimated. Thus R. A. Fisher in his fundamental work *The Genetical Theory of Natural Selection*, published in 1930, assumed selective advantages of up to about one per cent. in wild populations. It is now clear that this value should considerably exceed sixty per cent., for that level proves to be quite common.

Faced with selection-pressures such as these, it is not unreasonable to envisage a single inter-breeding community forming distinct local races with no geographical barrier, past or present, between them: the "sympatric evolution" of Mayr (1947). This has remained a hypothetical concept so far, except when due to polyploidy; a phenomenon largely restricted to plants, since it requires vegetative reproduction to carry the new form over the initial stages of its spread. It seems worthwhile, therefore, to describe the first occasion on which a continuous population is known to have become subdivided into two groups without any form of isolation to assist the process; for information on that occurrence can only be obtained so far from a single article (Creed, Dowdeswell, Ford, McWhirter, 1959). It was detected in the Meadow Brown, *Maniola jurtina* (Satyridae), one of the commonest and most widespread butterflies in Britain. This has one generation in a year, the imagines normally fly from mid-June to mid-August, and its larvae feed upon various common grasses.

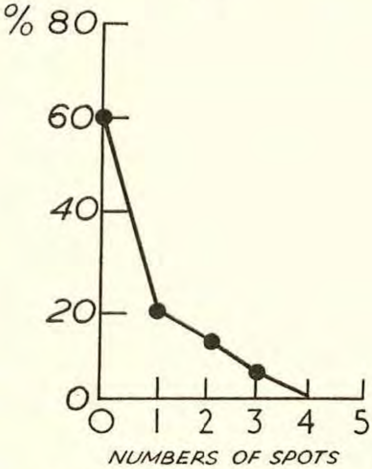
We required some feature which could be used to measure variability in this species, and picked upon the spots on the underside of the hindwings. These are small and black, occasionally with white centres, and are placed submarginally. They may be absent, or present in any number up to five per wing, and are usually symmetrical on the two sides. They are controlled by polygenes which affect the two sexes differently. In the males the individuals with two spots on each wing nearly always constitute the commonest class, or "mode" (Figure 3); in the females the spotless ones do so over a large part of Southern England (Figure 4), though elsewhere they differ considerably in this respect. Thus that sex is generally the more diagnostic and we shall confine our attention to it in this account.

FIG. 3



MALES

FIG. 4



FEMALES, West Devon Eastwards

It is astonishing that this sexual difference in spotting in one of our commonest butterflies had passed unnoticed by collectors and was detected only when the insect was first used for experimental purposes in 1946.

It is unlikely that the spots influence the survival of the butterfly, but breeding experiments combined with ecological work have demonstrated that the polygenes controlling them have other effects which do so. Thus the spotting allows us to detect important differences in physiology or habits.

Within each race spot-values are powerfully stabilized by selection against environmental differences. Thus that with a single mode at O spots in the females (Figure 4) occurs from West Devon to the North Sea and at least as far to the north as the Mersey and the Wash: probably much further for, though we have little information from Scotland, it is the form found in Sutherland. Throughout this extensive range, spotting appears to be unaffected by the environment. It is the same in coastal and inland districts, in the Atlantic climate of Devon and the semi-Continental one of East Suffolk. It is the same, too, on chalk downs, with their alkaline soil and exposed conditions, as in acid marshes and woodlands.

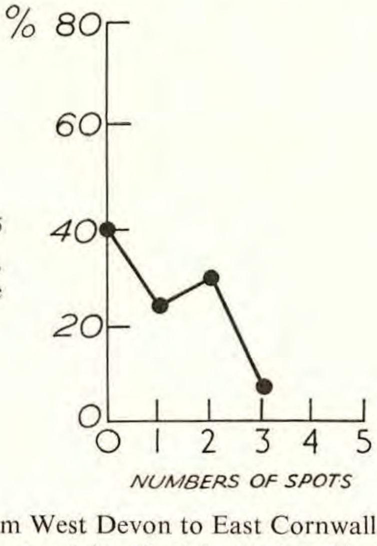


FIG. 5
FEMALES,
East Cornish Type

As we pass from West Devon to East Cornwall a different type of spotting is encountered in *Maniola jurtina:* bimodal with a larger mode at O and a lesser at 2 (Figure 5). This extends to central

Cornwall and indeed still further West where, however, the insect becomes rarer; while a distinct form, unimodal at 2 spots in the female as in the male, occurs in Penwith (the Land's End Peninsula, beyond the Hayle—Marazion isthmus).

The East Cornish type, with its bimodal females, is also stabilized against the diversities of its own area. It is the same in the centre of the county as on the coast, and the same both east and west of the granite intrusion of Bodmin Moor which constitutes a barrier to the insect.

In 1952 we had begun the study of the transects between the main English and the East Cornish races, obtaining information on their occurrence both in the southern coastal strip below Dartmoor and thirteen miles inland along the line from Okehampton in Devon to the Lewannick district just west of Launceston in Cornwall. The change from one type to the other took place over a distance of twelve miles in the latter, that is the more northerly of the transsects, and over about five miles in the southern one. To our surprise, we had obtained no evidence of a cline in either: the population being completely English (unimodal) at the eastern, and completely Cornish (bimodal) at the western, end of each. In the next season which we could devote to this work, that of 1956, we decided to investigate this subject more thoroughly, choosing for that purpose the more northerly transect in order to avoid difficulties introduced by the suburbs of Plymouth. Its two ends, twelve miles apart, proved still to be respectively Southern English (near Lydford) and East Cornish (on the river Inny near Lewannick). We therefore divided the intervening range into three blocks of approximately equal lengths. Starting from the eastern end, we accordingly advanced four miles to Chillaton, where the *jurtina* population was still Southern English. We therefore went forward a further four miles to the western plain of the river Tamar, which here constitutes the county boundary. To our surprise the butterflies gave no indication that we were now so near the Cornish stabilization, proving indeed to be completely of the Southern English type.

Accordingly we proceeded halfway along the remaining four mile strip between the Tamar and the terminal locality of the transect. This took us close to the hamlet of Larrick. Here we made our way westwards up the valley of a little stream which rose in the heights above the river Inny two miles distant, near Lewannick, where the

Maniola jurtina population was Cornish. As the situation had become critical, we scored our captures roughly as we went, sitting down to do so accurately at intervals. This we did after working along the stream for four fields. Though only about a mile and a half from the known Cornish stabilization, the result was completely Southern English; it might as well have been obtained in Suffolk. We then crossed into the next field westwards and soon became aware that a change had at last occurred in the butterflies we were catching. Here, and on some rough ground beyond, we amassed a sufficient sample for statistical analysis and found that the *Maniola jurtina* were completely Cornish. A stabilization which had persisted across Southern England had at this point readjusted to a new, also stable, form (the East Cornish). It did so at a single field hedge across which the insects were flying freely.

Strange as this occurrence appeared, it called at this stage for no new concept for its most likely interpretation seemed merely to involve a situation already well known. As a first hypothesis, then, it was assumed that though no geographical discontinuity exists between the two races today, one had done so in the past and they had only subsequently extended their ranges and met. That is to say, the Cornish and Southern English forms had evolved in isolation to such an extent that, on crossing, their combined gene-complex was ill adapted. Thus the hybrid specimens though constantly produced would be as constantly eliminated. The two types therefore would not merge into one another but remain sharply distinct at the boundary between them. This is a situation well known in ecological genetics. We therefore planned to test its application to this instance by a joint programme of breeding and cytology; for if explicable along these lines, one would expect to find unpaired loops in the chromosomes of the hybrids absent from those of either of the stabilized races. That investigation never became necessary. The samples obtained the following year demonstrated that the situation was yet more remarkable than we had supposed and excluded an interpretation along the more conventional lines just indicated.

The results of 1957 repeated those of 1956 except for that section of our transect which included the eastern part of the Larrick locality and the western flood plain of the Tamar. The populations here, which had been of the English type the year before, were now completely Cornish. It was a matter of much interest to determine if the change from one to the other remained clear-cut at the new position. We therefore crossed the Tamar to collect in the eastern flood plain, here only 160 yards wide, and obtained an intermediate population there. That locality was bounded by hanging woods, above which the normal English stabilization began and continued. The intermediate area was clearly of negligible extent in comparison with the great range of the two standard populations on either side of it: the one extending westwards to the far side of Bodmin Moor and the other eastwards across England to the North Sea.

The sharp distinction between the two forms has persisted subsequently at the same site as in 1957; that is to say, on either side of the narrow eastern flood plain of the Tamar. In 1959 we tested once more the southern of the two transects studied in 1952: that between Dartmoor and the sea. Here also we obtained evidence of an abrupt transition from the Southern English to the East Cornish spotting. Furthermore, the latter had spread two to three miles eastwards, as it had done further north.

Considering these facts, it is clear that the sudden change in a continuous population from one stabilization to another could not be due to a past discontinuity and subsequent extension of range. In 1956 butterflies of the Southern English form were laying their eggs at East Larrick and the west Tamar localities, yet the spotting had become Cornish there the generation following. We cannot picture a wave of *Maniola jurtina* advancing from the west, the insects further east recoiling before it. On the contrary, the two forms must be determined by powerful selective forces. Yet their stabilization over a wide diversity of country excludes the simple concept that different genotypes are favoured in different environments. We are indeed forced to suppose that this butterfly has two distinct ways in which it can adjust itself to closely similar conditions when the areas occupied by each type are large enough to build up a balanced gene-complex, provided that the selective forces involved are very great. When we compare the 1957 generation with the previous one, that of 1956, in the region from East Larrick to the West Tamar flood plain, the selection operating against individuals with one spot rather than 2 or more can be calculated. It proved to be 65 per cent., with 95 per cent. fiducial limits at 82 and 31 per cent.

Here we have evidence that under powerful selection a single race can become subdivided into two without isolation: a situation, though doubtless an exceptional one, which can be added to those that promote sufficiently rapid evolutionary change for the process to be examined in natural populations. Several others have been identified and used for that purpose. Attention must be drawn to one of these here, since the study of butterflies and moths has contributed greatly to its analysis. This is polymorphism, and it is defined (Ford, 1940*a*) as the occurrence together in the same habitat of two or more discontinuous forms of a species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation.

Polymorphism, then, is a form of variability, but one in which we are concerned neither with the distinctions between geographical races nor with continuous variation such, for instance, as that in human height, which can take all values from one extreme to another. The definition moreover excludes the segregation of rare recessive and heterozygous conditions thrown into the population at intervals by mutation and constantly eliminated by selection; of these, the albinos encountered occasionally in many animals, and a severe human disorder, Huntington's chorea, provide examples.

The sharply distinct forms constituting a polymorphism must evidently be controlled by some switch-mechanism, which must nearly always be genetic so as to allow of its evolution. This is in fact provided by genes or super-genes which, considered from an adaptive point of view, may at any one time be either advantageous, disadvantageous or of neutral survival value.

Now every genetic unit which proves permanently useful will be spread by natural selection until its allelomorph is reduced to the status of a rare mutant, when it can no longer promote that form of variation which constitutes polymorphism. On the other hand, when unequivocally disadvantageous, its advance is checked by counter-selection at an early stage. It can indeed be maintained only by recurrent mutation, so that it also is incapable of generating a polymorphism. It might be thought, however, that many polymorphisms are determined by genes or super-genes of no importance to the organism, a view which a superficial survey of their effects certainly seems to endorse. This possibility also is excluded, for Fisher (1930*b*) has shown that for a gene to be effectively neutral compared with its allelomorph, the balance of advantage and disadvantage concerned must be very exact. Also the spread of such a gene, if derived from a single mutation, is extremely slow: so slow indeed that the number of individuals which possess it cannot greatly exceed the number of generations since its occurrence. Moreover, mutation is too rare to hasten the process materially. That is to say, long before such "neutral" genes have gained any substantial footing in a population, the accurate balance needed to ensure their neutrality will have been upset by changes in the environment, the gene-complex or both.

Thus if any unifactorial character occurs in even as much as one per cent. of a population, it must have spread to that extent because it has some advantage. But, as we have seen, a permanent polymorphism cannot arise if that advantage persists unchecked. It can do so indeed only by reason of a balance of advantages and disadvantages, and this is most usually attained when the heterozygote is favoured compared with either homozygote: a situation which must be generated even if there is some obvious ecological reason for maintaining diversity, as with heterostyled plants. The reason for this is bound up with the evolution of dominance.

We owe to Fisher (1928) the principle that advantageous qualities *become* dominant and disadvantageous ones *become* recessive, owing to selection magnifying the intensity of the one and minimising that of the other, as first demonstrated in wild material in the common Currant Moth, *Abraxas grossulariata* (Ford, 1940*b*). It is established also that genes have multiple effects and, since mutation is a random process relative to the needs of the organism, if one of them proves beneficial and becomes dominant, the others are likely to be harmful and become recessive. Consequently the heterozygote will have advantages only, while the two homozygotes will each have some advantages and disadvantages, thus leading automatically to heterozygous superiority and so to polymorphism.

The Lepidoptera have provided several of the essential steps required to test and develop this conclusion and, in more general terms, the importance of selection in maintaining polymorphic characters in a community. Two examples of the way in which they have done so may usefully be mentioned here.

The Scarlet Tiger, *Panaxia dominula*, is a day-flying moth with a striking colour-pattern. The forewings are bronze green with white spots, some of them yellowish, the hindwings scarlet spotted with black. There is a single generation in the year, the perfect insect appearing on the wing in July.

This is a very local species, restricted to the southern half of England, though sometimes abundant where it occurs. I am uncertain if it has been obtained recently in Somerset but it has been taken near Sherborne since 1950. It is nearly always found by river banks or in damp sites and it has been studied intensively since 1939 in an isolated marsh of fifteen acres at Cothill, Berkshire, five miles from Oxford. Indeed the colony there has been more fully quantified than any other population of animals in the world.

In this one locality *Panaxia dominula* is polymorphic for a gene affecting the pattern. All three genotypes are distinct. The rare homozygote, known as *bimacula*, is very unlike normal *dominula*, for the white spots on the forewings are absent except the two basal ones while the red on the hindwings is largely obscured by black. The heterozygote, *medionigra*, is much nearer in appearance to the ordinary form than to *bimacula* but seems always to be identifiable; for the central white spot in the forewings is small or absent and there is an additional black one, sometimes reduced to a few scales only, in the middle of the hind pair.

By the technique of marking, release and recapture (Fisher and Ford, 1947) the size of the population in this marsh has been assessed year by year while, at the same time, the frequency of the three forms has been determined, so allowing an estimate of the percentage of the *medionigra* gene (that responsible for *medionigra* when heterozygous and for *bimacula* when homozygous) at the locus concerned. The results up to 1958 are given in the table on page 40.

Now under Mendelian inheritance it can be calculated to what extent a mutant gene may spread fortuitously, provided its frequency and the size of the population in which it occurs be known. These two requisites had not heretofore been fulfilled in any wild population, but they are available for that of *Panaxia dominula* at Cothill. Consequently, it is possible to determine if the fluctuations in gene-frequency noticed from one year to another are such as can arise by chance. The analysis shows that they are much too large for this: they must be due to selection, changing in magnitude and direction from year to year, and controlling the relative numbers of the polymorphic phases concerned (Fisher and Ford, 1947; Sheppard, 1956). This is a type of test widely applicable, and *Panaxia dominula* provided the initial instance of it.

TABLE 1.

Year	*Gene-frequency per cent*	*Population Size*
1939	9.2	?
1940	11.1	?
1941	6.8	2,000 to 2,500
1942	5.4	1,200 to 2,000
1943	5.6	1,000
1944	4.5	5,000 to 6,000
1945	6.5	4,000
1946	4.3	6,000 to 8,000
1947	3.7	5,000 to 7,000
1948	3.6	2,600 to 3,800
1949	2.9	1,400 to 2,000
1950	3.7	3,500 to 4,700
1951	2.5	4,000 to 6,000
1952	3.6	6,000 to 8,000
1953	2.6	5,000 to 11,000
1954	2.9	10,000 to 12,000
1955	1.1	1,500 to 2,500
1956	3.0	7,000 to 15,000
1957	4.6	14,000 to 18,000
1958	3.7	12,000 to 18,000

THE POPULATION-SIZE OF THE COLONY OF THE SCARLET TIGER AT COTHILL, BERKSHIRE, AND THE FREQUENCY IN IT OF THE *MEDIONIGRA* GENE

The phenomenon of Industrial Melanism in moths supplies additional information on polymorphism. Over sixty species have in the last hundred years become black in the manufacturing regions of Britain and a similar change has taken place in other countries. Those affected all rest fully exposed on tree trunks or rocks and derive protection from their resemblance to bark or lichen. Kettlewell (1955, 1956 *a* & *b*) has shown that they are preyed upon by insectivorous birds, Hedge Sparrows, Redstarts, Yellow Hammers, Spotted Flycatchers and others. These selectively eliminate in large numbers the inappropriately coloured forms: the blackish specimens in the ordinary countryside and the pale ones in industrial areas where they no longer match the background now that it is begrimed with soot. Not only have the dark forms become common in recent times, passing in some species from rare varieties to occupying 95 to 98 per cent. of the population in these smoke-polluted districts, but they have evolved. Their black colour has become more intense and uniform, and in several of them a scatter of light scales, present when first the dark insects began to spread, has been eliminated.

Yet the situation is more complex than this. The melanics are nearly always dominant to the normal pale specimens in the species in which they have successfully established themselves, and in them the heterozygotes are at a physiological advantage compared with either homozygote. They are, in fact, the hardier, being better able to withstand partial starvation and polluted food (Ford, 1940*b*). Evidently heterozygous advantage has developed here and it has done so for the reasons already explained.

But that phenomenon is not one of isolated instances. On the contrary, its occurrence can be deduced as a generalization of the widest kind. Therefore it is applicable also to long-established instances of polymorphism in general, and consequently to one of the most polymorphic of animals; that is to say, to Man.

It was, basically, on account of these considerations that it was possible to predict, in advance of the facts, that the frequencies of the human blood groups are determined by powerful selection pressures (Ford, 1940*a*). Bearing this in mind, and the fact that the serological types do not influence mating preferences (the choice of husband and wife), it became clear that the various blood groups must be associated with liability to develop specific diseases (Ford, 1945). Six years after that prediction, Struthers (1951) showed that a significant excess of individuals who die of bronchial pneumonia during the first two years of life belong to group A of the O, A, B blood group series. Soon afterwards Aird, Bentall and Roberts (1953) established that cancer of the stomach is also significantly commoner in the same group, while duodenal ulcer is more frequent in those who do not secrete the O, A, B, blood group antigen into the saliva (McConnell, 1960). Such individuals constitute about 22 per cent. of the population in England, the ability or inability to do so being itself a polymorphism. Numerous other instances of the kind are now being detected, so opening up a new branch of medical research, the importance of which in diagnosis and conceivably in treatment is yet to be at all fully explored.

When one sets out to study butterflies and moths one might not expect to travel the path which leads to new concepts in medicine, but so it was, and there is a principle of importance here. Such results illustrate that scientific research must never be planned wholly along utilitarian lines. Administrators have sometimes wished that it might be; were they allowed their way, they would in the long run defeat their own ends.

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