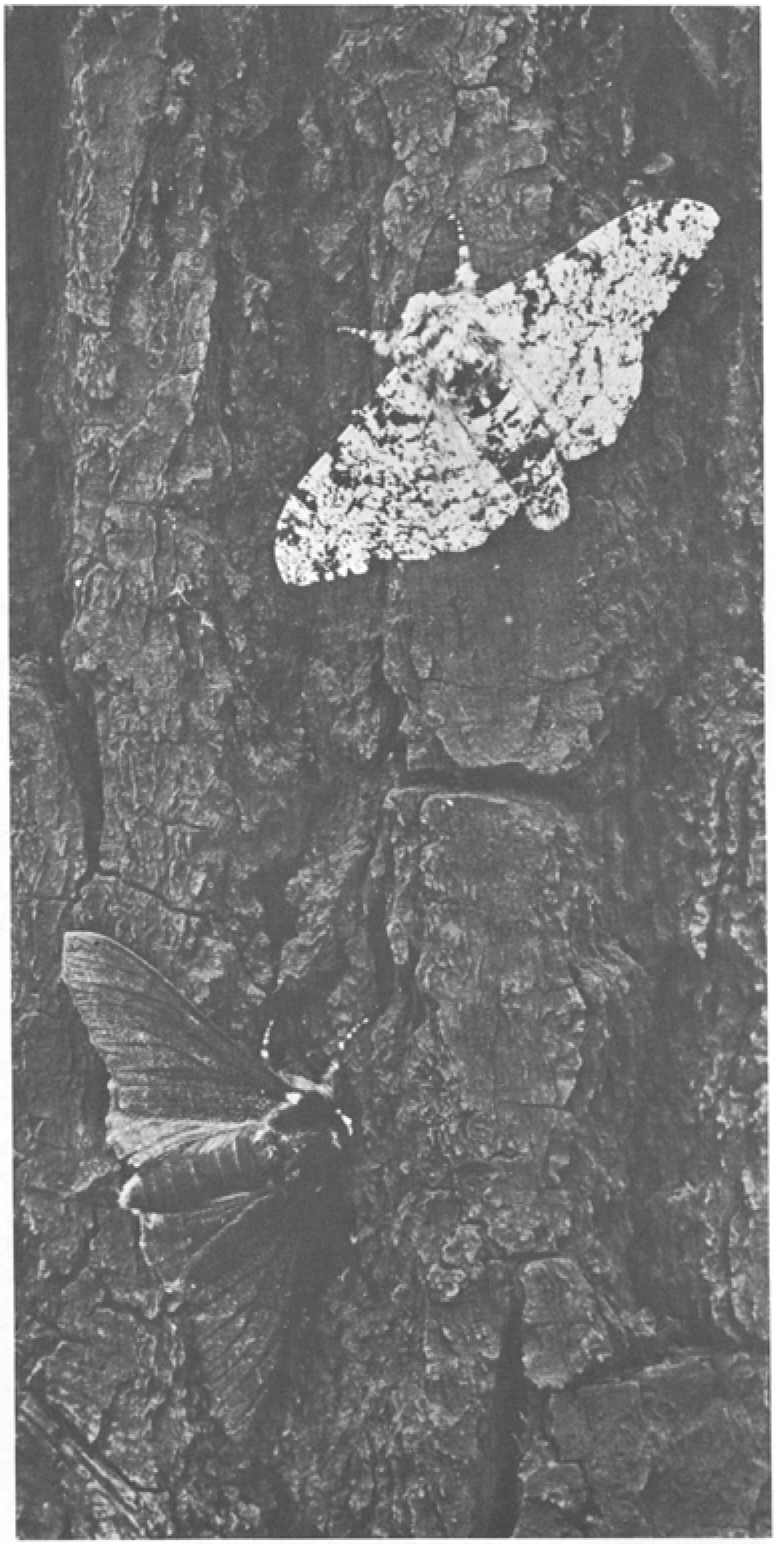
3 Darwin's Missing Evidence

In his time certain species of maths were light in color. Today in many areas these species are largely dark. If he had noticed the change occurring, he would have observed evolution in action.

Charles Darwin's *Origin of Species*, the centenary of which we celebrate in 1959, was the fruit of 26 years of laborious accumulation of facts from nature. Others before Darwin had believed in evolution, but he alone produced a cataclysm of data in support of it. Yet there were two fundamental gaps in his chain of evidence. First, Darwin had no knowledge of the mechanism of heredity. Second, he had no visible example of evolution at work in nature.

It is a curious fact that both of these gaps could have been filled during Darwin's lifetime. Although Gregor Mendel's laws of inheritance were not discovered by the community of biologists until 1900, they had first been published in 1866. And before Darwin died in 1882, the most striking evolutionary change ever witnessed by man was taking place around him in his own country.

The change was simply this. Less than a century ago moths of certain species were characterized by their light coloration, which matched such backgrounds as light tree trunks and lichencovered rocks, on which the moths passed the daylight hours sitting motionless. Today in many areas the same species are predominantly dark! We now call this reversal "industrial melanism."



DARK AND LIGHT FORMS of the peppered moth were photographed on the trunk of an oak blackened by the polluted air of the English industrial city of Birmingham. The light form (*Biston betularia*) is clearly visible; the dark form (*carbonaria*) is well camouflaged.

It happens that Darwin's lifetime coincided with the first great man-made change of environment on earth. Ever since the Industrial Revolution commenced in the latter half of the 18th century, large areas of the earth's surface have been contaminated by an insidious and largely unrecognized fallout of smoke particles. In and around industrial areas the fallout is measured in tons per square mile per month; in places like Sheffield in England it may reach 50 tons or more. It is only recently that we have begun to realize how widely the lighter smoke particles are dispersed, and to what extent they affect the flora and fauna of the countryside.

In the case of the flora the smoke particles not only pollute foliage but also kill vegetative lichens on the trunks and boughs of trees. Rain washes the pollutants down the boughs and trunks until they are bare and black. In heavily polluted districts rocks and the very ground itself are darkened.

Now in England there are some 760 species of larger moths. Of these more than 70 have exchanged their light color and pattern for dark or even all-black coloration. Similar changes have occurred in the moths of industrial areas of other countries: France, Germany, Poland, Czechoslovakia, Canada and the U. S. So far, however, such changes have not been observed anywhere in the tropics. It is important to note here that industrial melanism has occurred only among those moths that fly at night and spend the day resting against a background such as a tree trunk.

These, then, are the facts. A profound change of color has occurred among hundreds of species of moths in industrial areas in different parts of the world. How has the change come about? What underlying laws of nature have produced it? Has it any connection with one of the normal mechanisms by which one species evolves into another?

In 1926 the British biologist Heslop Harrison reported that the industrial melanism of moths was caused by a special substance which he alleged was present in polluted air. He called this substance a "melanogen," and suggested that it was manganous sulfate or lead nitrate. Harrison claimed that when he fed foliage impregnated with these salts to the larvae of certain species of light colored moths, a proportion of their offspring were black. He also stated that this "induced melanism" was inherited according to the laws of Mendel.

Darwin, always searching for missing evidence, might well have accepted Harrison's Lamarckian interpretation, but in 1926 biologists were skeptical. Although the rate of mutation of a hereditary characteristic can be increased in the laboratory by many methods, Harrison's figures inferred a mutation rate of 8 per cent. One of the most frequent mutations in nature is that which causes the disease hemophilia in man; its rate is in the region of .0005 per cent, that is, the mutation occurs about once in 50,000 births. It is, in fact, unlikely that an increased mutation rate has played any part in industrial melanism.

At the University of Oxford during the past seven years we have been attempting to analyze the phenomenon of industrial melanism. We have used many different approaches. We are in the process of making a survey of the present frequency of light and dark forms of each species of moth in Britain that exhibits industrial melanism. We are critically examining each of the two forms to see if between them there are any differences in behavior. We have fed large numbers of larvae of both forms on foliage impregnated with substances in polluted air. We have observed under various conditions the mating preferences and relative mortality of the two forms. Finally we have accumulated much information about the melanism of moths in parts of the world that are far removed from industrial centers, and we have sought to link industrial melanism with the melanics of the past.

Our main guinea pig, both in the field and in the laboratory, has been the peppered moth *Biston betularia* and its melanic form *carbonaria*. This species occurs throughout Europe, and is probably identical with the North American *Amphidasis cognataria*. It has a one-year life cycle; the moth appears from May to August. The moth flies at night and passes the day resting on the trunks or on the underside of the boughs of roughbarked deciduous trees such as the oak. Its larvae feed on the foliage of such trees from June to late October; its pupae pass the winter in the soil.

The dark form of the peppered moth was first recorded in 1848 at Manchester in England. Both the light and dark forms appear in each of the photographs at right and on the next page. The background of each photograph is noteworthy. In the photograph on the next page the background is a lichenencrusted oak trunk of the sort that today is found only in unpolluted rural districts. Against this background the light form is almost invisible and the dark form is conspicuous. In the photograph at right the background is a bare and blackened oak trunk in the heavily polluted area of Birmingham. Here it is the dark form which is almost invisible, and the light form which is conspicuous. Of 621 wild moths caught in these Birmingham woods in 1953, 90 per cent were the dark form and only 10 per cent the light. Today this same ratio applies in nearly all British industrial areas

SAME TWO FORMS of the peppered moth were photographed against the lichen-encrusted trunk of an oak in an unpolluted area. Here it is the dark form which may be clearly seen. The light form, almost invisible, is just below and to the right of the dark form.

and far outside them.

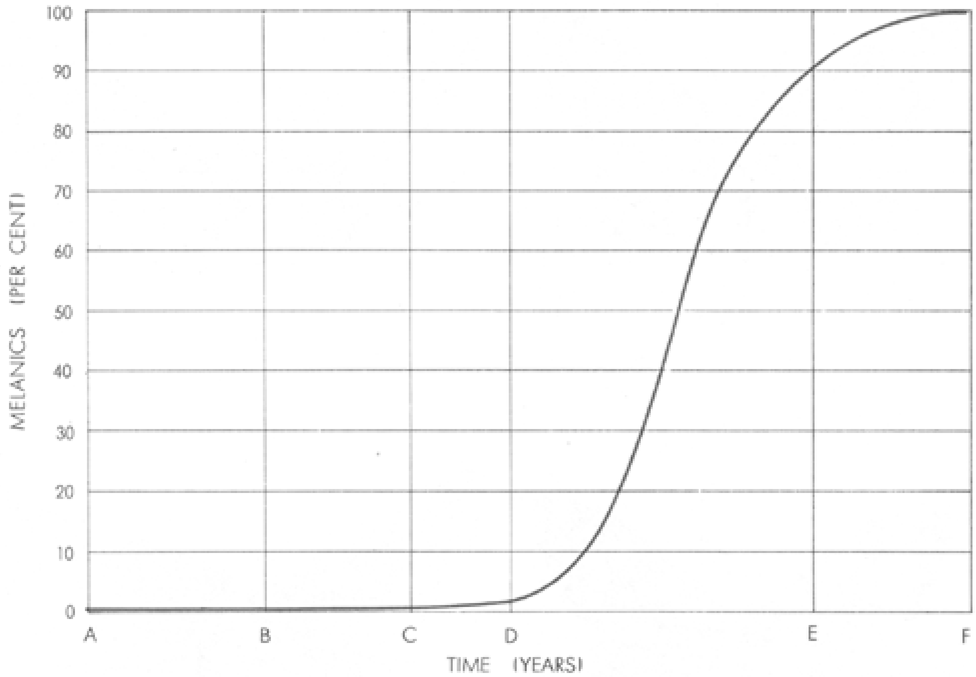
We decided to test the rate of survival of the two forms in the contrasting types of woodland. We did this by releasing known numbers of moths of both forms. Each moth was marked on its underside with a spot of quick-drying cellulose paint; a different color was used for each day. Thus when we subsequently trapped large numbers of moths we could identify those we had released and established the length of time they had been exposed to predators in nature.

In an unpolluted forest we released 984 moths: 488 dark and 496 light. We recaptured 34 dark and 62 light, indicating that in these woods the light form had a clear advantage over the dark. We then repeated the experiment in the polluted Birmingham woods, releasing 630 moths: 493 dark and 137 light. The result of the first experiment was completely reversed; we recaptured proportionately twice as many of the dark form as of the light.

For the first time, moreover, we had witnessed birds in the act of taking moths from the trunks. Although Britain has more ornithologists and bird watch ers than any other country, there had been absolutely no record of birds actually capturing resting moths. Indeed, many ornithologists doubted that this happened on any large scale.

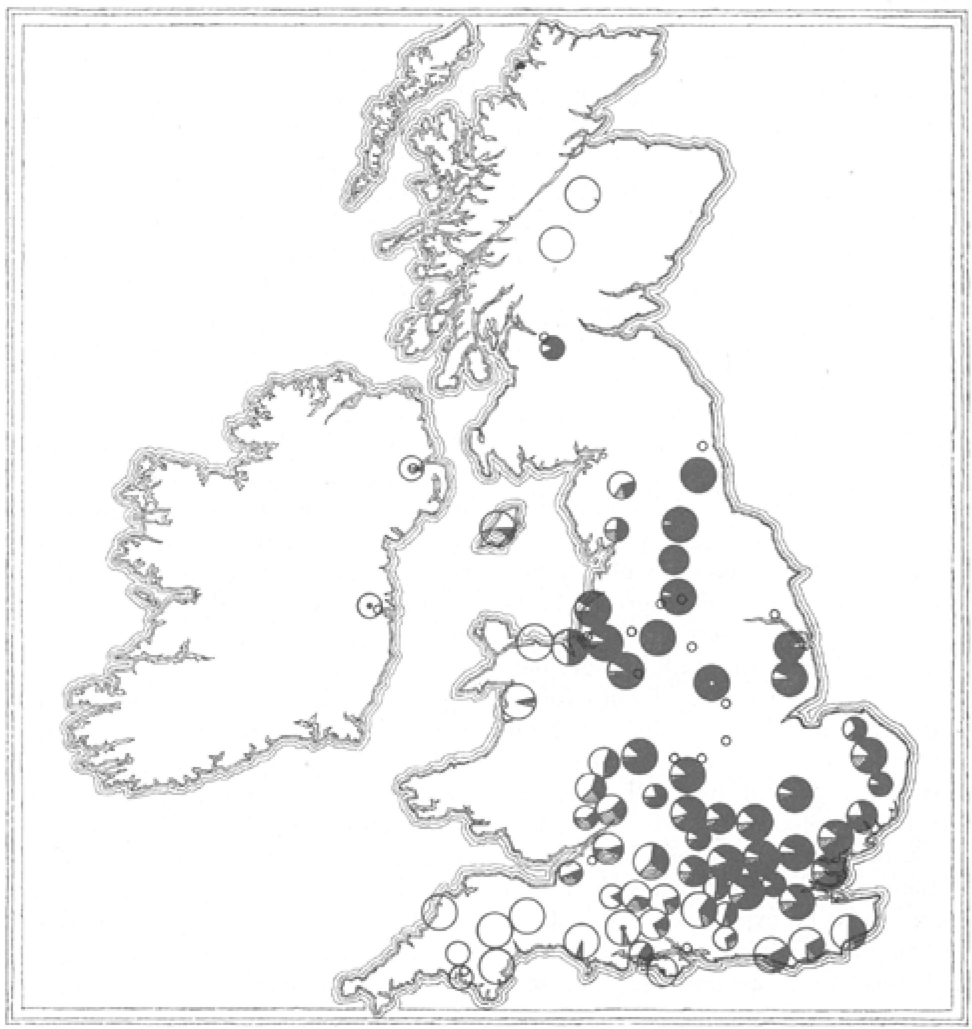
The reason for the oversight soon became obvious. The bird usually seizes the insect and carries it away so rapidly that the observer sees nothing unless he is keeping a constant watch on the insect. This is just what we were doing in the course of some of our experiments. When I first published our findings, the editor of a certain journal was sufficiently rash as to question whether birds took resting moths at all. There was only one thing to do, and in 1955 Niko Tinbergen of the University of Oxford filmed a repeat of my experiments. The film not only shows that birds capture and eat resting moths, but also that they do so selectively.

These experiments lead to the following conclusions. First, when the environment of a moth such as *Biston betularia* changes so that the moth cannot hide by day, the moth is ruthlessly eliminated by predators unless it mutates to a form that is better suited to its new environment. Second, we now have visible proof that, once a mutation has occurred, natural selection alone can be responsible for its rapid spread. Third, the very fact that one form of moth has replaced another in a comparatively short span of years indicates that this evolutionary mechanism is remarkably flexible.



SPREAD OF MUTATION from the light form to the dark (melanic) is expressed by this curve, discussed in detail in the text. The mutation occurs in the period AB, spreads slowly during BD and spreads rapidly during DE. During EF the light form is either gradually eliminated, as indicated by the curve, or remains at a level of about 5 per cent of the population.

The present status of the peppered moth is shown in the map on the opposite page. This map was built up from more than 20,000 observations made by 170 voluntary observers living in various parts of Britain. The map makes the following points. First, there is a strong correlation between industrial centers and a high percentage of the dark form of the moth. Second, populations consisting entirely of the light form are found today only in western England and northern Scotland. Third, though the counties of eastern England are far removed from industrial centers, a surprisingly high percentage of the dark form is found in them. This, in my opinion, is due to the long-standing fallout of smoke particles carried from central England by the prevailing southwesterly winds.



PROPORTION OF FORMS of the peppered moth at various locations in the British Isles is indicated on this map. The open area within a colored circle represents the proportion of the light form *Biston betularia* recorded; the solid colored area, the proportion of the dark form *carbonaria*; the hatched colored area, the proportion of another dark form, *insularia*. Small black circles on the map indicate the location of major industrial centers.

Now in order for the dark form of a moth to spread, a mutation from the light form must first occur. It appears that the frequency with which this happens-that is, the mutation rate-varies according to the species. The rate at which the light form of the peppered moth mutates to the dark form seems to be fairly high; the rate at which the mutation occurs in other species may be very low. For example, the light form of the moth *Procus literosa* disappeared from the

Sheffield area many years ago, but it has now reappeared in its dark form. It would seem that a belated mutation has permitted the species to regain lost territory. Another significant example is provided by the moth *Tethea ocularis*, Prior to 1947 the dark form of this species was unknown in England. In that year, however, many specimens of the dark form were for the first time collected in various parts of Britain; in some districts today the dark form now comprises more than 50 per cent of the species. There is little doubt that this melanic arrived in Britain not by mutation but by migration. It had been known for a considerable time in the industrial areas of northern Europe, where presumably the original mutation occurred.

The mutation that is responsible for industrial melanism in moths is in the majority of cases controlled by a single gene. A moth, like any other organism that reproduces sexually, has two genes for each of its hereditary characteristics: one gene from each parent. The mutant gene of a melanic moth is inherited as a Mendelian dominant; that is, the effect of the mutant gene is expressed and the effect of the other gene in the pair is not. Thus a moth that inherits the mutant gene from only one of its parents is melanic.

The mutant gene, however, does more than simply control the coloration of the moth. The same gene (or others closely linked with it in the hereditary material) also gives rise to physiological and even behavioral traits. For example, it appears that in some species of moths the caterpillars of the dark form are hardier than the caterpillars of the light form. Genetic differences are also reflected in mating preference. On cold nights more males of the light form of the peppered moth appear to be attracted to light females than to dark. On warm nights, on the other hand, significantly more light males are attracted to dark females.

There is evidence that, in a population of peppered moths that inhabits an industrial area, caterpillars of the light form attain full growth earlier than caterpillars of the dark form. This may be due to the fact that the precipitation of pollutants on leaves greatly increases late in the autumn. Caterpillars of the dark form maybe hardier in the presence of such pollution than caterpillars of the light form. In that case natural selection would favor light-form caterpillars which mature early over light-form caterpillars which mature late. For the hardier caterpillars of the dark form, on the other hand, the advantages of later feeding and longer larval life might outweigh the disadvantages of feeding on increasingly polluted leaves. Then natural selection would favor those caterpillars which mature late.

Another difference between the behavior of *B. betularia* and that of its dark form *carbonaria* is suggested by our experiments on the question of whether each form can choose the "correct" background on which to rest during the day. We offered light and dark backgrounds of equal area to moths of both forms, and discovered that a significantly large proportion of each form rested on the correct background. Before these results can be accepted as proven, the experiments must be repeated on a larger scale. If they are proven, the behavior of both forms could be explained by the single mechanism of "contrast appreciation." This mechanism assumes that one segment of the eye of a moth senses the color of the background and that another segment senses the moth's own color; thus the two colors could be compared. Presumably if they were the same, the moth would remain on its background; but if they were different, "contrast conflict" would result and the moth would move off again. That moths tend to be restless when the colors conflict is certainly borne out by recent field observations.

It is evident, then, that industrial melanism is much more than a simple change from light to dark. Such a change must profoundly upset the balance of hereditary traits in a species, and the species must be a long time in restoring that balance. Taking into account all the favorable and unfavorable factors at work in this process, let us examine the spread of a mutation similar to the dark form of the peppered moth. To do so we must consult the diagram at the top of the preceding page.

According to the mutation rate and the size of the population, the new mutation may not appear in a population for a period varying from one to 50 years. This is represented by AB on the diagram. Let us now assume the following: that the original successful mutation took place in 1900, that subsequent new mutations failed to survive, that the total local population was one million, and that the mutant had a 30-per-cent advantage over the light form. (By a 30-per-cent advantage for the dark form we mean that, if in one generation there were 100 light moths and 100 dark, in the next generation there would be 85 light moths and 115 dark.)

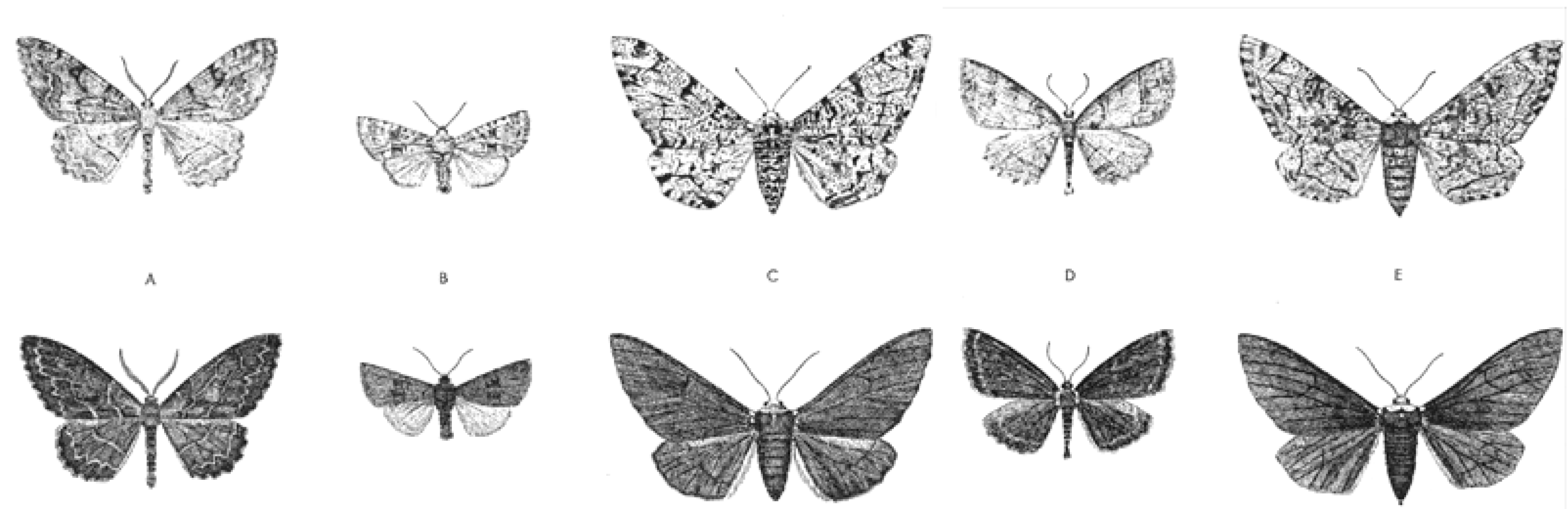
On the basis of these assumptions there would be one melanic moth in 1,000 only in 1929 (BC). Not until 1938 would there be one in 100 (BD). Once the melanics attain this level, their rate of increase greatly accelerates.

In the period between 1900 and 1938 (BD) natural selection is complicated by other forces. Though the color of the dark form gives it an advantage over the light, the new trait is introduced into a system of other traits balanced for the light form; thus the dark form is at first at a considerable physiological disadvantage. In fact, when moths of the dark form were crossed with moths of the light form 50 years ago, the resulting broods were significantly deficient in the dark form. When the same cross is made today, the broods contain more of the dark form than one would expect. The system of hereditary traits has become adjusted to the new trait.

There is evidence that other changes take place during the period BD. Specimens of the peppered moth from old collections indicate that the earliest melanics were not so dark as the modern dark form: they retained some of the white spots of the light form. Today a large proportion of the moths around a city such as Manchester are jet black. Evidently when the early melanics inherited one gene for melanism, the gene was not entirely dominant with respect to the gene for light coloration. As the gene complex adjusted to the mutation, however, the new gene became almost entirely dominant.

When the dark form comprises about 10 per cent of the population, it may jump to 90 per cent in as little as 15 or 20 years. This is represented by period DE on the graph. Thereafter the proportion of the dark form increases at a greatly reduced rate.

Eventually one of two things must happen: either the light form will slowly be eliminated altogether, or a balance will be struck so that the light form continues to appear as a small but definite proportion of the population. This is due to the fact that the moths which inherit one gene for dark coloration and one for light (heterozygotes) have an advantage over the moths which inherit two genes for dark coloration (homozygotes). And when two heterozygotes mate, a quarter of their offspring will have two genes for light coloration, *i.e.*, they will be light. Only after a very long period of time, therefore, could the light forms (and with them the gene for light coloration) be entirely eliminated. This period of removal, represented by EF on the diagram, might be more than 1,000 years. Indications so far suggest, however, that complete removal is unlikely, and that a balance of the two forms would probably occur. In this balance the light form would represent about 5 per cent of the population.



FIVE SPECIES OF MOTH that have both light and dark forms are depicted in their actual size. In each case the light form is at the top and the dark form is at the bottom. The species are: *Cleora repandata* (A), *Procus literosa* (B), *Biston betularia* (C), *Ectropis consonaria* (D) and *Amphidasis cognataria* (E). All of the species are European except the last, which occurs in North America and may be identical with *Biston betularia*.

The mechanisms I have described are without doubt the explanation of industrial melanism: normal mutation followed by natural selection resulting in an insect of different color, physiology and behavior. Industrial melanism involves no new laws of nature; it is governed by the same mechanisms which have brought about the evolution of new species in the past.

There remains, however, one major unsolved problem. Why is it that, in almost all industrial melanics, the gene for melanism is dominant? Many geneticists would agree that dominance is achieved by natural selection, that it is somehow related to a successful mutation in the distant past. With these thoughts in mind I recently turned my attention away from industrial centers and collected moths in one of the few remaining pieces of ancient Caledonian pine forest in Britain: the Black Wood of Rannoch. Located in central Scotland far from industrial centers, the Black Wood is probably very similar to the forests that covered Britain some 4,000 years ago. The huge pines of this forest are only partly covered with lichens, Here I found no fewer than sever species of moths with melanic forms.

I decided to concentrate on the species *Cleora repandata*, the dark form of which is similar to the dark form of the same species that has swept through central England. This dark form, like the industrial melanics, is inherited as a Mendelian dominant. Of just under 500 specimens of *C. repandata* observed, 10 per cent were dark.

C. repandata spends the day on pine trunks, where the light form is almost invisible. The dark form is somewhat more easily seen. By noting at dawn the spot where an insect had come to rest, and then revisiting the tree later in the day, we were able to show that on some days more than 50 per cent of the insects had moved. Subsequently we found that because of disturbances such as ants or hot sunshine they had had to fly to another tree trunk, usually about 50 yards away. I saw large numbers of these moths on the wing, and three other observers and I agreed that the dark form was practically invisible at a distance of more than 20 yards, and that the light form could be followed with ease at a distance of up to 100 yards. In fact, we saw birds catch three moths of the light form in flight. It is my belief that when it is on the wing in these woods the dark form has an advantage over the light, and that when it is at rest the reverse is true.

This may be one of many ways in which melanism was useful in the past. It may also explain the balance between the light and dark forms of *Cleora pandata* in the Black Wood of Rannoch. In this ease a melanic may have been preserved for one evolutionary reason but then have spread widely for another.

The melanism of moths occurs in many parts of the world that are not industrialized, and in environments that are quite different. It is found in the mountain rain forest of New Zealand's South Island, which is wet and dark. It has been observed in arctic and subarctic regions where in summer moths must fly in daylight. It is known in very high mountains, where dark coloration may permit the absorption of heat and make possible increased activity. In each case recurrent mutation has provided the source of the change, and natural selection, as postulated by Darwin, has decided its destiny.

Melanism is not a recent phenomenon but a very old one. It enables us to appreciate the vast reserves of genetic variability which are contained within each species, and which can be summoned when the occasion arises. Had Darwin observed industrial melanism he would have seen evolution occurring not in thousands of years but in thousands of days-well within his lifetime. He would have witnessed the consummation and confirmation of his life's work.

Evolution and the Fossil Record	H. B. D. Kettlewell	1959	pgs. 28-33