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Postindustrial Melanism in the Peppered Moth

L. M. COOK, G. S. MANI, M. E. VARLEY

New data show the geographical pattern of frequency of the melanic morph *carbonaria* of the peppered moth, *Biston betularia*, in 1983–84. These frequencies are compared with data from 1952 to 1970. After 20 years of smoke control, the area of high melanic frequency has contracted to the northeast. The change indicates a disadvantage to *carbonaria* of about 12 percent compared with 20 years ago. Computer simulations, which do not include the assumption of heterozygote advantage, provide a good match to the surface for the period 1952 to 1970, and also the 1983–84 surface. Experiments on visual predation have been criticized as giving unrepresentative estimates of selection but they permit satisfactory simulations to be made.

THE RESPONSE OF THE PEPPERED moth, *Biston betularia*, to environmental changes brought about by industrialization remains one of the most fully documented cases of microevolutionary change. The first survey backed up by quantitative data was published by Kettlewell; it was based on data obtained between 1952 and 1956 and on the use of a variety of moth traps and capture methods. The results were presented in the familiar "pie diagrams" showing the frequencies of the two melanic types, *carbonaria* and *insularia*, and of the typicals. Similar surveys were carried out in 1957 to 1964 and in 1965 to 1970. All three sets of results were listed together by Kettlewell (*1*), and the map of morph frequencies was updated by Sheppard (*2*). A detailed survey was carried out by Clarke and Sheppard (*3*), Whittle *et al.* (*4*), and Bishop (*5*) of the transition from a high melanic frequency in Liverpool to a low frequency in rural northern Wales, an area where *insularia* is rare so that the cline measures the ratio of *carbonaria* to typical. By 1972, smoke control legislation and replacement of old coal-burning housing stock had reduced atmospheric pollution. There was also a drop in *carbonaria* frequency (*6*). In order to provide a baseline for future comparison, a survey was carried out that extended the northern Wales–Liverpool transect to the Pennines east of Manchester (*7*). Data from individual trap sites were mapped with a computer program that interpolates values between points, to provide contours or three-dimensional maps. The surface produced may be altered by varying a smoothing factor, which was chosen to give a good fit to the data available for the northern Wales cline. Over 90 percent *carbonaria* in Cheshire dropped to less than 5 percent in Wales to the southwest of a narrow transition region (*7*).

One of the purposes of the study was to investigate the possibility that heterozygotes for the melanic gene, indistinguishable in appearance from the melanic homozygote, have a greater net fitness than either homozygote, as had been suggested (*1, 8, 9*). It was concluded that the evidence could as well be explained by a migration-selection hypothesis, with the same fitness being ascribed to the two melanic genotypes. The implications of the model were investigated theoretically (*10–12*), and additional evidence was available from breeding experiments (*13*). Using all the available evidence, Mani (*12*) obtained a good fit to the United Kingdom data on melanic morph frequency without the assumption of heterozygote advantage.

Since the early 1970's study of *B. betularia* has been part of the Foundation Year biology program in the Open University. Students are asked to collect moths in standard traps over 5-day periods. Records are credited to the Open University Study Center nearest to their collecting site. Since 1983 specimens of each morph collected have been sent to L. M. Cook for checking. The survey covers most of England and Wales and part of southern Scotland. We present here results for *carbonaria* frequency, based on a total of 1825 moths recorded at 190 locations.

To illustrate morph frequencies, we have used the same computer program and the same smoothing factor that were used in the earlier survey. A spurious sense of continuity is given to point records, but the position of contours is objectively determined once program parameters have been chosen. Frequency surfaces for Kettlewell's survey are

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L. M. Cook, Department of Zoology, University of Manchester, Manchester M13 9PL, United Kingdom.
G. S. Mani, Department of Physics, University of Manchester, Manchester M13 9PL, United Kingdom.
M. E. Varley, Department of Biology, Open University, Walton Hall, Milton Keynes MK7 6AA, United Kingdom.

compared with those for 1983–84 (Fig. 1). In discussing the changes observed, the notation in (*14*) is used.

If the fitness of the two melanic genotypes is the same, so that $w_1 = w_2$, then w may be estimated from the change over n generations, since $P_n/Q_n = w^n P_0/Q_0$, and log (P_n/Q_n) on n is linear with a slope of log w. Contour maps were produced separately for the three series of data presented by Kettlewell (*1*). Sixty-one points at 50-km intervals over England, Wales, and southern Scotland were then taken from the resulting surfaces, and their values compared between the different surveys. For the transition from 1952 to 1957 (time elapsed, 5 generations) the geometric mean of w was 0.98 ± 0.04. For 1952 to 1970 (time elapsed, 18 generations) the geometric mean was 0.99 ± 0.02. These changes cannot be tested for significance since they are not based on independent records. Standard errors measuring variation between estimates from the 61 locations are given, however, to indicate the amount of variation observed. The estimates are sufficiently near to unity to suggest that there is no detectable selection operating over the period elapsed. We have therefore pooled the data to indicate the pattern at the end of the period of high atmospheric pollution (Fig. 1a). The 1983–84 data provide a frequency surface in the same way (Fig. 1b). Considering 15 generations to have elapsed between the two data sets, we calculate w to be 0.88 ± 0.02 from the 50-km point data. On average, since the introduction of smoke control, there has been 12 percent selection against melanics.

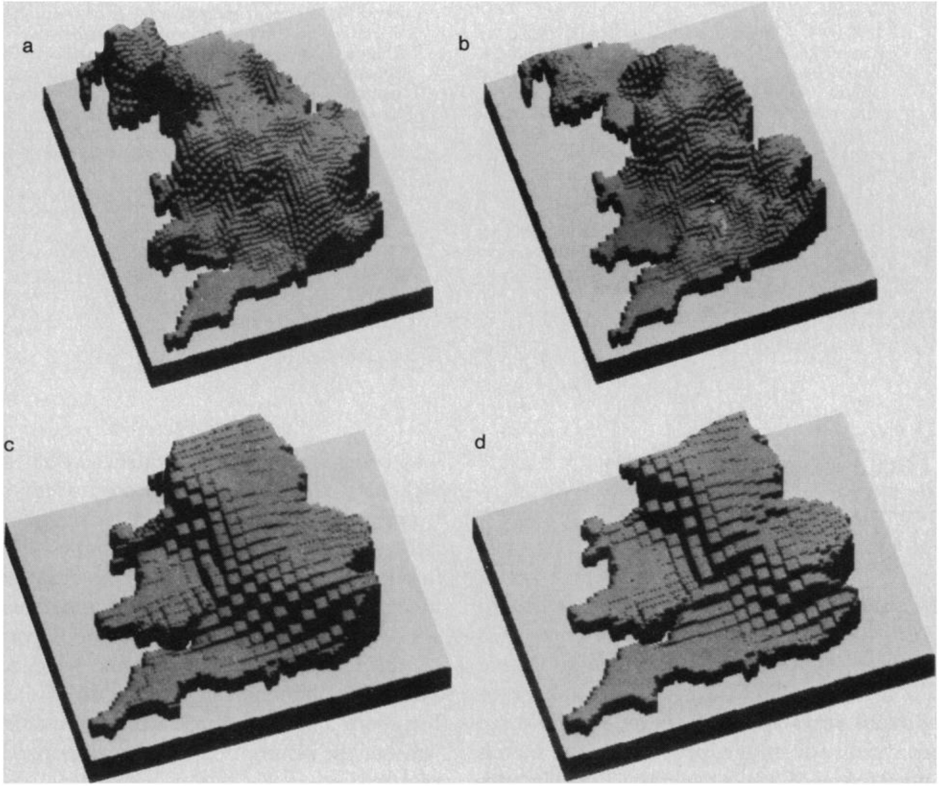

Fig. 1. Computer-generated surfaces for the frequency of *carbonaria* in the peppered moth (a) in 1952 to 1970 and (b) in 1983–84 (*14*). The vertical scale ranges from 0 percent in the southwestern peninsula to 99 percent at the highest point to the northeast. The cline running diagonally across England and Wales shifts to the northeast. Data are incomplete for northern England and southern Scotland, so change there is unconfirmed. Simulations of the early survey (c) and of the later one (d) show the patterns generated by the use of a set of parameters derived from experiments by several workers.

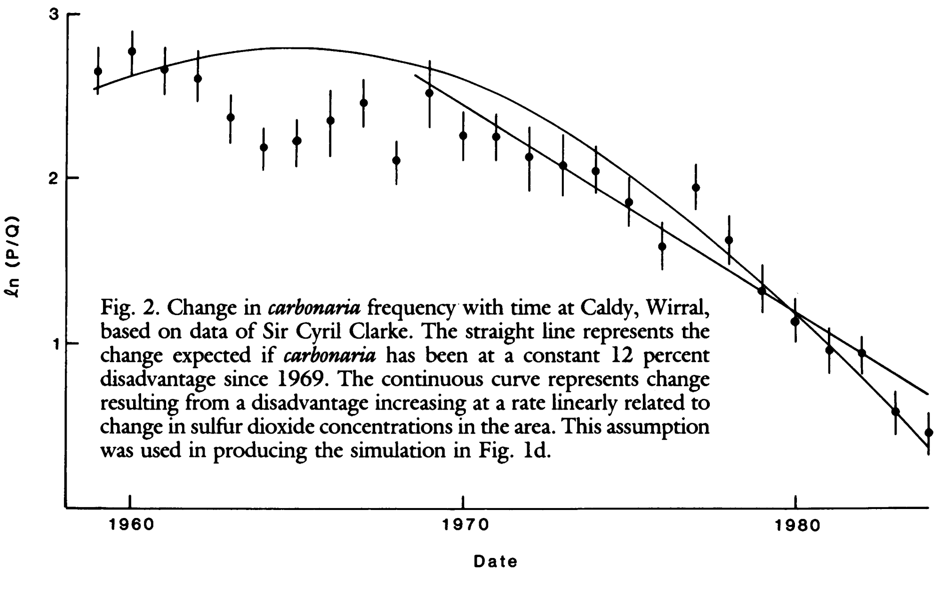

Fig. 2. Change in *carbonaria* frequency with time at Caldy, Wirral, based on data of Sir Cyril Clarke. The straight line represents the change expected if *carbonaria* has been at a constant 12 percent disadvantage since 1969. The continuous curve represents change resulting from a disadvantage increasing at a rate linearly related to change in sulfur dioxide concentrations in the area. This assumption was used in producing the simulation in Fig. 1d.

The area of high melanic frequency in northern England is still present but has contracted in range. The result is that some localities have experienced very substantial declines in melanic frequency, while in others there is little change. One site at which the morph frequency cline has contracted is at Caldy, Wirral, where collections have been made by Sir Cyril Clarke from 1959 to the present (*15*). The change observed is presented in Fig. 2 as the logarithm of the ratio of *carbonaria* to noncarbonaria. For the last 16 years the change is more or less constant, and, if a regression line is fitted to these points, provides an estimate of w of 0.88 ± 0.01, as does the comparison of Kettlewell's and the Open University data. Selection against *carbonaria* may have been very low from 1959 to 1968 and thereafter reached a more or less steady level of 12 percent, as the general survey also suggests.

It is possible that w_1 and w_2 are not equal. Clarke and Sheppard (*3*) inferred a higher fitness for the heterozygote than the melanic homozygote from examination of changes in morph frequency. Bishop *et al.* disagreed with this interpretation (*7*) and consider the existing data to be compatible with the assumption that w_1 and w_2 are the same. If it is assumed that the gene frequency q is the square root of Q (which is an approximation), so that $p = 1 - q$, then for any pair of generations 0, 1 the quantity $P_1Q_0/Q_1p_0 = 2w_2 - p_0(2w_2 - w_1)$. Considering the Caldy data, we find $w_1 = 1.09$ and $w_2 = 0.76$. The difference is in the reverse direction from the one proposed, so that there is no evidence of the existence of heterozygote advantage.

The two surveys have been compared with large-scale computer simulations designed to test the effectiveness with which morph frequency pattern can be predicted. A model was developed (*10, 12*) in which England and Wales were represented by a grid of 360 square cells of linear dimension 22.5 km. Values for the minimum set of parameters required were obtained from the literature and from modeling the northern Wales cline (*11*). Mani (*12*) presented comparisons with the Kettlewell data for three transects. Figure 1c shows the complete surface, for comparison with Fig. 1a. The agreement is sufficiently close as to indicate that a set of assumptions derived from a small part of the area (the cline in the northwest) may be used to explain the morph frequency distribution over the whole of England and Wales, and that these assumptions need not include heterozygote advantage.

For the period from the 1970's to the present, fitness of *carbonaria* and *insularia* is assumed to have decreased linearly by an amount estimated from the change in recorded atmospheric sulfur dioxide on the Wirral (*15*). This produced a good match to the change in morph frequency at Caldy, and consequently the same change is assumed throughout the country. The result of the simulation projected to 1984 is shown in Fig. 1d. In this case also there is a satisfactory match to the observed data, with the plateau of high *carbonaria* frequency retreating to the northeast, although the predicted frequency in the London area is higher than the frequency observed. With the selective intensities involved in the model, such discrepancies are not surprising. The selection is associated with industrial pollution and is assumed to act through the agency of visual predation. Mikkola (*16, 17*) has pointed out, rightly, that more observations are needed before we can have a true picture of the role of visual selection, and that experiments to measure visual selection have design deficiencies. He questions whether the evidence even gives useful information on selective predation. The selective pressures assumed in these simulations are based on the visual selection experiments, however, and for the most part have a good predictive value. In our opinion, this suggests that the experiments do give a correct indication of the order and direction of the selective pressure involved.

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18. This survey could not have been made without the participation of Open University students. We are grateful to A. C. Arnold for help with the graphics.

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Expression of an Epidermal Antigen Used to Study Tissue Induction in the Early *Xenopus laevis* Embryo

REBECCA M. AKERS, CAREY R. PHILLIPS, NORMAN K. WESSELLS*

A monoclonal antibody (Epi 1) has been produced that recognizes an antigen expressed in epidermal cells of *Xenopus laevis* embryos. The Epi 1 antigen appears in embryonic epidermis at the end of gastrulation and is not expressed in nonepidermal structures derived from ectoderm (for example, neural tube or cement gland). The capacity to express the Epi 1 antigen is restricted to cells of the animal hemisphere prior to the midblastula stage of development (stage 8), and tissue interactions during gastrulation inhibit the expression of the Epi 1 antigen in neural ectoderm. This epidermal antigen will be a valuable marker for studies of ectodermal commitment.

THE FATES OF MANY EMBRYONIC cells are determined during the time between fertilization and gastrulation. In some tissues, such as the nervous system, cell fate is thought to be influenced by control signals arising in other parts of the embryo; these interactions are referred to as "induction" (*1–3*). Little is known about the molecular events underlying induction. This is due in part to the fact that the experimental analysis of this process relies on the appearance of morphological features appropriate to particular tissue types. Tissue differentiation may be far removed in time from the actual induction events and may involve complex patterns of cellular behavior that are not amenable to study by molecular techniques. One strategy is to identify molecules that are expressed in a tissue-specific fashion in the early embryo and to study the factors controlling the appearance of these molecules during development. We have produced a monoclonal antibody that distinguishes between epidermal ectoderm and neural ectoderm at the onset of neurulation. The antigen recognized by this antibody is restricted to epidermal ectoderm, and its expression in neural ectoderm appears to be inhibited by tissue interactions. This antibody may therefore be useful in molecular studies of neural induction.

A membrane preparation obtained from late neurula (stages 19 to 22) *Xenopus laevis* embryos (*4*) was used to immunize mice for the production of monoclonal antibodies (*5*). Several antibodies demonstrating tissue-specific binding were isolated and characterized. One of these, referred to as Epi 1, recognizes an antigen expressed in epidermal tissue. Immunocytochemical preparations of embryos processed with the Epi 1 antibody exhibited intense staining in the ventral and lateral surface ectoderm, whereas the dorsal ectoderm overlying the archenteron lacked detectable staining (Fig. 1, A and D). The boundaries of the unstained region correspond to the lateral borders of the developing neural plate (Fig. 1B). Immunocytochemical and Western blot analysis revealed that this antigen appears in the embryo at the time of blastopore closure (stage 13) and persists in the epidermis through the feeding larva stage. At no time during embryonic or larval development does the Epi 1 antigen appear in the nervous system or other nonepidermal structures derived

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R. M. Akers and N. K. Wessels, Department of Biological Sciences, Stanford University, Stanford, CA 94305.
C. R. Phillips, Department of Zoology, University of California, Berkeley 94720.

*To whom correspondence should be addressed.