Decline of melanic moths

SIR — Industrial melanism has been best documented in *Biston betularia*, the peppered moth. Melanic variants, unknown before 1848, all but replaced paler forms by the turn of the century in populations near British industrial centres. Distant rural populations, however, changed little[1]. The changes have been attributed to habitat modifications and differential predation on the moths by birds[1].

Conditions began to reverse following the creation of smokeless zones in the United Kingdom initiated by the Clean Air Act of 1956. Beginning in 1959 the *Biston* population at Caldy common, 18 km west of Liverpool, has been sampled each year[2]. There the frequency of the melanic form has dropped from a high of 94.2% in 1960 to its current (1994) low of 18.7% (65 melanic of 348). Similar reversals are well documented elsewhere in Britain, but the Caldy Common sample is by far the largest, with nearly 18,000 specimens taken to date.

Melanism has also been reported in the American subspecies of the peppered moth, *B. betularia cognataria*. Melanics have remained at low frequencies in rural Massachusetts between 1958 and 1977 (ref. 3); and in the Virginia mountains (1968–94, D. A. West, personal communication); whereas in central Pennsylvania melanics were once appreciably high in frequency and fell from 52 to 38% between 1971 and 1986 (ref. 4). The only North American location where melanic *Biston* has been recorded at frequencies comparable to British samples (exceeding 90%) is in southern Michigan[5]. Between 1959 and 1961, a combined total of 576 *Biston* have been collected at the E. S. George Reserve, a rural field station near Detroit. The sample sizes ranged from 24 to 173 over five generations (in Michigan the species is bivoltine), with no statistical difference in the distributions of melanics to non-melanics ($G_4 = 1.433$, $P > 0.75$). In all, 515 of the 576 were melanic[5].

To determine if the frequency of melanic *Biston* has changed since the last census was taken at that location more than 30 years ago, we ran moth traps there for 7 weeks during last summer. Because of comparable sample sizes and the sample intervals, the most appropriate comparison is between the last sample taken (second brood, 1961) and the current sample. That 1961 sample, with 22 melanics and 2 non-melanics ($n = 24$), and the 1994 sample of 4 melanics and 21 non-melanics ($n = 25$), differ very significantly ($G_1 = 31.99$, $P << 0.001$).

The 1994 Michigan and the 1994 Liverpool samples do not differ in the frequency of melanics ($G_1 = 0.115$, $P > 0.5$). Although there are no intermediate sample points from the George Reserve,

the nearly matching drop in the frequency of melanics from above 90% to below 20% at these two locations over the same 35-year time interval is either an extraordinary coincidence, or parallel evolution.

A "parallel" Clean Air Act in 1963, with subsequent amendments, also led to reductions in atmospheric pollution in the United States[6]. Despite general improvements in most indicators of air quality, southeastern Michigan falls within the acid-rain belt[6]. However, the George Reserve was not conspicuously blackened before clean air legislation, nor has the habitat changed in this regard since then (D.F.O., unpublished data). It has remained a woodland habitat of mixed deciduous trees, many of them dark-barked oaks (*Quercus* spp.) and black walnuts (*Juglans nigra*). Furthermore, there has been no perceptible change in the lichen flora in that region of Michigan over the past 30 years (H. Crum, personal communication); thus, it appears that the rise and subsequent fall in melanism in the *Biston* population on the George

Reserve is not related to lichen succession. Although the evolution of melanism in this southern Michigan population seems to have paralleled the changes in British populations in both directions, common causes for the changes are not obvious. Clearly, more extensive sampling of American *Biston* is needed.

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Hydrophobicity and phylogeny

SIR — Mitochondrial DNA (mtDNA) sequences are routinely used to infer the pattern and timing of evolutionary divergences[1]. Various methods for estimating phylogenies are used, each based on a different model of evolution. All these methods assume some model of evolutionary change, whether explicitly, as is the case for the likelihood procedures, or impli-citly, as is the case for other methods[2,3]. A lack of correspondence between an underlying model and actual evolutionary processes can lead to failure of a method. Unfortunately, several of the underlying models fail to adequately explain mtDNA sequence evolution[4,5]. This suggests that factors beyond those accounted for by the models are acting on mtDNA sequences. Incorporating these additional influences into our models will enhance our ability to correctly recover phylogenies by improving the correspondence between the model of evolution and actual evolutionary processes. Here

we describe the mechanistic basis of a constraint acting on mtDNA sequences that represents one such influence.

It is well known that sites within DNA sequences differ in their relative freedom to vary[6]. In protein-encoding genes, third positions of codons typically vary more than first positions which, in turn, vary more than second positions, owing primarily to the differential degeneracy of the genetic code at these positions. It is often assumed that more rapidly evolving sites are more likely to result in chance matches (homoplasy) than are more slowly evolving sites when distant phylogenetic comparisons are made[7]. This may not necessarily be the case. If the 'character state space' (the number of possible states a site can exhibit) of a slowly evolving site is highly constrained, it could retain less phylogenetic information than a more rapidly evolving site for which more character states are available, because the probability that multiple substitutions will result in chance matches (homoplasy) across taxa increases as character-state space becomes more tightly constrained.

Constraints on character state space in DNA sequences are often reflected as deviations from a 1:1:1:1 ratio of the four bases A, G, C and T. This condition is termed base compositional bias. We have investigated compositional bias at all three codon positions for the 13 protein-encoding genes in each of 12 taxa (see figure caption). There does not seem to be a

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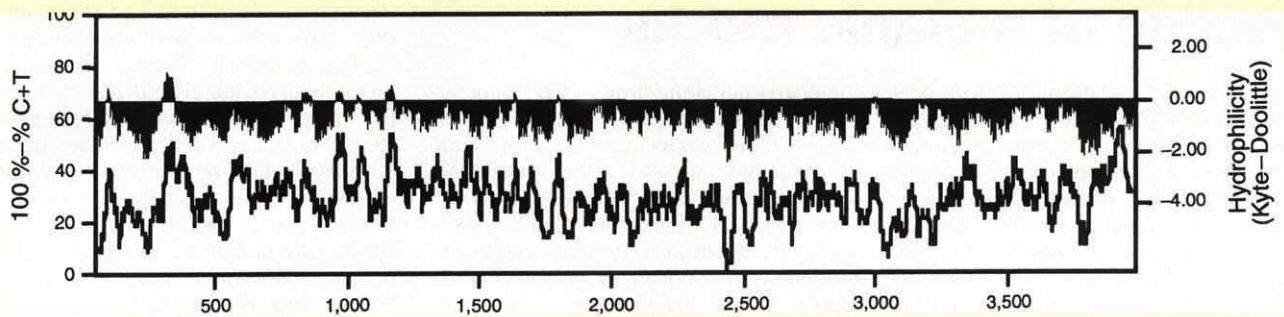
Correspondence between base composition at second positions of codons for the 13 human mitochondrial protein-encoding genes (lower histogram) and the hydrophilicity of corresponding amino acids calculated by the Kyte–Doolittle method (upper histogram) using the MacVector Program (IBI). Base compositions were computed by averaging C and T frequencies using a sliding window of 40 bases for the entire sequence. Similar figures and patterns of base compositional bias at the three codon positions of protein-encoding genes were also determined for rat, mouse, cow, possum, chicken, frog, carp, lancet, sea urchin (2 species) and Drosophila (data not shown).

strong bias for or against any of the four bases at the first position in these taxa. At second positions there is a striking compositional bias for C and T. Finally, there is a strong bias for A (or against G) at third positions. The different base compositions probably reflect different constraints operating at each of the codon positions.

We have found that the bias for T and C at second positions is directly correlated with hydrophobicity (see figure). Of the 26 triplets encoding hydrophobic residues, 24 (92%) have T or C at the second position. Mitochondrial proteins contain a high proportion of hydrophobic residues, that are frequently clumped together into domains. These domains probably represent membrane-spanning segments that must be hydrophobic to ensure their conformational stability[8-10]. This functional requirement for hydrophobicity effectively constrains character-state space at second positions to one of two states, T or C, causing sec-

ond-position sites to cycle between these two states over evolutionary time. Ironically, these positions, hitherto regarded as perhaps the most reliable for inferring evolutionary histories of distantly related taxa, may actually carry less phylogenetic signal and be less reliable than the more fast-evolving first positions whose compositional bias is less skewed.

Here we have identified one constraint that influences mtDNA sequence evolution. If we are to build realistic models of DNA sequence evolution that better allow us to retrieve phylogenetic signal from such data, it will be important to identify others.

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Complex bacterial patterns

SIR — Budrene and Berg[1] have studied patterns of spots, stripes and rings formed by motile cells of *Escherichia coli* grown in a thin layer of semi-solid agar. Similar patterns are produced by *Salmonella typhimurium* (Y. Blat and M. Eisenbach, personal communication). Here we propose that the interplay of a few common cooperative strategies underlies this diverse set of phenomena. We use two kinds of models to demonstrate that these strategies (the generic features), rather than the details of the models, are crucial for producing the observed patterns.

The most familiar strategy for ensuring cooperative behaviour is attractive chemotaxis. Indeed, Budrene and Berg[1] proposed that a chemoattractant mediates the observed spot formation in *E. coli*. This is easily confirmed by using a model of growth that includes nutrient diffusion and consumption, and bacterial

growth and bacterial motion[2]. The addition of a diffusing attractant c that is constantly emitted by the bacteria, together with bacterial motion towards its gradient, leads to the creation of spots. We show results of numerical simulation of a continuous model including these features (*a* in the figure).

This simple model is insufficient, however, to explain several crucial observations[1]. In the experiments, spots appear sequentially in the wake of a spreading broad ring and later 'lock' into position as the bacteria turn non-motile. To capture these effects one must introduce additional mechanisms. First, we explicitly include in the model a 'triggering' field w (a field that must reach a threshold before attractant emission is activated). The value of this threshold may depend on the ambient chemoattractant concentration. In detail, if $c > c_0$, emission occurs when $w > w_0$ and otherwise, when

$w > w_1$ (where $w_1 > w_0$). Biologically, w represents the hazardous waste products produced by the bacteria. Also, bacteria in the full model differentiate into a nonmotile state when starved for a sufficiently long time. To observe (in the simulations) different patterns, we vary the model's parameters related to the bacterial response to the triggering field and to the precise nature of the chemoattractant signalling. The final continuous model is then in good agreement with many of the features seen in experiment, including the possible radial organization of spots or stripes (*b* in the figure)

An alternative model — the 'communicating walkers' approach[3] (introduced in the study of branching patterns during growth of *Bacillus subtilis*) — leads to the same results. The pattern shown in *c* of the figure was produced by the discrete model described in ref. 3 which we have modified to apply to *E. coli* in semi-solid agar. The 'freezing' of a walker (which stops moving after an interval of time during which not enough food is available) represents the observed differentiation into a non-motile state of the individual bacterium. Augmenting the bacterial motion and nutrient dynamics are the following additional features. First, a 'triggering' field (representing the waste products). The field is produced by the bacteria at a rate proportional to the rate of food consumption and is decomposed by the bacteria at a fixed rate. Second, a chemoattractant field. The emission of the chemoattractant is stimulated when the triggering field exceeds a threshold value or when the concentration of the attractant in the surrounding of the walkers exceeds a minimum value. Once a walker starts to produce attractant, it does so at a fixed rate for an interval of time such that a fixed amount is released.

The walkers approach has its computational advantages and disadvantages relative to the continuum approach. As each walker represents a coarse-graining of about 10^4–10^5 bacteria (numerically, one cannot handle 10^{10} particles, which is the number of bacteria in a colony), it has an