

Releasing "ice-minus" bacteria

SIR — Two strategies for the biological control of those bacteria that act as seeds for ice-crystal formation (and the consequent crop damage) have recently been presented. Researchers at the University of Colorado propose spraying fields with bacterial viruses (bacteriophage)¹, while researchers at the University of California propose spraying fields with genetically engineered bacteria whose "ice-nucleation gene" has been deleted^{1,2}. Most of the media attention has gone to the latter stratagem, largely because of the controversy surrounding the release of recombinant DNA organisms into the environment^{2,3}. However, successful implementation of these strategies may be limited by ecological and evolutionary factors subsequent to the deployment of predators (bacteriophage) or competitors (recombinant bacteria). The application of bacteriophage to populations of sensitive bacteria engenders intense selection for phage-resistant mutants⁴ whose increase could defeat future, if not present, control. The application of recombinant "ice-minus" bacteria requires that they out-compete naturally occurring ice-nucleating forms², whereas the arbitrary deletion of an evolved gene would likely be disadvantageous, or at best neutral.

An integrated strategy might overcome these difficulties, and might even begin to counter some of the fears associated with releasing recombinant bacteria. Bacteriophage infect bacterial cells by first adsorbing to some surface protein or other component of the cell wall; phage are generally very specific in their site of adsorption⁵. By comparing the sensitivities of "ice-minus" and "ice-plus" isogenic bacteria to a diverse set of bacteriophage, one might identify a phage whose site of adsorption is the ice-nucleating surface protein. By applying this bacteriophage in concert with the "ice-minus" recombinant bacteria, one would provide the "ice-minus" phage-resistant bacterium with a strong selective advantage over the "ice-plus" phage-sensitive natural counterpart. Moreover, as phage-resistant mutants appeared among the naturally occurring bacteria, they too would probably be "ice-minus" since resistance to phage typically arises via the alteration or loss of function of the gene encoding the specific surface protein to which the phage adsorbs⁵; even if such phage-resistant mutants were not "ice-minus", selection favouring their increase would be ameliorated by high densities of the phage-resistant recombinant competitor. Finally, with this strategy, it should be possible to release "disarmed" strains of "ice-minus" recombinant bacteria — recombinant strains bearing genes (or deletions of genes) which ensure their competitive inferiority outside the range of simultaneous application of bacteriophage, yet whose phage-resistance

renders them selectively favoured within the range of simultaneous phage application. Thus, the spread of recombinant bacteria would be prevented by virtue of their competitive inferiority coupled with their inability to support the very phage whose presence is necessary to permit their establishment.

Is there a precedent for this strategy? Williams Smith and Huggins⁶ successfully treated experimental bacterial infections in mice using bacteriophage, precluding the rise of phage-resistant bacterial mutants in a manner similar to that proposed above. First, pathogenicity of the target bacterium was shown to depend on the presence of a particular surface antigen⁷.

Second, a bacteriophage was isolated whose site of adsorption is that surface antigen. Third, it was shown that the bacteria became resistant to the phage through the loss of that surface antigen. In essence, Williams Smith and Huggins chose phage for which resistance was incompatible with pathogenicity of the target bacterium. Whether phage can be isolated for which resistance is incompatible with ice-nucleation by the target bacteria remains to be seen.

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How "gradual"?

SIR — The recent article by Rhodes¹ on "Gradualism, punctuated equilibrium and the *Origin of Species*" is a major event in clarifying Darwin's views on both rates of evolution and "gradualism". The claim that "gradualism" refers specifically to long-term regular changes in the geological record² has led to much confusion. It is now clear¹ that Darwin's views on gradualism should be understood on a shorter biological or ecological time scale³, and that they do not require (nor could they explain) slow, long-term, orthogenetic changes in the fossil record.

There is one point that has not been covered and which helps to establish how (apart from misquotation³) the misunderstanding arose. It may also help to prevent such errors in future. This is that terms such as "fast", "slow" and particularly "gradual" have no scientific relevance unless they are used with respect to a specified time scale.

The term "temporal solipsism" has been

used by Kamen⁴ to describe the confusion that arises when conclusions are applied to the wrong time scale. His example is photosynthesis and he pointed out that it can be studied over many different time scales. Radiation physicists study quantum processes that occur within 10^{-15} - 10^{-9} seconds, or even longer. This "era" extends over at least six orders of magnitude, and overlaps with the time frame of photochemistry which studies events from 10^{-9} to about 10^{-4} or 10^{-3} s. Biochemists study events occurring from about 10^{-3} up to 10 s, and physiologists extend the range up to 10^4 s. Crop physiologists, farmers and ecologists extend the domain to at least 10^9 s. But long-term evolutionary changes such as the relative sensitivity of carboxylating enzymes to CO_2 and O_2 (ref. 5), development of C_4 photosynthesis and crassulacean acid metabolism, extend into the domain of more than 10^{16} s.

Given this extraordinary range of 10^{-15} - 10^{16} s, it is clear that "fast", "slow" and "gradual" are meaningless unless a temporal frame of reference is given. The interpretation suggested is that some palaeontologists have shown "temporal solipsism" by assuming that gradualism referred specifically to a geological time scale. It is clear^{1,3} that Darwin, in writing a book for a general readership, was referring to a biological or ecological time scale^{1,3} in discussing small, gradual changes between generations. Such a process does not predict orthogenetic trends in the fossil record.

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Be kind to flies

SIR — E.G. Gray's method for swatting flies¹ may work² but must always result in the death of the fly which cannot be justified unless the insect in question really is a health hazard. Last Christmas I bought my arachniphobe family a simple device called a "Spider Scoop". This consists of a transparent plastic dome mounted scissor-like on a broad plastic base. The dome is put over the offending arthropod and the base gently brought into place trapping the animal inside it. The principle resembles that of the card and beaker technique³. I recommend this method of fly trapping as efficient, clean and kind.

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