Putative oligocene spores

Cano & Borucki (3) have reported an exciting addition to the compilation of claims for exceptional bacterial longevity by Kennedy et al. (4). They isolated live Bacillus sphaericus from a dead bee that had become entrapped in Dominican amber in the oligocene era, between 25 and 40 million years ago. B. sphaericus is a plausible find since endospore-forming bacteria are normal inhabitants of the guts of modern bees. Their strain, which presumably survived as spores, differed from the type strain and modern mosquito-pathogenic strains of B. sphaericus in rRNA gene sequence, again a plausible feature, but this is a poorly studied and heterogeneous 'species' comprising at least 18 species-ranked taxa (1) and it is premature to accept on this basis that their strain belongs to an extinct taxon.

Many of the reports listed by Kennedy et al. (4) did not rigidly exclude contamination from the external environment by modern bacteria, but Cano & Borucki (3), clearly aware of this hazard, were meticulously careful in their controls and showed convincingly that their B. sphaericus strain came from inside the bee and from nowhere else. A careful reading of their paper discloses that this was the limit of their scientific claim.

However, press releases and even microbiologists have taken that finding to imply that the spores which gave rise to their cultures were as old as the bee. This can only be true if the bee's internal commensals died or sporulated when the bee died. But it is more likely that the microbes inhabiting its corpse multiplied at the expense of nutrients released by the decaying internal organs of the bee. Subject to the first scenario discussed later, when all the digestible parts of the bee were used up, cryptic growth would ensue: the microbes themselves would start to die, making renewed nutrients available to the survivors, which would then multiply, but in due course die, and so on. Generation would follow generation as the nutrients which originally constituted the bee were recycled, the total population declining gradually. This is the normal behaviour of bacterial populations in closed environments; the population will persist, though ultimately it will decline to extinction. Kennedy et al. (4) categorized such processes as 'in situ reproduction'; few of the reports they compiled had taken it into account.

Cano & Borucki (3) did not address this matter, and an important question therefore remains: for how long did a bacterial microcosm persist within their entombed bee? At least three relevant scenarios can be envisaged.

(i) The decline in any closed population will be enormously accelerated if the environment rapidly dehydrates or becomes toxic. In the particular case of B. sphaericus, which is an obligate aerobe, rapid anoxia would put a stop to its reproduction in situ. So brief a period of reproduction would have been trivial in the present context, consistent with the spores being as old as the bee. Cano & Borucki (3) offered no relevant information.

(ii) Such decline can in principle be slowed considerably if the environment is incompletely closed, i.e. if gases and water vapour, though not organic nutrients and foreign bacteria, can be exchanged with the external environment. Cycles of sporulation and regrowth such as B. sphaericus can undergo would be expected to protract racial survival. Information on the permeability of amber to gases seems not to be readily available, but it cannot be assumed to be gas-tight: it is a hardened mastic, rubber and the like are permeable to non-polar gases, and sometimes to water vapour, albeit slowly. Like most obligate aerobes, B. sphaericus might adjust readily to oxygen-limited conditions. An infinitesimal trickle of oxygen would probably have favoured prolonged survival of a declining, incompletely closed, population of B. sphaericus, other conditions being favourable.

But could vapour phase exchange alone have enabled a slow and protracted turnover of a B. sphaericus population over millions of years, such that the parents of Cano & Borucki's isolates were relatively modern descendants of the original strain? Even making favourable assumptions based on present physiological knowledge, the prospect seems unlikely. Thus, the amount of water and organic matter initially available in the bee could hardly have given rise to more than 10^5 cells. Assuming that a declining oxygen-limited population has a cryptic growth factor of 10 (i.e. that 10 cells must die to support the doubling of one survivor) and accepting a maximum longevity of 10,000 years for a population which sporulates regularly [an overestimate based on Bartholomew & Paik (2) and Parduhn & Watterson (5)], the most favourably placed microcosm would have died out completely before a hundred thousand years had passed; a negligible time in the context of 25–40 million. Only if B. sphaericus spores have a maximum survival time of some 2 million years would this scenario approach plausibility.

(iii) However, had extraneous organic nutrient also crept into to the bee's amber tomb, from...
the amber itself or through sub-microscopic fissures or faults in its structure, reproduction in situ might have sustained the race of bacteria entombed within the bee almost indefinitely. This is the most plausible alternative scenario to a spore’s lifetime of 2–40 million years.

Considerations of the kind outlined above are not trivial because, if the parent spores which gave rise to Cano & Borucki’s cultures were genuinely of an age comparable to that of the bee, or even but a tenth of that, then biologists’ current views on the ‘tenacity of life’ (4), on the stability of DNA, and on the resistance of spores to radiation damage, as well as aspects of bacterial evolution, must all be radically revised. There can be no objection in principle to such a revision, but at present it must be delayed. The evidence that their strain of *B. spharicus* is indigenous to the dead bee appears to be sound, but the implications regarding the longevity of *B. spharicus* spores must be regarded with reserve until the physicochemical micro-environment within the bee’s amber coffin during those millions of years is clearer.

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