Reply

Pathogenic microbial ancient DNA: a problem or an opportunity?

We agree with Donoghue & Spigelman (2005) that, although pathogen studies hold great potential, any discussion requires a critical assessment of the results to date. However, we did note, as did Pääbo *et al.* (2004), that the field of ancient pathogen DNA still lacks a series of well-controlled and rigorous studies that address technical issues and reliability criteria. This is unfortunate, as the rapid evolutionary rate of many pathogens offers a unique means to establish the authenticity of ancient pathogen sequences—since they should clearly be ancestral to modern genetic diversity (e.g. Reid *et al.* 1999). Such characterisation of pathogen evolutionary rates would also be of major importance to studies of the origin and spread of diseases, and so the relative lack of such investigation is particularly regrettable.

Very few ancient pathogen studies have followed established aDNA protocols such as independent replication and cloning. Only 3 out of 47 studies have performed independent replication at all (Drancourt & Raoult 2005), and none of the studies (Spigelman *et al.* 2002; Fletcher *et al.* 2003a,b; Donoghue *et al.* 2005) mentioned by Donoghue & Spigelman (2005) as following aDNA criteria included cloning. Yet these studies have used mobile insertion elements (e.g. IS 6110 in tuberculosis) or conserved loci (e.g. 16S) to detect the presence of pathogens, and very similar or identical sequences have been reported from environmental bacteria (Gilbert *et al.* 2004). For example, Rollo & Marota (1999) report that direct sequencing of ancient microbial DNA produced a sequence resembling (for example) *Treponema pallidum* (the causative agent of venereal syphilis) even in the absence of real *T. pallidum*, simply due to the presence of diverse bacterial DNA in the experiment. In addition, the limited knowledge of current microbial diversity (only 1–5% of extant microbial diversity is believed to be known) makes it very difficult to use short aDNA sequences to establish unequivocally the presence of a given pathogen, as even supposed ‘obligate’ species of humans or plants may possess currently undetected close relatives in the surrounding environment (Hebsgaard *et al.* 2005). Lastly, where specific studies have set out to replicate reports of ancient pathogenic bacterial DNA it has not been possible (Gilbert *et al.* 2004).

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The accompanying comment can be viewed on pages 641–642 or at <http://dx.doi.org/doi:10.1098/rspb.2005.3261>.