Introduction: species and speciation in micro-organisms

After 3 billion years of evolution, only 5000 bacterial species have been described compared with over a million animal species that have evolved in only 600 million years. There are a number of reasons for this striking anomaly, including the procedures required to formally define species of bacteria and archaea and the breadth of these species compared with those in higher organisms (Staley 2006). A very different picture is obtained from surveys of sequences sampled from even the simplest of environments, which indicate an immense uncharacterized prokaryotic diversity (Curtis *et al.* 2006). In particular, the study of microbial evolution based on small ribosomal subunit RNA sequence analyses has forever changed our understanding of biological diversity. The resulting phylogenetic Tree of Life has shown that the perceived lack of bacterial species is not simply owing to lower levels of biodiversity relative to the multicellular eukaryotes. Rather, it is now clear that there are vast previously unrecognized reservoirs of genotypes stemming largely from uncultured species. Further, this observation is not confined to the prokaryotes, and the immense complexity of sequence diversity found in microbial eukaryotes has only recently started to be explored. However, increases in our ability to discover, and inter-relate, genotypes has not been matched by our ability to make sense of the data. Microbiologists have been questioning the present species concepts and definitions used in microbiology, namely the morphological species concept for eukaryotic micro-organisms and the species definition for bacteria and archaea (there is generally no accepted species concept) based on DNA–DNA reassociation. The key issue is whether closely related isolates of bacteria (and other micro-organisms) cluster into discrete groups that can be identified, circumscribed and assigned as species. Then, if species can be identified, the challenge is to identify what processes cause micro-organisms to speciate and the phenomena that generate cohesion within species.

Our aim in convening this discussion meeting was to draw key workers together from diverse fields of microbial population biology, systematics and ecology to discover whether we could identify patterns and processes that might lead us to a unifying species concept for bacteria and archaea that could incorporate modern approaches and new sources of data. We also included microbial eukaryote biologists, as the mechanisms underlying speciation are better understood in eukaryotes and it is important to identify whether parallels exist that can be struck across the entire Tree of Life.

One contribution of 15 to a Discussion Meeting Issue ‘Species and speciation in micro-organisms’.

Although most microbiologists view speciation as an evolutionary process, it has not been possible to study the process in appropriate detail until recently (Staley 2006). Species have typically been defined by taxonomists using the sequence of a single locus (usually 16S rRNA), or a crude measure of overall genome similarity, and using very few isolates of each taxon (Gevers *et al.* 2006). However, bacterial population biologists and ecologists are now characterizing hundreds or thousands of isolates of single taxa, and of closely related taxa, using the sequences of multiple house-keeping loci (Hanage *et al.* 2006a) and whole genomes (Konstantinidis *et al.* 2006). Such high-throughput multilocus sequence-based approaches (and population genomics) are starting to be used as a tool to explore speciation in both environmental organisms and pathogens (Hanage *et al.* 2006a; Polz *et al.* 2006; Ward *et al.* 2006; Whitaker 2006), and both prokaryotes and microbial eukaryotes, in order to provide data that are amenable to population genetic analysis as well as phylogenetic analysis (Koufopanou *et al.* 2006; Taylor *et al.* 2006). These new data are providing a much more critical analysis of whether species can be defined by this approach (Fenchel & Finlay 2006; Hanage *et al.* 2006a) as well as the role of ecological niches (Cohan 2006; Ward *et al.* 2006) and biogeography on speciation (Whitaker 2006).

Central to an understanding of the processes that underlie microbial speciation are methodologies, both theoretical and practical, for recognizing species (Cohan 2006; Gevers *et al.* 2006). While this is a controversial area, progress is being made. Within the microbial eukaryotes, sequencing global samples of individuals of fungi and protists has shown that a vast diversity of genotypes exists. This diversity is contained within relatively few morphologically recognized species that are globally distributed. However, it has become increasingly apparent that strongly supported lineages can occur within morphologically described species (Koufopanou *et al.* 2006; Whitaker 2006), although this is disputed for the protists (Fenchel & Finlay 2006). That these ‘phylogenetic species’, which cannot be distinguished by morphology, are not simply the product of neutral genetic drift between geographically separate populations, is shown by the presence of strengthened barriers to mating between sympatric, compared to allopatric, members of the different clades. These experiments provide clear evidence for the evolution of post-fertilization barriers to mating, at least in some well-studied microbial eukaryotes. Therefore, hitherto unrecognized clades of microbial eukaryotes are behaving as good biological species such that when extrinsic barriers to gene flow are absent, strong intrinsic barriers have evolved to keep individuals from forming one global panmictic population (Taylor *et al.*

2006). The challenge is now to identify the loci that control these barriers to mating in order to understand the underlying mechanisms of speciation, as well as establishing whether these observations are specific to fungi, or can be generalized to protists and, perhaps, bacteria and archaea.

Allied to developments in experimental approaches to species definition and species concepts are recent advancements in theoretical approaches. Two obvious differences between microbes and multicellular organisms are that (i) population sizes tend to be much larger and (ii) rates of homologous recombination (HR) can vary greatly, and lateral transfer can spread genes across great phylogenetic distances (Gogarten & Townsend 2005). While there is a good understanding of the biological mechanisms that underpin the ability of prokaryotes to accept small chromosomal replacements from their close relatives (a process described as localized sex), it is not at all clear how such mechanisms of HR impact on the formation of bacterial species. A subset of theoretical arguments has focused on purely neutral models, where the breakdown of HR is a function of neutral sequence divergence (Cohan 1995). Here, Hanage *et al.* (2006b) and Falush *et al.* (2006) use neutral models to show that stable genotypic clusters can evolve in the absence of selection if recombination rates reduce very sharply with increasing sequence divergence, and the latter authors argue that these models can explain the patterns of divergence that are seen across a genus such as *Salmonella*. On the other hand, Hanage *et al.* (2006b) argue that speciation is unlikely to occur by this mechanism alone, since neutral sympatric speciation requires a much steeper reduction in recombination rate with divergence than is suggested by the empirical data. Therefore, the challenge is to determine the extent to which the variable levels (and types) of recombination in micro-organisms impact on our ability to produce a new unified concept of species.

The meeting provided an opportunity for participants to showcase recent developments in the studies of microbial taxonomy and speciation. Although their expertise and backgrounds varied enormously, common agreement was obtained on two issues. First, recent developments in sequencing technology and genome analyses are and will continue to provide the necessary tools for understanding microbial speciation. Second, unravelling the puzzle of microbial speciation remains one of the most elusive, important and exciting areas of microbiological research.

We would like to thank the participants, and discussants, for their contributions and the Royal Society for its generous financial support. We would also like to thank all the staff at the Royal Society for their help in arranging this meeting.

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July 2006

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REFERENCES

- Cohan, F. M. 1995 Does recombination constrain neutral divergence among bacterial taxa? *Evolution* **49**, 164–175. (doi:10.2307/2410302)
- Cohan, F. M. 2006 Toward a conceptual and operational union of bacterial systematics, ecology, and evolution. *Phil. Trans. R. Soc. B* **361**, 1985–1996. (doi:10.1098/rstb.2006.1918)
- Curtis, T. P., Head, I. M., Lunn, M., Woodcock, S., Schloss, P. D. & Sloan, W. T. 2006 What is the extent of prokaryotic diversity? *Phil. Trans. R. Soc. B* **361**, 2023–2038. (doi:10.1098/rstb.2006.1921)
- Falush, D., Torpdahl, M., Didelot, X., Conrad, D. F., Wilson, D. J. & Achtman, M. 2006 Mismatch induced speciation in *Salmonella*: model and data. *Phil. Trans. R. Soc. B* **361**, 2045–2053. (doi:10.1098/rstb.2006.1925)
- Fenchel, T. & Finlay, B. J. 2006 The diversity of microbes: resurgence of the phenotype. *Phil. Trans. R. Soc. B* **361**, 1965–1973. (doi:10.1098/rstb.2006.1924)
- Gevers, D., Dawyndt, P., Vandamme, P., Willems, A., Vancanneyt, M., Swings, J. & De Vos, P. 2006 Stepping stones towards a new prokaryotic taxonomy. *Phil. Trans. R. Soc. B* **361**, 1911–1916. (doi:10.1098/rstb.2006.1915)
- Gogarten, P. & Townsend, J. P. 2005 Horizontal gene transfer, genome innovation and evolution. *Nat. Rev. Microbiol.* **3**, 679–687. (doi:10.1038/nrmicro1204)
- Hanage, W. P., Fraser, C. & Spratt, B. G. 2006a Sequences, sequence clusters and bacterial species. *Phil. Trans. R. Soc. B* **361**, 1917–1927. (doi:10.1098/rstb.2006.1917)
- Hanage, W. P., Spratt, B. G., Turner, K. M. E. & Fraser, C. 2006b Modelling bacterial speciation. *Phil. Trans. R. Soc. B* **361**, 2039–2044. (doi:10.1098/rstb.2006.1926)
- Konstantinidis, K. T., Ramette, A. & Tiedje, J. M. 2006 The bacterial species definition in the genomic era. *Phil. Trans. R. Soc. B* **361**, 1929–1940. (doi:10.1098/rstb.2006.1920)
- Koufopanou, V., Hughes, J., Bell, G. & Burt, A. 2006 The spatial scale of genetic differentiation in a model organism: the wild yeast *Saccharomyces paradoxus*. *Phil. Trans. R. Soc. B* **361**, 1941–1946. (doi:10.1098/rstb.2006.1922)
- Polz, M. F., Hunt, D. E., Preheim, S. P. & Weinreich, D. M. 2006 Patterns and mechanisms of genetic and phenotypic differentiation in marine microbes. *Phil. Trans. R. Soc. B* **361**, 2009–2021. (doi:10.1098/rstb.2006.1928)
- Staley, J. T. 2006 The bacterial species dilemma and the genomic-phylogenetic species concept. *Phil. Trans. R. Soc. B* **361**, 1899–1909. (doi:10.1098/rstb.2006.1914)
- Taylor, J. W., Turner, E., Townsend, J. P., Dettman, J. R. & Jacobson, D. 2006 Eukaryotic microbes, species recognition and the geographic limits of species: examples from the kingdom Fungi. *Phil. Trans. R. Soc. B* **361**, 1947–1963. (doi:10.1098/rstb.2006.1923)
- Ward, D. M., Bateson, M. M., Ferris, M. J., Köhl, M., Wieland, A., Koeppel, A. & Cohan, F. M. 2006 Cyanobacterial ecotypes in the microbial mat community of Mushroom Spring (Yellowstone National Park, Wyoming) as species-like units linking microbial community composition, structure and function. *Phil. Trans. R. Soc. B* **361**, 1997–2008. (doi:10.1098/rstb.2006.1919)
- Whitaker, R. J. 2006 Allopatric origins of microbial species. *Phil. Trans. R. Soc. B* **361**, 1975–1984. (doi:10.1098/rstb.2006.1927)