

LETTER TO THE EDITOR**Radical changes to chlamydial taxonomy are not necessary just yet – reply**

The authors of ‘Radical Change’ (Schachter *et al.*, 2001) question the basis for the reclassification of the order *Chlamydiales* using molecular markers (Everett *et al.*, 1999). They prefer to keep the previous classification system or to develop a classification that relies clearly on biological markers. In support of their view, they infer conclusions from a review by Tanner *et al.* (1999). However, Mike Tanner and Norman Pace agree with and fully support our reclassification of chlamydiae into two genera and nine species, and would have used this as the basis of their chapter, if it were not already ‘in press’ when the reclassification was published (personal communication). The previous one-order, one-family, one-genus classification system (Page, 1966) was based on the presence of a unique developmental cycle among chlamydiae. The separation of two species (Page, 1968) was based on biological markers and served well for many years, although it became obviously strained by new findings. Specific transformation of the Page classification began a dozen years ago, when DNA–DNA hybridization was first applied to the study of chlamydiae (Cox *et al.*, 1988). This led to the creation of *C. pneumoniae* and *C. pecorum* without revision of the corresponding criteria for distinguishing *C. psittaci* or *C. trachomatis* (Grayston *et al.*, 1989; Fukushi & Hirai, 1992). The new species were introduced as pathogens only of humans or ruminants, respectively, and rapid identification relied on criteria which Page (1966) had rejected as too arbitrary for taxonomic purposes (serology and host-specificity). Today, *C. pneumoniae* and *C. pecorum* are found in marsupials, *C. pneumoniae* in frogs and horses, and *C. pecorum* in swine (Berger *et al.*, 1999; Schiller *et al.*, 1997; Wardrop *et al.*, 1999). Thus, the question is not whether we need the new classification system, but on what characters a revision should be based.

Biological markers identified by Page and others continue to be valuable guides for identifying cultured isolates, but assays for them are not easy to perform reliably. Unlike molecular data for DNA and proteins, which provide definitive differences between the species (Bush & Everett, 2001), biological markers can change as understanding of the organisms changes (Everett *et al.*, 1999; Matsumoto *et al.*, 1998; Ojcius *et al.*, 1998). We can clearly illustrate the utility of molecular markers for chlamydial identification and the lack of resolution provided by biological markers by using an example from our own research. We have a number of

swine isolates that, according to the previous classification system, must be sensitive to sulfadiazine and accumulate glycogen in inclusions in order to be included in *C. trachomatis* along with their genetically close relatives. However, many of the swine strains are resistant to sulfadiazine and most are meagre producers of glycogen. The swine strains are, however, readily distinguished from isolates of *C. trachomatis* by natural host ranges, by phylogenetic analysis, and by using PCR and PCR-RFLP tests developed from sequence data for both MOMP and ribosomal genes (Bush & Everett, 2001; Everett & Andersen, 1999; Kaltenböck *et al.*, 1997). Thus, existing biological markers may distinguish some species, but there are simply too few biological markers to definitively identify all species.

The authors of ‘Radical Change’ describe our proposal for a new genus as arbitrary because they believe the separation is based entirely on ribosomal sequence analysis. The availability of 16S and 23S rRNA sequence data allowed us to use the Palys method of identifying sequence similarity clusters in the *Chlamydiales* (Palys *et al.*, 1997). The former species *C. psittaci* and *C. trachomatis* clearly partition into clusters of species using this method to examine genetic and ecological differences. Ribosomal sequence analysis is increasingly being used to determine chlamydial interrelationships (Everett *et al.*, 1999; Fritsche *et al.*, 2000; Horn *et al.*, 2000; Pudjiatmoko *et al.*, 1997; Takahashi *et al.*, 1997), and 16S rDNA analysis is the standard for placing organisms in the context of phylogeny (Ludwig & Schleifer, 1999; Stackebrandt & Goebel, 1994). Our rooted rRNA phylogeny shows that the divergence of chlamydial species occurred through a series of distinct evolutionary steps, resulting in differentiable groups which we recognize as families, genera and species. The *Chlamydiaceae* clearly split into two monophyletic clades early in the evolution of the family (Bush & Everett, 2001). It has been also observed that genera are often separated by 16S rDNA differences of 95% or more (Ludwig *et al.*, 1998). However, classification at the level of species and genus requires more than a determination of the primary structure of 16S rDNA, of *ompA*, or of any other single gene. Thus it is significant that the *Chlamydia* and *Chlamydophila* genera differ in their having partial chromosome deletions and rearrangements (Everett *et al.*, 1999; Myers *et al.*, 2000; Read *et al.*, 2000).

We have recently found the same evolutionary history for the MOMP, GroEL, 60 kDa cysteine-rich protein, KDO-transferase, small cysteine-rich lipoprotein (Bush & Everett, 2001) and RNase P RNA (Herrmann *et al.*, 2000) genes as for the rRNA. A number of DNA-based tests have already been reported using these genes for identification of the various lineages. The new taxonomy is thus not only useful, appropriate and convenient, but it provides a starting point for studying forces that affect the evolution of individual genes and selective pressures responsible for divergence of the nine clearly distinct *Chlamydiaceae* lineages. DNA sequence markers allow us to resolve clinical questions (Cox *et al.*, 1998) and to obtain results that are easily comparable from one laboratory to another without problematic culture and transport of isolates. Comparisons of species, sequences and other data through the Internet and GenBank are already available and allow rapid strain identification in terms that can be easily understood and used by the diagnostician, the taxonomist and the epidemiologist.

The new classification provides criteria for the designation of new families and genera in the order *Chlamydiales* that are already being applied and will continue to be needed as our knowledge of this group expands (Horn *et al.*, 2000). As proposed, the trivial epithet, 'chlamydiae' still applies to all *Chlamydiales*. In the *Chlamydiaceae*, the name of the new genus, *Chlamydophila*, is sufficiently similar to *Chlamydia* to be recognizable and has been applied at both GenBank and ATCC. The nine new and emended *Chlamydiaceae* species show a high level of congruence with host ranges, growth characteristics in tissue culture, virulence differences, and the types of disease they cause. While the old biological markers for these lineages undergo re-evaluation, potential new biological markers such as motility can be carefully characterized. Genetic markers which have been clearly laid out (Everett *et al.*, 1999; Bush & Everett, 2001) can be relied upon to evaluate the taxon-specificity of these biological markers and to distinguish chlamydial groups.

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