Title

Horizontal gene transfer among microbial genomes: new insights from complete genome analysis

Journal

Current Opinion in Genetics & Development, 10(6)

ISSN

0959-437X

Author

Eisen, Jonathan A

Publication Date

2000-12-01

DOI

10.1016/s0959-437x(00)00143-x

Peer reviewed

Horizontal gene transfer among microbial genomes: new insights from complete genome analysis

Jonathan A Eisen

The determination and analysis of complete genome sequences has led to the suggestion that horiztonal gene transfer may be much more extensive than previously appreciated. Many of these studies, however, rely on evidence that could be generated by forces other than gene transfer including selection, variable evolutionary rates, and biased sampling.

Addresses
The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, Maryland 20850, USA; e-mail: jeisen@tigr.org; WWW: http://www.tigr.org/~jeisen

Current Opinion in Genetics & Development 2000, **10**:606–611

0959-437X/00/$ – see front matter

Abbreviation
HGT horzontal gene transfer

Introduction

> 'The view commonly entertained by naturalists is that species, when intercrossed, have been specially endowed with sterility, in order to prevent their confusion. This view certainly seems at first highly probable, for species living together could hardly have been kept distinct had they been capable of freely crossing'
>
> Charles Darwin, *On the Origin of Species*.

Analysis of complete genome sequences is providing insights into many issues concerning microbe evolution. One such area involves the transfer of genetic material between distinct evolutionary lineages — a phenomena known as horizontal, or lateral, gene transfer. (I prefer the use of 'transfer' instead of 'exchange' to refer to the process as, in most known cases, the process is unidirectional and rarely involves reciprocal exchanges of DNA. In addition, although the process can involve many genes or parts of genes, for the sake of simplicity I refer to it simply as 'gene transfer'.) This contrasts with what is considered the 'normal' process of inheritance — the transmission of traits from parents to offspring, also known as vertical inheritance. The occurrence of horizontal gene transfer (HGT), which blurs the boundaries between species even more than the hybridization referred to by Darwin quoted above, has been generally accepted for many years [1]. Examples include transfers from organellar to nuclear genomes in eukaryotes and of plasmids between bacterial species. Analysis of complete genome sequences has led to suggestions that the extent of HGT is far greater than was previously appreciated [2•,3•,4–9]. The reliability of some of these estimates, however, has been called into question recently by many researchers [10••]. In this review, I discuss the methods that have been used to infer past occurrences of HGT from analysis of genome sequences, and then some of the recently proposed examples.

Why study horizontal gene transfer?

There are a variety of reasons for better understanding the type and extent of HGT. As HGT results in chimeric species — that is, organisms in which different portions of the genome have different histories — attempts to infer the evolutionary history of species must take such transfer into account. Understanding HGT is also valuable in studying evolutionary processes such as the origins of new functions. In addition, HGT apparently plays an active role in many biological processes including the emergence and spread of virulence and resistance to antibiotics and the long-term maintenance of organelles [11]. The inference of HGT can also be useful in predicting gene functions, an integral part of most genome-sequencing projects. For example, predicting the targetting of nuclear genes to organelles can be aided by determining if those genes have an organellar ancestry.

Steps in horizontal gene transfer

HGT is more than just the transfer of genes — it should really be considered a multi-step process (Figure 1). Understanding HGT and the design of methods to detect it requires an understanding of all of these steps. I summarize some of the important parts of each step here. First, the gene to be transferred evolves within its donor lineage (Figure 1a). Two types of evolution are of particular importance to the study of HGT: co-evolution with other genes in the genome, and incorporation of genome-wide features of the donor lineage such as codon usage, GC content, splicing signals, and promoters (referred to here as 'genome phenotype'). At some point, the gene is then transferred to another lineage either via a vector (e.g. a virus) or by direct (e.g. mating) or indirect (e.g. competence) DNA exchange (Figure 1b). The gene must also be in or get into a format that allows long-term maintenance and replication (e.g. either as a self-replicating extrachromosomal element or by insertion into existing elements) (Figure 1c). The mechanisms of transfer and maintenance influence the patterns of HGT. For example, mismatch repair systems in some species prevent homologous recombination based insertions of distantly related genes but have no effect on insertion via non-homologous recombination [12]. A frequently overlooked component of HGT is the spread of a transferred gene in the recipient population (Figure 1e). Although this spread can occur by neutral means, it is generally thought to be driven by selective forces (e.g. selection for antibiotic resistance). For the

selection to work, the gene must function in the recipient. Thus, the extent and type of adaptation in the donor lineage as well as the distance between donor and recipient (both in terms of general molecular biology such as genetic code and genome phenotype such as codon usage bias) greatly influences the frequency of HGT. Finally, after a gene has been transferred it will begin to adapt in many features to its new lineage, a process referred to as 'amelioration' [13].

Methods for inferring horizontal gene transfer

A variety of methods have been developed to infer the occurrence of HGT. The utility of each method depends, in part, on the nature of all the steps in HGT that have occurred. In the following sections I discuss these methods as well as some of their strengths and weaknesses.

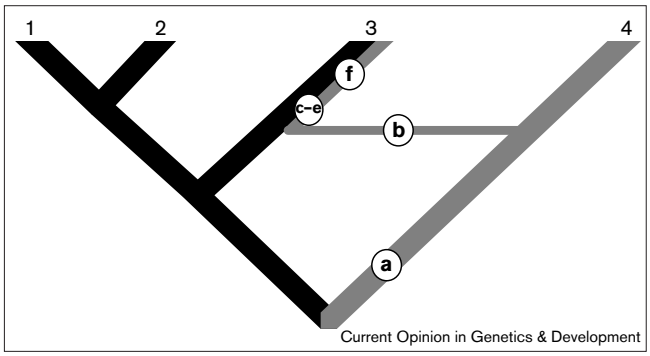
Comparing phylogenetic trees of different genes

One method that has been used to determine whether HGT has occurred involves inferring evolutionary trees for many genes in many genomes. HGT should cause different genes to have different trees. It is important to recognize that phylogenetic reconstruction methods are not perfect. Many factors in addition to HGT can lead to differences in inferred trees of different genes, including poor data; misuse of phylogenetic reconstruction methods; inaccurate alignments [14]; convergence; the misidentification of orthologs versus paralogs; and different species sampling for different genes [15]. Even when used correctly, phylogenetic reconstruction methods do not always produce the correct history [16]. In addition, phylogenetic methods do not always work well in inferring exchanges among very closely related species. Therefore there are valid reasons for using other approaches to infer HGT. Nevertheless, phylogenetic reconstruction methods remain the only way to reliably infer historical events from gene sequences. In addition, of all the methods used to infer HGT, phylogenetic methods are the only ones that are based on a large body of work. For example, phylogenetic methods are designed to accommodate variation in evolutionary rates and patterns within and between taxa. It is unfortunate that many studies of HGT have completely ignored the uses of phylogenetic reconstruction.

Patterns of best matches to different species

A common alternative method for inferring HGT involves using similarity search techniques to determine the 'best match' for each gene in a genome. HGT is frequently invoked for those genes that have best matches to supposedly distant species. The main advantages of these best-match methods are their speed and automatability — but their limitation is accuracy. For example, evolutionary rate variation can lead to two distantly related genes being detected as best matches (if the rate of evolution was very slow in their lineages) or two closely related genes not matching well (if they have evolved rapidly) [17]. In addition, there has not yet been any work that has determined how many unusual best matches one might expect by chance alone. Other problems with best-match methods include being easily misled by multidomain proteins, ignoring the possibility of gene loss, and not allowing reliable identification of orthology. Finally, this method is also significantly biased by genome size in that the number of best matches to a particular species is dependent in part on the total number of open reading frames in that species and not just evolutionary relatedness [18].

Figure 1



Stages in horizontal gene transfer. This figure depicts a hypothetical scenario of the evolution of four species (1–4). The main lines show the vertical evolution of these species. During the evolution, six major steps occur. First **(a)** genes in these species evolve and part of this evolution is adaptation to the replication, transcription, and translation systems of that species. At some point **(b)**, a set of genes is transferred from the lineage of species 4 to the lineage of species 3 (indicated by a gray horizontal line). After this transfer, the genes must **(c)** be maintained and replicated, **(d)** probably are under strong selection and **(e)** must spread within the population. **(f)** Finally, the transferred genes begin to ameliorate to their new lineage.

Distribution patterns of genes

Lateral gene transfer can result in the addition of novel genes in a particular species — thus, in theory, gene transfers could be identified by detecting genes with uneven distribution patterns (e.g. if a particular gene is present in gram-positive bacteria and all plants but not other bacteria or eukaryotes). Although such an approach can be effective, it is frequently misleading. For example, uneven distribution patterns can also be caused by gene loss or rapid sequence divergence (such that homologs are not detected in some lineages even when they are present). In addition, analysis of distribution patterns cannot be used to infer transfers involving homologous recombination of parts of genes.

Identifying regions of the genome with unusual compositions

As discussed above, there is significant uniformity in genome phenotype within genomes. As it takes time for a transferred gene to ameliorate to the recipient genome's phenotype, 'foreign' genes in a genome can be detected by identifying genes with unusual phenotypes (e.g. nucleotide composition or codon usage) [4,13,19]. An advantage of this approach is that it only requires the genome sequence of

Table 1

Methods of detecting horizontal gene transfer.

Observation	For what HGTs does it not occur?	Other possible causes	Comments
Unusual distribution patterns.	If recipient already has gene.	Sampling bias, gene loss, rapid divergence and ancestral polymorphisms.	Only works for HGT of novel genes.
Unusual nulceotide composition (e.g. codon usage, GC content).	If donor/recipient similar. If transfer was a long time ago.	Selection and mutation bias. Replication direction.	Only need a single genome. Rapid.
High hit to distant species.	If transfer is among close relatives.	Selection. Rate variation. Gene loss.	Fast but error prone.
Incongruent phylogenetic trees.	If transfer is among close relatives.	Bad trees. Missed paralogs.	Accurate but slow.
Clustering of any of the above in genome.	If genes were not transferred together. If gene order has been rearranged.	Selection.	–

one species and is not prone to the problems of phylogenetic reconstruction. A disadvantage is that unusual compositions can also be caused by factors other than HGT, such as selection, mutation bias, and even the direction of transcription relative to the replication origin [20•]. The use of compositional analysis to detect HGT requires detailed examination of multiple features for any particular genome to identify which characteristics are most likely caused by HGT [21]. This method cannot detect transfers between species with similar compositions nor those that have occurred a long enough time ago such that amelioration has been completed. In some cases, unusual composition may be a reflection more of the biology of vectors (e.g. phage and plasmid replication) and not adaptation to a particular host genome.

Combined approaches

As each type of evidence commonly used to infer HGT can be explained by other forces (see Table 1), it can also be useful to combine multiple lines of evidence. A good example of a combined approach comes from studies of gene transfer from chloroplast genomes to nuclear genomes [22]. This analysis has included the use of distribution patterns to identify cases of gene loss from organelles and corresponding gain in nuclear genomes; phylogenetic reconstruction methods to prove that these nuclear genes are evolutionarily derived from organellar genomes and composition analysis to document the amelioration of these genes with the nuclear genome. The limitation of combined approaches is that each method is designed to detect transfers of diferent types and ages.

Examples of horizontal gene transfer proposed on the basis of genome analysis

Thermophilic bacteria and Archaea

Analysis of the complete genomes of the bacteria *Aquifex aeolicus* and *Thermotoga maritima* and comparison with other complete genomes has led to the suggestion that large numbers of genes have been transferred between thermophilic bacteria and Archaea [23,24•]. In both cases, best-match methods have revealed that a high percentage of each proteome was most similar to Archaeal genes rather than bacterial genes — ~20% for *A. aeolicus* [23] and 25% for *T. maritima* [24•]. As indicated above, however, best matches can be misleading. The high percentage of Archaeal-like genes in these genomes could be caused by any of the following: a high rate of evolution in the mesophilic bacteria, the loss of these genes from mesophilic bacteria, or convergence [10••]. The authors present additional evidence supporting the possibility of HGT of some of the Archaeal-like genes including clustering in the genome, nucleotide-composition anomalies, and evolutionary trees showing some genes branching next to Archaeal genes [23,24•,25] but the results remain inconclusive. For example, a large fraction of the Archaeal-like genes had no additional support for transfer, indicating that the best-match method may be significantly misleading [26].

Organellar to nuclear transfers

The understanding of organellar to nuclear gene transfers has been greatly increased in the past few years with the completion of more genome sequences, both organelles and eukaryotes [22,27,28]. For example, tracing gene loss from chloroplast genomes [29] is now being supplemented with analysis of nuclear genome of plants, which will allow the identification of genes with chloroplast origins [30•]. Additional complete chloroplast genomes are also helping in this analysis [31–33].

Transfers involving pathogen genomes

A variety of studies have suggested the occurrence of extensive HGT in the history of many of the pathogen species for which complete genome sequences are now available. For example, analysis of nucleotide composition and codon usage has identified many regions of possible HGT in *Escherichia coli* (up to 18% of the genome) [4]. It will be interesting to repeat this type of analysis with pathogenic strains of *E. coli* and to try to identify the source of

this foreign DNA. Genome analysis suggests that a large percentage of the *Xylella fastidiosa* genome (~7%) appears to be derived from λ-like phage [34]. The finding that only a modest proportion of the genes in the *Campylobacter jejuni* genome had matches to genes from other ε-Proteobacteria, led to the suggestion that this species may have acquired many genes by HGT [35•]. In contrast, genome analysis indicates that HGT appears to have played little role in shaping Chlamydial genomes: most of the differences within and between *C. pneumoniae* and *C. trachomatis* strains involve chromosomal rearrangements and sequence divergence [36•,37].

Genome analysis has also lent support to one of the more interesting possible cases of HGT — that between the *Haemophilus influenzae* and *Neisseria meningitidis* lineages. This would be interesting because although these species are only moderately closely related, they occupy similar niches in the human ecosystem. The possibility of HGT between these two species was suggested initially on the basis of individual gene analysis [38]. Analysis of the complete genome of *N. meningitidis* (best matches and distribution of uptake signal sequences) supports this possibility [18,39].

Transfers involving whole genetic elements (small chromosomes, megaplasmids, plasmids)

Analysis of complete genomes is revealing that many of the smaller genetic elements in microbes may have separate origins from the main chromosomes. For example, the linear plasmids of *Borrelia* appear to be prone to HGT among strains or species [40]. Other proposed cases of discordant evolution of smaller genetic elements versus chromosomes include the *Xylella fastidiosa* plasmid [34], the megaplasmids and plasmid of *D. radiodurans* [41] and the smaller chromosome of *V. cholerae* [42]. The source of these smaller elements in each species remains unclear. For *D. radiodurans* and *V. cholerae*, any transfer probably occurred long ago because the smaller genetic elements have undergone extensive exchange with the main chromosome in each species. Interestingly, phylogenetic analysis suggests that the megaplasmids of Rhizobia are only transferred among closely related strains or species and not across long distances [43].

Is there a core recalcitrant to transfer?

Despite the apparent occurrence of extensive gene transfers in the history of microbes, it appears that there is a 'core' to each evolutionary lineage that is recalcitrant to transfer. Recent evidence for this comes from the construction of 'whole-genome trees' that are based on analysis of the presence and absence of particular homologs or orthologs in different complete genomes [44••]. Although these are not really trees, they do represent a measure of the average of the evolutionary patterns including gene loss and transfer as well as phylogeny — of all genes in a genome. That these average trees are nearly identical to the trees of rRNA genes suggests that those HGT events that occur are either constrained by phylogenetic relationships indicated by the rRNA tree or only occur for a moderate portion of the genome. Initial studies indicate that both appear to be true. For example, phylogenetic analysis suggests that the more universal genes can be exchanged within [45] but not between major microbial lineages [23]. The fact that these rRNA lineages share many genes [45,46] supports the core concept, as does evidence that only particular classes of genes are prone to transfer (see below).

What types of genes are transferred?

Analysis of complete genome sequences is helping determine what types of genes are most prone to transfer. Two recent studies [45,47] using phylogenetic analysis of many genes, conclude that genes which interact with many other genes (informational genes in [47]) are less prone to gene transfer than those with fewer interactions (operational genes in [47]). This was referred to as the 'complexity hypothesis' [47]. It is still not clear if complexity of interactions is the key factor here. For example, it is possible that sequence conservation of genes between species also influences the likelihood of transfer [25], which is probably what allows even rRNA genes to be transferred [48,49]. In addition, because informational genes are more likely to be required for survival, they are probably less likely to be lost from a species. Therefore, there will be little opportunity to replace them with a homologous gene. In addition, some genes may even facilitate their own transfer. For example, genes could avoid the need for amelioration by carrying the features needed for their own expression. Possible examples include self-regulatory transcription factors (which could bind to their own promoter), amino-acyl tRNA synthetases (which have been shown to be prone to transfer [50,51] and could help avoid codon usage problems), splicing factors, recombinases, and DNA polymerases.

Conclusions and future directions

The analyses described here, as well as many others not reported for space reasons, are beginning to reveal a great deal about HGT and its influence of gene and genome evolution. Some of the more interesting proposals include the following: that HGT may be more important than mutation in evolving new functions [4], that the tree of life may be unresolvable because of the extent of HGT [3•], and that what we consider phylogeny may be defined in a large part by HGT [2•]. However interesting these proposals are, I recommend caution before jumping on the HGT bandwagon. In most cases, the proposals for HGT involve only one type of evidence, such as nucleotide composition bias or unusual distribution patterns. As each of these can be caused by factors other than HGT, it will be important to see if they are supported by other lines of evidence. Furthermore, in most of the proposed cases, there is little information regarding what the likely donor lineage was or when in the course of evolution the transfer likely occurred. Even when donors and recipients have been proposed, there is rarely supporting

evidence regarding the absence of the genes from relatives of the recipient lineage that diverged prior to the transfer. For a complete picture of any proposed case of HGT it will be important to have information regarding the vectors, what, if any, selective forces were involved in the transfer, and what was the extent of amelioration.

With all these caveats aside, it seems likely that HGT has been frequent in the course of microbial evolution. It remains to be seen just how frequent HGTs have been and what the rules that govern the whole process are. As we learn more about the mechanisms and rules of HGT, we will be better equipped to model the process and develop more rigorous tests of its occurrence. Only then will we be able to determine the role HGT has played in shaping microbial evolution and biology.

Acknowledgements

I thank all the people at TIGR for support, assistance, and helpful discussions.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Davies J: **Origins and evolution of antibiotic resistance.** *Microbiologia* 1996, **12**:9-16.

2. • Doolittle WF: **Phylogenetic classification and the universal tree.** *Science* 1999, **284**:2124-2129.

Discussion of how the concept of classification of species may need to be revised in light of extensive horizontal gene transfer.

3. • Martin W: **Mosaic bacterial chromosomes: a challenge *en route* to a tree of genomes.** *Bioessays* 1999, **21**:99-104.

A review of HGT in microorganisms and the difficulties that extensive gene transfer presents to attempts to study the evolution of species. Outlines the need to search for principles governing genetic exchange.

4. Lawrence JG, Ochman H: **Molecular archaeology of the *Escherichia coli* genome.** *Proc Natl Acad Sci USA* 1998, **95**:9413-9417.

5. Doolittle WF: **Lateral genomics.** *Trends Cell Biol* 1999, **9**:M5-M8.

6. Doolittle WF: **The nature of the universal ancestor and the evolution of the proteome.** *Curr Opin Struct Biol* 2000, **10**:355-358.

7. Gogarten JP, Murphey RD, Olendzenski L: **Horizontal gene transfer: pitfalls and promises.** *Biol Bull* 1999, **196**:359-361 [discussion 361-362].

8. Lawrence JG: **Selfish operons: the evolutionary impact of gene clustering in prokaryotes and eukaryotes.** *Curr Opin Genet Dev* 1999, **9**:642-648.

9. Lawrence JG: **Gene transfer, speciation, and the evolution of bacterial genomes.** *Curr Opin Microbiol* 1999, **2**:519-523.

10. •• Kyrpides NC, Olsen GJ: **Archaeal and bacterial hyperthermophiles: horizontal gene exchange or common ancestry?** *Trends Genet* 1999, **15**:298-299.

Excellent commentary on the pitfalls of using sequence similarity methods to infer HGT.

11. Doolittle WF: **You are what you eat: a gene transfer ratchet could account for bacterial genes in eukaryotic nuclear genomes.** *Trends Genet* 1998, **14**:307-311.

12. Matic I, Taddei F, Radman M: **Genetic barriers among bacteria.** *Trends Microbiol* 1996, **4**:69-72.

13. Lawrence JG, Ochman H: **Amelioration of bacterial genomes: rates of change and exchange.** *J Mol Evol* 1997, **44**:383-397.

14. Gatesy J, Desalle R, Wheeler W: **Alignment-ambiguous nucleotide sites and the exclusion of systematic data.** *Mol Phylog Evol* 1993, **2**:152-157.

15. Eisen JA: **The RecA protein as a model molecule for molecular systematic studies of bacteria: comparison of trees of RecAs and 16s rRNAs from the same species.** *J Mol Evol* 1995, **41**:1105-1123.

16. Philippe H, Forterre P: **The rooting of the universal tree of life is not reliable.** *J Mol Evol* 1999, **49**:509-523.

17. Eisen JA: **Phylogenomics: improving functional predictions for uncharacterized genes by evolutionary analysis.** *Genome Res* 1998, **8**:163-167.

18. Tettelin H, Saunders NJ, Heidelberg J, Jeffries AC, Nelson KE, Eisen JA, Ketchum KA, Hood DW, Peden JF, Dodson RJ *et al.*: **Complete genome sequence of *Neisseria meningitidis* serogroup B strain MC58.** *Science* 2000, **287**:1809-1815.

19. Mrazek J, Karlin S: **Detecting alien genes in bacterial genomes.** *Ann NY Acad Sci* 1999, **870**:314-329.

20. • Lafay B, Lloyd AT, McLean MJ, Devine KM, Sharp PM, Wolfe KH: **Proteome composition and codon usage in spirochaetes: species-specific and DNA strand-specific mutational biases.** *Nucleic Acids Res* 1999, **27**:1642-1649.

One of the first detailed comparative analyses of proteome composition and codon usage between two related genomes (*T. pallidum* and *B. burgdorferi*). They found that the primary factor influencing codon usage is the direction a gene is transcribed relative to the direction of replication. This suggests that unusual nucleotide composition cannot be used as a reliable indicator of HGT and that gene-finding programs could be improved if they incorporated information on direction of replication.

21. Ochman H, Lawrence JG: **Phylogenetics and the Amelioration of the Bacterial Genome.** In Escherichia coli *and Salmonella. Cellular and Molecular Biology, edn 2*. Edited by Neidhardt FC. Washington DC: American Society of Microbiology; 1999:2627-2637.

22. Palmer JD, Adams KL, Cho Y, Parkinson CL, Qiu YL, Song K: **Dynamic evolution of plant mitochondrial genomes: mobile genes and introns and highly variable mutation rates.** *Proc Natl Acad Sci USA* 2000, **97**:6960-6966.

23. Aravind L, Tatusov RL, Wolf YI, Walker DR, Koonin EV: **Evidence for massive gene exchange between archaeal and bacterial hyperthermophiles.** *Trends Genet* 1998, **14**:442-444.

24. • Nelson KE, Clayton RA, Gill SR, Gwinn ML, Dodson RJ, Haft DH, Hickey EK, Peterson JD, Nelson WC, Ketchum KA *et al.*: **Evidence for lateral gene transfer between Archaea and bacteria from genome sequence of *Thermotoga maritima*.** *Nature* 1999, **399**:323-329.

In addition to presenting the second complete genome of a bacterial hyperthermophile, the authors present evidence that there have been even more exchanges between this species and Archaea than for *Aquifex aeolicus.*

25. Worning P, Jensen LJ, Nelson KE, Brunak S, Ussery DW: **Structural analysis of DNA sequence: evidence for lateral gene transfer in *Thermotoga maritima*.** *Nucleic Acids Res* 2000, **28**:706-709.

26. Logsdon JM, Faguy DM: **Thermotoga heats up lateral gene transfer.** *Curr Biol* 1999, **9**:R747-R751.

27. Adams KL, Song K, Roessler PG, Nugent JM, Doyle JL, Doyle JJ, Palmer JD: **Intracellular gene transfer in action: dual transcription and multiple silencings of nuclear and mitochondrial cox2 genes in legumes.** *Proc Natl Acad Sci USA* 1999, **96**:13863-13868.

28. Gardner MJ, Tettelin H, Carucci DJ, Cummings LM, Aravind L, Koonin EV, Shallom S, Mason T, Yu K, Fujii C *et al.*: **Chromosome 2 sequence of the human malaria parasite *Plasmodium falciparum*.** *Science* 1998, **282**:1126-1132.

29. Martin W, Stoebe B, Goremykin V, Hansmann S, Hasegawa M, Kowallik KV: **Gene transfer to the nucleus and the evolution of chloroplasts.** *Nature* 1998, **393**:162-165.

30. • Lin X, Kaul S, Rounsley S, Shea TP, Benito MI, Town CD, Fujii CY, Mason T, Bowman CL, Barnstead M *et al.*: **Sequence and analysis of chromosome 2 of the plant *Arabidopsis thaliana*.** *Nature* 1999, **402**:761-768.

The authors here provide evidence for HGT of a nearly intact mitochondrial genome to chromosome II. In addition, the authors use evolutionary analysis to identify likely chloroplast targeted proteins.

31. Lemieux C, Otis C, Turmel M: **Ancestral chloroplast genome in *Mesostigma viride* reveals an early branch of green plant evolution.** *Nature* 2000, **403**:649-652.

32. Turmel M, Otis C, Lemieux C: **The complete chloroplast DNA sequence of the green alga *Nephroselmis olivacea*: insights into**

the architecture of ancestral chloroplast genomes. *Proc Natl Acad Sci USA* 1999, **96**:10248-10253.

33. Zhang Z, Green BR, Cavalier-Smith T: **Single gene circles in dinoflagellate chloroplast genomes.** *Nature* 1999, **400**:155-159.

34. Silvestri ML, Siqueira WJ, de Souza AA, de Souza AP, Terenzi MF, Truffi D, Tsai SM, Tsuhako MH, Vallada H, Van Sluys MA *et al.*: **The genome sequence of the plant pathogen *Xylella fastidiosa*.** *Nature* 2000, **406**:151-157.

35. • Parkhill J, Wren BW, Mungall K, Ketley JM, Churcher C, Basham D, Chillingworth T, Davies RM, Feltwell T, Holroyd S *et al.*: **The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences.** *Nature* 2000, **403**:665-668.

In addition to presenting the complete genome sequence of the second species in the ε-Proteobacteria, the authors show how genome sequences can be used to identify hypervariable loci. Most remarkably, they find extensive polymorphisms in the library used for sequencing showing that the mutation rate in this species, especially at microsatellite loci, is very high.

36. • Read TD, Brunham RC, Shen C, Gill SR, Heidelberg JF, White O, Hickey EK, Peterson J, Utterback T, Berry K *et al.*: **Genome sequences of *Chlamydia trachomatis* MoPn and *Chlamydia pneumoniae* AR39.** *Nucleic Acids Res* 2000, **28**:1397-1406.

A detailed comparison of four closely related genomes including two genomes sequenced by the authors and two previously published genomes. Shows how comparisons of closely related species can be used to identify recent events such as inversions, duplications, and deletions.

37. Shirai M, Hirakawa H, Kimoto M, Tabuchi M, Kishi F, Ouchi K, Shiba T, Ishii K, Hattori M, Kuhara S *et al.*: **Comparison of whole genome sequences of *Chlamydia pneumoniae* J138 from Japan and CWL029 from USA.** *Nucleic Acids Res* 2000, **28**:2311-2314.

38. Kroll JS, Wilks KE, Farrant JL, Langford PR: **Natural genetic exchange between *Haemophilus* and *Neisseria*: intergeneric transfer of chromosomal genes between major human pathogens.** *Proc Natl Acad Sci USA* 1998, **95**:12381-12385.

39. Saunders NJ, Hood DW, Moxon ER: **Bacterial evolution: bacteria play pass the gene.** *Curr Biol* 1999, **9**:R180-R183.

40. Casjens S, Palmer N, van Vugt R, Huang WM, Stevenson B, Rosa P, Lathigra R, Sutton G, Peterson J, Dodson RJ *et al.*: **A bacterial genome in flux: the twelve linear and nine circular extrachromosomal DNAs in an infectious isolate of the Lyme disease spirochete *Borrelia burgdorferi*.** *Mol Microbiol* 2000, **35**:490-516.

41. White O, Eisen JA, Heidelberg JF, Hickey EK, Peterson JD, Dodson RJ, Haft DH, Gwinn ML, Nelson WC, Richardson DL *et al.*: **Genome sequence of the radioresistant bacterium *Deinococcus radiodurans* R1.** *Science* 1999, **286**:1571-1577.

42. Heidelberg JF, Eisen JA, Nelson WC, Clayton RA, Gwinn ML, Dodson RJ, Haft DH, Hickey EK, Peterson JD, Umayam L *et al.*: **The genome sequence of *Vibrio cholerae*, the etiologic agent of cholera.** *Nature* 2000, **406**:477-484.

43. Wernegreen JJ, Riley MA: **Comparison of the evolutionary dynamics of symbiotic and housekeeping loci: a case for the genetic coherence of rhizobial lineages.** *Mol Biol Evol* 1999, **16**:98-113.

44. •• Snel B, Bork P, Huynen MA: **Genome phylogeny based on gene content.** *Nat Genet* 1999, **21**:108-110.

One of the first attempts to build a 'whole genome tree'. The authors show that a tree based on gene presence/absence is very similar to the rRNA tree for the same species, suggesting that there is some average phylogeny of a genome that is accurately reflected in the rRNA of life.

45. Makarova KS, Aravind L, Galperin MY, Grishin NV, Tatusov RL, Wolf YI, Koonin EV: **Comparative genomics of the Archaea (Euryarchaeota): evolution of conserved protein families, the stable core, and the variable shell.** *Genome Res* 1999, **9**:608-628.

46. Graham DE, Overbeek R, Olsen GJ, Woese CR: **An archaeal genomic signature.** *Proc Natl Acad Sci USA* 2000, **97**:3304-3308.

47. Jain R, Rivera MC, Lake JA: **Horizontal gene transfer among genomes: the complexity hypothesis.** *Proc Natl Acad Sci USA* 1999, **96**:3801-3806.

48. Asai T, Zaporojets D, Squires C, Squires CL: **An *Escherichia coli* strain with all chromosomal rRNA operons inactivated: complete exchange of rRNA genes between bacteria.** *Proc Natl Acad Sci USA* 1999, **96**:1971-1976.

49. Yap WH, Zhang Z, Wang Y: **Distinct types of rRNA operons exist in the genome of the actinomycete *Thermomonospora chromogena* and evidence for horizontal transfer of an entire rRNA operon.** *J Bacteriol* 1999, **181**:5201-5209.

50. Brown JR, Doolittle WF: **Gene descent, duplication, and horizontal transfer in the evolution of glutamyl- and glutaminyl-tRNA synthetases.** *J Mol Evol* 1999, **49**:485-495.

51. Wolf YI, Aravind L, Grishin NV, Koonin EV: **Evolution of aminoacyl-tRNA synthetases — analysis of unique domain architectures and phylogenetic trees reveals a complex history of horizontal gene transfer events.** *Genome Res* 1999, **9**:689-710.