ELSEVIER

For better or worse: genomic consequences of intracellular mutualism and parasitism

Jennifer J Wernegreen

Bacteria that replicate within eukaryotic host cells include a variety of pathogenic and mutualistic species. Early genome data for these intracellular associates suggested they experience continual gene loss, little if any gene acquisition, and minimal recombination in small, isolated populations. This view of reductive evolution is itself evolving as new genome sequences clarify mechanisms and outcomes of diverse intracellular associations. Recently sequenced genomes have confirmed a trajectory of gene loss and exceptional genome stability in long-term, nutritional mutualists and certain pathogens. However, new genome data for the Rickettsiales and Chlamydiales indicate more repeated DNA, a greater abundance of mobile DNA elements, and more labile genome dynamics than previously suspected for ancient intracellular lineages. Surprising discoveries of conjugation machinery in the parasite *Rickettsia felis* and the amoebae symbiont *Parachlamydia* sp. suggest that DNA transfer might play key roles in some intracellular taxa.

Addresses
Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543, USA

Corresponding author: Wernegreen, Jennifer J (jwernegreen@mbl.edu)

Current Opinion in Genetics & Development 2005, **15**:572–583

This review comes from a themed issue on
Genomes and evolution
Edited by Stephen J O'Brien and Clarie M Fraser

Available online 17th October 2005

0959-437X/$ – see front matter

DOI 10.1016/j.gde.2005.09.013

Introduction

In bacterial evolution, the transition from a free-living existence to a close relationship with eukaryotic cells represents a frequent theme. Certain bacterial symbionts have taken such associations to the extreme by completely abandoning any semblance of a free-living phase and replicating solely within the domain of a host cell. Throughout the history of life, these obligately intracellular bacteria have acted as major evolutionary catalysts, being involved in the origin of organelles and the diversification of eukaryotes. Present-day intracellular associations include a range of parasites, mutualists and commensal symbionts that play important roles in the ecology and physiology of their hosts (see Glossary for many of the terms mentioned in the Introduction) [1].

Owing to their medical and ecological importance, intracellular bacteria have been targets of numerous genome sequencing projects that have provided insights into the consequences of this specialized lifestyle (Table 1; Box 1). We have learned that, typically, these species have drastically reduced genomes that encode a streamlined metabolism, show rapid DNA sequence evolution and strong nucleotide compositional biases, and exhibit lower levels of genome flux (i.e. gene acquisition from foreign sources, and intragenomic changes such as inversions and translocations). The integration of population genetic processes with knowledge of bacterial physiology and ecology has helped to clarify mechanisms that might explain these common features.

In particular, current views of 'reductive evolution' emphasize that fundamental evolutionary processes — natural selection, mutation and genetic drift — might affect intracellular species differently than they do free-living ones [2,3]. For instance, genome streamlining might reflect relaxed purifying selection on metabolic functions that are dispensable in a resource-rich intracellular niche. In addition, strong effects of nucleotide mutations in intracellular bacteria might elevate rates of gene disruption, followed by erosion owing to a deletion bias in bacteria [4]. Many intracellular endosymbionts show few if any signs of gene acquisition. This is thought to reflect their generally low levels of repeats and mobile DNA, reduced recombination functions, and limited opportunities for DNA exchange among sequestered species [5]. Moreover, reduction of effective population sizes (N_e) owing to bottlenecks upon transmission [6] is expected to increase the rates of fixation of slightly deleterious mutations [7]. Lack of gene exchange would exacerbate this effect by preventing the recovery of beneficial alleles or entire gene regions that are lost [3,8].

This reductive evolution model offers a valuable framework to explain commonalities among intracellular bacteria, to identify informative exceptions, and to generate predictions that can be tested with new sequence data. The abundance of excellent reviews on this topic illustrates the utility of this conceptual framework in assimilating a wealth of new genome information and in guiding development of the field [9–15]. In this review, I discuss insights from recent — between 2004 and July 2005 — genome analyses of obligately intracellular bacteria that replicate solely within a host cell. These data

Glossary

Commensal symbiont: A symbiont that benefits from an association without conferring a serious disadvantage or advantage to the host.
Genetic drift: This describes the changes in the frequencies of alleles or genotypes as a result of chance alone. This stochastic effect plays an especially important role in small populations, in which drift can accelerate the fixation of slightly deleterious mutations [7].
Genome flux: A broad term describing changes in gene content or order owing to gene acquisition by horizontal transfer from foreign donors or recombination among related strains or species. This also includes intragenomic changes within a given genome, such as inversions, duplications, translocations and deletions.
Mobile DNA: Elements such as phage DNA, transposons, conjugative plasmids, insertion sequences and other DNA segments that move among or within genomes, typically without the need of extensive DNA sequence matches for homologous recombination. Often considered as selfish DNA that propagates at the expense of hosts and depends on occasional horizontal transmission for its maintenance.
Mutualist: A symbiont that provides a benefit to the host and, in turn, benefits from the association.
Obligately intracellular: An organism that replicates exclusively within a host cell.
Parasite: A symbiont that propagates by causing some degree of harm to the host.
Reductive evolution: A conceptual framework that considers the evolutionary and molecular mechanisms that drive genome streamlining in most intracellular bacteria. Current views suggest that gene loss reflects relaxed selection on dispensable traits, elevated mutation pressure, and even the loss of beneficial functions mutations as a result of genetic drift in small bacterial populations. Furthermore, reduced recombination documented in some intracellular associates might prevent the recovery of lost alleles or gene regions.
Symbiont: Any species that lives in close association with another. Broadly speaking, symbiosis includes obligate and facultative relationships that are parasitic, mutualistic or commensal. Among symbionts, endosymbionts are those that live within the tissues or cells of their hosts for part or all of their life cycles. Endosymbionts that can replicate within host cells are termed intracellular. Of these intracellular associates, certain highly specialized ones have lost the ability to replicate outside of host cells and are obligately intracellular — the focus of this review.
Type III secretion: An assemblage of ~20 proteins that spans the cell membrane, transports proteins out of the cell and mediates the delivery of specific proteins that suppress defenses or otherwise facilitate cell invasion.
Type IV secretion: Derived repeatedly from conjugation systems [83••], this is a secretion pathway that exports distinct DNA or protein substrates that cause various physiological changes in host cells during infection.

Box 1 Diverse lifestyles and host effects of intracellular bacteria.

Bacteria 'make their living' within host cells through a variety of strategies. At one end of the spectrum, intracellular parasites represent unwelcome invaders that spread at their hosts' expense and offer models to understand how bacteria exploit cellular functions. Fully sequenced representatives (Table 1) include *Mycobacterium leprae* and *Coxiella burnetii*, which have adopted an obligately intracellular lifestyle quite recently, and older intracellular lineages such as *Phytoplasma*, a plant parasite and close relative of the epicellular *Mycoplasmas*, in addition to parasites within the families Rickettsiaceae and Anaplasmataceae. In addition to vertebrate pathogens, Anaplasmataceae includes the invertebrate endosymbiont *Wolbachia*, typically a parasite in insects that hijacks host reproduction to increase the production of infected females. Given that other members of *Wolbachia* are mutualists involved in development and oogenesis in nearly all filarial nematodes, this genus shows a natural lifestyle variation that facilitates comparisons between mutualists and parasites.

The exclusive intracellularity across known Rickettsiaceae and Anaplasmataceae implies that they adopted this lifestyle at least 400 mya, a conservative estimate of divergence between the two families [27]. The even more ancient Chlamydiales acquired an intracellular lifestyle ~700 mya [28••], and today include parasitic members of the Chlamydiaceae that cause respiratory, ocular and genital infections, in addition to the recently sequenced *Parachlamydia* sp., a mutualistic symbiont of free-living amoebae and occasional opportunistic pathogen of humans. Although they share an obligately intracellular lifestyle, the wide diversity in tissue tropism, host ranges, life-history nuances, and phenotypic effects of intracellular parasites remain poorly understood.

Infections don't always turn out poorly for the host. At the other end of the symbiotic spectrum, mutualists provide benefits that increase the fitness of their hosts. In addition to the nematode and amoebae hosts mentioned above, insects as a group frequently associate with beneficial intracellular bacteria. These symbionts occur within specialized host cells, undergo maternal transmission to offspring and have co-evolved with hosts in stable associations that date back tens to hundreds of millions of years [69]. For example, estimated divergence times of host insects imply that the *Blochmannia*–ant and *Wigglesworthia*–tsetse associations originated at least 30 mya, and the even older *Buchnera*–aphid symbiosis was established ~150–200 mya. Such endosymbionts provide essential nutrients to about 10–15% of insects, most of which feed on nutritionally unbalanced diets (e.g. plant sap or blood). By enabling their hosts to exploit otherwise inadequate food sources and habitats, the acquisition of these mutualists can be viewed as a key innovation in the evolution of their hosts [70,71]. These 'primary' endosymbionts often co-exist with *Wolbachia* and facultative ('secondary') endosymbionts [72,73••]. Mutualistic symbionts and reproductive parasites might offer new tools for the modification, suppression, containment or eradication of arthropod populations of medical and/or agricultural importance (e.g. [74–76,86••]).

enable us to explore expectations of reductive evolution models — namely, that intracellular bacterial genomes are (i) severely reduced, (ii) specialized to their particular host association, and (iii) show patterns of genome dynamics that differ from those of free-living species.

Genome size reduction

The influx of genome data supports the general trend that strictly intracellular bacteria have very reduced genomes, typically in the range of 1 Mb or less (Figure 1). At 416 kb, the tiny genome of *Buchnera* BCc, associated with the cedar aphid *Cinara cedri*, is the smallest known for bacteria (A Latorre, unpublished). As expected from their small genomes, metabolisms of intracellular bacteria are simpler than those of free-living or facultatively intracellular species with larger genomes. This reduction often involves massive deletions early in the transition to intracellularity (see Update). For example, reconstructions of early *Buchnera* evolution indicate deletions of large DNA segments, some of which encoded 20 open reading frames (ORFs) or more, in addition to exceptionally high levels of gene rearrangements compared with other γ-Proteobacteria [16,17•]. Sequence data from facultatively or recently intracellular species provide a window into these

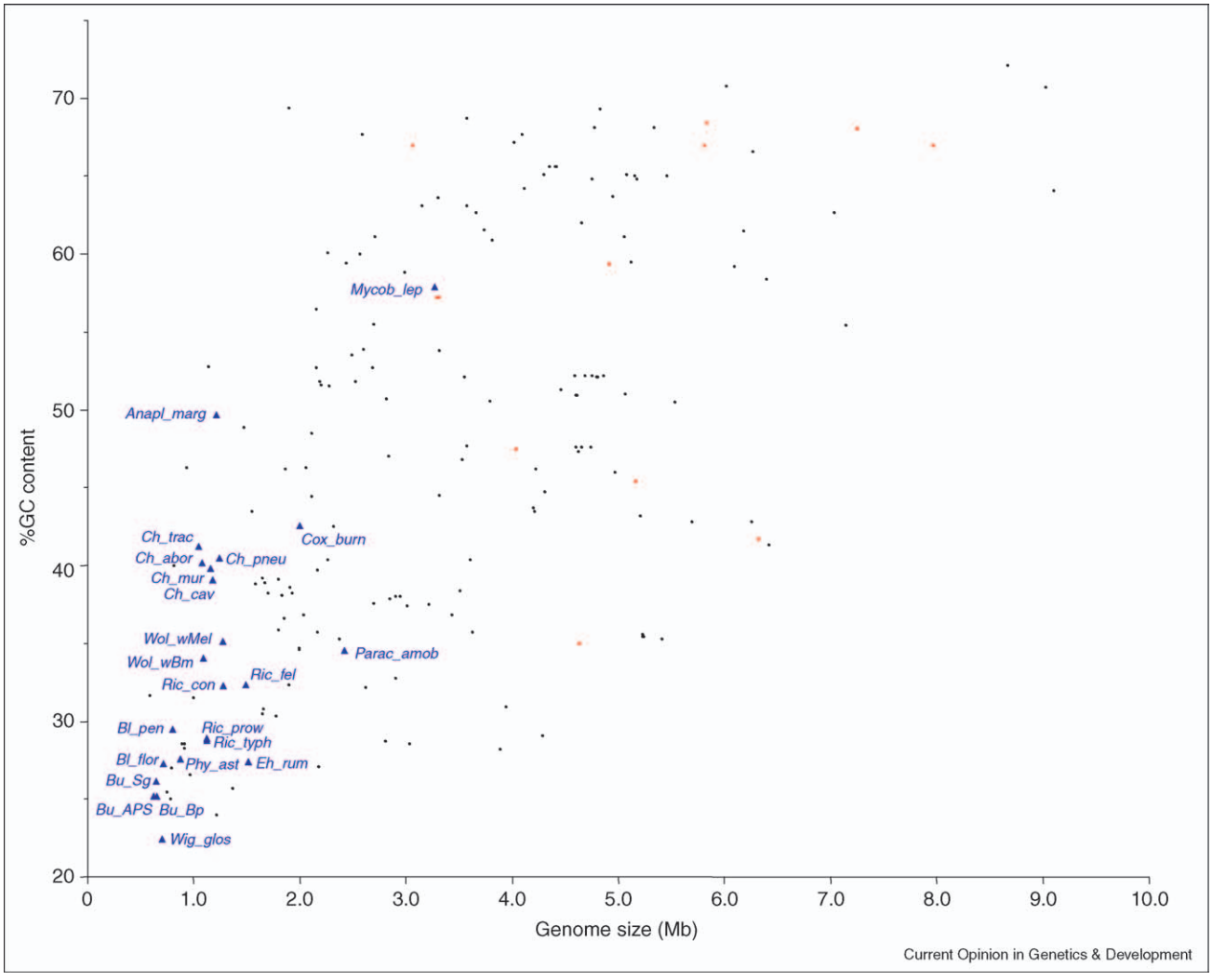
Table 1

Obligately intracellular bacteria with full genome sequence data (as of July 2005) or for which genome projects are in progress.

	Genome size (Mb)	Genome released	Host	Host effects
γ-Proteobacteria				
Enterobacteriales				
**Buchnera aphidicola* APS	0.66	2000	Aphid, *Acyrthosiphon pisum*	Nutritional mutualist
**Buchnera aphidicola* Sg	0.64	2002	Aphid, *Schizaphis graminum*	Nutritional mutualist
**Buchnera aphidicola* Bp	0.62	2003	Aphid, *Baizongia pistaciae*	Nutritional mutualist
**Buchnera aphidicola* BCc	0.42[a]	In progress, University of Valencia	Aphid, *Cinara cedri*	Nutritional mutualist
**Wigglesworthia glossinidia*	0.7	2002	Tsetse fly, *Glossina brevipalpis*	Nutritional mutualist
**Blochmannia floridanus*	0.71	2003	Ant, *Camponotus floridanus*	Nutritional mutualist
**Blochmannia pennsylvanicus*	0.79	2005	Ant, *Camponotus pennsylvanicus*	Nutritional mutualist
**Baumannia cicadellinicola*	0.69[b]	In progress, University of Arizona and TIGR	Sharp shooter, *Homalodisca coagulata*	Likely nutritional mutualist
Legionellales				
Coxiella burnetii	2.03	2003	Reptiles, birds, and mammals	Q fever
α-Proteobacteria				
Rickettsiales				
Rickettsiaceae				
Rickettsia conorii	1.27	2000	Mammals, through insect vectors	Rocky Mountain spotted fever
Rickettsia prowazekii	1.11	1998	Mammals, through insect vectors	Typhus
Rickettsia typhi	1.11	2003	Mammals, through insect vectors	Murine typhus
Rickettsia felis	1.46	2005	Mammals, through insect vectors	Spotted fever
Anaplasmataceae				
Anaplasma marginale	1.2	2004	Mammals, through insect vectors	Bovine anaplasmosis, human granulocytic ehrlichiosis
Ehrlichia ruminantium (2 strains)	1.5–1.52	2005	Wild ruminants, through tick host	Heartworm disease
Wolbachia wMel	1.27	2004	Fruit fly, *Drosophila melanogaster*	Cytoplasmic incompatibility
**Wolbachia* wBm	1.08	2005	Filarial nematode, *Brugia malayi*	Worm development and fertility
Wolbachia wAna		2005 (95% of genome recovered from Trace Archive, [62])	Fruit fly, *Drosophila ananassae*	Cytoplasmic incompatibility
Wolbachia wRi		2005 (75–80% of genome recovered from Trace Archive [62])	Fruit fly, *Drosophila simulans*	Cytoplasmic incompatibility
Wolbachia wUni		In progress, EUWOL (European *Wolbachia* Consortium)	Parasitoid wasp, *Muscidifurax uniraptor*	Induction of parthenogenesis
Wolbachia wVul	1.6–1.7	In progress, EUWOL	Isopod, *Armadillidium vulgare*	Induction of feminization
Wolbachia wRi	1.5–1.6	In progress, EUWOL	Fruit fly, *Drosophila simulans*	Cytoplasmic incompatibility
Wolbachia–Culex	~1.5	In progress (http://www.sanger.ac.uk/Projects/W_pipientis/)	Mosquito, *Culex quinquefasciatus*	Cytoplasmic incompatibility
**Wolbachia–Onchocerca*	~1.1	In progress (http://www.sanger.ac.uk/Projects/Wolbachia/)	Nematode, *Onchocerca volvulus*	Worm development and fertility
Mollicutes				
Acholeplasmatales				
Phytoplasma asteris	0.86	2003	Plants, through insect vector	Stunted plant growth and other symptoms
Actinobacteria				
Actinomycetales				
Mycobacterium leprae	3.27	2001	Humans and other vertebrates	Leprosy
Chlamydiae group				
Chlamydiales				
**Parachlamydia sp.*	2.41	2004	Free-living amoebae	
Chlamydiaceae				
Chlamydia muridarum	1.08	2000	Rodents	Mouse lung or genital tract infections
Chlamydia trachomatis	1.04	1998	Human and other mammals	Chronic genital and ocular infections
Chlamydophila abortus	1.14	2005	Ruminants and swine	Ruminant abortion
Chlamydophila caviae	1.18	2003	Guinea pig	Guinea pig inclusion conjunctivitis (GPIC)
Chlamydophila pneumoniae (4 strains)	1.23	1999–2003	Human and other mammals	Pneumonia, bronchitis and pharyngitis

The list of genomes for which sequencing is in progress is intended to illustrate the rapid growth of this data and is not exhaustive. * Mutualistic association. [a] Updated from Gil *et al*. [84]. [b] Updated from Moran *et al*. [85]. Initials after endosymbiont strains often refer to the invertebrate host species from which the bacteria were isolated.

Figure 1



Genome size and %GC content of bacterial chromosome sequences, illustrating the small genome size and AT-richness of obligate intracellular associates. Intended as an update to similar published figures (e.g. [77]), this graph includes genomes that were publicly available as of July 2005. Genomes of multiple, closely related strains are presented with a single point. Blue triangles represent obligately intracellular species. Red points represent those species that possess two or more chromosomes (the mark reflects values for single chromosomes).

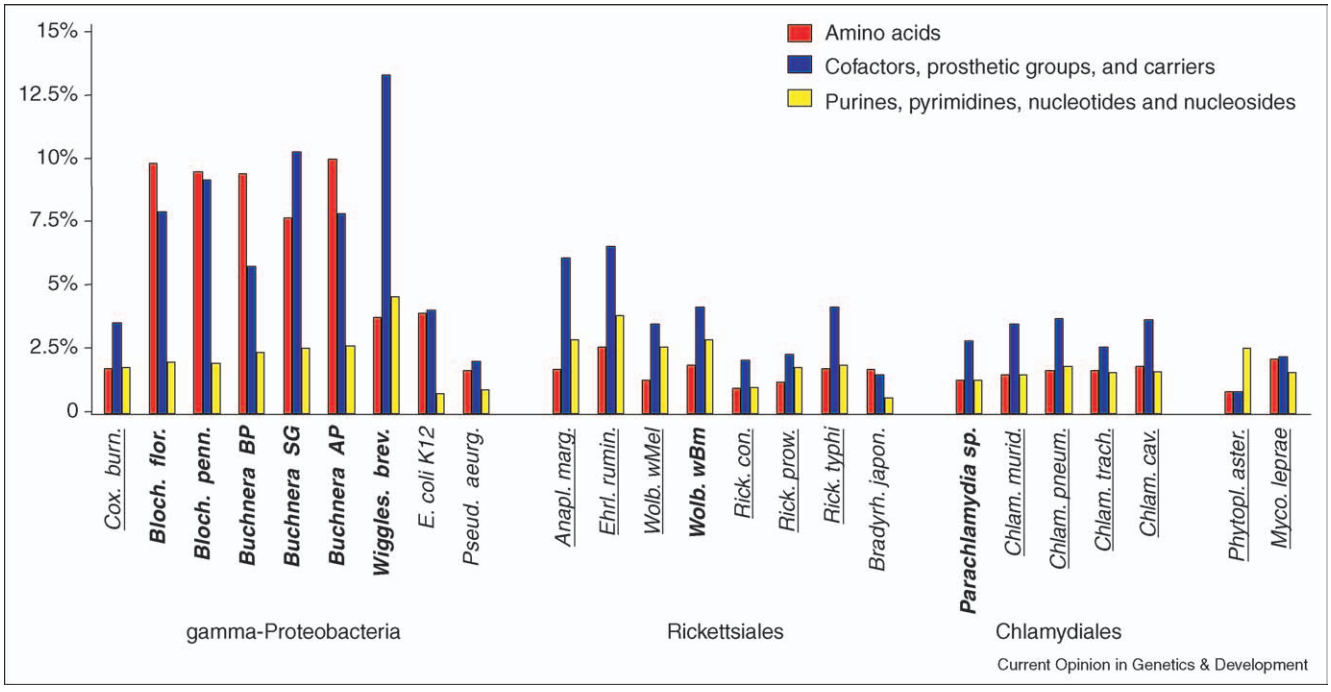
early stages of genome turbulence, when the proliferation of insertion sequences (ISs) and other mobile DNA elements might catalyze instability [9]. In addition, the larger genomes, numerous pseudogenes, and/or dispersed repeats in recent associates (e.g. *Coxiella burnetii*, *Mycobacterium leprae* and the *Sitophilus oryzae* [weevil] primary endosymbiont [SOPE]) also support an initial instability [18,19,20••].

Genome size variation within endosymbiont groups indicates that streamlining has continued in the context of intracellular associations but in a much more gradual fashion. For example, in contrast to large early deletions, subsequent gene loss in *Buchnera* has tended to occur through gene disruption and gradual erosion, with inactivated genes requiring ~40–60 million years to erode completely [21•]. This continued genome shrinkage leads to further metabolic loss. For instance, reduction in *Buchnera* BCc is caused by the loss of protein-coding genes, in comparison with the numbers in other *Buchnera* species, and not by the shortening of ORFs or intergenic regions [22••].

Specialization to the intracellular niche

What are the metabolic implications of severe genome reduction? Consistent with the prediction that many deleted genes were dispensable in a host cell, small intracellular genomes tend to lose genes for metabolic diversity but retain those encoding transcription, translation and other basic processes that are important regard-

Figure 2



Percentage of genes encoding particular biosynthetic functions. Y axis indicates proportion of ORFs involved in biosynthesis of (i) amino acids (red), (ii) cofactors, prosthetic groups, and carriers (blue), and (iii) purines, pyrimidines, nucleotides and nucleosides (yellow). Species in bold are obligately intracellular mutualists; those underlined are obligately intracellular pathogens. *Escherichia coli*, *Pseudomonas aeruginosa* and *Bradyrhizobium japonicum* retain a free-living phase and are included for comparison. Full names of other bacteria are listed in Table 1. Values for *E. coli* and the γ-proteobacterial nutritional mutualists were based on re-analysis of genome sequences [37]. Data for other species was downloaded from the Comprehensive Microbial Resource at The Institute for Genomic Research (TIGR, http://www.tigr.org/) [78], for a more consistent comparison across genomes, but might differ from original genome papers. Readers interested in particular taxa are encouraged to refer to the original genome-publications. In the rare cases that TIGR counted largely overlapping, putative ORFs as two separate genes, these were counted as a single ORF for the purposes of this figure.

less of ecological niche [2]. The preferential retention of informational genes also holds within endosymbiont groups. Analysis of partial genome regions indicates that, compared with its *Buchnera* relatives, *Buchnera* BCc has undergone a more extensive loss of metabolic than informational functions [22••].

The nutrient trade balance

Although all parasites and mutualists rely on their host for certain nutrients, they differ in the degree of their dependency. As expected from their roles in supplementing the diet of the host, primary, γ-proteobacterial mutualists of insects retain a wide spectrum of biosynthetic genes to fulfill symbiont functions, devoting a higher fraction of their genomes to biosynthesis than do free-living bacteria or pathogens (Figure 2) [11,15]. By contrast, intracellular parasites apparently rely on their eukaryotic host cell for many amino acids, cofactors, nucleotides and other compounds.

Though more subtle, the same contrast holds among species of the Rickettsiales and Chlamydiales orders, in which mutualists encode a wider array of biosynthetic functions than do parasites. The relatively large (2.41 Mb) genome of *Parachlamydia* complicates direct comparison of genome proportions with its ~1–1.2 Mb parasitic relatives, but its retention of twice as many amino acid and cofactor biosynthetic genes suggests it imposes fewer metabolic demands on its host cell. Within Rickettsiales, the mutualistic *Wolbachia* wBm, unlike *Rickettsia*, retains the ability to synthesize riboflavin and other coenzymes [23••]. Biosynthesis of riboflavin and heme might be key functions of the symbiont, because, to date, neither pathway has been detected in the *Brugia malayi* genome [24]. *Wolbachia* wBm also retains complete pathways for biosynthesis of purines and pyrimidines, and might supplement the nematode's nucleotide pools during oogenesis and embryogenesis [23••]. Although the parasitic *Wolbachia* wMel shares many of these same functions, the nematode mutualist devotes a larger fraction of its smaller genome to these potentially host-beneficial traits.

Shared infection strategies of mutualists and pathogens

One of the surprises from molecular studies of host-associated bacteria has been the discovery of 'virulence'

genes in mutualists [25,26]. As expected, obligately intracellular parasites typically encode numerous mechanisms to infect various tissue and cell types and to evade an ever-adapting host immune system. These mechanisms include Type III secretion (in Chlamydiales), Type IV secretion (*Coxiella* and Rickettsiales) [see Glossary], and paralogous families of polymorphic surface proteins (e.g. in *R. felis*, *Anaplasma* and *Ehrlichia*) [27].

Certain obligately intracellular mutualists also possess such so-called pathogenicity genes. The discovery of Type III secretion in *Parachlamydia* highlights parallels with related parasites and implies that the ancestor of this group could infect cells [28••]. In a similar manner to certain insect endosymbionts [29], this amoebae associate might deliver specific proteins into host cells to facilitate invasion. This exciting discovery pushes the origin of infectious chlamydia back to ~700 million years ago (mya), arguably the oldest intracellular group that today spans diverse host associations and effects [30,31•]. In a similar fashion to its pathogenic relatives, *Parachlamydia* imports ATP from the host cytosol using an ATP/ADP translocase, an 'energy-parasite' transport system unique to Rickettsiales, Chlamydiales, and plant plastids [32•]. *Parachlamydia* apparently acquired Type IV secretion through horizontal gene transfer, making it the only chlamydia to possess this pathway [28••].

Wolbachia spp. might also use common infection strategies across diverse interaction types. As with *Wolbachia* wMel and other parasitic α-Proteobacteria, *Wolbachia* wBm possesses Type IV secretion in its stable mutualistic association with nematodes [23••]. The shared presence of ankyrin repeats in both *Wolbachia* genomes — although there are fewer in *Wolbachia* wBm — might mediate attachment of endosymbionts to the cytoskeleton, modulation of host gene expression, or other activities essential to their intracellular lifestyle. The roles of these and other *Wolbachia* genes in shaping its varied host interactions are promising new areas of research [33,34] (see Update).

Even the long-term insect mutualists possess genes once considered to be in the purview of parasitism. The urease gene cluster, which encodes significant virulence factors in some bacterial and fungal pathogens [35], is retained in *Blochmannia*, apparently enabling this ant symbiont to hydrolyze insect host waste (urea) to ammonia used in amino acid biosynthesis [36,37]. The overexpression of GroEL in certain insect mutualists [38] also occurs in some pathogens, in which this and other heat-shock proteins are major antigens and candidates for vaccine development [39–41]. The functions of GroEL in mutualists remain uncertain, but it might help the folding of endosymbiont proteins that have experienced deleterious amino acid changes [42], mediate viral transmission through certain insect hosts [43], or counter irreversible oxidative modifications during stationary phase [44], in which endosymbionts probably spend much of their life cycle. Other parallels with pathogens include the retention of outer membrane proteins and flagellar genes, the latter of which might be involved in secretion [45]. These parallels suggest that 'pathogenicity' functions might have evolved in the context of beneficial interactions and today play generally important roles for host-associated bacteria.

Deleterious deletions

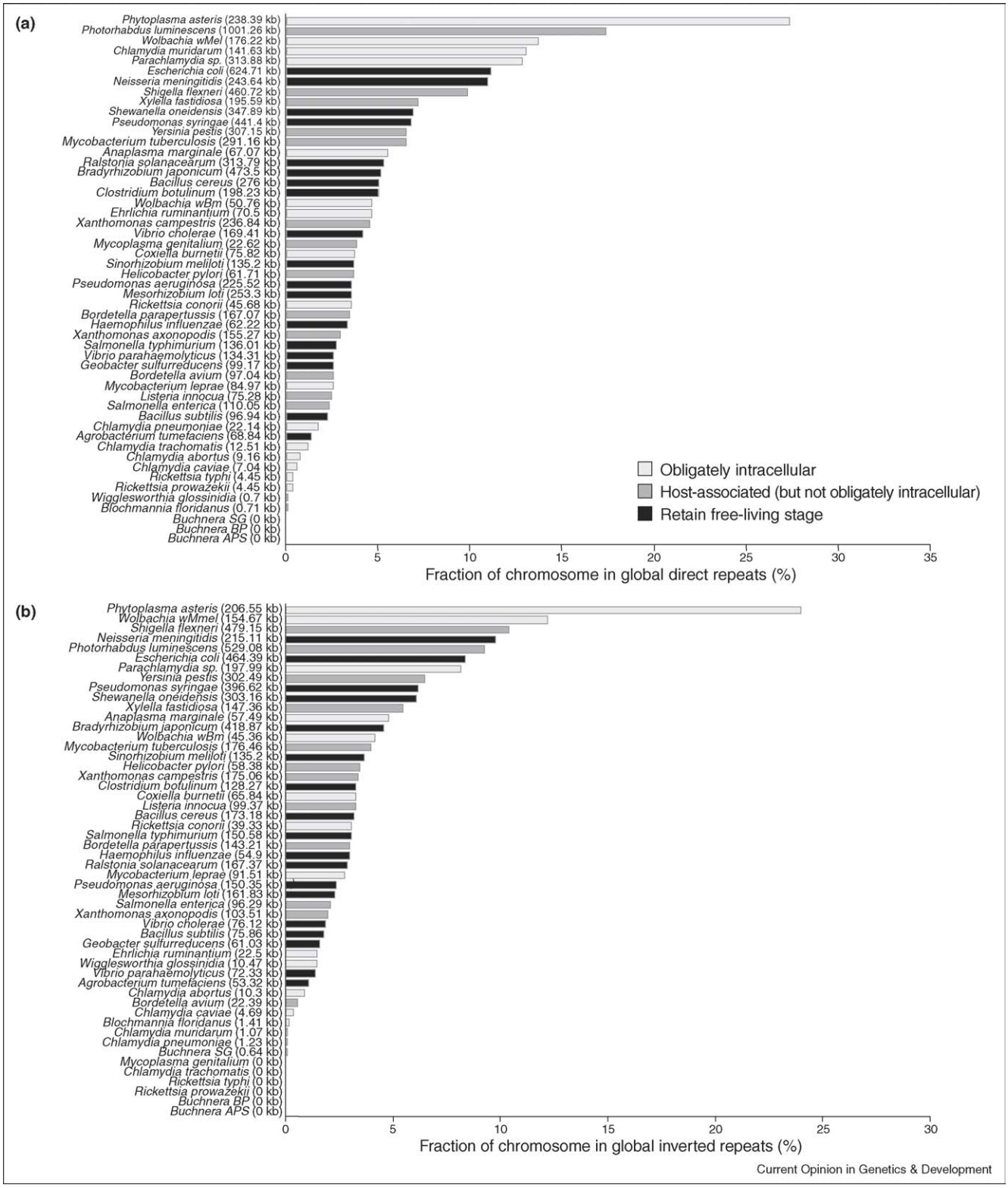
In contrast to the above examples of adaptation to an intracellular lifestyle, it is more difficult to infer cases of deleterious deletions that are fixed by genetic drift, another component of the reductive evolution model. Candidates for harmful deletions include the loss of several DNA repair functions, a convergent pattern across intracellular pathogens and mutualists. Notably, in *Buchnera* many DNA repair loci were lost in large, early deletions that included numerous genes of varied functions, a pattern that is difficult to reconcile with adaptive fine-tuning of gene content [17•]. The loss of repair functions might contribute to the drop in %GC content associated with genome reduction (Figure 1). Namely, AT bias might reflect greater exposure of an underlying GC→AT mutational pressure in small genomes that lack many DNA repair functions (but see the study by Rocha and Danchin [46] for a metabolic hypothesis for AT bias).

Genome dynamics within a host cell

As noted above, initial genome turbulence of intracellularity is thought to be followed by genetic stability associated with the consumption of recombination genes and repeated DNA in large deletions, reduced opportunities for gene exchange in a sequestered environment, and extinction of mobile DNA species that require horizontal transmission for their maintenance.

Is recent genome data consistent with genetic stability of long-term intracellular associates? In many cases, the answer is a resounding yes. Although horizontal gene transfer can be important in the evolution of new host associations [47], strictly intracellular associates often show little evidence of laterally acquired genes. In *Buchnera*, this stability extends to intragenomic dynamics, with no gene acquisition, inversions or translocations throughout 50–70 million years of evolution within aphids [48], and near-perfect conservation of gene order since the establishment of this association 150–200 mya [49] (biosynthetic plasmids have mediated the few exceptions to genome stability in this group [50,51•]). Within the ant mutualist *Blochmannia*, lineages that diverged ~20 mya exhibit a similar pattern of chromosome stasis [37]. Such stability also characterizes certain long-term intracellular pathogens, for which rare cases of horizontal transfer (e.g. a potentially acquired 12 kb sequence in *Rickettsia typhi* [52]) appear to be the exceptions, synteny between

Figure 3



Fraction of bacterial chromosomes devoted to **(a)** global direct repeats and **(b)** global inverted repeats. Although certain obligately intracellular bacteria have few if any repeats, others have repeat densities that match or exceed those of facultatively intracellular or free-living species. Values represent only long repeats ($\geq$100 nucleotides long, with at least an 80% match among copies), a category that mediates large-scale inversions, duplications and deletions. Values were obtained from the CBS (Centre for Biological Sequence Analysis) Genome Atlas Database

species implies few intragenomic rearrangements [48,53], and repeated DNA is often scarce (Figure 3) [5].

However, recent data indicate that DNA transfer might play a key role in shaping other intracellular associates. The *R. felis* genome revealed the first conjugative plasmid discovered in intracellular bacteria, and experimental analysis demonstrated conjugative pili and mating [54••]. *R. felis* also stands out among *Rickettsia* in having abundant transposases, a >six-fold higher density of repeats, paralogous gene families encoding surface proteins, and evidence for frequent inversions and translocations that disrupt synteny. Of the ORFs lacking in other *Rickettsia* spp., 91 have closest matches outside of the α-Proteobacteria, implying that a sizable fraction of this genome might be horizontally acquired. Notably, in the transmission of *R. felis* through flea vectors, co-infection with *Bartonella henselae*, *Bartonella quintana* or *Wolbachia* might enable opportunities for gene transfer [54••].

Genetic machinery for DNA transfer also occurs in *Parachlamydia* spp., each of which possesses a genomic island encoding all *tra* genes essential for F-like conjugative DNA transfer, the first evidence for a putative conjugative system in the Chlamydiales [28••,55•]. Conjugation might occur within the amoebae host containing numerous bacteria tightly packed in vacuoles [56]. Although base composition analysis revealed few signs of recent lateral gene acquisition, the apparent acquisitions of F-like conjugative DNA transfer and, as noted above, of Type IV secretion might be important in shaping host interactions [28••].

In addition to conjugation machinery, certain intracellular bacteria possess more mobile DNA, such as bacteriophage and transposable elements, than previously suspected [57] (see Update). Although missing from nutritional mutualists, such elements persist in long-term intracellular species such as *Parachlamydia* spp., *R. felis*, *Phytoplasma asteris*, *Wolbachia* wMel and, to a lesser extent, *Wolbachia* wBm [23••,54••,58••,59,60]. These and other intracellular bacteria also have surprisingly abundant repeated DNA sequences, devoting a relatively large fraction of their chromosomes to global direct and inverted repeats that can mediate large-scale intragenomic rearrangements (Figure 3). The growing list of examples of disrupted synteny implies frequent inversions and translocations [23••,28••,58••,61•].

Other examples of genome flux come from re-analysis of available genomes, often within a phylogenetic context. Detailed phylogenetic analysis showed fifteen cases of gene transfer into the Chlamydiaceae from plant, fungal, archaebacterial and bacterial donors [62]. Recombination among closer relatives was demonstrated by Gomes and colleagues [63•], who showed that transfer had occurred between the rodent-associated *Chlamydia muridarum* and *Chlamydia caviae*, and demonstrated frequent recombination among *Chlamydia trachomatis* strains at genes encoding polymorphic membrane proteins (pmps), a Chlamydiaceae-specific family of proteins, members of which are expressed on the cell surface. The same study discovered the first IS element in this group. Because subtle variations in gene content can lead to important differences in host ranges and phenotypic effects, just a handful of genes might influence pathogenic signatures [64]. Thus, even occasional gene transfer might be biologically significant.

At a more local genomic level, recombination among tandem repeats or among paralogous gene copies enables certain parasites to respond to the adaptive immune response of vertebrate hosts. Many intracellular parasites commit a high percentage of their tiny genomes to paralogous families of polymorphic surface molecules, suggesting that host immunity is among the 'highest priority' of the challenges they face [27] (see Update). By recombining various pseudogenes into a single expression site, *Anaplasma* spp. have generated sequential diversity of membrane proteins, thereby achieving a persistent infection [65•]. As crucial players in the generation of surface-coat antigenic variation, such pseudogenes are quite distinct from those reflecting genome erosion in Rickettsiales, *M. leprae*, *C. burnetii* and other parasites. In *Ehrlichia*, continual duplications among numerous tandemly repeated genes counter genome reduction by creating new loci that are important in immune evasion [66•]. In short, despite the exceptional stability of some intracellular associates, it is increasingly clear that intracellular lifestyle does not necessarily constrain genome flux.

Conclusions

The rapidly growing database of bacterial genomes has enhanced our understanding of processes that shape the outcomes of intimate bacterial–eukaryotic relationships. Models of reductive evolution offer a valuable framework to understand forces shaping the metabolic capabilities of obligately intracellular bacteria and their potential for genetic change. Recent genome data have upheld many expectations from these models, including severe genome-size reduction and metabolic specialization of intra-

(Figure 3 Legend Continued) [79,80] and were based on calculation methods described elsewhere [81,82]. Because searches were performed across entire chromosomes, values represent global, rather than just local, repeats. The location of the repeats along the chromosome can be visualized in the Genome Atlas or Repeat Atlas databases (http://www.cbs.dtu.dk/services/GenomeAtlas/). The Y-axis lists the bacterial species name and, in parentheses, the length of DNA involved in repeats. The *Rickettsia felis* genome, not yet released when this figure was developed, has an exceptionally high fraction of repeated DNA [54••].

cellular lineages. As predicted, many intracellular genomes are exceptionally stable, showing little evidence of gene acquisition by lateral transfer and few if any intragenomic changes that disrupt synteny among related strains.

Recent data have also revealed some exciting surprises that illustrate diverse modes of genome evolution within host cells. Discoveries of Type III and Type IV secretion in mutualists highlight parallels among infection strategies and add to the growing evidence that 'virulence' genes can play crucial roles in beneficial interactions. In addition, genomes are teaching us that intracellular associates can experience various forms of genome flux, ranging from gene acquisition from phylogenetically distant donors, to intragenomic lability with frequent inversions and rearrangements, to specific recombination-mechanisms that generate antigenic diversity. This rather surprising component of reductive evolution has prompted new research into the impact of gene transfer and recombination in these species, including the roles of mobile elements that manage to persist in certain anciently intracellular groups.

Completion of additional genomes will provide a richer context to assess mechanisms of evolution and to make predictions about functions that mediate host associations. Testing these predictions will depend on the development of new experimental approaches to clarify processes involved in various stages of bacterial infection, persistence, and transmission to new hosts. Promising experimental methods often build upon genome data, such as the use of microarrays to assess gene expression [67••] and applications of an ever-growing knowledge of natural mobile DNA sequences to develop tools for the genetic manipulation of uncultivable bacteria [68].

Update

Since the July submission of this review, several important papers in endosymbiont genomics have been published (owing to space limitations, only a few have been added below). These include an exploration by Nilsson *et al.* [87••] of deletion rates and patterns in experimental cultures of *Salmonella enterica*. The authors detected very large deletions, similar in magnitude to those considered important in the early evolution of endosymbiont genomes.

Full sequences of extrachromosomal DNA (ecDNA) of *Sodalis glossinidius*, including three plasmids and a bacteriophage of two *S. glossinidius* isolates, revealed transposases, conjugation functions and evidence for recent gene acquisition [88••]. These findings illustrate the importance of gene exchange in the evolution of some intracellular associates.

In addition, several recent studies have explored the evolution of α-proteobacterial endosymbionts [89••–91••]. These articles include an overview of genome plasticity in mutualistic and pathogenic α-Proteobacteria [89••], an investigation of the roles of ankyrin domain genes in shaping distinct reproductive alterations caused by *Wolbachia* [90••], and a population genetic study showing a recent global replacement of *Wolbachia* throughout *Drosophila melanogaster* lab stocks and field populations [91••].

Acknowledgements

I thank Richard R Lawler and Kostas Bourtzis for helpful comments on the manuscript, David W Ussery for sharing DNA repeat calculations and helpful advice, and many investigators for providing information about genome projects in progress. My apologies to colleagues whose work I could not highlight, owing to space constraints. I gratefully acknowledge support from the National Institutes of Health (R01 GM62626-01), National Science Foundation (DEB 0089455) and the NASA Astrobiology Institute (NNA04CC04A).

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. Moran NA: **The ubiquitous and varied role of infection in the lives of animals and plants**. *Am Nat* 2002, **60**:S1-S8.
2. Andersson SG, Kurland CG: **Reductive evolution of resident genomes**. *Trends Microbiol* 1998, **6**:263-268.
3. Moran NA: **Accelerated evolution and Muller's rachet in endosymbiotic bacteria**. *Proc Natl Acad Sci USA* 1996, **93**:2873-2878.
4. Mira A, Ochman H, Moran NA: **Deletional bias and the evolution of bacterial genomes**. *Trends Genet* 2001, **17**:589-596.
5. Frank AC, Amiri H, Andersson SG: **Genome deterioration: loss of repeated sequences and accumulation of junk DNA**. *Genetica* 2002, **115**:1-12.
6. Mira A, Moran NA: **Estimating population size and transmission bottlenecks in maternally transmitted endosymbiotic bacteria**. *Microb Ecol* 2002, **44**:137-143.
7. Ohta T: **Slightly deleterious mutant substitutions in evolution**. *Nature* 1973, **246**:96-98.
8. Muller J: **The relation of recombination to mutational advance**. *Mutat Res* 1964, **106**:2-9.
9. Moran NA, Plague GR: **Genomic changes following host restriction in bacteria**. *Curr Opin Genet Dev* 2004, **14**:627-633.
10. Subtil A, Dautry-Varsat A: ***Chlamydia:* five years A.G. (after genome)**. *Curr Opin Microbiol* 2004, **7**:85-92.
11. Gil R, Latorre A, Moya A: **Bacterial endosymbionts of insects: insights from comparative genomics**. *Environ Microbiol* 2004, **6**:1109-1122.
12. Klasson L, Andersson SG: **Evolution of minimal-gene-sets in host-dependent bacteria**. *Trends Microbiol* 2004, **12**:37-43.
13. Renesto P, Ogata H, Audic S, Claverie JM, Raoult D: **Some lessons from *Rickettsia* genomics**. *FEMS Microbiol Rev* 2005, **29**:99-117.
14. Batut J, Andersson SG, O'Callaghan D: **The evolution of chronic infection strategies in the alpha-proteobacteria**. *Nat Rev Microbiol* 2004, **2**:933-945.
15. Zientz E, Dandekar T, Gross R: **Metabolic interdependence of obligate intracellular bacteria and their insect hosts**. *Microbiol Mol Biol Rev* 2004, **68**:745-770.

16. Moran NA, Mira A: **The process of genome shrinkage in the obligate symbiont *Buchnera aphidicola***. *Genome Biol* 2001, **2**: RESEARCH0054.

17. • Belda E, Moya A, Silva FJ: **Genome rearrangement distances and gene order phylogeny in γ-Proteobacteria**. *Mol Biol Evol* 2005, **22**:1456-1467.
By comparing 30 γ-proteobacterial genomes, this study quantified the number of inversions necessary to explain current gene order. *Buchnera* and *Wigglesworthia* have experienced relatively high levels of gene rearrangements. Given the conservation of gene order within *Buchnera*, this implies a very high rearrangement rate early in the history of this mutualist.

18. Seshadri R, Paulsen IT, Eisen JA, Read TD, Nelson KE, Nelson WC, Ward NL, Tettelin H, Davidsen TM, Beanan MJ *et al.*: **Complete genome sequence of the Q-fever pathogen *Coxiella burnetii***. *Proc Natl Acad Sci USA* 2003, **100**:5455-5460.

19. Cole ST, Eiglmeier K, Parkhill J, James KD, Thomson NR, Wheeler PR, Honore N, Garnier T, Churcher C, Harris D *et al.*: **Massive gene decay in the leprosy bacillus**. *Nature* 2001, **409**:1007-1011.

20. •• Dale C, Jones T, Pontes M: **Degenerative evolution and functional diversification of type-III secretion systems in the insect endosymbiont *Sodalis glossinidius***. *Mol Biol Evol* 2005, **22**:758-766.
This study characterizes two Type III secretion systems in a maternally transmitted endosymbiont of tsetse flies. The authors found that both systems are closely related to extant pathogenicity islands but have undergone functional diversification in this mutualism. This study demonstrates the specific expression of these systems during cellular invasion and the subsequent proliferation of the endosymbiont.

21. • Gomez-Valero L, Latorre A, Silva FJ: **The evolutionary fate of nonfunctional DNA in the bacterial endosymbiont *Buchnera aphidicola***. *Mol Biol Evol* 2004, **21**:2172-2181.
This analysis traces the history of gene erosion during the divergence among *Buchnera* lineages. The finding that inactivated genes require 40–60 million years to disintegrate completely suggests that continued genome reduction within intracellular groups is a gradual process.

22. •• Perez-Brocal V, Latorre A, Gil R, Moya A: **Comparative analysis of two genomic regions among four strains of *Buchnera aphidicola*, primary endosymbiont of aphids**. *Gene* 2005, **345**:73-80.
This preliminary analysis of two sequence regions of *Buchnera* BCc, which has the smallest known bacterial genome, showed a significant reduction in the number of genes encoding metabolic functions. This full genome sequence for this strain is in progress and will shed light on functions retained in this tiny genome.

23. •• Foster J, Ganatra M, Kamal I, Ware J, Makarova K, Ivanova N, Bhattacharyya A, Kapatral V, Kumar S, Posfai J *et al.*: **The *Wolbachia* genome of *Brugia malayi*: endosymbiont evolution within a human pathogenic nematode**. *PLoS Biol* 2005, **3**:e121.
This genome sequence revealed that mutualistic *Wolbachia* retain numerous metabolic pathways that might function in its beneficial association with nematodes, such as synthesis of riboflavin, heme and nucleotides. Although this species shares many metabolic traits with *Wolbachia* wMel, the mutualist lacks phage DNA and has lower levels of repeated DNA.

24. Ghedin E, Wang S, Foster JM, Slatko BE: **First sequenced genome of a parasitic nematode**. *Trends Parasitol* 2004, **20**:151-153.

25. Hacker J, Hentschel U, Dobrindt U: **Prokaryotic chromosomes and disease**. *Science* 2003, **301**:790-793.

26. Goebel W, Gross R: **Intracellular survival strategies of mutualistic and parasitic prokaryotes**. *Trends Microbiol* 2001, **9**:267-273.

27. Palmer GH: **The highest priority: what microbial genomes are telling us about immunity**. *Vet Immunol Immunopathol* 2002, **85**:1-8.

28. •• Horn M, Collingro A, Schmitz-Esser S, Beier CL, Purkhold U, Fartmann B, Brandt P, Nyakatura GJ, Droege M, Frishman D *et al.*: **Illuminating the evolutionary history of chlamydiae**. *Science* 2004, **304**:728-730.
The genome sequence of *Parachlamydia sp*., a symbiont of free-living amoebae, has many virulence factors found in parasitic chlamydia, including a Type III secretion system. This finding suggests that, 700 mya, the last common ancestor of this group had already adopted an intracellular lifestyle. The authors propose that ancient chlamydia might have originated mechanisms to exploit eukaryotic cells.

29. Dale C, Plague GR, Wang B, Ochman H, Moran NA: **Type III secretion systems and the evolution of mutualistic endosymbiosis**. *Proc Natl Acad Sci U S A* 2002, **99**:12397-12402.

30. Corsaro D, Venditti D: **Emerging chlamydial infections**. *Crit Rev Microbiol* 2004, **30**:75-106.

31. • Everett K, Thao M, Horn M, Dyszynski G, Baumann P: **Novel chlamydiae in whiteflies and scale insects: endosymbionts '*Candidatus* Fritschea bemisiae' strain Falk and '*Candidatus* Fritschea eriococci' strain Elm**. *Int J Syst Evol Microbiol* 2005, **55**:1581-1587.
Combining genetic data and microscopy, this study characterizes chlamydial endosymbionts in whiteflies and scale insects. A detailed phylogenetic analysis provides an excellent overview of the genetic diversity and lifestyle variation in this bacterial group.

32. • Schmitz-Esser S, Linka N, Collingro A, Beier CL, Neuhaus HE, Wagner M, Horn M: **ATP/ADP translocases: a common feature of obligate intracellular amoebal symbionts related to Chlamydiae and Rickettsiae**. *J Bacteriol* 2004, **186**:683-691.
This study screened bacterial endosymbionts of free-living amoebae and paramecia for the presence of ATP/ADP translocase genes, which are necessary for the transport of ATP across a membrane in exchange for ADP. The discovery of these genes solely in the Rickettsiales and the Chlamydiales, including *Parachlamydia*, is consistent with current views about their distribution. Phylogenetic analysis suggests these translocases originated in the Chlamydiales and were horizontally transferred to Rickettsiales and plants.

33. McGraw EA, O'Neill SL: ***Wolbachia pipientis*: intracellular infection and pathogenesis in *Drosophila***. *Curr Opin Microbiol* 2004, **7**:67-70.

34. Brownlie JC, O'Neill SL: ***Wolbachia* genomes: insights into an intracellular lifestyle**. *Curr Biol* 2005, **15**:R507-R509.

35. Burne RA, Chen YY: **Bacterial ureases in infectious diseases**. *Microbes Infect* 2000, **2**:533-542.

36. Gil R, Silva FJ, Zientz E, Delmotte F, Gonzalez-Candelas F, Latorre A, Rausell C, Kamerbeek J, Gadau J, Holldobler B *et al.*: **The genome sequence of *Blochmannia floridanus*: comparative analysis of reduced genomes**. *Proc Natl Acad Sci USA* 2003, **100**:9388-9393.

37. Degnan PH, Lazarus AB, Wernegreen JJ: **Genome sequence of *Blochmannia pennsylvanicus* indicates parallel evolutionary trends among bacterial mutualists of insects**. *Genome Res* 2005, **15**:1023-1033.

38. Fares MA, Moya A, Barrio E: **GroEL and the maintenance of bacterial endosymbiosis**. *Trends Genet* 2004, **20**:413-416.

39. Bencina D, Slavec B, Narat M: **Antibody response to GroEL varies in patients with acute *Mycoplasma pneumoniae* infection**. *FEMS Immunol Med Microbiol* 2005, **43**:399-406.

40. Renesto P, Azza S, Dolla A, Fourquet P, Vestris G, Gorvel JP, Raoult D: **Proteome analysis of *Rickettsia conorii* by two-dimensional gel electrophoresis coupled with mass spectrometry**. *FEMS Microbiol Lett* 2005, **245**:231-238.

41. Hechard C, Grepinet O, Rodolakis A: **Molecular cloning of the *Chlamydophila abortus groEL* gene and evaluation of its protective efficacy in a murine model by genetic vaccination**. *J Med Microbiol* 2004, **53**:861-868.

42. Fares MA, Ruiz-Gonzalez MX, Moya A, Elena SF, Barrio E: **Endosymbiotic bacteria: GroEL buffers against deleterious mutations**. *Nature* 2002, **417**:398.

43. Morin S, Ghanim M, Zeidan M, Czosnek H, Verbeek M, van den Heuvel JF: **A GroEL homologue from endosymbiotic bacteria of the whitefly *Bemisia tabaci* is implicated in the circulative transmission of tomato yellow leaf curl virus**. *Virology* 1999, **256**:75-84.

44. Fredriksson A, Ballesteros M, Dukan S, Nystrom T: **Defense against protein carbonylation by DnaK/DnaJ and proteases of the heat shock regulon**. *J Bacteriol* 2005, **187**:4207-4213.

45. Akman L, Yamashita A, Watanabe H, Oshima K, Shiba T, Hattori M, Aksoy S: **Genome sequence of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia***. *Nat Genet* 2002, **32**:402-407.

46. Rocha EP, Danchin A: **Base composition bias might result from competition for metabolic resources**. *Trends Genet* 2002, **18**:291-294.

47. Ochman H, Moran NA: **Genes lost and genes found: evolution of bacterial pathogenesis and symbiosis**. *Science* 2001, **292**:1096-1099.

48. Tamas I, Klasson L, Canback B, Naslund AK, Eriksson AS, Wernegreen JJ, Sandstrom JP, Moran NA, Andersson SG: **50 million years of genomic stasis in endosymbiotic bacteria**. *Science* 2002, **296**:2376-2379.

49. Van Ham RC, Kamerbeek J, Palacios C, Rausell C, Abascal F, Bastolla U, Fernandez JM, Jimenez L, Postigo M, Silva FJ *et al.*: **Reductive genome evolution in *Buchnera aphidicola***. *Proc Natl Acad Sci USA* 2003, **100**:581-586.

50. Van Ham RC, Gonzalez-Candelas F, Silva FJ, Sabater B, Moya A, Latorre A: **Postsymbiotic plasmid acquisition and evolution of the repA1-replicon in *Buchnera aphidicola***. *Proc Natl Acad Sci USA* 2000, **97**:10855-10860.

51. • Sabater-Munoz B, van Ham RC, Moya A, Silva FJ, Latorre A: **Evolution of the leucine gene cluster in *Buchnera aphidicola:* insights from chromosomal versions of the cluster**. *J Bacteriol* 2004, **186**:2646-2654.

This study describes an exceptional case of gene rearrangements in *Buchnera*. The authors discovered that variable position of a leucine biosynthesis gene cluster (*leuABCD*) reflects independent chromosomal insertions from ancestral plasmid-encoded genes.

52. McLeod MP, Qin X, Karpathy SE, Gioia J, Highlander SK, Fox GE, McNeill TZ, Jiang H, Muzny D, Jacob LS *et al.*: **Complete genome sequence of *Rickettsia typhi* and comparison with sequences of other rickettsiae**. *J Bacteriol* 2004, **186**:5842-5855.

53. Mira A, Klasson L, Andersson SG: **Microbial genome evolution: sources of variability**. *Curr Opin Microbiol* 2002, **5**:506-512.

54. •• Ogata H, Renesto P, Audic S, Robert C, Blanc G, Fournier PE, Parinello H, Claverie JM, Raoult D: **The genome sequence of *Rickettsia felis* identifies the first putative conjugative plasmid in an obligate intracellular parasite**. *PLoS Biol* 2005, **3**:e248.

The genome of this flea-associated pathogen is substantially different from those of its *Rickettsia* relatives. In addition to the exciting discovery of a conjugative plasmid, this study revealed numerous transposases and repeated DNA in this genome. The authors also show evidence for two types of pili, hemolytic activity, β-lactamase activity and actin-polymerization-driven intracellular motility.

55. • Greub G, Collyn F, Guy L, Roten CA: **A genomic island present along the bacterial chromosome of the *Parachlamydiaceae* UWE25, an obligate amoebal endosymbiont, encodes a potentially functional F-like conjugative DNA transfer system**. *BMC Microbiol* 2004, **4**:48.

This re-analysis of the *Parachlamydia sp*. genome sequence identified a genomic island that has atypical GC-content and is flanked by inverted repeats. This island encodes an F-like conjugative DNA transfer mechanism involved in sex pilus retraction and mating pair stabilization, and might provide a genetic tool for chlamydia research.

56. Horn M, Wagner M: **Bacterial endosymbionts of free-living amoebae**. *J Eukaryot Microbiol* 2004, **51**:509-514.

57. Bordenstein SR, Reznikoff WS: **Mobile DNA in obligate intracellular bacteria**. *Nat Rev Microbiol* 2005, **3**:688-699.

58. •• Wu M, Sun LV, Vamathevan J, Riegler M, Deboy R, Brownlie JC, McGraw EA, Martin W, Esser C, Ahmadinejad N *et al.*: **Phylogenomics of the reproductive parasite *Wolbachia pipientis* wMel: A streamlined genome overrun by mobile genetic elements**. *PLoS Biol* 2004, **2**:E69.

This genome, the first published for *Wolbachia*, has exceptionally high levels of repetitive DNA and mobile elements that might mediate genome flux. Initially considered highly unusual for reduced genomes, similar patterns have since been discovered in other long-term intracellular α-Proteobacteria such as *Rickettsia felis*, *Ehrlichia ruminantium*, *Anaplasma marginale* and, to a lesser extent, the mutualistic *Wolbachia* wBm.

59. Oshima K, Kakizawa S, Nishigawa H, Jung HY, Wei W, Suzuki S, Arashida R, Nakata D, Miyata S, Ugaki M *et al.*: **Reductive evolution suggested from the complete genome sequence of a plant-pathogenic phytoplasma**. *Nat Genet* 2004, **36**:27-29.

60. Lee IM, Zhao Y, Bottner KD: **Novel insertion sequence-like elements in phytoplasma strains of the aster yellows group are putative new members of the IS3 family**. *FEMS Microbiol Lett* 2005, **242**:353-360.

61. • Salzberg SL, Hotopp JC, Delcher AL, Pop M, Smith DR, Eisen MB, Nelson WC: **Serendipitous discovery of *Wolbachia* genomes in multiple *Drosophila* species**. *Genome Biol* 2005, **6**:R23.

The authors discovered sequences for three new *Wolbachia* species in the Trace Archive (a publicly available repository for unanalyzed sequence data; http://www.ncbi.nlm.nih.gov/Traces). The bacteria are associated with *Drosophila ananassae*, *Drosophila simulans* and *Drosophila mojavensis*, and available sequences encompass 95%, 75–80% and 6–7% of the *Wolbachia* genomes, respectively. Comparisons with published *Wolbachia* genomes confirmed high levels of genome flux in this group, as numerous rearrangements, insertions and hundreds of novel genes were detected.

62. Ortutay C, Gaspari Z, Toth G, Jager E, Vida G, Orosz L, Vellai T: **Speciation in *Chlamydia:* genomewide phylogenetic analyses identified a reliable set of acquired genes**. *J Mol Evol* 2003, **57**:672-680.

63. • Gomes JP, Bruno WJ, Borrego MJ, Dean D: **Recombination in the genome of *Chlamydia trachomatis* involving the *polymorphic membrane protein C* gene relative to *ompA* and evidence for horizontal gene transfer**. *J Bacteriol* 2004, **186**:4295-4306.

This phylogenetic analysis of numerous *C. trachomatis* strains demonstrated clear evidence for frequent, whole-gene recombination among polymorphic membrane proteins that are expressed on the cell surface. The authors suggest that genome plasticity might produce adaptive changes in tissue tropism and pathogenesis.

64. Read TD, Myers GS, Brunham RC, Nelson WC, Paulsen IT, Heidelberg J, Holtzapple E, Khouri H, Federova NB, Carty HA *et al.*: **Genome sequence of *Chlamydophila caviae* (*Chlamydia psittaci* GPIC): examining the role of niche-specific genes in the evolution of the Chlamydiaceae**. *Nucleic Acids Res* 2003, **31**:2134-2147.

65. • Brayton KA, Kappmeyer LS, Herndon DR, Dark MJ, Tibbals DL, Palmer GH, McGuire TC, Knowles DP. Jr: **Complete genome sequencing of *Anaplasma marginale* reveals that the surface is skewed to two superfamilies of outer membrane proteins**. *Proc Natl Acad Sci USA* 2005, **102**:844-849.

The genome of this tick-borne mammalian pathogen revealed two gene families that underlie its rapid generation of antigenic variation. Pseudogenes in this species do not reflect genome erosion; rather, they play important roles in generating variation in surface coat proteins.

66. • Collins NE, Liebenberg J, de Villiers EP, Brayton KA, Louw E, Pretorius A, Faber FE, van Heerden H, Josemans A, van Kleef M *et al.*: **The genome of the heartwater agent *Ehrlichia ruminantium* contains multiple tandem repeats of actively variable copy number**. *Proc Natl Acad Sci USA* 2005, **102**:838-843.

In contrast to the stable genomes of some intracellular bacteria, this pathogen has numerous tandemly repeated and duplicated sequences that mediate frequent translocations, inversions and duplications that have given rise to new genes.

67. •• Moran NA, Dunbar HE, Wilcox JL: **Regulation of transcription in a reduced bacterial genome: nutrient-provisioning genes of the obligate symbiont *Buchnera aphidicola***. *J Bacteriol* 2005, **187**:4229-4237.

Using full-genome microarrays, this study showed that transcription in *Buchnera* changes little in response to host dietary changes, suggesting that loss of regulation-genes might constrain the ability to respond to environmental changes.

68. Qin A, Tucker AM, Hines A, Wood DO: **Transposon mutagenesis of the obligate intracellular pathogen *Rickettsia prowazekii***. *Appl Environ Microbiol* 2004, **70**:2816-2822.

69. Buchner P: *Endosymbiosis of Animals with Plant Microorganisms*. New York: Interscience Publishers, Inc; 1965.

70. Baumann P, Moran N, Baumann L: **Bacteriocyte-associated endosymbionts of insects**. In *The Prokaryotes, A Handbook on the Biology of Bacteria; Ecophysiology, Isolation, Identification, Applications.* Edited by Dworkin M. Springer-Verlag; Release 3.1, 2000.

71. Moran N, Telang A: **Bacteriocyte-associated symbionts of insects**. *Bioscience* 1998, **48**:295-304.

72. Aksoy S, Rio RV: **Interactions among multiple genomes: tsetse, its symbionts and trypanosomes**. *Insect Biochem Mol Biol* 2005, **35**:691-698.

73. Moran NA, Russell JA, Koga R, Fukatsu T: **Evolutionary**
•• **relationships of three new species of enterobacteriaceae living as symbionts of aphids and other insects**. *Appl Environ Microbiol* 2005, **71**:3302-3310.
This combination of phylogenetic analysis and electron microscopy describes three new species of secondary endosymbionts that coexist with *Buchnera* within aphid hosts.

74. Brownstein JS, Hett E, O'Neill SL: **The potential of virulent *Wolbachia* to modulate disease transmission by insects**. *J Invertebr Pathol* 2003, **84**:24-29.

75. Rio RV, Hu Y, Aksoy S: **Strategies of the home-team: symbioses exploited for vector-borne disease control**. *Trends Microbiol* 2004, **12**:325-336.

76. Sinkins SP, Godfray HC: **Use of *Wolbachia* to drive nuclear transgenes through insect populations**. *Proc Biol Sci* 2004, **271**:1421-1426.

77. Moran NA: **Microbial minimalism: genome reduction in bacterial pathogens**. *Cell* 2002, **108**:583-586.

78. Peterson JD, Umayam LA, Dickinson T, Hickey EK, White O: **The Comprehensive Microbial Resource**. *Nucleic Acids Res* 2001, **29**:123-125.

79. Hallin PF, Ussery DW: **CBS Genome Atlas Database: a dynamic storage for bioinformatic results and sequence data**. *Bioinformatics* 2004, **20**:3682-3686.

80. Ussery DW, Binnewies TT, Gouveia-Oliveira R, Jarmer H, Hallin PF: **Genome update: DNA repeats in bacterial genomes**. *Microbiology* 2004, **150**:3519-3521.

81. Skovgaard M, Jensen LJ, Friis C, Stærfeldt H-H, Worning P, Brunak S, Ussery DW: **The atlas visualisation of genome-wide information**. *Methods Microbiol* 2002, **33**:49-63.

82. Jensen LJ, Friis C, Ussery DW: **Three views of microbial genomes**. *Res Microbiol* 1999, **150**:773-777.

83. Frank AC, Alsmark CM, Thollesson M, Andersson SG: **Functional**
•• **divergence and horizontal transfer of type IV secretion systems**. *Mol Biol Evol* 2005, **22**:1325-1336.
Using a phylogenetic approach, the authors illustrate the importance of horizontal transfer in the evolution and functional divergence of type IV secretion pathways. Repeatedly, transfer events have corresponded to shifts from the ancestral state of conjugation to secretion of effector molecules.

84. Gil R, Sabater-Munoz B, Latorre A, Silva FJ, Moya A: **Extreme genome reduction in *Buchnera* spp.: toward the minimal genome needed for symbiotic life**. *Proc Natl Acad Sci USA* 2002, **99**:4454-4458.

85. Moran NA, Dale C, Dunbar H, Smith WA, Ochman H: **Intracellular symbionts of sharpshooters (Insecta: Hemiptera: Cicadellinae) form a distinct clade with a small genome**. *Environ Microbiol* 2003, **5**:116-126.

86. Zabalou S, Riegler M, Theodorakopoulou M, Stauffer C, Savakis C,
•• Bourtzis K: **Wolbachia-induced cytoplasmic incompatibility as a means for insect pest population control**. *Proc Nat Acad Sci USA* 2004, **101**:15042-15045.
This study illustrates the potential use of *Wolbachia*-induced reproductive incompatibilities to suppress insect host populations. Using embryonic cytoplasm transfer, the authors infected Mediterranean fruit fly (medfly) hosts with *Wolbachia* from a related fruit fly species. This new infection was successfully established and shown to cause complete cytoplasmic incompatibility in the medfly host. Moreover, the release of infected males in cage experiments caused the suppression of lab populations.

87. Nilsson AI, Koskiniemi S, Eriksson S, Kugelberg E, Hinton JC,
•• Andersson DI: **Bacterial genome size reduction by experimental evolution**. *Proc Natl Acad Sci USA* 2005, **102**:12112-12116.
Taking an experimental approach, the authors examined rates, sizes and chromosomal locations of gene deletions during serial passage of *Salmonella enterica* cultures. Deletion rates were particularly high ($\sim$2.5 bp/chromosome/generation) in a strain with disrupted mismatch repair functions (*mutS*$^{-}$). The large sizes (up to 202 kb) of many deletions corroborate the view that massive deletion events impacted the early evolution of certain intracellular bacteria.

88. Darby AC, Lagnel J, Matthew CZ, Bourtzis K, Maudlin I,
•• Welburn SC: **Extrachromosomal DNA of the symbiont *Sodalis glossinidius***. *J Bacteriol* 2005, **187**:5003-5007.
Three plasmids and a bacteriophage were sequenced from two strains of *Sodalis glossinidius*, a secondary endosymbiont of tsetse flies. Annotation of plasmid genes revealed two putative symbiotic islands showing signatures of recent horizontal acquisition. In addition, the presence of pilus genes suggests that conjugation is important in the evolution of this symbiont. These sequence data illustrate the important role of gene transfer in some intracellular bacteria, and further support the idea that parasites and mutualists, such as *Sodalis*, often use similar genes to mediate host interactions.

89. Sallstrom B, Andersson SG: **Genome reduction in the**
•• **α-Proteobacteria**. *Curr Opin Microbiol* 2005, **8**:579-585.
This review highlights evidence for genome plasticity, including computational estimates of gene losses, acquisitions and duplications, among medically, agriculturally and environmentally significant α-Proteobacteria. The authors emphasize the importance of recombination in generating antigenic variability within pathogen populations, and note common patterns of genome reduction in intracellular species. This review also notes examples of genome reduction in 'real-time' during the course of a pathogen's infection of a single host individual and during lab cultivation of a plant symbiont.

90. Iturbe-Ormaetxe I, Burke GR, Riegler M, O'Neill SL:
•• **Distribution, expression, and motif variability of ankyrin domain genes in *Wolbachia pipientis***. *J Bacteriol* 2005, **187**:5136-5145.
The authors address the molecular mechanisms shaping the diversity of reproductive alterations that *Wolbachia* causes in hosts, with a focus on the potential roles of ankyrin (ANK) domain genes. These domains, known to mediate protein–protein interactions, are abundant in the *Wolbachia* genome. Comparisons of ANK domain genes with different effects across *Wolbachia* species revealed significant variation that might affect the functions of encoded proteins.

91. Riegler M, Sidhu M, Miller WJ, O'Neill SL: **Evidence for a global**
•• ***Wolbachia* replacement in *Drosophila melanogaster***. *Curr Biol* 2005, **15**:1428-1433.
Using polymorphic molecular markers to distinguish closely related *Wolbachia* strains, the authors sampled *Drosophila melanogaster* hosts from stock collections and field populations. They discovered that one *Wolbachia* strain has replaced all others in this fruit fly species during the past century. It is difficult to explain this global replacement by cytoplasmic incompatibility, but it might be linked to the recent invasion by P elements in this host and an establishment of a maternally transmitted repressive P cytotype.