Lifestyle evolution in symbiotic bacteria: insights from genomics

Nancy A. Moran and Jennifer J. Wernegreen

Bacteria that live only in eukaryotic cells and tissues, including chronic pathogens and mutualistic bacteriocyte associates, often possess a distinctive set of genomic traits, including reduced genome size, biased nucleotide base composition and fast polypeptide evolution. These phylogenetically diverse bacteria have lost certain functional categories of genes, including DNA repair genes, which affect mutational patterns. However, pathogens and mutualistic symbionts retain loci that underlie their unique interaction types, such as genes enabling nutrient provisioning by mutualistic bacteria-inhabiting animals. Recent genomic studies suggest that many of these bacteria are irreversibly specialized, precluding shifts between pathogenesis and mutualism.

Nancy Moran and Jennifer Wernegreen are at the Dept of Ecology and Evolutionary Biology, and Center for Insect Science, University of Arizona, Tucson, AZ 85721, USA. Jennifer Wernegreen is currently at the JBP Center for Comparative Molecular Biology and Evolution, The Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543, USA (nmoran@u.arizona.edu; jwernegreen@mbl.edu).

Increasingly, ecologists and evolutionary biologists are appreciating the huge diversity of bacteria that complete their life cycles within, or closely associated with, eukaryotic cells. These interactions encompass a wide spectrum of effects on hosts, from invasive pathogenesis to obligate associations in which hosts depend on infection for their own survival or reproduction. Although some bacterial associates can be difficult to categorize, many can be unambiguously labeled as mutualists (defined as associates that increase the fitness of the individual host) or as parasites (associates that decrease the fitness of the infected host) (Box 1).

Does this diversity of effects on hosts result from individual microbial lineages shifting frequently among different lifestyles or does it reflect independent origins from free-living bacteria of many eukaryote-dependent lineages, each fixed in a particular mode of interaction? More specifically, do mutualistic endosymbionts arise from parasitic bacteria or vice versa and, if such shifts occur, how frequent are they? Several recent studies appear relevant to these questions.

Box 1. Definitions of terms relating to bacteria associated with eukaryotes

Parasitism: an association that imposes a net negative effect on the fitness of the individual host.

Mutualism: an association that imposes a net positive effect on the fitness of the individual host.

Symbionts: bacteria that live within a host's body and especially within host cells. (Here, we use the term to refer generally to such bacteria, regardless of the effects on hosts; some authors use the term specifically for mutualistic bacteria.)

Bacteriocyte association: bacterial symbionts are confined to host cells that appear specialized for this function, presumably because the infection is mutualistic and hosts have evolved to promote it.

Vertical or maternal transmission: infection of hosts that occurs through transfer from mother to progeny, often through infection of eggs or embryos within the mother's body.

Resident genome: genome of a bacterium that lives in close, often intracellular, association with a eukaryotic host. After Ref. 23.

Pathogenicity island: a cluster of genes that confers pathogenic potential and that has been acquired through horizontal transfer from another bacterium.

First, evolutionary ecologists have developed theoretical expectations regarding the evolutionary transitions among different interaction types. Second, by using DNA sequences to place mutualists and parasites on the bacterial phylogeny, we can identify transitions between the two. Third, recent developments in microbial genomics have created the exciting possibility of fully cataloging the functional capabilities of symbionts (Box 1) and parasites – organisms that could not even be differentiated a few years ago. Indeed, because their genomes are small and thus amenable to full sequencing, we can expect to know much more about the ecological and functional capacities of bacteria living in animal hosts than we know about the hosts themselves. This genomic information will allow us to determine not only whether an interaction type is labile within lineages, but also when and why.

Here, we point out that although evolutionary ecologists have postulated frequent transitions between mutualism and parasitism, phylogenetic studies suggest conservatism of interaction types for many bacterial associates (Box 1) of eukaryotes. Furthermore, complete gene inventories from bacterial genome studies might reveal a basis for these constraints: irreversible genome shrinkage, with differential gene loss in mutualistic and parasitic bacterial groups. Finally, we suggest that the field of molecular population genetics can provide explanations for why symbionts undergo genome reduction, as well as predictions regarding its distribution. Our focus is on animal-associated bacteria; currently, most full genome sequences are from these organisms.

Should we expect labile interactions?

Numerous investigators have proposed that mutualism and parasitism (defined here in terms of effects on host fitness) are dynamic alternatives to a partner in a biological interaction[1–7]. Conditions favoring overlap in host and symbiont reproductive interests, such as vertical transmission (Box 1) and long-term persistence in an individual host, push towards mutualism. Conditions favoring selfishness, such as a high rate of horizontal infection and competition within hosts, push towards parasitism and exploitation. Because these conditions will depend on environmental factors, such as host abundance and host behaviors, we might expect that closely related bacterial

 0169-5347/00/$ – see front matter PII: S0169-5347(00)01902-9

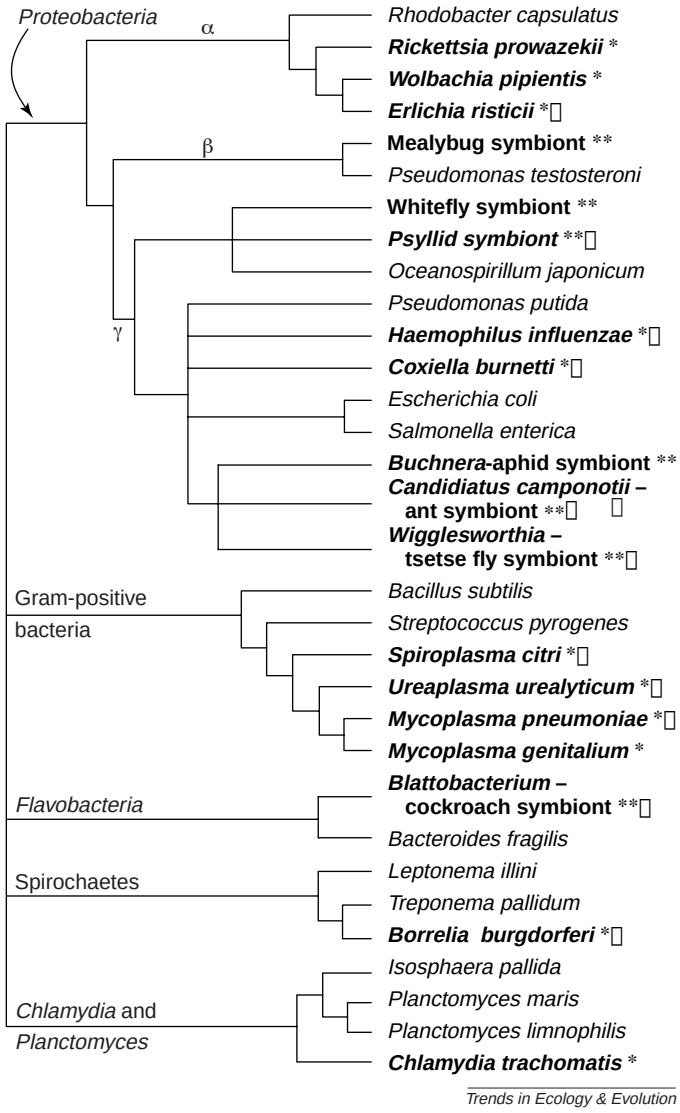

Fig. 1. Phylogenetic placement of pathogenic and symbiotic bacterial clades within the Eubacteria[12,14,15,17]. Key: *, chronic pathogen; **, bacteriocyte-associated mutualist; symbiotic bacteria are indicated by bold typeface; α, β and γ refer to major subdivisions of the Proteobacteria.

Table 1. Estimated ages of bacterial symbionts of animals

Bacterial symbionts	Estimated age of clade (My)
Pathogens[a]	
Mycoplasmas	276–553
Mycoplasma-like organisms (MLOs)	99–198
Mycoplasma hominus group	133–265
Mycoplasma pneumoniae group	141–283
Spiroplasma	133–265
Rickettsia (including *Orentia*)	115–230
Wolbachia	25–50
Rickettsia and *Wolbachia*	188–375
Borrelia	29–58
Chlamydia	52–105
Bacillus subtilis group (includes related spp.)	107–215
Mutualistic symbionts[b]	
Buchnera (aphids)	150–250
Candidiatus camponotii (ants)	>50
Wigglesworthia (tsetse fly)	40
Blattobacterium (cockroaches and termites)	135–300
Psyllid symbionts	100–200

[a]Estimates for pathogens based on molecular clock calculations using mean pairwise divergence of 16S rRNA through the basal node of the clade, with calibration of 0.02–0.04 substitutions/site/50 My (million years. This range corresponds to rates estimated for free-living bacteria and for bacteriocyte associates (Box 1) that cospeciate with hosts[15].
[b]Estimates for bacteriocyte associates are based on host fossils and evidence for synchronous symbiont–host cospeciation[15,17].

species or geographically separated populations would occupy different positions on a continuum between mutualism and parasitism[5]. Furthermore, we expect that the same organism would sometimes both benefit and manipulate its partner, if both effects increase its own spread. Lability in the degree of mutualism and/or antagonism has been demonstrated for several systems involving plant pollinators[6] and fungal endophytes[8]. In particular among intracellular bacteria, mutualistic symbionts are sometimes hypothesized to have arisen as attenuated pathogens[9]. One of our chief concerns is whether these models, which lack details of functional capacities and constraints of symbionts, are adequate for understanding how interaction type evolves within a symbiont lineage.

Have bacterial symbionts switched between mutualism and parasitism?

Frequent transitions between parasitism and mutualism would produce a mosaic of interaction types within clades of animal-associated bacteria, resulting in closely related parasitic and mutualistic species sometimes inhabiting the same or related hosts. Molecular phylogenetic studies allow us to test this prediction by placing noncultivable symbiotic bacteria in a phylogenetic context; this development began with the work of Woese[10] and continues with the exploration of prokaryotic diversity in specialized habitats, including eukaryotic hosts[11,12]. Contrary to predictions from theory, these phylogenies suggest that most bacterial symbionts that form chronic infections in animals belong to deeply branching clades that are strictly parasitic or strictly mutualistic (Fig. 1). Some parasitic groups, of formerly ambiguous phylogenetic status, have been found to form well defined clades of strict parasites; examples include the Mollicutes (mycoplasmas and spiroplasmas), and the rickettsiae and relatives. Similarly, phylogenetic studies have revealed several clades consisting solely of mutualists inhabiting invertebrate hosts[13–17]. Among bacteria that are intimately associated with animal cells and tissues, mutualistic and parasitic lineages are rarely found to be close relatives based on current phylogenies.

Furthermore, both mutualistic and intimate parasitic clades are often ancient. For numerous invertebrate groups with mutualistic bacteria, phylogenetic matching of hosts and symbionts indicates that the infection occurred in a common ancestor of modern host species. The minimum ages inferred from host fossils imply that these associations are consistently ancient, ranging up to 300 million years old (My) (Ref. 15; Table 1). Ages for parasite clades can be estimated using molecular clocks that are calibrated based on other groups and even the most conservative calculations give ages ranging into the hundreds of millions of years[18] (Table 1). Because the interaction type might have originated before the common ancestor for each of these clades, these dates might be underestimates of the age of the interaction.

Table 2. Genome sizes of bacteria associated with animals[a]

Species	Genome size (Mb)[c]
Bacillus subtilis[b]	4.22
Ureaplasma urealyticum	0.75
Mycoplasma genitalium	0.58
Mycoplasma pneumoniae	0.81
Rhodobacter capsulatus	3.70
Rickettsia prowazekii	1.10
Erlichia risticii	1.40
Escherichia coli[b]	4.64
Haemophilus influenzae	1.83
Buchnera aphidicola	0.630–0.645
Coxiella burnetti	1.60
Treponema pallidum	1.14
Borrelia burgdorferi	1.45
Chlamydia trachomatis	1.04

[a]Data taken from Refs 24, 30 and from http://wit.integratedgenomics.com/GOLD/prokaryagenomes.html
[b]*Bacillus subtilis* and *Escherichia coli* are free-living bacteria included for comparison.
[c]Abbreviation: Mb, Megabase.

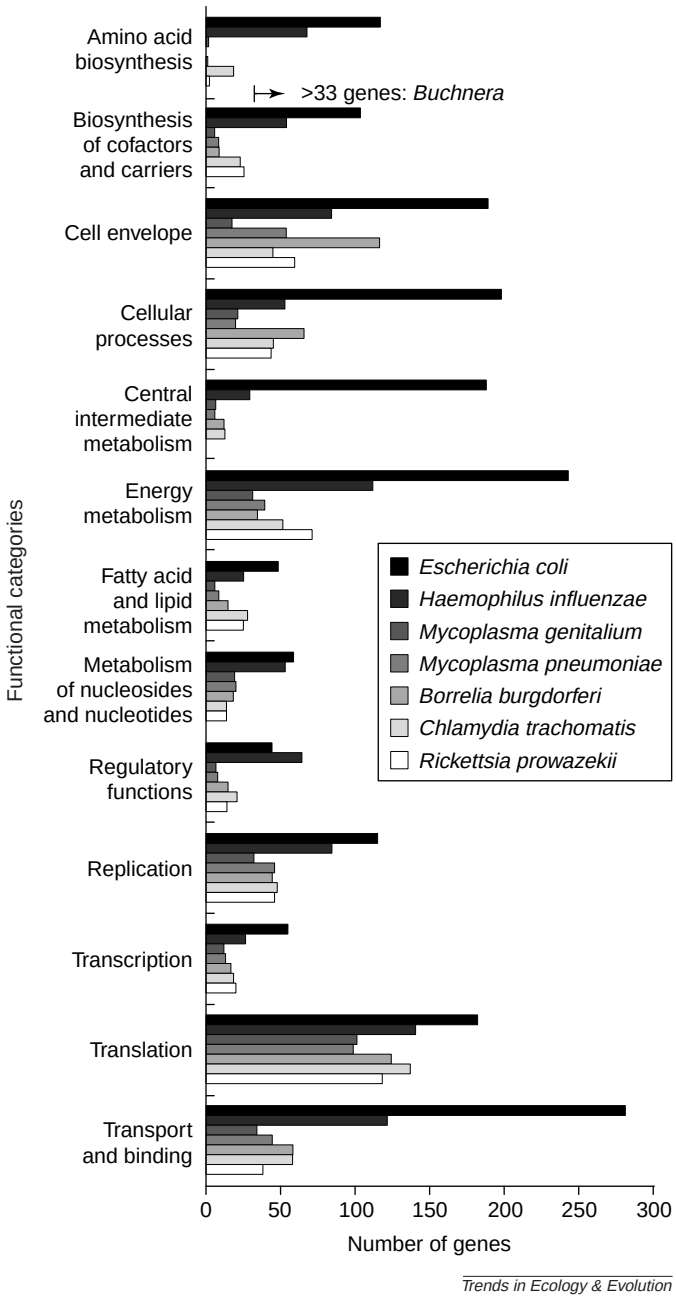

Fig. 2. The numbers of loci in different functional categories, such as energy metabolism and regulatory functions, in selected free-living and pathogenic bacteria for which full genomes are available[31,43–49].

Exceptions: labile interactions

Only rarely does current evidence suggest that both mutualistic and parasitic lineages arose from an ancestor already intimately associated with eukaryotes. The most prominent case is that of the mitochondria, which are specialized mutualists related to *Rickettsia* and other parasitic bacteria[19]. Other instances of close relationships between parasitic and mutualistic symbionts, consistent with a shift in interaction type, occur in the Flavobacteria (which contains an obligate mutualist of cockroaches[13], as well as related male-killing parasites in ladybird beetles[20]) and in *Wolbachia* (which includes reproductive parasites of arthropods[21] and apparent mutualists of nematodes[22]). Nonetheless, constraints on functional capabilities are implied by the fact that benefits to the fecundity or survivorship of female hosts are not ubiquitous in these, and other, groups of maternally transmitted bacteria. Under almost any set of realistic conditions, selection favors such benefits; however, studies of reproductive parasites in insects have usually failed to detect them[20,21].

Does genome content prevent switches between mutualism and parasitism?

The monophyly and antiquity of mutualistic and parasitic bacterial clades indicate that symbiotic interactions between bacteria and animals might be more constrained than evolutionary models predict. Such models are based on the implicit presumption that partners retain the genetic potential for a full spectrum of interaction types. Is this assumption true for intimate bacterial associates of animals? Evidence from full genome sequences, summarized in the next section, suggests a basis for constraints on shifts in symbiotic interactions: the irreversible nature of certain kinds of genome evolution. Recent findings indicate that this lifestyle results in major genomic changes, including massive loss of genes underlying a variety of functional capabilities. These losses are expected to limit the evolutionary options for particular bacterial lineages.

The syndrome of 'resident' genomes

Andersson and Kurland[23] recognized a suite of characteristic features typical of 'resident' genomes (Box 1) – their term for bacterial genomes that function under the domain of a host cell. These features include reduced size, adenine–thymine (AT) bias, fast sequence evolution and frequent gene rearrangements. Whereas initially the syndrome was remarked for a phylogenetically diverse set of pathogenic bacteria[23,24], recent findings indicate that it also applies to maternally transmitted mutualistic bacteria[14,24–28]. For example, *Buchnera aphidicola*, an obligate associate of aphids and the best studied mutualistic symbiont, has extreme AT bias[14,27] and fast sequence evolution at all loci[26–28]. (See http://link.springer.de/link/service/books/10125 for a complete summary of molecular evolution in *Buchnera* and other insect symbionts.) Similar patterns of AT bias and fast evolution are seen in maternally transmitted symbionts from various other hosts[15–17,26,29]. As in pathogenic bacteria, *Buchnera* shows

Table 3. Presence and absence of DNA repair and recombinase loci in pathogenic, mutualistic and free-living bacteria based on full genome sequences[a,b]

		Proteobacteria				Gram-positive bacteria			Spirochaete
Repair genes grouped by type		*Escherichia coli*[c]	*Buchnera aphidicola*[d]	*Haemophilus influenzae*[e]	*Rickettsia prowazekii*[e]	*Bacillus subtilis*[c]	*Mycoplasma pneumoniae*[e]	*Mycoplasma genitalium*[e]	*Borrelia burgdorferi*[e]
Direct damage reversal									
	phrB	+	+	–	–	–	–	–	–
	ada	+	–	–	–	+	–	–	–
	ogt	+	–	–	–	–	–	–	–
Base excision repair									
	ung	+	+	+	–	+	+	+	+
	tag	+	–	+	–	–	–	–	–
	alkA	+	–	–	–	+	–	–	–
	mutM	+	–	+	–	+	–	–	–
	mutY	+	+	+	–	–	–	–	–
	nth	+	+	+	+	+	–	–	+
	nfo	+	+	–	–	–	+	+	–
Mismatch repair									
	mutS	+	+	+	+	+	–	–	+
	mutL	+	+	+	+	–	–	–	+
	mutH	+	–	+	–	–	–	–	–
	recJ	+	–	+	+	–	–	–	+
	uvrD	+	–	+	+	–	–	–	+
Oxidative damage repair									
	mutT	+	+	+	–	+	–	–	–
Recombinase pathways									
	recA	+	–	+	+	+	+	+	+
	recB	+	+	+	+	–	–	–	+
	recC	+	+	+	+	–	–	–	+
	recD	+	+	+	+	–	–	–	+
	recF	+	–	+	+	+	–	–	–
	recN	+	–	+	+	+	–	–	–
Nucleotide excision repair									
	uvrA	+	–	+	+	+	+	+	+
	uvrB	+	–	+	+	+	+	+	+
	uvrC	+	–	+	+	+	+	+	+
	mfd	+	+	+	+	–	–	–	+

[a]Data taken from Refs 31, 43–48, 50.
[b]Key: +, present; –, absent.
[c]Free-living bacteria.
[d]Mutualistic symbiont of aphid (*Acyrthosiphon pisum*).
[e]Pathogen.

extreme genome reduction, with estimates of about 630–650 kb (Refs 24,30), which approaches the known lower limit of bacterial genome size (580 kb for the pathogen *Mycoplasma genitalium*[31]) (Table 2).

Lost genes: convergence between pathogens and mutualists

Because bacterial genomes consist mostly of functional genes, genome reduction implies gene loss. Intimate pathogens converge not only in genome size but also in the functional roles of genes lost (Fig. 2; Table 3); their tiny genomes consist mainly of genes underlying the basic functions of cell growth and division, such as replication, transcription, translation and energy metabolism. These organisms might possess 'minimal' cellular genomes[25], a possibility supported by the rather consistent lower limit of bacterial genome size – around 600 kb.

For diverse groups of pathogenic bacteria, as well as *Buchnera*, lost loci include a large proportion of those encoding DNA repair and recombinase functions (Fig. 2; Table 3)[23]. An implication is that mutation rates and patterns will differ from those of large genome bacteria. In particular, repair gene loss might underlie the AT bias of small genomes[26]. Another implication of the parallel loss of genes underlying recombination pathways is that small genome bacteria show little or no recombination within or between species, a condition expected to affect the evolutionary lability of these lineages.

Specificity to sequestered habitats and loss of recombinase pathways are both likely to limit the extent to which intimate pathogens and mutualists undergo recombination with unrelated bacteria. This limitation could accelerate gene loss by rendering silencing mutations irreversible.

Biosynthetic capabilities: differences between pathogens and mutualists

Small genome pathogenic bacteria have also lost genes for intermediate metabolism and biosynthetic pathways[23,25,31] (Fig. 2), an observation explainable by the fact that many metabolites are available within the host-cell environment[25].

In particular, amino acid biosynthetic genes are almost entirely eliminated in pathogenic bacteria, which obtain them from hosts (Fig. 2). Here, we find a striking contrast with *Buchnera*, the only mutualist for which information is available: in spite of massive genome reduction, genes for amino acid biosynthesis are retained[14]. Of 101 open-reading frames (ORFs) characterized for *Buchnera*, 33 show clear homology to biosynthetic genes for essential amino acids (the set of amino acids that animals cannot produce themselves)[14]. Retention of these genes is linked to the fact that a primary benefit of *Buchnera* for aphid hosts is the provision of essential amino acids[1,14]. Selection at the host level must have favored retention of these and, almost certainly, other biosynthetic genes, while *Buchnera* adopted its mutualistic lifestyle. The fact that *Buchnera* retains an arsenal of host-beneficial biosynthetic pathways is strong evidence that it did not evolve from a small genome pathogen of aphids; such an ancestor would not have retained the biosynthetic capacities that underlie the mutualism.

Could *Buchnera* have evolved from a parasitic ancestor by reacquiring genes for amino acid biosynthesis, thus enabling a transition to mutualism? Gene transfer among unrelated bacteria is increasingly recognized as an important process in bacterial evolution[32,33]. In the case of *Buchnera* or other intracellular mutualists, reacquisition is improbable, both because symbionts are physically segregated from other organisms and because effective reacquisition requires simultaneous uptake of multiple genes, as required for pathway functionality. Acquisition of a single pathway might occur because genes for particular pathways are sometimes grouped into an operon in the donor organism[34]. However, mutualistic functionality would often require the unlikely event of simultaneous uptake of multiple pathways; for example, animals feeding on phloem sap or vertebrate blood depend on symbiont provisioning for several nutrients[35–37]. Reacquisition can definitely be ruled out for *Buchnera*, for which phylogenetic evidence indicates a common history of amino acid biosynthetic genes and other loci[14]. Genome size is remarkably constant among *Buchnera* lineages that diverged up to 100 million years ago (Mya) (Ref. 30); this stability of genome size suggests static gene inventories in these symbionts, in contrast with free-living bacteria.

How general is the inference that mutualistic bacteria cannot be derived from intimate pathogens? The answer will depend, in part, on whether mutualism of other bacteria is based on functional contributions that involve multiple genes and biosynthetic pathways. This is probably generally true when nutritional contributions are the basis of a mutualism, as appears to be the case for most mutualisms between bacteria and animals[35–37]. Animals lack the biosynthetic pathways that are retained in most other organisms and symbiosis can enable animal hosts to use specialized niches lacking required nutrients. Examples include insects feeding on phloem sap, which is low in essential amino acids[35]; various arthropods feeding on vertebrate blood, which lacks B vitamins[38]; and chemoautotrophic marine invertebrates that obtain energy from symbionts[38]. In each of these situations, the nutritional contributions required for effective mutualism depend on genes typically lost from small genome pathogens.

Conversely, can mutualists evolve into pathogens? Pathogens require special capabilities to invade host tissues, to evade host defenses and to survive, at least briefly, outside host tissues during horizontal transmission to new hosts. Although these capabilities might sometimes be transferred among bacterial strains[39], it is not clear whether mutualists with strict asexuality and fastidious habitat requirements within cells or tissues are able to acquire genes required for long-term success as pathogens.

From population genetics: explanations?

From the previous discussion, it appears that mutualistic and parasitic bacteria share a common syndrome of genomic reduction and accelerated sequence evolution. Why? For symbionts that form chronic infections, reduced effective population size and reduced recombination have been hypothesized to increase levels of genetic drift and to decrease the effectiveness of selection; this effect has been proposed for pathogens[23,40] and mutualistic symbionts[26–29], including organelles[41]. As a result, a large pool of mildly deleterious mutations, which would be effectively eliminated in free-living bacteria, might drift to fixation. Small population size is imposed by restrictions to individual hosts in which bacteria occur largely as pure clones from single infections. In mutualistic bacteria, single infections are often the result of maternal transmission; host progeny receive only the limited diversity of bacteria present in their mother. In horizontally transmitted pathogens, lineages might also go through bottlenecks at each infection, thus limiting genetic variation within each host. In such bacteria, the primary determinant of bacterial population size will be the population size of animal hosts, which are orders of magnitude smaller than those of free-living bacteria[26]. The effects of drift and limited recombination on sequence evolution are supported by the concentration of substitutions at sites under selection[26–28,41], sequence changes that result in decreased stability of gene products[29] and the lack of adaptive codon bias[28].

Although some gene loss might be entirely neutral or even favorably selected in intracellular bacteria, the loss of presumably beneficial genes (such as repair loci) might be a result of the fixation of deleterious deletions by drift. Loss of loci encoding recombination enzymes or insertion sequences could compromise processes that underlie successful incorporation of foreign genes. Thus, gene loss in intracellular symbionts might not be balanced by gene uptake, by contrast to the situation in free-living bacteria.

Other interactions: parallels and differences

Lability of host interactions among opportunistic animal pathogens and plant symbionts

Our focus has been on bacterial associates of eukaryotes that fit the resident genome syndrome proposed by Andersson and Kurland[23]. These are chronic pathogens or mutualists that spend their life cycles closely associated with host cells. Many bacteria show more labile associations with eukaryotes; in these, shifts between mutualism and parasitism appear to be achieved relatively frequently through the transfer of genes affecting interactions with hosts. For example, the acquisition of 'pathogenicity islands' (sets of genes allowing invasion of host cells) (Box 1) transforms animal commensals into invasive pathogens[39]. Similarly, in bacterial associates of plants, acquisition and loss of certain genes mediates shifts between mutualistic, parasitic and free-living lifestyles[42].

In addition to frequent horizontal transfer, opportunistic animal associates and plant symbionts also differ from intracellular small genome bacteria in their ability to grow as free-living strains and to constantly reinfect individual hosts. Free-living stages will confer larger genetic population sizes and retention of genes for metabolic diversity. Not surprisingly, these more opportunistic associates of eukaryotes do not show the strong effect of drift nor the genome reduction that characterizes resident genomes of obligate symbionts and pathogens.

Prospects

In summary, phylogenetic and genomic data support the hypothesis that many clades of intimate bacterial symbionts are strictly mutualistic or parasitic. Transitions between these interaction types might be restricted owing to irreversible loss of genes and associated functional capabilities. Further testing of this proposal will be possible as phylogenetic relationships, genome complements and phenotypic effects of additional symbiont lineages are characterized. For example, a large proportion of insect species contain maternally transmitted bacteria[15,36,37] and most are completely uncharacterized despite the rapid increase in knowledge from molecular studies during the past decade. New molecular techniques, such as *in situ* hybridization and gene microarrays, promise to shed light on symbiont distributions within and between host individuals, symbiont genome complements and symbiont patterns of gene expression in hosts. Combined, these approaches will illuminate links between genome evolution and symbiont phenotypes.

Acknowledgements

We thank H. Ishikawa for unpublished information on the gene content of Buchnera*. Work underlying this article was supported by NSF DEB-9978518 (N.A.M.) and an NIH training grant to the Center for Insect Science (J.J.W.). H. Ochman, members of the Moran lab and anonymous reviewers provided helpful comments.*

References

1 Douglas, A.E. (1998) Host benefit and the evolution of specialization in symbiosis. *Heredity* 81, 599–603
2 Frank, S.A. (1997) Models of symbiosis. *Am. Nat.* 150, S80–S99
3 Herre, E.A. *et al.* (1999) The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends Ecol. Evol.* 14, 49–53
4 Lipsitch, M. *et al.* (1995) The population-dynamics of vertically and horizontally transmitted parasites. *Proc. R. Soc. London Ser. B* 260, 321–327
5 Nuismer, S.L. *et al.* (1999) Gene flow and geographically structured coevolution. *Proc. R. Soc. London Ser. B* 266, 605–609
6 Pellmyr, O. and Leebens-Mack, J. (1999) Forty million years of mutualism: evidence for Eocene origin of the yucca–yucca moth association. *Proc. Natl. Acad. Sci. U. S. A.* 96, 9178–9183
7 Yamamura, N. (1993) Vertical transmission and evolution of mutualism from parasitism. *Theor. Popul. Biol.* 44, 95–109
8 Saikkonen, K. *et al.* (1998) Fungal endophytes: a continuum of interactions with host plants. *Annu. Rev. Ecol. Syst.* 29, 319–343
9 Corsaro, D. *et al.* (1999) Intracellular life. *Crit. Rev. Microbiol.* 25, 39–79
10 Woese, C.R. (1987) Bacterial evolution. *Microbiol. Rev.* 51, 221–271
11 Hugenholtz, P. *et al.* (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *J. Bacteriol.* 180, 4765–4774
12 Maidak, B.L. *et al.* (1999) A new version of the RDP (ribosomal database project). *Nucleic Acids Res.* 27, 171–173
13 Bandi, C. *et al.* (1995) The establishment of intracellular symbiosis in an ancestor of cockroaches and termites. *Proc. R. Soc. London Ser. B* 259, 293–299
14 Baumann, P. *et al.* (1997) The evolution and genetics of aphid endosymbionts. *BioScience* 47, 12–20
15 Moran, N.A. and Telang, A. (1998) The evolution of bacteriocyte-associated endosymbionts in insects. *BioScience* 48, 295–304
16 Peek, A.S. *et al.* (1998) Accelerated evolutionary rate in sulfur-oxidizing endosymbiotic bacteria associated with the mode of symbiont transmission. *Mol. Biol. Evol.* 15, 1514–1523
17 Spaulding, A.W. and von Dohlen, C.D. (1998) Phylogenetic characterization and molecular evolution of bacterial endosymbionts in psyllids (Hemiptera: Sternorrhyncha). *Mol. Biol. Evol.* 15, 1506–1513
18 Stothard, D.R. *et al.* (1994) Ancestral divergence of *Rickettsia bellii* from the spotted fever and typhus groups of *Rickettsia* and antiquity of the genus *Rickettsia*. *Int. J. Syst. Bacteriol.* 44, 798–804
19 Lang, B.F. *et al.* (1999) Mitochondrial genome evolution and the origin of eukaryotes. *Annu. Rev. Genet.* 33, 351–397
20 Hurst, G.D.D. *et al.* (1999) *Adonia variegata* (Coleoptera: Coccinellidae) bears maternally inherited Flavobacteria that kill males only. *Parasitology* 118, 125–134
21 O'Neill, S.L. *et al.*, eds (1998) *Influential Passengers: Inherited Microorganisms and Arthropod Reproduction*, Oxford University Press
22 Bandi, C. *et al.* (1999) Effects of tetracycline on the filarial worms *Brugia pahangi* and *Dirofilaria immitis* and their bacterial endosymbionts *Wolbachia*. *Int. J. Parasitol.* 29, 357–364
23 Andersson, S.G.E. and Kurland, C.G. (1998) Reductive evolution of resident genomes. *Trends Microbiol.* 6, 263–268
24 Charles, H. and Ishikawa, I. (1999) Physical and genetic map of the genome of *Buchnera*, the primary endosymbiont of the pea aphid, *Acyrthosiphon pisum*. *J. Mol. Evol.* 48, 142–150
25 Maniloff, J. (1996) The minimal cell genome: 'on being the right size'. *Proc. Natl. Acad. Sci. U. S. A.* 93, 10004–10006
26 Moran, N.A. (1996) Accelerated evolution and Muller's ratchet in endosymbiotic bacteria. *Proc. Natl. Acad. Sci. U. S. A.* 93, 2873–2878
27 Clark, M.A. *et al.* (1999) Sequence evolution in bacterial endosymbionts having extreme base composition. *Mol. Biol. Evol.* 16, 1586–1598
28 Wernegreen, J.J. and Moran, N.A. (1999) Evidence for genetic drift in endosymbionts (*Buchnera*): analyses of protein-coding genes. *Mol. Biol. Evol.* 16, 83–97
29 Lambert, J.L. and Moran, N.A. (1998) Deleterious mutations destabilize ribosomal RNA of endosymbionts. *Proc. Natl. Acad. Sci. U. S. A.* 95, 4458–4462
30 Wernegreen, J.J. *et al.* (2000) Decoupling of genome size and sequence divergence in a symbiotic bacterium. *J. Bacteriol.* 182, 3867–3869
31 Fraser, C.M. *et al.* (1995) Minimal gene complement of *Mycoplasma genitalium*. *Science* 270, 397–403
32 Doolittle, W.F. (1999) Phylogenetic classification and the universal tree. *Science* 284, 2124–2128
33 Lawrence, J.G. and Ochman, H. (1998) Molecular archaeology of the *Escherichia coli* genome. *Proc. Natl. Acad. Sci. U. S. A.* 95, 9413–9417
34 Lawrence, J.G. and Roth, J.R. (1996) Selfish operons: horizontal transfer may drive the evolution of gene clusters. *Genetics* 143, 1843–1860
35 Douglas, A.E. (1998) Nutritional interactions in insect–microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. *Annu. Rev. Entomol.* 43, 17–37
36 Buchner, P. (1965) *Endosymbiosis of Animals with Plant Microorganisms*, Interscience
37 Douglas, A.E. (1989) Mycetocyte symbiosis in insects. *Biol. Rev. Camb. Philos. Soc.* 64, 409–434
38 Lee, R.W. *et al.* (1999) Pathways of inorganic nitrogen assimilation in chemoautotrophic bacteria–marine invertebrate symbioses: expression of host and symbiont glutamine synthetase. *J. Exp. Biol.* 202, 289–300
39 Groisman, E.A. and Ochman, H. (1997) How *Salmonella* became a pathogen. *Trends Microbiol.* 5, 343–349
40 Bergstrom, C.T. *et al.* (1999) Transmission bottlenecks as determinants of virulence in rapidly evolving pathogens. *Proc. Natl. Acad. Sci. U. S. A.* 96, 5095–5100
41 Lynch, M. and Blanchard, J.L. (1998) Deleterious mutation accumulation in organelle genomes. *Genetica* 102/103, 29–39
42 Sullivan, J.T. and Ronson, C.W. (1998) Evolution of rhizobia by acquisition of a 500-kb symbiosis island that integrates into a phe-tRNA gene. *Proc. Natl. Acad. Sci. U. S. A.* 95, 5145–5149
43 Andersson, S.G.E. *et al.* (1998) The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. *Nature* 396, 133–140
44 Blattner, F.R. *et al.* (1997) The complete genome sequence of *Escherichia coli* K-12. *Science* 277, 1453–1474
45 Fleischmann, R.D. *et al.* (1996) Whole-genome random sequencing and assembly of *Haemophilus influenzae*. *Science* 269, 496–512
46 Fraser, C.M. *et al.* (1997) Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature* 390, 580–586
47 Himmelreich, R. *et al.* (1996) Complete sequence analysis of the genome of the bacterium *Mycoplasma pneumoniae*. *Nucleic Acids Res.* 24, 4420–4449
48 Kunst, F. *et al.* (1997) The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*. *Nature* 390, 249–256
49 Stephens, R.S. *et al.* (1998) Genome sequence of an obligate intracellular pathogen of humans: *Chlamydia trachomatis*. *Science* 282, 754–759

Reference added in proof

50 Shigenobu, S. *et al.* Mutualism as revealed at the genomic level: the whole genome sequence of *Buchnera* spp. APS, an endocellular bacterial symbiont of aphids. *Nature* (in press)