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MINIREVIEW

Defying Muller's Ratchet: Ancient Heritable Endobacteria Escape Extinction through Retention of Recombination and Genome Plasticity

Mizue Naito,[a] Teresa E. Pawlowska[b]

Graduate Field of Microbiology, Cornell University, Ithaca, New York, USA[a]; School of Integrative Plant Science, Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, New York, USA[b]

ABSTRACT **Heritable endobacteria, which are transmitted from one host generation to the next, are subjected to evolutionary forces that are different from those experienced by free-living bacteria. In particular, they suffer consequences of Muller's ratchet, a mechanism that leads to extinction of small asexual populations due to fixation of slightly deleterious mutations combined with the random loss of the most-fit genotypes, which cannot be recreated without recombination. Mycoplasma-related endobacteria (MRE) are heritable symbionts of fungi from two ancient lineages, *Glomeromycota* (arbuscular mycorrhizal fungi) and *Mucoromycotina*. Previous studies revealed that MRE maintain unusually diverse populations inside their hosts and may have been associated with fungi already in the early Paleozoic. Here we show that MRE are vulnerable to genomic degeneration and propose that they defy Muller's ratchet thanks to retention of recombination and genome plasticity. We suggest that other endobacteria may be capable of raising similar defenses against Muller's ratchet.**

MOLECULAR EVOLUTION PATTERNS IN HERITABLE ENDOBACTERIA

Heritable endobacteria are an exceptional group of bacteria that reside within eukaryotic host cells and are transmitted vertically from one host generation to the next. They occupy the cytoplasmic niches of a variety of hosts, including arthropods, nematodes, and fungi (1, 2). These associations of bacteria and eukaryotic hosts often endow the partners with novel capabilities that could not be achieved in separation from each other, leading to evolutionary innovations. The extent of interdependence between the host and endobacteria can vary based on factors such as age of association and degree of coevolution: i.e., endosymbionts exhibit either facultative or obligate dependence on the host, and their impact is essential, nonessential, or antagonistic to the host's survival. In addition, the antiquity and nature of the association between the partners determine whether endosymbiont transmission is exclusively vertical or punctuated by instances of horizontal transmission.

Vertically transmitted endobacteria undergo unique molecular evolution, resulting in genome structures not normally seen in their free-living relatives, with the exception of a select few marine bacteria (3). Endosymbiont genomes are reduced in size and exhibit accelerated sequence evolution (4). These features are consequences of living in the cytoplasmic niche of a host that is unlike a free-living environment; the intracellular compartment offers not only protection and absence of competition, but a metabolically rich milieu where many genes in the endobacterial genomes become redundant or unnecessary (5). Furthermore, exclusively vertical transmission has several consequences that affect endosymbiont population structure. In particular, population bottlenecks mark every transmission, since only a portion of the endosymbiont population is passed to the next generation of the host. Moreover, endosymbiont populations are subdivided by being restricted to individual host lineages. They do not engage in recombination and exhibit limited genetic diversity. These factors contribute to the reduction of endosymbiont effective population sizes relative to free-living bacteria. Based on the nearly neutral theory of molecular evolution, such population dynamics are predicted to limit the strength of purifying selection, resulting in the accumulation and fixation of slightly deleterious mutations in the endosymbiont genome (6), manifested by an overall increase in the ratio of nonsynonymous to synonymous substitutions in protein coding genes (7). Continual accumulation of slightly deleterious mutations over time leads to inactivation of the affected genes, including DNA repair machinery, which further accelerates mutation buildup in the genome. Due to deletional bias prevailing in bacterial genomes, mutation-compromised genes are eliminated, eventually resulting in the reduced and degenerate genomes typical for ancient heritable endobacteria (8).

The genes that are ultimately lost in reduced endobacterial genomes come from all functional categories—most notably metabolism, cell envelope biosynthesis, transcriptional regulation, and DNA repair and recombination (8). The genes that are retained, in addition to those important for symbiosis, are involved in essential cellular functions, such as DNA replication, RNA transcription, and protein translation. However, even within these essential categories, only minimal repertoires of genes required for basic functionality are maintained, with most endobacteria displaying the loss of accessory subunits otherwise present in these systems (5).

In heritable bacteria, changes in genome coding capacity and stability appear to complement gene content modifications associated with the transition to a life in the eukaryotic cell (8). Early

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Address correspondence to Teresa E. Pawlowska, tep8@cornell.edu.

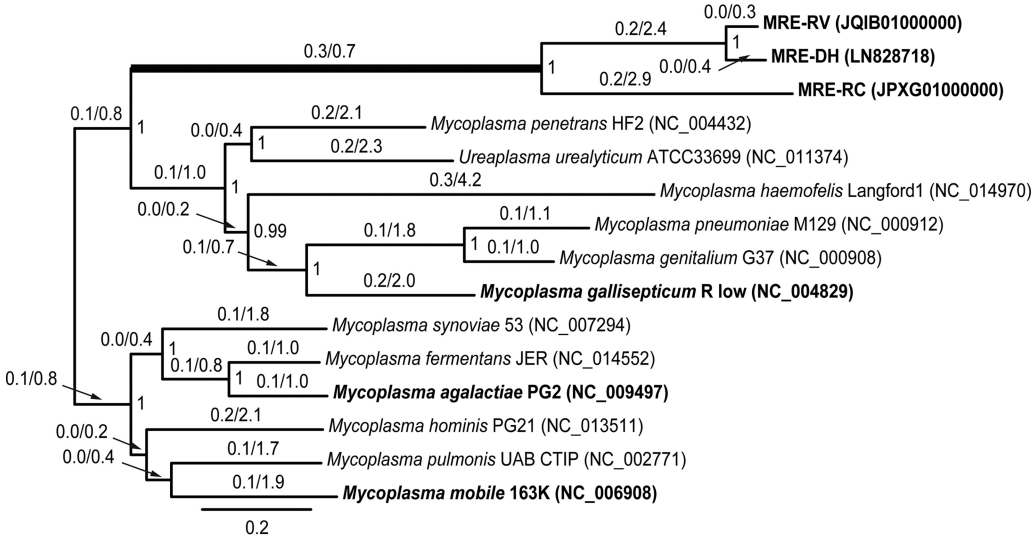

FIG 1 Bayesian phylogeny of MRE reconstructed based on the concatenated nucleotide sequences of the following genes: *dnaG*, *infC*, *nusA*, *rplA*, *rplB*, *rplC*, *rplE*, *rplF*, *rplM*, *rplN*, *rplP*, *rplT*, *rpmA*, *rpsB*, *rpsC*, *rpsE*, *rpsJ*, *rpsS*, and *smpB*. MRE were sampled from *Dentiscutata heterogama* (MRE-DH), *Racocetra verrucosa* (MRE-RV), and *Rhizophagus clarus* (MRE-RC). Bayesian posterior probabilities greater than 0.90 are shown at nodes; Bayesian analyses were conducted using MrBayes v.3.2 (64) under the nucleotide substitution model GTR + Γ + I with 1,000,000 generations and a 250,000 burn-in. The numbers above branches are the estimates of *dN*/*dS* ratios obtained using the codeml module of PAML v.4.8 (65), assuming a two-ratio model. The thickened branch leading to MRE indicates that, according to the likelihood ratio model testing conducted in codeml, the *dN*/*dS* ratio along it is significantly different from the background *dN*/*dS* ratio for all other branches ($\chi^2 = 36.12$, $P < 0.001$). Taxa in boldface were subjected to Tajima 1D relative rate tests (26) (Table 1).

stages of genome contraction are characterized by the abundance of pseudogenes, mobile genetic elements (MGEs), and genomic rearrangements. In contrast, the advanced stages found in ancient endobacteria with a reduced gene set are typified by the near absence of pseudogenes, the lack of MGEs, and relatively stable yet progressively deteriorating genomes.

MULLER'S RATCHET AND POPULATION EXTINCTION

Rapid fixation of slightly deleterious mutations and genome degeneration are not the only hazards that bedevil small asexual populations of heritable endobacteria. In the absence of recombination, they are vulnerable to extinction, as, by chance, the most-fit genotypes can be lost. This continual decrease in the mean fitness of a population was described by Hermann Joseph Muller (9) and is known as Muller's ratchet. Each consecutive loss of the most-fit genotype advances the ratchet toward population collapse, as these genotypes cannot be recreated without recombination. Consequently, Muller's ratchet is a powerful force that contributes to genome erosion in heritable endobacteria (7), ultimately leading to disappearance of endosymbiont lineages (10). While recombination is the principal mechanism capable of disabling the ratchet, evolutionary theory suggests that several other factors could decelerate its advance, including compensatory evolution, in which fitness losses caused by earlier mutations are restored by subsequent mutations (11), horizontal acquisition of foreign DNA (12), and host-level selection that maintains a network of endosymbiont populations associated with individual host lineages (13).

GENOME EVOLUTION IN THE MYCOPLASMA-RELATED ENDOBACTERIA OF FUNGI

Recently, genomic data (with GenBank accession numbers in brackets) were generated for a novel group of heritable endobacteria, the mycoplasma-related endobacteria (MRE), associated with four distinct *Glomeromycota* host species: MRE-CE (*Claroideoglomus etunicatum* [JPXH00000000]), MRE-DH (*Dentiscutata heterogama* [LN828718]), MRE-RC (*Rhizophagus clarus* [JPXG01000000]), and MRE-RV (*Racocetra verrucosa* [JQIB01000000]) (14, 15). MRE reside in the cytoplasm of fungi representing all major lineages of *Glomeromycota* (arbuscular mycorrhizal fungi [AMF]) and the *Endogone* lineage of *Mucoromycotina* (2, 16–18). AMF are asexual soil fungi that form obligate symbioses (mycorrhizae) with the majority of terrestrial plants (19). Fossil records date AMF to the Ordovician (~460 million years ago [MYA]) (20) and indicate that the mycorrhizal symbiosis existed as early as in the Devonian (~400 MYA) (21). In contrast, the genus *Endogone* encompasses a group of poorly understood fungi with diverse lifestyles (16). However, their ancestors are believed to have colonized the early Devonian plants simultaneously with AMF (22). The role of MRE in the biology of their fungal hosts is unknown.

Origin of the MRE-fungus symbiosis. MRE are members of the *Mollicutes*, a group characterized by minimal genomes, highly reduced metabolic capabilities, and the lack of a peptidoglycan cell wall. Phylogenetic reconstructions place MRE in the *Mycoplasma pneumoniae* group of the family *Mycoplasmataceae* (14) (Fig. 1), suggesting that MRE arose as a consequence of a host switch from animals to fungi. Such origin implies that vertically transmitted MRE associated with fungi share some genomic features with horizontally transmitted animal-associated mycoplasmas. Detection of MRE in both *Glomeromycota* and the *Endogone* lineage of *Mucoromycotina*, as well as molecular phylogeny data offering marginally significant support for codivergence between MRE and these two groups of fungi, gave rise to the hypothesis that the origin of the MRE association with fungi predates the divergence between *Glomeromycota* and *Mucoromycotina* during the early Pa-

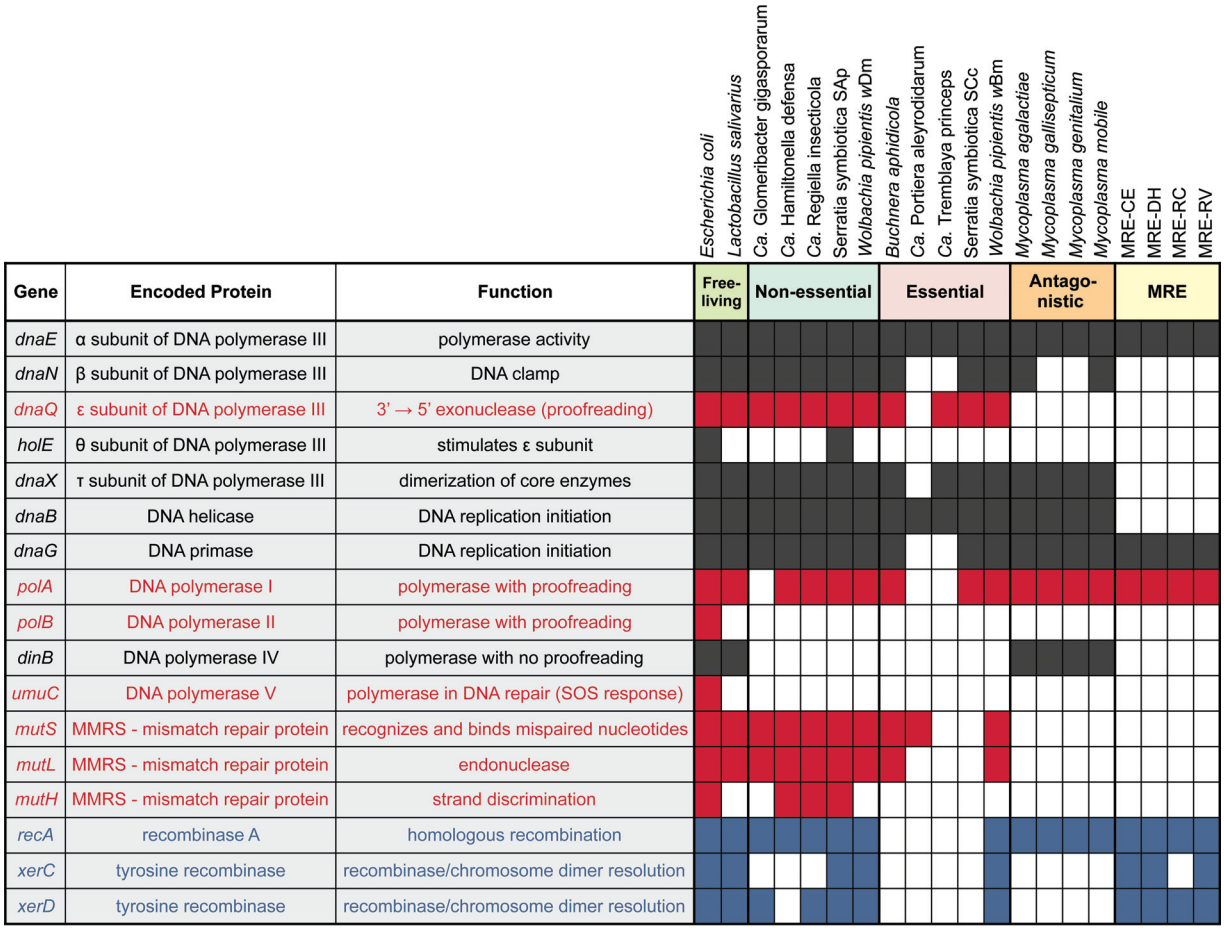

FIG 2 Loss and retention of select genes involved in DNA replication (black), repair (red), and recombination (blue). The four populations of the obligate endobacteria MRE are compared with free-living bacteria and other endobacteria, categorized as nonessential, essential, and antagonistic symbionts. Colored squares indicate the presence of the gene, and white squares indicate loss of the gene. The non-MRE bacterial strains used (with GenBank accession numbers in parentheses) are *Escherichia coli* O157:H7 strain Sakai (BA000007.2), *Lactobacillus salivarius* UCC118 (CP000233.1), "*Candidatus* Glomeribacter gigasporarum" BEG34 (CAFB00000000.1), "*Candidatus* Hamiltonella defensa" 5AT (CP001277.1), "*Candidatus* Regiella insecticola" LSR1 (ACYF00000000.1), *Serratia symbiotica* SAp (AENX00000000.1), *Wolbachia pipientis* endosymbiont of *Drosophila melanogaster* wDm (AE017196.1), *Buchnera aphidicola* APS (BA000003.2), "*Candidatus* Portiera aleyrodidarum" BT-QVLC (CP003867.1), "*Candidatus* Tremblaya princeps" PCVAL (CP002918.1), *Serratia symbiotica* SCc (CP002295.1), *Wolbachia pipientis* endosymbiont of *Brugia malayi* WBM (AE017321.1), *Mycoplasma agalactiae* PG2 (CU179680.1), *Mycoplasma gallisepticum* S6 (CP006916.2), *Mycoplasma genitalium* 6282 (CP003771.1), and *Mycoplasma mobile* 163K (AE017308.1).

leozoic (16, 18). Alternatively, ancestral MRE could have invaded and spread concomitantly into representatives of these two groups of fungi when they cocolonized Devonian plants during early terrestrialization (22). Regardless of which scenario is accurate, the association between MRE and fungi may be one of the oldest heritable symbioses on the planet.

MRE intrahost diversity. Unexpectedly for heritable endobacteria, which tend to be genetically uniform within their hosts (8), MRE populations display unusually high diversity levels (2, 17, 18). There appear to be two sources of MRE intrahost diversity: (i) rapid accumulation of mutations and (ii) horizontal transmission between hosts. (i) Like in other mycoplasmas, mutation accumulation in MRE genomes can be attributed to the losses of DNA polymerase II (*polB*), the proofreading subunit of DNA polymerase III (*dnaQ*), and the methyl-directed mismatch repair system (MMRS) (*mut* genes) (Fig. 2) (14). The losses of *dnaQ*, *polB*, and MMRS genes have been shown to cause hypermutator phenotypes in other bacteria due to the inability to fix errors in their DNA sequences (23–25). To assess the extent of mutation accumulation in MRE, we compared the rates of evolution between MRE and *Mycoplasma gallisepticum*, which also represents the *M. pneumoniae* clade of the *Mycoplasmataceae* (Fig. 1), by conducting Tajima 1D relative rate test (26), implemented in MEGA7 (27), on DNA sequences at 19 protein coding loci (Table 1). With a mutation rate of 1.02×10^{-5} substitutions per site per year, *M. gallisepticum* is considered to be one of the fastest-evolving bacteria (28). We found that MRE genomes evolve more rapidly than that of *M. gallisepticum* (Table 1), which places MRE among the ultrafast-evolving microbes. (ii) The second source of MRE intrahost diversity appears to be horizontal transmission, inferred from analyses of codivergence patterns between MRE and AMF (18). The exact mechanisms of horizontal transmission are unknown. MRE dispersal across AMF of one species could be facilitated by fusions between hyphae of different fungal strains (29). MRE transmission across different AMF species could occur when hyphae are damaged due to grazing by soil fauna (30). In either

TABLE 1 MRE exhibit molecular evolution rate acceleration relative to other mycoplasmas[a]

Ingroup (GenBank accession no.)	Outgroup (GenBank accession no.)	Relative rate statistic[b]
MRE-DH (LN828718), *M. gallisepticum* R low (NC_004829)	*M. mobile* 163K (NC_006908)	123.65*
MRE-RC (JPXG01000000), *M. gallisepticum* R low (NC_004829)	*M. mobile* 163K (NC_006908)	126.42*
MRE-RV (JQIB01000000), *M. gallisepticum* R low (NC_004829)	*M. mobile* 163K (NC_006908)	128.82*
MRE-DH (LN828718), *M. gallisepticum* R low (NC_004829)	*M. agalactiae* PG2 (NC_009497)	140.48*
MRE-RC (JPXG01000000), *M. gallisepticum* R low (NC_004829)	*M. agalactiae* PG2 (NC_009497)	137.44*
MRE-RV (JQIB01000000), *M. gallisepticum* R low (NC_004829)	*M. agalactiae* PG2 (NC_009497)	142.19*

[a] Results are as indicated by the results of Tajima's 1D relative rate test (26) for DNA sequences at 19 protein coding loci listed in Fig. 1.
[b] The 1D relative rate statistic distribution is the same as the distribution of χ^2. *, significant at $P < 0.0001$. If the value is significant, the null hypothesis of equal rates of sequence evolution can be rejected.

case, horizontal transmission could potentially facilitate genetic exchanges among distinct MRE genotypes.

Are MRE genomes degenerate? In terms of the size and extent of metabolic dependence on the host, MRE genomes are comparable to the genomes of other closely related animal-associated mycoplasmas. However, unlike in other *Mycoplasma* species, MRE transmission is predominantly vertical, which exposes MRE intrahost populations to demographic bottlenecks of about 1,000 cells that populate individual AMF spores (17). Such recurrent bottlenecks may contribute to a decline in effective population size and magnification of genetic drift relative to natural selection, which, in turn, could make MRE genomes vulnerable to progressive degeneration and expose their populations to Muller's ratchet. In fact, the significant increase in the molecular evolution rate experienced by MRE relative to horizontally transmitted *M. gallisepticum* (Table 1) suggests that vertical transmission impacts MRE evolution. To assess the specific consequences of vertical transmission on MRE, we focused on (i) the ratio of the rate of nonsynonymous nucleotide substitutions to the rate of synonymous substitutions (*dN*/*dS*) in protein coding genes and (ii) the abundance of putative pseudogenes in the MRE genomes. (i) The genome-wide increases of *dN*/*dS* ratios in heritable endobacteria compared to those in free-living relatives are commonly used as an indicator of accumulation of slightly deleterious mutations (7). Our analysis of *dN*/*dS* ratios along the branches of the *Mycoplasmataceae* phylogeny reconstructed using sequences of 19 protein coding genes (Fig. 1) revealed that the *dN*/*dS* ratio along the branch leading to MRE is significantly higher than the background *dN*/*dS* ratio along all other branches of this phylogeny ($\chi^2 = 36.12$, $P < 0.001$). It is unlikely that this pattern represents adaptation to a new host, as the increased nonsynonymous substitution rate is apparent across several broadly conserved genes, including sequences encoding ribosomal proteins. Instead, this observation is consistent with the increased accumulation of slightly deleterious mutations after MRE had switched from horizontal to vertical transmission. Interestingly, *dN*/*dS* ratios along terminal branches leading to individual MRE genomes do not appear to be elevated, which suggests that, over time, MRE might have experienced refinement of the mechanisms that contribute to purging of slightly deleterious mutations. (ii) Proliferation of pseudogenes is a hallmark of rapid genome erosion due to accumulation of deleterious mutations (8). In the absence of proteomic data, putative pseudogenes can be identified by comparing open reading frames (ORFs) with their homologues in closely related taxa to detect changes that abolish their original function (31). We followed this approach in MRE and considered ORFs that were reduced in length by 50% relative to their homologues to harbor premature stop codons and ORFs that were over 150% longer than their homologues to have lost start/stop codons. This approach revealed that in MRE, putative pseudogenes constitute between 21 and 30% of all non-orphan ORFs (Table 2). These numbers are comparable to those observed in heritable nonessential mutualists of insects, such as *Serratia symbiotica* SAp in pea aphids, which originated 90 MYA, and 26% of its 2.8-Mb genome is made up by pseudogenes (32). The presence of pseudogenes in MRE suggests that their genomes are experiencing active degeneration. However, the evolutionary context of pseudogene formation in MRE appears to be different from that of other heritable endobacteria. In particular, the *Mycoplasma* parentage of the MRE lineage suggests that the genomes of MRE ancestors were already reduced in size at the time of their transition from animal to fungal hosts, an event that was also likely associated with a switch from horizontal to vertical transmission. Consequently, in contrast to bacteria in which pseudogenization represents early stages of progressive genome contraction, pseudogenization in MRE appears to be a steady-state process responsible for deactivation of genes continually acquired through horizontal transfer, not unlike in other mycoplasmas in which horizontal gene acquisition is one of the mechanisms that counter genome erosion (33). Collectively, the *dN*/*dS* values in protein coding genes and the abundance of putative pseudogenes in the MRE genomes suggest that the MRE

TABLE 2 Putative pseudogenes in the MRE genomes[a]

ORF type	No. (%) of ORFs		
	MRE-CE	MRE-RV	MRE-RC
Non-orphan ORFs	323 (100)	526 (100)	390 (100)
ORFs with putative premature stop	39 (12.1)	61 (11.6)	89 (22.8)
ORFs with putative loss of stop/start	36 (11.1)	49 (9.3)	30 (7.7)

[a] Putative pseudogenes were identified by comparing MRE non-orphan open reading frames (ORFs) with their homologues. ORFs that were over 50% shorter (putative premature stop codon) and longer (putative loss of stop/start codon) are included.

lineage experienced a period of accelerated genome degradation followed by a refinement of mechanisms that allow for purging of slightly deleterious mutations, leading to present-day apparently steady-state genome erosion.

Genetic recombination in MRE. Unlike most ancient heritable endobacteria (8), MRE harbor various DNA recombination systems (14) (Fig. 2). The putative role of recombination in shaping the MRE population structure was indicated initially by the presence of recombination signatures in rRNA gene sequences sampled from MRE associated with diverse AMF hosts distributed globally (17, 18). To further explore the contribution of recombination to MRE evolution, we conducted coalescent population modeling using ClonalFrame (34). We estimated the per-site effect of recombination relative to mutation: i.e., the ratio of rates at which nucleotides become substituted as a result of recombination versus mutation (r/m) based on nucleotide sequences at 13 protein coding loci (*rplA*, *rplB*, *rplC*, *rplM*, *rplN*, *rplP*, *rplT*, *rpmA*, *rpsB*, *rpsC*, *rpsE*, *rpsJ*, and *smpB*) sampled from MRE-DH, MRE-RC, and MRE-RV. The MCMC analyses included 200,000 generations after an initial 50,000-generation burn-in; convergence was assessed using the Gelman-Rubin statistic with a cutoff of 1.1 (34). We found that in MRE, the r/m was 1.3 with a 95% confidence interval of 0.68 to 2.23. Given that the mutation rate is exceptionally high in MRE, and the recombination rate appears to exceed it, recombination is likely to play an important role in MRE evolution.

Mobile genetic elements and genome plasticity in MRE. Similar to other mycoplasmas (35), MRE harbor a substantial number of MGEs, such as phages, insertion sequence elements (14), and integrative and conjugative elements (36). For example, between 1 and 8% of non-orphan genes in the MRE genomes encode transposases (14). Moreover, some of the MRE genomes show evidence of historical invasion by plectrovirus (14), a phage known to infect *Spiroplasma* species (35). The preponderance of MGEs in MRE presents a contrast with most other ancient heritable endobacteria with highly reduced genomes from which such elements appear to be absent (8) and suggests that MRE genomes may experience MGE-mediated genomic rearrangements (36).

One of the most striking features of MRE is the extreme plasticity of their genomes suggested by extensive chromosomal rearrangements apparent in MRE genomic assemblies (14). There appear to be two major sources of chromosomal rearrangements in MRE: recombination facilitated by simple sequence repeats (SSRs) and MGE activity (14). Both SSRs and MGEs often mark disruptions in gene synteny. The degree of plasticity in MRE genomes testifies to the activities of MGEs and recombination machineries. As these two mechanisms underlying MRE chromosomal rearrangements are common in other mycoplasmas (35, 37), MRE must have retained them even after the host switch from animals to fungi and the transition from horizontal to predominantly vertical transmission.

MRE SIMILARITIES TO OTHER HERITABLE ENDOBACTERIA

The trajectory of degenerative genome contraction in heritable endobacteria—with early stages characterized by a rapid loss of gene content, proliferation of pseudogenes, MGEs, and genomic rearrangements and advanced stages typified by minimal, relatively stable but gradually decaying genomes—was reconstructed from genomic comparisons across endobacteria of arthropods with different genome sizes and antiquities (8). For example, *Buchnera aphidicola*, which originated 160 to 280 MYA, is one of the oldest lineages with reduced and decayed genomes (38). *Buchnera* is an essential nutritional mutualist of aphids, and its transmission is exclusively vertical. The absence of MGEs from the 641-kb *Buchnera* genomes is believed to be responsible for the lack of genomic rearrangements between two strains that diverged over 50 MYA (39). Such lack of MGEs represents a stark contrast from the substantial mobilomes of nonessential mutualists with mixed transmission in which vertical transfers are occasionally punctuated by horizontal transfers. For example, the genomes of two sister species, "*Candidatus* Hamiltonella defensa" and "*Candidatus* Regiella insecticola," are 2.1 and 2.0 Mb in size, with MGEs making up 34 and 20% of all coding sequences, respectively (40). While the antiquity of endosymbionts with mixed transmission is notoriously difficult to establish (41), the minimal age of these two nonessential defensive mutualists was estimated to be 100 million years (42). A similar pattern is apparent across different strains of *Serratia symbiotica* that form distinct types of mutualisms with their aphid hosts. For example, *S. symbiotica* SCc is an essential nutritional mutualist of unknown antiquity (43). Its 1.8-Mb genome lacks MGEs and does not show evidence of being able to support recombination (43). In contrast, *S. symbiotica* SAp, the previously mentioned nonessential defensive mutualist of aphids, which dates back to ~90 MYA, is considered to represent early stages of genome contraction (32). Its 2.8-Mb genome is capable of recombination and contains 4% MGEs (32).

With their origin at over 400 MYA (18), the ultrarapid evolution rate, and the incessant interplay of genome erosion and restoration, MRE appear to defy predictions of the degenerative genome contraction model. Importantly, this departure from the degenerative evolution model is not a consequence of host factors, which could be expected to differentiate fungus-associated MRE from the arthropod-associated endobacteria, such as *Buchnera* or *Serratia* SCc. The absence of such host effects is evidenced by the intrahost genetic diversity that distinguishes MRE not only from most arthropod-associated endobacteria but also from another heritable endosymbiont of AMF, "*Candidatus* Glomeribacter gigasporarum," which displays genetic homogeneity of intrahost populations (17, 41).

Recent studies driven by rapid accumulation of genomic data from diverse endobacteria suggest that departures from the degenerative model of endosymbiont evolution may be also found in other organisms. For example, genome rearrangements were detected in highly reduced and decayed genomes of essential mutualists of insects, "*Candidatus* Portiera aleyrodidarum" in whiteflies (44) and "*Candidatus* Tremblaya princeps" in mealybugs (45); both of these associations date back to 100 to 200 MYA (46). In the 358-kb genome of "*Ca.* Portiera aleyrodidarum" BT, large-scale structural polymorphisms exist even within individual insects, and this variation is likely mediated by recombination across identical repeats that are maintained by gene conversion (44). Similarly, the 139-kb genome of "*Ca.* Tremblaya princeps" exists within single insects in two forms, differentiated by a genomic inversion present in both orientations (45). Likewise, MGEs have been suggested to be important in the essential mutualist of nematodes, *Wolbachia pipientis w*Bm, due to their ability to act as gene conversion sites or sites of homologous recombination (47). *Wolbachia w*Bm dates back to 50 to 55 MYA (48). It is capable of recombination, and its 1.1-Mb genome contains 2.4% apparently inactive MGEs (47, 49). In addition to ancient mutualists with

exclusively vertical transmission, MRE share several similarities with *Wolbachia* reproductive parasites of arthropods. Genomes of these arthropod-associated *Wolbachia* are also reduced and degenerate (50) while retaining the ability to recombine (51) and undergo extensive rearrangements (52), a process often mediated by MGEs (53). Like MRE, arthropod-associated *Wolbachia* strains engage occasionally in horizontal transmission, and distinct *Wolbachia* genotypes have been observed to coexist in host individuals (51). However, with the estimated age of 50 to 55 million years (48), the association of *Wolbachia* with their arthropod hosts is considerably younger than the symbiosis between MRE and fungi (18). Moreover, *Wolbachia* genomes retain a larger repertoire of DNA repair mechanisms than those present in MRE (Fig. 2). Collectively, while these examples suggest that other heritable endobacteria may share some evolutionary mechanisms with MRE, none of these lineages appears to be equally ancient.

MAINTENANCE OF RECOMBINATION AND GENOME PLASTICITY TO OVERCOME MULLER'S RATCHET

We equate the maintenance of recombination machinery in the MRE genome to the evolution of sex in eukaryotic systems. Despite its inherent costs, sex is maintained in the majority of eukaryotes, with models explaining its significance that range from the creation of novel genotypes to resist pathogen infection in the Red Queen hypothesis, through acceleration of adaptation by eliminating competition among beneficial mutations in the Fisher-Muller hypothesis, to the prevention of population extinction via Muller's ratchet in finite populations (54). Though the exact mechanism is unclear, MRE are capable of genomic recombination (17, 18), perhaps through the uptake of DNA from dead MRE cells present in the same host cytoplasmic niche. Given that some highly divergent MRE populations appear to be products of horizontal transmission (18), such DNA acquisitions could generate novel high-fitness genotypes. Yet, being subjected to predominantly vertical transmission, the MRE genomes inevitably erode through Muller's ratchet (Fig. 1; Table 2). Nevertheless, they appear to be able to purge the deleterious mutations and generate new genotypes through DNA recombination and genome plasticity (Fig. 3). Furthermore, theoretical modeling indicates that recombination-mediated horizontal gene transfer of foreign DNA in bacterial populations can stall the advance of Muller's ratchet, even if the foreign genes contain more deleterious mutations than the recipient cells (12). If uptake and recombination of foreign DNA are frequent enough, a diverse bacterial population can resist Muller's ratchet better than a genetically homogenous population of the same size.

It is unclear how MRE are able to prevent their recombination machinery from being eroded, as seen in the majority of other heritable endobacteria. This may be a general feature of the genome structure in all mycoplasmas. Their limited gene set and rapid mutation accumulation may be sources of strong selective pressure to maintain recombination genes. Rapid genetic change afforded by active recombination machinery is also likely favored given a mycoplasma lifestyle of antagonistic dependence on eukaryotic hosts whose evolving defensive responses necessitate continuous evasion (55). Accordingly, despite their minimal genomes, *Mycoplasma* species appear to have retained their sexual competence and ability for horizontal gene transfer among cells sharing the same niche (56). For example, a recent study has shown that *Mycoplasma agalactiae* is able to exchange and transfer

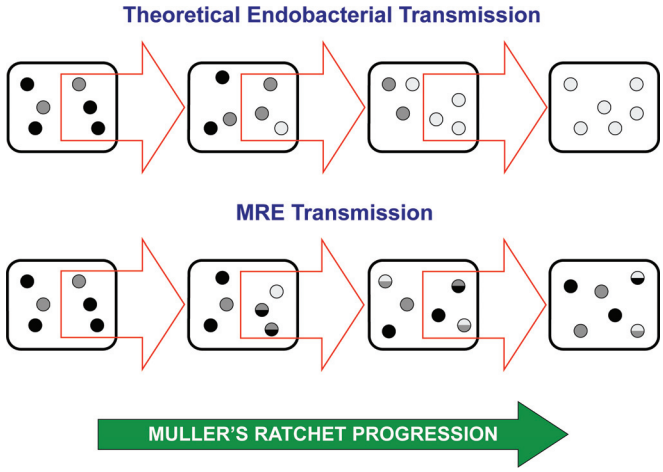

FIG 3 MRE transmission and Muller's ratchet progression compared to theoretical predictions for heritable endobacteria. Due to transmission bottlenecks and fixation of slightly deleterious mutations, heritable endobacteria are expected to degenerately progress to a relatively homogeneous population of unfit individuals. MRE escape Muller's ratchet by retention of recombination and genome plasticity to purge some of the slightly deleterious mutations and maintain a genetically diverse population. Intrahost selection is expected to eliminate low-fitness genotypes generated by recombination. Black outlined rectangles represent host cells, with red arrows indicating transmission bottlenecks. Endobacteria are represented as small circles, with darker shading depicting the most-fit individuals to lighter shading depicting the least-fit individuals. Circles with two-tone color depict recombination events in MRE.

nearly every fragment of its chromosome through conjugation, including long stretches of DNA containing up to 80 genes (57). Whether a similar mechanism operates in MRE remains to be investigated.

While recombination is considered to be an unequivocally beneficial evolutionary force, understanding the role of MGEs in evolution is more nuanced (58). MGEs are selfish entities that impose on the host cell costs of their replication. More importantly, integration events into new sites of the host genome may disrupt important regulatory and metabolic functions (59). Despite these downsides, the presence of MGEs is viewed as advantageous in mycoplasmas (35). It allows for acquisition of new genetic information and contributes to genomic rearrangements associated with novel phenotypes (60). In MRE, facilitation of chromosomal rearrangements seems to be the defining role of MGEs (14). Furthermore, homologous MGEs scattered throughout the MRE genomes could act as sites of recombination or gene conversions (36). Such recombinogenic roles of MGEs have also been observed in other bacteria (47, 61, 62). Consequently, the potential of MGEs to create sites of homology, in conjunction with the retention of recombination genes, provides MRE with a mechanism for escaping Muller's ratchet to maintain evolutionary longevity.

Mycoplasmas are now seen as a highly adaptable rather than a degenerate group of bacteria driven only by reductive evolution (56, 57, 63). Likewise, MRE appear to have maintained horizontal transmission and recombination abilities as well as MGE activity that allow for maintenance of a dynamic population structure. Consequently, we propose that MRE utilize bacterial sex and genome plasticity as a mechanism for decelerating Muller's ratchet, a phenomenon likely linked to their mollicute ancestry and relat-

edness to the mycoplasmas. In particular, it cannot be excluded that, like other mycoplasmas, MRE are antagonists of their hosts, a lifestyle that favors rapid genetic change to either avoid or frustrate host defenses.

CONCLUSIONS

Our exploration of the novel lineage of ancient heritable endobacteria of fungi revealed evidence of genomic degeneration accompanied by active maintenance of recombination genes and genome plasticity, which is responsible for intrahost genetic diversity. We propose that these diversity-generating mechanisms hinder the impending advance of Muller's ratchet (Fig. 3), a luxury not possible in other endobacteria that have lost, through genome erosion, their recombinant abilities and other mechanisms underlying genome plasticity.

It is clear that genome evolution in endobacteria is influenced by multiple factors, and thus the trajectory of endobacterial populations may not be as simple and predictable as once believed. The dynamics of endobacterial populations have profound effects not only on their evolutionary fate but also on the hosts that harbor them. MRE are exceptional endosymbionts that appear to have retained bacterial sex and genome plasticity and, as a consequence, are able to prevent the effects of Muller's ratchet that imperil other ancient heritable endobacteria.

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