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Large-Scale Intersubspecific Recombination in the Plant-Pathogenic Bacterium *Xylella fastidiosa* Is Associated with the Host Shift to Mulberry

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Homologous recombination plays an important role in the structuring of genetic variation of many bacteria; however, its importance in adaptive evolution is not well established. We investigated the association of intersubspecific homologous recombination (IHR) with the shift to a novel host (mulberry) by the plant-pathogenic bacterium *Xylella fastidiosa*. Mulberry leaf scorch was identified about 25 years ago in native red mulberry in the eastern United States and has spread to introduced white mulberry in California. Comparing a sequence of 8 genes (4,706 bp) from 21 mulberry-type isolates to published data (352 isolates representing all subspecies), we confirmed previous indications that the mulberry isolates define a group distinct from the 4 subspecies, and we propose naming the taxon *X. fastidiosa* subsp. *morus*. The ancestry of its gene sequences was mixed, with 4 derived from *X. fastidiosa* subsp. *fastidiosa* (introduced from Central America), 3 from *X. fastidiosa* subsp. *multiplex* (considered native to the United States), and 1 chimeric, demonstrating that this group originated by large-scale IHR. The very low within-type genetic variation (0.08% site polymorphism), plus the apparent inability of native *X. fastidiosa* subsp. *multiplex* to infect mulberry, suggests that this host shift was achieved after strong selection acted on genetic variants created by IHR. Sequence data indicate that a single ancestral IHR event gave rise not only to *X. fastidiosa* subsp. *morus* but also to the *X. fastidiosa* subsp. *multiplex* recombinant group which infects several hosts but is the only type naturally infecting blueberry, thus implicating this IHR in the invasion of at least two novel native hosts, mulberry and blueberry.

Recognition of the potential importance of homologous recombination in the evolution of bacteria has been steadily growing since the work of Smith et al. (1, 2). Recombination of homologous donor DNA into the bacterial chromosome appears to be ubiquitous, although the relative importance of recombination versus mutation in driving evolutionary change varies widely among species (2–5). Homologous recombination typically involves relatively small pieces of DNA (often <1 kb [1, 6, 7]), which suggests entry of donor DNA into the bacterial cell via transformation. However, not all species with significant levels of recombination, such as *Escherichia coli*, are typically competent for transformation (7–10), and it is also possible that large pieces of DNA entering the cell via conjugation may be cleaved into smaller pieces before recombination occurs.

Regardless of the processes that make donor DNA available, it is clear that homologous recombination in bacteria is an important mechanism of genetic exchange. Like sex in eukaryotes, recombination blends genetic variation and as such acts as a cohesive process that can inhibit the subdivision of taxa (11), but when the donor DNA comes from a genetically differentiated population, recombination can dramatically increase genetic diversity. However, the critical issue raised by Smith et al. (2) of why the relative rate of recombination is so variable across taxa remains unresolved. This issue is unlikely to be resolved until we understand more about the benefits of homologous recombination.

The possible benefits remain largely speculative, since adaptive consequences of recombination between close relatives are difficult to detect. Adaptive shifts may be important in maintenance of transformation (12), and there is some evidence that homologous recombination may be important in promoting a number of adaptive responses. These include maintaining variability of surface proteins in animal pathogens to avoid host defenses (3, 13, 14), transfer of virulence genes in plant-pathogenic bacteria (15), and evolution of new taxa (16) by, for example, facilitating adaptation to novel hosts (17).

Homologous recombination can most easily be studied when genetic transfer has occurred between genetically distinct but closely related taxa. Under such circumstances, it may be possible to detect not only recombinant events but also sources of incorporated DNA and, potentially, to identify adaptive consequences of the exchange. This scenario is found in the plant-pathogenic bacterium *Xylella fastidiosa*, where intersubspecific homologous recombination (IHR) is well documented (18–23).

X. fastidiosa infects xylem vessels of a wide range of plant host species in the Americas (24, 25). *X. fastidiosa* has been divided into four subspecies, three of which are found in the United States (26, 27). These groups are genetically distinct, with values of DNA-DNA hybridization between them of less than 70% (27), sequence differences of 2% or more at synonymous sites (26), and distinct 16S rRNA gene and 16S-23S rRNA gene spacer sequences (28, 29). These differences reflect estimated divergence times of more than 15,000 years (21, 26). Furthermore, each subspecies has a distinct and largely nonoverlapping set of plant hosts (22): in the United

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States, *X. fastidiosa* subsp. *fastidiosa* causes Pierce's disease of grape, *X. fastidiosa* subsp. *sandyi* causes oleander leaf scorch, and *X. fastidiosa* subsp. *multiplex* causes leaf scorch disease on a range of trees, including oak, elm, and peach. In South America, *X. fastidiosa* subsp. *pauca* infects citrus and coffee (see reference 30).

X. fastidiosa is competent for transformation (31), and some isolates carry conjugative plasmids (32), so sympatry of subspecies potentially creates conditions conducive for both the occurrence and detection of IHR. Sympatry of *X. fastidiosa* subspecies appears to be relatively recent: while *X. fastidiosa* subsp. *multiplex* is probably native to the United States, there is compelling evidence that the other two subspecies found in the United States were introduced (20). *X. fastidiosa* subsp. *sandyi* has been known in the United States for only about 30 years, while *X. fastidiosa* subsp. *fastidiosa* has presumably been present since the first known outbreak of Pierce's disease ca. 130 years ago. Furthermore, it appears that a similar situation exists in South America. While *X. fastidiosa* subsp. *pauca* is native to South America, there is evidence of the introduction of a second subspecies into Argentina and/or Brazil causing plum leaf scald, first observed in 1935 (33).

Analysis of sequences indeed demonstrated large-scale recombination of *X. fastidiosa* subsp. *fastidiosa* sequences into *X. fastidiosa* subsp. *multiplex* in the United States (23), and, in Brazil, there has been substantial recombination into *X. fastidiosa* subsp. *pauca* of sequences from a distinct taxon, tentatively identified as *X. fastidiosa* subsp. *multiplex* (21). However, large-scale introgression is not the rule. Analyses of genomes of U.S. isolates of *X. fastidiosa* subsp. *fastidiosa* show very limited introgression of *X. fastidiosa* subsp. *multiplex* (20, 31); moreover, large-scale introgression into *X. fastidiosa* subsp. *multiplex* is restricted to a well-defined set of genotypes, suggesting that it may have been initiated by very few events (23). (A similar pattern may also be true of *X. fastidiosa* subsp. *pauca*; see reference 21.) The majority of *X. fastidiosa* subsp. *multiplex* isolates show little evidence of IHR, and the data available suggest that even intrasubspecific recombination is limited (22).

Thus, the picture emerging in *X. fastidiosa* is one of limited successful homologous recombination on a short time scale, with bursts of large-scale exchange occurring very infrequently. This raises the possibility that, by substantially increasing the available genetic variability, these large-scale events facilitate rapid evolutionary change that can result in colonization of new plant hosts. This scenario has been proposed as the mechanism underpinning the invasion of blueberry (and possibly blackberry) by recombinant forms of *X. fastidiosa* subsp. *multiplex* (23) and, more speculatively, the infection of citrus and coffee by *X. fastidiosa* subsp. *pauca* in Brazil (21).

Another candidate for this scenario is the form of *X. fastidiosa* infecting mulberry, a form that does not appear to fit within the framework of the four subspecies so far identified. Kostka et al. (34) first observed the disease of mulberry leaf scorch (MLS) in the Washington, DC, area on the native red mulberry (*Morus rubra*), and further study revealed infected trees along the east coast as far north as New York City, NY. Since that time, MLS has been observed in Nebraska (35) and also in California on the introduced white mulberry (*Morus alba*) (35). Previous genetic assessment showed that, although the 16S rRNA gene sequence of mulberry isolates is consistent with that of *X. fastidiosa* subsp. *fastidiosa*, based on analyses of randomly amplified polymorphic DNAs (RAPDs) and 16S-23S rRNA gene spacer sequences, these types cluster as a distinct group (29, 35, 35).

Here we used multilocus sequence typing (MLST) to evaluate the genetic relationship of the mulberry isolates to the 4 subspecies and to establish its hybrid ancestry via IHR, supporting the hypothesis that IHR facilitates host shifts. We show that this ancestry is shared with the recombinant group of *X. fastidiosa* subsp. *multiplex*; however, the recombinant group has largely introgressed into *X. fastidiosa* subsp. *multiplex*, and we propose that the continued genetic distinctiveness of the mulberry type merits recognition as a new subspecies, *X. fastidiosa* subsp. *morus*.

MATERIALS AND METHODS

Isolates used. The study examined DNA sequences from 20 isolates from mulberry, *Morus* spp. (16 sequenced in this study plus 4 sequenced independently [36]), plus 1 isolate from heavenly bamboo, *Nandina domestica*, known to be genetically similar (37). These 21 isolates, referred to as a group as the mulberry isolates, included examples from California, Kentucky, and Washington, DC (Table 1). They were all typed using the MLST scheme developed for *X. fastidiosa* and using 7 housekeeping loci (see reference 19 for details). A sequence from one additional locus, the cell surface gene *pilU*, was also obtained.

Genetic relationships of the isolates within *X. fastidiosa*. The DNA sequences were compared to previously published MLST and *pilU* data from 352 isolates of *X. fastidiosa*, defining 65 sequence types (STs), where an ST is a unique genotype based on the MLST, as follows: 110 isolates (17 STs) of *X. fastidiosa* subsp. *fastidiosa* (from Costa Rica as well as the United States) (19, 20), 21 isolates (1 ST) of *X. fastidiosa* subsp. *sandyi* (19), 143 isolates (32 STs) of *X. fastidiosa* subsp. *multiplex* (22, 23), and 78 isolates (5 STs) of *X. fastidiosa* subsp. *pauca* from Brazil (21).

To show the genetic relationship of the isolates from mulberry (and *Nandina*) to all previously published STs, we created a distance tree using a concatenation of the 8 sequenced loci for all of the known STs using the PHYLIP programs DNADIST and NEIGHBOR (38). In the analysis, the two known indels (6 bp and 30 bp) were given weights equivalent to 1 and 3 transversions, respectively. A distance tree was used since the IHR involved in the history of *X. fastidiosa* made a phylogenetic tree inappropriate; however, a maximum-likelihood phylogenetic tree (using the PHYLIP program DNAML) was evaluated for completeness and comparison.

Inferring the history of mulberry and recombinant-type isolates. Using maximum parsimony, we tested the hypothesis that the STs defined by the mulberry isolates were related to the STs of the recombinant-group *X. fastidiosa* subsp. *multiplex* (22) though an ancestral introgression of *X. fastidiosa* subsp. *multiplex* into *X. fastidiosa* subsp. *fastidiosa*, with subsequent divergence due to additional *X. fastidiosa* subsp. *multiplex* introgression. We created a maximum-parsimony tree using the PARS program in PHYLIP, with each allele as a character. Since our hypothesis emphasized a single ancestral *X. fastidiosa* subsp. *fastidiosa* strain, with potentially multiple *X. fastidiosa* subsp. *multiplex* donors, we weighted the loci that included alleles containing *X. fastidiosa* subsp. *fastidiosa* sequence more than those loci identified as having only *X. fastidiosa* subsp. *multiplex* sequence. We used a 2-fold weighting scheme; higher values produced identical results. The collection of equally parsimonious trees was reduced by applying the assumption that all nonrecombinant *X. fastidiosa* subsp. *fastidiosa* alleles absent from the current U.S. population of *X. fastidiosa* subsp. *fastidiosa* had been vertically transmitted through the tree.

We evaluated the origins of all alleles found in the mulberry isolates using two tests. For chimeric alleles, we tested for recombination breakpoints using the targeted introgression test (23). In most cases, the alleles were not chimeric and their ancestry was obvious; however, in each case a ratio test was used to support this conclusion (23).

TABLE 1 *Xylella fastidiosa* mulberry-type isolates used in this study

Identifier	ST	Alias	Host of origin	Host location[a] (city, county, state or district)	Reference or source
NAN0033	ST30	NI065	Nandina (*Nandina domestica*)	Redlands, San Bernardino County, CA	37
MUL0034	ST30	MLS059	White mulberry (*Morus alba*)	Redlands, San Bernardino County, CA	39
MUL0035	ST30	MLS012	White mulberry	Redlands, San Bernardino County, CA	39
MUL0084	ST29	ATCC 35868[b]	Red mulberry (*Morus rubra*)	Washington, DC	42
MUL0087	ST29	ATCC 35869	Red mulberry	Washington, DC	42
MUL0089	ST31		White mulberry	Riverside, Riverside County, CA	This study
MUL0117	ST29	33BAT	White mulberry	Lexington, Fayette County, KY	J. Hartman[c]
MUL0147	ST29	FK-51	Red mulberry	Washington, DC	34
MUL0148	ST29	FK-79	Red mulberry	Washington, DC	34
MUL0182	ST29	113BA	White mulberry	Louisville, Jefferson County, KY	J. Hartman
MUL0191	ST30	MLS024	White mulberry	Riverside, Riverside County, CA	39
MUL0192	ST30	MLS063	White mulberry	Redlands, San Bernardino County, CA	39
MUL0232	ST31		White mulberry	Riverside, Riverside County, CA	This study
MUL0267	ST31		White mulberry	Riverside, Riverside County, CA	This study
MUL0436	ST31		White mulberry	Riverside, Riverside County, CA	This study
MUL0451	ST31		White mulberry	Riverside, Riverside County, CA	This study
MUL0454	ST31		White mulberry	Riverside, Riverside County, CA	This study
MUL0504	ST62	Riv11	White mulberry	Riverside, Riverside County, CA	36
MUL0505	ST62	Riv16	White mulberry	Riverside, Riverside County, CA	36
MUL0506	ST62	Riv19	White mulberry	Riverside, Riverside County, CA	36
MUL0507	ST62	Riv25	White mulberry	Riverside, Riverside County, CA	36

[a] Complete location information is provided where available.
[b] ATCC, American Type Culture Collection (Manassas, VA).
[c] John Hartman, Department of Plant Pathology, University of Kentucky.

Nucleotide sequence accession numbers. The MLST data are available at the MLST website (http://pubmlst.org), where the 4 new mulberry-type STs have been added. The gene sequences for the new MLST alleles analyzed in this study are available at GenBank under the following accession numbers: for *petC* allele 5, FJ610170; for *MalF* allele 6, FJ610177; for *cysG* allele 8, FJ610186; for *holC* allele 5, FJ610199; for *holC* allele 8, FJ610203; and for *nuoL* allele 6, FJ610212.

RESULTS AND DISCUSSION

Genetic composition of the mulberry type. MLST of the 21 "mulberry" isolates (20 from mulberry plus 1 from heavenly bamboo) defined 4 STs, and all isolates carried allele 1 at the non-MLST *pilU* locus (Table 2). No indels were present in any of the six new alleles found in these isolates. The 4 STs were very closely related, with only 4 variable nucleotide sites (of 4,161 bp of the MLST plus 545 bp from *pilU*, i.e., 0.08% of sites). All of the isolates from the eastern United States (4 from the native species *M. rubra* in Washington, DC, and 2 from the introduced species *M. alba* in Kentucky) had the same genotype (ST29), while ST30, ST31, and ST62 defined 5, 6, and 4 of the California samples, respectively, with all from *M. alba* except 1 (ST30) from heavenly bamboo (*N. domestica*). ST62 differed from ST31 by one base and from the eastern ST29 by one base; however, ST30 more closely resembled the eastern ST29 (2 bases different) than ST62 and ST31 (3 and 4 bases different). All 4 of the base pair differences separating the 4 STs were nonsynonymous, so it was not possible to estimate the age of their clade using the method of Schuenzel et al. (26), which is based on the number of synonymous changes.

The STs of the mulberry isolates differed from the next most similar ST, the recombinant-group *X. fastidiosa* subsp. *multiplex* ST28, by 0.42% (20 of 4,706 bp), while the closest nonrecombinant ST was ST18, an *X. fastidiosa* subsp. *fastidiosa* form from Costa Rica that differed by 0.77% (36 bp). The distance tree

TABLE 2 The MLST sequence types (STs) of the mulberry-type *X. fastidiosa* isolates, showing the alleles at each locus and their phylogenetic origins[a]

ST	Allele no. for each MLST locus								State or city location(s)
	leuA	*petC*	*malF*	*cysG*	*holC*	*nuoL*	*gltT*	*pilU*	
ST29	4 (fas-rg[b])	3 (mult)	6 (fas-u)	18 (rec-rg[c])	5 (fas-u)	4 (mult-rg[d])	3 (mult)	1 (fas)	KY, Washington, DC
ST30	4 (fas-rg)	**5** (mult-u)	6 (fas-u)	**8** (rec-u)	5 (fas-u)	4 (mult-rg)	3 (mult)	1 (fas)	CA
ST31	4 (fas-rg)	3 (mult)	6 (fas-u)	18 (rec-rg)	**8** (fas-u)	**6** (mult-u)	3 (mult)	1 (fas)	CA
ST62	4 (fas-rg)	3 (mult)	6 (fas-u)	18 (rec-rg)	5 (fas-u)	**6** (mult-u)	3 (mult)	1 (fas)	CA

[a] Bolded allele numbers define variant alleles within the mulberry type that in each case are derived from the alternate allele via a single base change. Origins: fas = *X. fastidiosa* subsp. *fastidiosa*; mult = *X. fastidiosa* subsp. *multiplex*; rec = a chimeric allele with an identified recombination breakpoint (see Table 3). An added "-u" (unique) indicates that the allele has only been found in the mulberry-type STs, while "-rg" (recombinant group) indicates that the allele has also been found in at least one recombinant-group *X. fastidiosa* subsp. *multiplex* ST (as defined in the remaining footnotes). The recombinant-group alleles are described in reference 23.
[b] Also found in ST32.
[c] Also found in ST28, ST40, and ST43.
[d] Also found in ST28 and ST32.

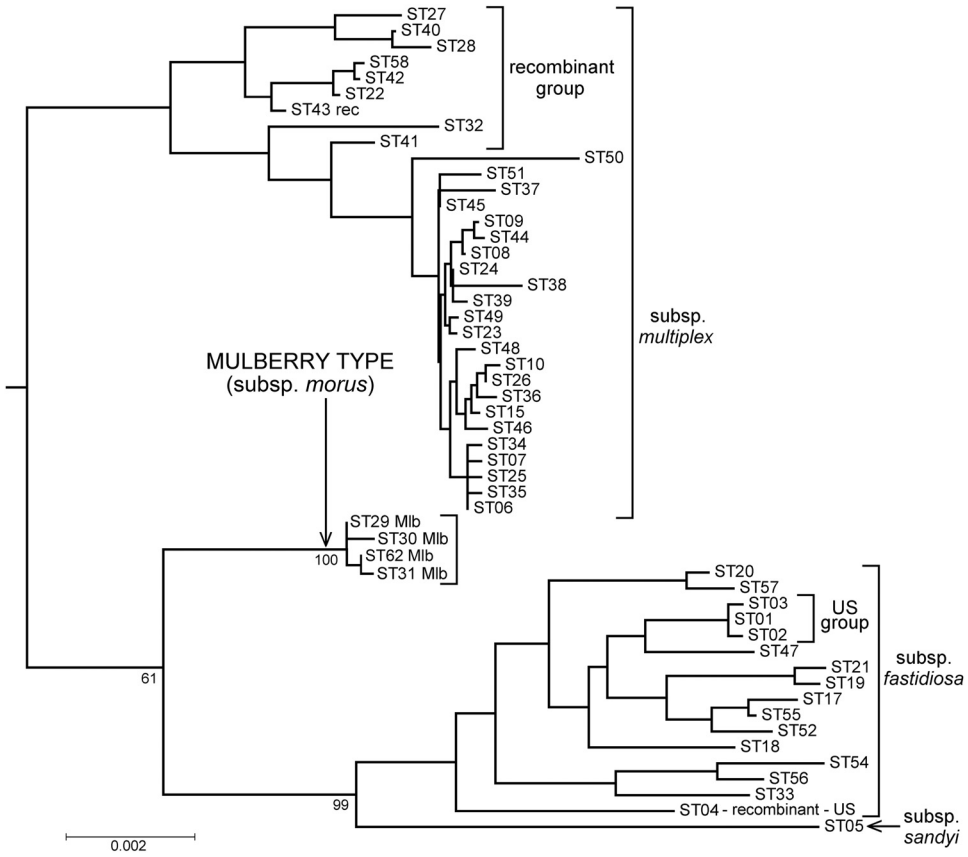

FIG 1 Distance tree showing the relationship of the mulberry-type sequence types (STs) to the three *X. fastidiosa* subspecies found in the United States based on a sequence from the 7 MLST loci plus *pilU*. Most STs of *X. fastidiosa* subsp. *fastidiosa* are from Central America, and those found in the United States are indicated. Bootstrap values at the important nodes neighboring the mulberry type are shown, with values in parentheses derived from the equivalent maximum-likelihood tree. The tree was rooted by the more distantly related *X. fastidiosa* subsp. *pauca* (isolates from Brazil).

(Fig. 1) shows that the mulberry isolates formed a group that is genetically distinct from all other known *X. fastidiosa* types, with 100% bootstrap support despite a geographical range spanning the United States from east to west. A maximum-likelihood tree revealed the same pattern (data not shown).

X. fastidiosa is currently divided into 4 subspecies (26, 27); 3 are shown in Fig. 1, while the fourth was used as an outgroup to root the tree shown, and none of these subspecies group closely with the isolates from mulberry. Thus, despite extensive sampling of a wide range of plant hosts across the United States, no other genetically similar isolates have been found, so there is no basis for classifying the mulberry isolates with any of the 4 preexisting subspecies. For this reason, we propose identifying this group as a new subspecies, *X. fastidiosa* subsp. *morus*, with the type defined by ST29 isolate MUL0084 (see Table 1). Chen et al. (35) and Hernandez-Martinez et al. (39) previously showed that the mulberry form of *X. fastidiosa* is genetically distinct from the recognized subspecific groupings using RAPDs, and our analysis strongly supports this observation.

Each of the preexisting subspecies of *X. fastidiosa* appears to have developed in geographical isolation, *X. fastidiosa* subsp. *multiplex* in North America, *X. fastidiosa* subsp. *fastidiosa* in Central America, and *X. fastidiosa* subsp. *pauca* in South America, with only the geographical origin of *X. fastidiosa* subsp. *sandyi* being unknown (20, 21). However, a detailed gene-by-gene analysis of *X. fastidiosa* subsp. *morus* demonstrated that it does not show this same pattern of phylogeographical divergence. Instead, it is clear that the origin of the group was via large-scale IHR (Table 3). All alleles at 4 loci (*leuA*, *malF*, *holC*, and *pilU*) were classified as *X. fastidiosa* subsp. *fastidiosa* alleles, and all alleles at 3 loci (*petC*, *nuoL*, and *gltT*) were classified as *X. fastidiosa* subsp. *multiplex* alleles, while the two alleles at the *cysG* locus were chimeric, having *X. fastidiosa* subsp. *multiplex* sequence at the 5′ end and *X. fastidiosa* subsp. *fastidiosa* sequence at the 3′ end. For all 8 loci, alternate classifications could be statistically rejected (Table 3).

The two chimeric *cysG* alleles had the same recombination breakpoint. Using *X. fastidiosa* subsp. *fastidiosa cysG* allele 14 as the reference allele (since it is the most similar allele over the whole length of the *cysG* sequence), a breakpoint between positions 48 and 258 was identified; however, using allele 12 narrows the range slightly to positions 71 to 258 (Table 3).

The lack of genetic diversity within *X. fastidiosa* subsp. *morus* suggests that it may be a relatively young subspecies. Consistent with this, mulberry leaf scorch was first recognized in the eastern United States in the 1980s (34) and was detected in the western United States around 2000 (39), although the disease is likely to have existed undetected for a long period prior to the 1980s. Also consistent with this view is that, although half of the 12 alleles found in the mulberry types are unique to this form of *X. fastidiosa*, all of these unique alleles are only one base pair away from

TABLE 3 Analysis of the subspecific origins of all alleles found in mulberry-type isolates, assigning them to *X. fastidiosa* subsp. *fastidiosa* or to *X. fastidiosa* subsp. *multiplex* or, given an identified recombination breakpoint, to both

Allele	No. of nucleotide differences from[a]:		Allele type(s)[c]	Total length	Ratio(s)[d]	*P*[e]
	X. fastidiosa subsp. *multiplex* allele no.:	*X. fastidiosa* subsp. *fastidiosa* U.S. allele no. (CR allele no.)[b]:				
leuA 4	3 = 8	1 = 3 (9 = 2)	FAS (rg)	708	8:2	<0.001
petC 3	3 = 0	1 = 5	MULT	533	0:5	0.002
petC 5	3 = 1	1 = 6	MULT (u)		1:6	0.005
malF 6	13 = 8	1 = 3 (10 = 1)	FAS (u)	730	8:2	<0.001
cysG 8	5 = 9	1 = 7 (14 = 4); 1 = 4 (14/12 = 1)	MULT (48/71–258); FAS (u)	600	0:3 vs 9:1	0.028
cysG 18	5 = 8	1 = 6 (14 = 3); 1 = 3 (14/12 = 0)	MULT (48/71–258); FAS (rg)		0:3 vs 8:0	0.012
holC 5	9 = 8	1 = 8 (19 = 1)	FAS (u)	379	8:1	<0.001
holC 8	3 = 9	1 = 9 (19 = 2)	FAS (u)		9:2	<0.001
nuoL 4	3 = 3	5 = 6	MULT (rg)	557	3:6	0.05
nuoL 6	3 = 4	5 = 7	MULT (u)		4:7	0.05
gltT 3	3 = 0	11 = 8	MULT	654	0:8	<0.001
pilU 1	3 = 17	1 = 0 (1 = 0)	FAS	545	17:0	<0.001

[a] Data represent the number of base pair differences between the mulberry-type allele and the most similar allele from non-IHR *X. fastidiosa* subsp. *multiplex* and from *X. fastidiosa* subsp. *fastidiosa*. Data are shown as follows: identification number of the most similar allele = number of base pair differences.

[b] In loci where any *X. fastidiosa* subsp. *fastidiosa* (FAS) sequence was detected, the distance from the closest *X. fastidiosa* subsp. *fastidiosa* allele found in Costa Rica is given in parentheses. In the case of a chimeric allele, these distances are also given (in square brackets) for the inferred *X. fastidiosa* subsp. *fastidiosa* portion of the allele.

[c] The inferred ancestor, *X. fastidiosa* subsp. *fastidiosa* or *X. fastidiosa* subsp. *multiplex* (MULT), is indicated with additional information as defined in Table 2. In chimeric alleles, the range containing the breakpoint is indicated in parentheses, with the variation that resulted from using two different reference alleles indicated by a slash (/).

[d] Ratios are expressed as follows: differences from the most similar *X. fastidiosa* subsp. *multiplex* allele/differences from the most similar *X. fastidiosa* subsp. *fastidiosa* allele (see footnote *a*). Data are expressed 5′ versus 3′ for cases in which there is a suspected breakpoint.

[e] Data corresponding to the origins of alleles were considered to be statistically significant if $P \leq 0.05$, based on the targeted introgression test (suspected chimeric alleles) or a ratio test (nonchimeric alelles), as described in reference 23. The ratio test was based on a comparison of the observed ratios of differences from 11:6 (presumed *X. fastidiosa* subsp. *multiplex* sequence testing the null hypothesis that it could be an *X. fastidiosa* subsp. *fastidiosa* sequence) or from 1:11 (presumed *X. fastidiosa* subsp. *fastidiosa* sequence testing the null hypothesis that it could be an *X. fastidiosa* subsp. *multiplex* sequence). These test ratios reflected the estimated pairwise mismatch of $\pi_{fas} = 0.006$, $\pi_{mult} = 0.001$, and $\pi_{total} = 0.011$ (see Materials and Methods). Analysis of the recombinant-group (rg) alleles was previously published in reference 23.

another known allele. In 4 of these (*petC* allele 5, *cysG* allele 8, *holC* allele 8, and *nuoL* allele 6), the difference is due to a derived base change seen in no other allele, suggesting that the mulberry types have been isolated only long enough to accumulate a few point mutations.

Linking the mulberry type and the recombinant group of *X. fastidiosa* subsp. *multiplex*. Three of the mulberry-type alleles (*leuA* allele 4, *cysG* allele 18, and *nuoL* allele 4) have been previously observed in the recombinant group of *X. fastidiosa* subsp. *multiplex* but nowhere else (see Table 2). The recombinant group is a set of STs that are genetically similar to the rest of *X. fastidiosa* subsp. *multiplex* (see Fig. 1), but they carry alleles that include some *X. fastidiosa* subsp. *fastidiosa* sequence (23). All recombinant-group STs have *X. fastidiosa* subsp. *multiplex* sequence at 4 loci (*petC*, *malF*, *nuoL*, and *gltT*) but have some recognizable *X. fastidiosa* subsp. *fastidiosa* sequence in 1 to 3 of the remaining 4 loci, *leuA*, *cysG*, *holC*, and *pilU*. Notably, all *X. fastidiosa* subsp. *morus* isolates carry *X. fastidiosa* subsp. *fastidiosa* sequence at these same 4 loci plus *malF* (Table 2).

A defining feature of the recombinant-group STs is that some (and perhaps all) of their *X. fastidiosa* subsp. *fastidiosa* sequence did not originate from the *X. fastidiosa* subsp. *fastidiosa* strains currently found in the United States. Instead, the sequence appears to be derived from *X. fastidiosa* subsp. *fastidiosa* variants found in Central America (23). The mulberry types show this same characteristic (Table 3). Of the five loci in mulberry-type STs that include *X. fastidiosa* subsp. *fastidiosa* sequence, four show a closer relationship to Costa Rica sequence than to any allelic sequence found in the United States, while there is no difference at *pilU*, since the most similar allele (*pilU* allele 1) is found in both locations (20). For *leuA* allele 4 and *cysG* allele 18 (also found in the recombinant group), the Costa Rica *X. fastidiosa* subsp. *fastidiosa* alleles provide a fit that is total of 4 bp better than that seen with alleles found in the United States (Table 3). Even more compelling is the example of *holC* allele 5, where the difference is 7 bp; the U.S. allele is 8 bp different, while the best-fit Costa Rica allele is only 1 bp different (Table 4).

Further tests of this ancestry are provided by variation at *holC* and *malF*. First, *holC* allele 5 and the allele derived from it by one base change (*holC* allele 8) are unique to the mulberry isolates. However, *holC* allele 5 appears to be the template from which recombinant-group *holC* allele 7 and allele 9 originated via recombination, as can be seen by noting the correspondence of their differing lightface data in Table 4 with those of *holC* 5. Second, in the recombinant group, *malF* is invariant for a widespread *X. fastidiosa* subsp. *multiplex* allele (*malF* allele 5), but the mulberry types are invariant for a unique *X. fastidiosa* subsp. *fastidiosa* allele (*malF* allele 6). This provides a new test of the link of the *X. fastidiosa* subsp. *fastidiosa* to Central American rather than U.S. sequence. Consistent with expectations, *malF* allele 6 is only 1 bp distant from Costa Rica *malF* allele 19 but is 3 bp distant from the most similar U.S. allele (Table 5). Adding up these differences for the most basal allele found in the mulberry type at each of the 5 loci reveals only 4-bp differences from Costa Rica alleles, whereas the differences from the U.S. *X. fastidiosa* subsp. *fastidiosa* alleles total 17 bp.

In summary, *X. fastidiosa* subsp. *morus* is similar to the recombinant group of *X. fastidiosa* subsp. *multiplex* in (i) the loci carry-

TABLE 4 Origins and interrelationships of the *holC* alleles found in the mulberry-type and recombinant-group isolates[a]

	Position in *holC* MLST sequence																
	0	0	0	0	0	0	0	0	0	0	1	1	1	2	3	3	3
	0	1	2	5	7	7	8	8	8	8	1	7	8	8	1	2	7
holC allele	9	8	7	6	7	8	3	6	7	9	0	6	2	7	7	3	2
Recomb. grp/*X. fastidiosa* subsp. *multiplex* 4	T	C	G	A	C	C	G	C	G*	G	A	A	C	C	T	C*	A*
Recomb. grp 7	T	C	G	G	T	A	A	T	A	G	G	A	T	C	C*	C*	A*
Recomb. grp 9	T	C	G	A	C	C	G	C	G*	G	A	A	C	C	T	T	G
Mulberry 8	T	A*	G	G	T	A	A	T	A	G	G	A	T	C	T	T	G
Mulberry 5	T	C	G	G	T	A	A	T	A	G	G	A	T	C	T	T	G
X. fastidiosa subsp. *multiplex* 3/6	T	C	G	A	C	C	G	C	G	G	A	A	C/T	C	C/T	C	A
X. fastidiosa subsp. *fastidiosa* 19	T	C	G	G	T	A	A	T	A	G	G	A	T	T	T	T	G
X. fastidiosa subsp. *fastidiosa* 1	G	C	G	A	C	C	G	C	A	A	G	A	T	T	T	T	G
X. fastidiosa subsp. *sandyi* 2	T	C	A	G	T	A	A	T	A	G	G	G	C	T	T	C	G

[a] The alleles are compared to the most similar *X. fastidiosa* subsp. *multiplex* alleles (which are indicated by shading), to the most similar *X. fastidiosa* subsp. *fastidiosa* allele found in the United States (allele 1), to the *X. fastidiosa* subsp. *sandyi* allele (also from the United States), and to the most similar *X. fastidiosa* subsp. *fastidiosa* allele found in Costa Rica (CR) but not in the United States (allele 19). Unshaded bases define sites different from those of the *X. fastidiosa* subsp. *multiplex* allele. Bases marked with an asterisk (*) in the mulberry-type and recombinant-group (recomb. grp) sequences are inconsistent with *X. fastidiosa* subsp. *fastidiosa* sequence, while all others are potentially consistent when all published *X. fastidiosa* subsp. *fastidiosa* is considered. Underlined bases are unique to *X. fastidiosa* subsp. *fastidiosa* and/or *X. fastidiosa* subsp. *sandyi*.

ing *X. fastidiosa* subsp. *fastidiosa* sequence, (ii) the alleles occurring at those loci, and (iii) the close relationship of the alleles to *X. fastidiosa* subsp. *fastidiosa* sequence found only in Central America. This high level of similarity suggests that the two groups share an origin. Previously, Nunney et al. (23) proposed that the recombinant group originated by a transfer of DNA from an *X. fastidiosa* subsp. *fastidiosa* donor to an *X. fastidiosa* subsp. *multiplex* recipient. The direction of the transfer was assumed on the basis of the close relationship of the recombinant group to the rest of *X. fastidiosa* subsp. *multiplex* (see Fig. 1). The sequence data from *X. fastidiosa* subsp. *morus* bring this assumption of direction into question. Instead, it is probable that the common origin involved introgression of *X. fastidiosa* subsp. *multiplex* into a unique *X. fastidiosa* subsp. *fastidiosa* strain that had been introduced into the United States from Central America. Under this hypothesis, *X. fastidiosa* subsp. *morus* is the relatively unaltered descendant of the ancestral hybrid, while repeated introgression from *X. fastidiosa* subsp. *multiplex* gave rise to the recombinant group of *X. fastidiosa* subsp. *multiplex*.

We examined the plausibility of this hypothesis through parsimony analysis, using alleles as characters, and the result is broadly supportive of the idea of a common origin of the mulberry-type and recombinant-group STs (Fig. 2). The initial analysis produced 7 equally parsimonious trees, but by assuming that the four *X. fastidiosa* subsp. *fastidiosa* alleles inconsistent with the current U.S. strains (*leuA* allele 4, *malF* allele 6, *cysG* allele 12, and *holC* allele 5) were themselves ancestral (as required under the hypothesis), the total was reduced to 2 trees. The only difference between these two trees involved the position of the clade corresponding to ST27, ST28, and ST40. In one tree (as shown in Fig. 2), the required recombination transfer of *cysG* allele 18 is minimized to one event (rather than two); alternatively, in the second tree, the clade branches from the ST58 lineage, which removes the necessity of a recombination transfer of *leuA* allele 6 (or its unlikely

TABLE 5 Origins and interrelationships of the *malF* alleles found in the mulberry-type and recombinant-group isolates[a]

	Position in *malF* MLST sequence																						
	0	0	0	0	0	2	2	3	3	3	3	3	4	4	4	4	4	4	4	5	5	5	5	6
	0	3	4	5	9	0	1	0	1	6	8	9	1	2	3	5	8	8	9	0	1	6	7	0
malF allele	3	8	4	6	9	6	3	6	6	4	1	3	3	4	6	6	1	6	8	4	3	1	3	0
Recomb. grp/*X. fastidiosa* subsp. *multiplex* 5	G	C	T	G*	C	C	G*	T	T	C	T	T	G	T*	T	C	T*	A*	G	A	G	A*	G	G
Mulberry 6	G	C	C	C	T	T	T	A	C	C	T	T	G	C	T	C	C	C	G	A	G	A*	G	G
X. fastidiosa subsp. *multiplex* 13/3	G	C/G	T	C/G	C	C	G	T	T	C	T	T	G	T	T	C	T	C/A	G	A	G	A	G	G
X. fastidiosa subsp. *fastidiosa* 10	G	C	C	C	T	T	T	A	C	C	T	T	G	C	T	C	C	C	G	A	G	G	G	G
X. fastidiosa subsp. *fastidiosa* 1	T	C	C	C	T	T	T	A	C	C	T	T	G	C	T	C	C	C	G	A	G	G	T	G
X. fastidiosa subsp. *sandyi* 2	G	C	T	C	C	C	T	A	C	T	C	C	A	C	C	T	T	C	A	G	A	G	G	A

[a] The alleles are compared to the most similar *X. fastidiosa* subsp. *multiplex* alleles (which are indicated by shading), to the most similar *X. fastidiosa* subsp. *fastidiosa* allele found in the United States (allele 1), to the *X. fastidiosa* subsp. *sandyi* allele (also from the United States), and to the most similar *X. fastidiosa* subsp. *fastidiosa* allele found in Costa Rica (CR) but not in the United States (allele 10). Unshaded bases define sites different from those of the *X. fastidiosa* subsp. *multiplex* allele. Bases marked with an asterisk (*) in the mulberry-type and recombinant-group (recomb. grp) sequences are inconsistent with *X. fastidiosa* subsp. *fastidiosa* sequence, while all others are consistent. Underlined bases are unique to *X. fastidiosa* subsp. *fastidiosa* and/or *X. fastidiosa* subsp. *sandyi*.

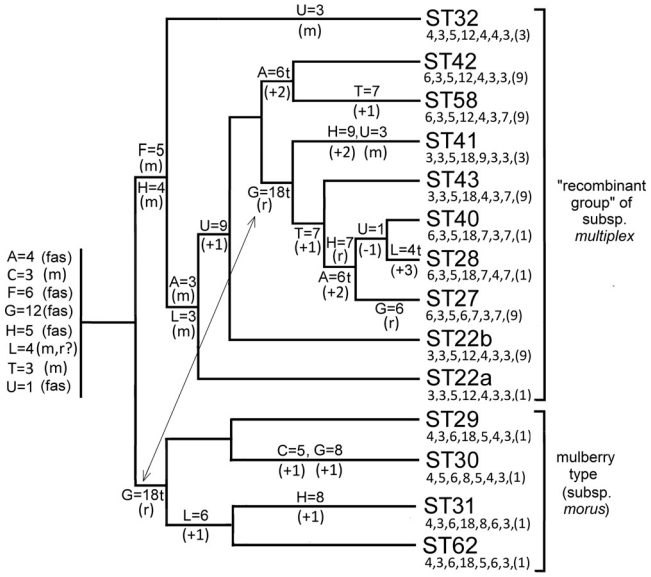

FIG 2 A maximum-parsimony tree of the mulberry-type STs and the STs making up the "recombinant group" of the subspecies multiplex (23). The diagonal line shows an inconsistency at *cysG*, which could be resolved only by recombination transfer (*t*) of the allele. Similar inconsistencies are apparent for A=6 and L=4. The gene names A, C, F, G, H, L, T, and U refer to the MLST loci *leuA*, *petC*, *malF*, *cysG*, *holC*, *nuoL*, and *gltT* and the non-MLST *pilU*. The numbers under each ST represent its defining alleles (plus *pilU* added parenthetically). Within the tree, allele numbers following a gene name show substitutions with an indication of their genesis: additional recombination with *X. fastidiosa* subsp. *multiplex* is indicated by *m* (insertion of a complete *X. fastidiosa* subsp. *multiplex* allele) or by *r* (the creation of a novel chimeric allele) and *i* base pair substitutions are indicated by $+i$ (with -1 indicating a possible reversion). The inferred basal genotype is also shown, indicating 5 loci from *X. fastidiosa* subsp. *fastidiosa* (*fas*).

second origination), which is an *X. fastidiosa* subsp. *multiplex* allele unique to the recombinant group.

Consistent with the common-origin hypothesis, parsimony requires very little postorigin modification within *X. fastidiosa* subsp. *morus*. Specifically, it requires only the basal acquisition of *cysG* allele 18, derived by recombination from presumed ancestral *cysG* allele 12 (Fig. 2). All other allelic changes are single base substitutions. The genesis of the recombinant group is more complex, consistent with the conclusions of Nunney et al. (23) and with the assumption of a history of continued introgression. Thus, the data suggest that the recombinant group has undergone sufficient additional recombination with *X. fastidiosa* subsp. *multiplex* that it has ceased to be a separate taxon. On the other hand, the mulberry isolates show no evidence of such introgression and thus have remained a distinct taxon meriting subspecific status. To this point, there is no evidence of intermediate genotypes that bridge the genetic space that now exists between the recombinant group and *X. fastidiosa* subsp. *morus* (see Fig. 1).

Nunney et al. (23) previously proposed that IHR-generated genetic variation facilitated invasion of new hosts, based on the observation that all isolates from blueberry (and perhaps blackberry, but the sample size was limited) were recombinant-group *X. fastidiosa* subsp. *multiplex*. This hypothesis is further supported by the invasion of mulberry by the chimeric *X. fastidiosa* subsp. *morus*. These examples raise three additional points of support. First, given the long-term geographical association of the native *X. fastidiosa* subsp. *multiplex* with these 3 native host plants, the failure to infect them suggests that the genetic variation required for successful invasion had been absent from the native subspecies. Second, contact of these plant hosts with two newly introduced subspecies (*X. fastidiosa* subsp. *fastidiosa* and *X. fastidiosa* subsp. *sandyi*) has failed to lead to infection of these plants; in all known cases of natural infection, these hosts were infected only by STs that had undergone large-scale IHR. Third, in each case, the STs found on these hosts show very little variation: blackberry, 1 ST; blueberry, 2 STs; and mulberry, 4 STs. This lack of within-host variation is consistent with host plants imposing strong host-specific selection on the bacterial genome. The data also suggest that host specificity is not determined by the lateral gene transfer of novel genetic material, since this would not impose the observed constraint on the genome. In addition, a similar pattern has been found in *X. fastidiosa* subsp. *pauca* in Brazil (21): evidence of large-scale IHR, combined with very limited genetic variation. From a sample of 55 citrus and 23 coffee isolates, only five STs were observed, with 85% of the citrus isolates having the same ST.

The data from *X. fastidiosa* show that massive recombination can occur between subspecies. We see this in the creation of *X. fastidiosa* subsp. *morus*, and a similar event may have been involved in the genesis of the *X. fastidiosa* subsp. *pauca* strain that infects citrus and coffee in South America (21). But how did this happen? It has been established that conjugative plasmids can occur in *X. fastidiosa* (32), including a candidate found in the mulberry type (36). Furthermore, high rates of transformation have been observed in the laboratory (31, 40). Which of these processes is involved in large-scale genomic exchange is not known.

These data raise a second issue: how, given the clear potential for genetic exchange, *X. fastidiosa* subsp. *morus* and also the ancestral *X. fastidiosa* subsp. *multiplex* and *X. fastidiosa* subsp. *fastidiosa* strains have not introgressed into an ill-defined network of isolates. There are two main, nonexclusive hypotheses that might explain how these taxa have remained distinct: "opportunity" and "host selection." The opportunity hypothesis is based on the distinct and almost completely nonoverlapping range of plant hosts of the subspecies (22), which could severely limit contact between them and hence limit opportunity for IHR. This hypothesis is strengthened if it could be established that genetic exchange typically occurs in the plant host. On the other hand, the opportunity hypothesis would be weakened if genetic exchange typically occurs in the insect vector, since different subspecies can colonize the same insect (e.g., the glassy-winged sharpshooter; see reference 41).

The host selection hypothesis proposes that different plant hosts impose strong host-specific selection such that, even if IHR occurs relatively frequently, most of the bacteria resulting from such exchange are maladapted and do not survive. Even moderate levels of recombination would be expected to generate high levels of genetic variability; however, very little genetic variability (0.08% site polymorphism) was observed within the mulberry type despite evidence of large-scale IHR and a broad geographical occurrence within the United States. This near monomorphism of the mulberry-type isolates suggests that plant host specialization places severe constraints on the genome; i.e., the shift to the new host seems to have eliminated all but a narrowly defined set of genotypes. If the host shift had been due to some other genetic change, such as the acquisition of new extrachromosomal genes,

then these genes would be expected to be seen in a number of different genetic backgrounds, which they are not.

Thus, in summary, *X. fastidiosa* subsp. *morus* provides an important example for understanding the role of homologous recombination in bacterial adaptive evolution. We have been able to associate a clear ecological shift with a high level of recombination. But we are left with a puzzle. The data are consistent with *X. fastidiosa* subsp. *morus* and the recombinant-group *X. fastidiosa* subsp. *multiplex* originating from a single large-scale IHR, with no unambiguous evidence of any similar events involving the strains of *X. fastidiosa* subsp. *fastidiosa* currently found in the United States. Was this initial event a conjugation, followed by DNA fragmentation within the bacterial cell which resulted in large-scale recombination, or was it associated with a period during which conditions promoted a high rate of transformation, conditions that no longer prevail or occur only rarely? At present, it is far from clear if one or both of these possibilities could account for the pattern of evolution illustrated in Fig. 2.

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REFERENCES

1. **Smith JM, Dowson CG, Spratt BG.** 1991. Localized sex in bacteria. Nature **349:**29–31. http://dx.doi.org/10.1038/349029a0.
2. **Smith JM, Smith NH, O'Rourke M, Spratt BG.** 1993. How clonal are bacteria. Proc. Natl. Acad. Sci. U. S. A. **90:**4384–4388. http://dx.doi.org/10.1073/pnas.90.10.4384.
3. **Feil EJ, Spratt BG.** 2001. Recombination and the population structures of bacterial pathogens. Annu. Rev. Microbiol. **55:**561–590. http://dx.doi.org/10.1146/annurev.micro.55.1.561.
4. **Hanage WP, Fraser C, Spratt BG.** 2006. Sequences, sequence clusters and bacterial species. Philos. Trans. R. Soc. Lond. B Biol. Sci. **361:**1917–1927. http://dx.doi.org/10.1098/rstb.2006.1917.
5. **Didelot X, Maiden MCJ.** 2010. Impact of recombination on bacterial evolution. Trends Microbiol. **18:**315–322. http://dx.doi.org/10.1016/j.tim.2010.04.002.
6. **Falush D, Kraft C, Taylor NS, Correa P, Fox JG, Achtman M, Suerbaum S.** 2001. Recombination and mutation during long-term gastric colonization by *Helicobacter pylori*: estimates of clock rates, recombination size, and minimal age. Proc. Natl. Acad. Sci. U. S. A. **98:**15056–15061. http://dx.doi.org/10.1073/pnas.251396098.
7. **Mau B, Glasner JD, Darling AE, Perna NT.** 31 May 2006. Genome-wide detection and analysis of homologous recombination among sequenced strains of *Escherichia coli*. Genome Biol. **7:**R44. http://dx.doi.org/10.1186/gb-2006-7-5-r44.
8. **Wirth T, Falush D, Lan RT, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MCJ, Ochman H, Achtman M.** 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol. Microbiol. **60:**1136–1151. http://dx.doi.org/10.1111/j.1365-2958.2006.05172.x.
9. **Guttman DS, Dykhuizen DE.** 1994. Clonal divergence in *Escherichia coli* as a result of recombination, not mutation. Science **266:**1380–1383. http://dx.doi.org/10.1126/science.7973728.
10. **Baur B, Hanselmann K, Schlimme W, Jenni B.** 1996. Genetic transformation in freshwater: *Escherichia coli* is able to develop natural competence. Appl. Environ. Microbiol. **62:**3673–3678.
11. **Bailly X, Olivieri I, Brunel B, Cleyet-Marel J-C, Bena G.** 2007. Horizontal gene transfer and homologous recombination drive the evolution of the nitrogen-fixing symbionts of *Medicago* species. J. Bacteriol. **189:**5223–5236. http://dx.doi.org/10.1128/JB.00105-07.
12. **Levin BR, Bergstrom CT.** 2000. Bacteria are different: observations, interpretations, speculations, and opinions about the mechanisms of adaptive evolution in prokaryotes. Proc. Natl. Acad. Sci. U. S. A. **97:**6981–6985. http://dx.doi.org/10.1073/pnas.97.13.6981.
13. **Nelson K, Selander RK.** 1994. Intergeneric transfer and recombination of the 6-phosphogluconate dehydrogenase gene (*gnd*) in enteric bacteria. Proc. Natl. Acad. Sci. U. S. A. **91:**10227–10231. http://dx.doi.org/10.1073/pnas.91.21.10227.
14. **Haake DA, Suchard MA, Kelley MM, Dundoo M, Alt DP, Zuerner RL.** 2004. Molecular evolution and mosaicism of leptospiral outer membrane proteins involves horizontal DNA transfer. J. Bacteriol. **186:**2818–2828. http://dx.doi.org/10.1128/JB.186.9.2818-2828.2004.
15. **Yan SC, Liu HJ, Mohr TJ, Jenrette J, Chiodini R, Zaccardelli M, Setubal JC, Vinatzer BA.** 2008. Role of recombination in the evolution of the model plant pathogen *Pseudomonas syringae* pv. tomato DC3000, a very atypical tomato strain. Appl. Environ. Microbiol. **74:**3171–3181. http://dx.doi.org/10.1128/AEM.00180-08.
16. **Guttman DS.** 1997. Recombination and clonality in natural populations of *Escherichia coli*. Trends Ecol. Evol. **12:**16–22. http://dx.doi.org/10.1016/S0169-5347(96)10057-4.
17. **Baldo L, Bordenstein S, Wernegreen JJ, Werren JH.** 2006. Widespread recombination throughout *Wolbachia* genomes. Mol. Biol. Evol. **23:**437–449. http://dx.doi.org/10.1093/molbev/msj049.
18. **Scally M, Schuenzel EL, Stouthamer R, Nunney L.** 2005. Multilocus sequence type system for the plant pathogen *Xylella fastidiosa* and relative contributions of recombination and point mutation to clonal diversity. Appl. Environ. Microbiol. **71:**8491–8499. http://dx.doi.org/10.1128/AEM.71.12.8491-8499.2005.
19. **Yuan X, Morano L, Bromley R, Spring-Pearson S, Stouthamer R, Nunney L.** 2010. Multilocus sequence typing of *Xylella fastidiosa* causing Pierce's disease and oleander leaf scorch in the United States. Phytopathology **100:**601–611. http://dx.doi.org/10.1094/PHYTO-100-6-0601.
20. **Nunney L, Yuan XL, Bromley R, Hartung J, Montero-Astua M, Moreira L, Ortiz B, Stouthamer R.** 2010. Population genomic analysis of a bacterial plant pathogen: novel insight into the origin of Pierce's disease of grapevine in the US. PLoS One **5:**e15488. http://dx.doi.org/10.1371/journal.pone.0015488.
21. **Nunney L, Yuan XL, Bromley RE, Stouthamer R.** 2012. Detecting genetic introgression: high levels of intersubspecific recombination found in *Xylella fastidiosa* in Brazil. Appl. Environ. Microbiol. **78:**4702–4714. http://dx.doi.org/10.1128/AEM.01126-12.
22. **Nunney L, Vickerman DB, Bromley RE, Russell SA, Hartman JR, Morano LD, Stouthamer R.** 2013. Recent evolutionary radiation and host plant specialization in the *Xylella fastidiosa* subspecies native to the United States. Appl. Environ. Microbiol. **79:**2189–2200. http://dx.doi.org/10.1128/AEM.03208-12.
23. **Nunney L, Hopkins DL, Morano LD, Russell SE, Stouthamer R.** 2014. Intersubspecific recombination in *Xylella fastidiosa* native to the United States: infection of novel hosts associated with an unsuccessful invasion. Appl. Environ. Microbiol. **80:**1159–1169. http://dx.doi.org/10.1128/AEM.02920-13.
24. **Purcell AH, Hopkins DL.** 1996. Fastidious xylem-limited bacterial plant pathogens. Annu. Rev. Phytopathol. **34:**131–151. http://dx.doi.org/10.1146/annurev.phyto.34.1.131.
25. **Sherald JL.** 2007. Bacterial leaf scorch of landscape trees: what we know and what we do not know. Arboric. Urban For. **33:**376–385. http://joa.isa-arbor.com/request.asp?JournalID=1&ArticleID=3015&Type=2.
26. **Schuenzel EL, Scally M, Stouthamer R, Nunney L.** 2005. A multigene phylogenetic study of clonal diversity and divergence in North American strains of the plant pathogen *Xylella fastidiosa*. Appl. Environ. Microbiol. **71:**3832–3839. http://dx.doi.org/10.1128/AEM.71.7.3832-3839.2005.
27. **Schaad NW, Postnikova E, Lacy G, Fatmi M, Chang CJ.** 2004. *Xylella fastidiosa* subspecies: *X. fastidiosa* subsp. *piercei*, subsp. nov., *X. fastidiosa* subsp. *multiplex* subsp. nov., and *X. fastidiosa* subsp. *pauca* subsp. nov. Syst. Appl. Microbiol. **27:**290–300. (Erratum, 27:763.) http://dx.doi.org/10.1078/0723-2020-00263.
28. **Hernandez-Martinez R, Costa HS, Dumenyo CK, Cooksey DA.** 2006. Differentiation of strains of *Xylella fastidiosa* infecting grape, almonds, and oleander using a multiprimer PCR assay. Plant Dis. **90:**1382–1388. http://dx.doi.org/10.1094/PD-90-1382.
29. **Su CC, Chang CJ, Yang WJ, Hsu ST, Tzeng KC, Jan FJ, Deng WL.** 2012. Specific characters of 16S rRNA gene and 16S-23S rRNA internal transcribed spacer sequences of *Xylella fastidiosa* pear leaf scorch strains. Eur. J. Plant Pathol. **132:**203–216. http://dx.doi.org/10.1007/s10658-011-9863-6.

30. **Almeida RPP, Nascimento FE, Chau J, Prado SS, Tsai CW, Lopes SA, Lopes JRS.** 2008. Genetic structure and biology of *Xylella fastidiosa* strains causing disease in citrus and coffee in Brazil. Appl. Environ. Microbiol. **74:**3690–3701. http://dx.doi.org/10.1128/AEM.02388-07.
31. **Kung SH, Almeida RPP.** 2011. Natural competence and recombination in the plant pathogen *Xylella fastidiosa*. Appl. Environ. Microbiol. **77:**5278–5284. http://dx.doi.org/10.1128/AEM.00730-11.
32. **Rogers EE, Stenger DC.** 2012. A conjugative 38 kB plasmid is present in multiple subspecies of *Xylella fastidiosa*. PLoS One **7:**e52131. http://dx.doi.org/10.1371/journal.pone.0052131.
33. **French WJ, Kitajima EW.** 1978. Occurrence of plum leaf scald in Brazil and Paraguay. Plant Dis. Rep. **62:**1035–1038.
34. **Kostka SJ, Tattar TA, Sherald JL, Hurtt SS.** 1986. Mulberry leaf scorch, new disease caused by a fastidious, xylem-inhabiting bacterium. Plant Dis. **70:**690–693. http://dx.doi.org/10.1094/PD-70-690.
35. **Chen JC, Hartung JS, Chang CJ, Vidaver AK.** 2002. An evolutionary perspective of Pierce's disease of grapevine, citrus variegated chlorosis, and mulberry leaf scorch diseases. Curr. Microbiol. **45:**423–428. http://dx.doi.org/10.1007/s00284-002-3785-7.
36. **Stenger DC, Lee MW, Rogers EE, Chen J.** 2010. Plasmids of *Xylella fastidiosa* mulberry-infecting strains share extensive sequence identity and gene complement with pVEIS01 from the earthworm symbiont *Verminephrobacter eiseniae*. Physiol. Mol. Plant Pathol. **74:**238–245. http://dx.doi.org/10.1016/j.pmpp.2010.03.003.
37. **Hernandez-Martinez R, de la Cerda KA, Costa HS, Cooksey DA, Wong FP.** 2007. Phylogenetic relationships of *Xylella fastidiosa* strains isolated from landscape ornamentals in southern California. Phytopathology **97:**857–864. http://dx.doi.org/10.1094/PHYTO-97-7-0857.
38. **Felsenstein J.** 1989. PHYLIP—phylogeny inference program (version 3.2). Cladistics **5:**164–166.
39. **Hernandez-Martinez R, Pinckard TR, Costa HS, Cooksey DA, Wong FP.** 2006. Discovery and characterization of *Xylella fastidiosa* strains in southern California causing mulberry leaf scorch. Plant Dis. **90:**1143–1149. http://dx.doi.org/10.1094/PD-90-1143.
40. **Kung SH, Retchless AC, Kwan JY, Almeida RPP.** 2013. Effects of DNA size on transformation and recombination efficiencies in *Xylella fastidiosa*. Appl. Environ. Microbiol. **79:**1712–1717. http://dx.doi.org/10.1128/AEM.03525-12.
41. **Costa HS, Guzman A, Hernandez-Martinez R, Gispert C, Cooksey DA.** 2006. Detection and differentiation of *Xylella fastidiosa* strains acquired and retained by glassy-winged sharpshooters (Hemiptera: Cicadellidae) using a mixture of strain-specific primer sets. J. Econ. Entomol. **99:**1058–1064. http://dx.doi.org/10.1603/0022-0493-99.4.1058.
42. **Wells JM, Raju BC, Hung HY, Weisburg WG, Mandelcopaul L, Brenner DJ.** 1987. *Xylella fastidiosa* gen. nov. sp. nov.: Gram-negative, xylem-limited, fastidious plant bacteria related to *Xanthomonas* spp. Int. J. Syst. Bacteriol. **37:**136–143. http://dx.doi.org/10.1099/00207713-37-2-136.