Rhizobium gone native: Unexpected plasmid stability of indigenous Rhizobium leguminosarum
(plasmid transfer/Trifolium/nodulation/symbiosis)

JENNIFER J. WERNEGREEN*†, ETHELYNDA E. HARDING‡, AND MARGARET A. RILEY*
*Department of Biology, Yale University, New Haven, CT 06511; and ‡Department of Biology, California State University, Fresno, CA 93740-0073

Communicated by Allan Campbell, Stanford University, Stanford, CA, March 5, 1997 (received for review October 3, 1996)

ABSTRACT Lateral transfer of bacterial plasmids is thought to play an important role in microbial evolution and population dynamics. However, this assumption is based primarily on investigations of medically or agriculturally important bacterial species. To explore the role of lateral transfer in the evolution of bacterial systems not under intensive, human-mediated selection, we examined the association of genotypes at plasmid-encoded and chromosomal loci of native Rhizobium, the nitrogen-fixing symbiont of legumes. To this end, Rhizobium leguminosarum strains nodulating sympatric species of native Trifolium were characterized genetically at plasmid-encoded symbiotic (sym) regions (nodulation AB and nodulation CIJT loci) and a repeated chromosomal locus not involved in the symbiosis with legumes. Restriction fragment length polymorphism analysis was used to distinguish genetic groups at plasmid and chromosomal loci. The correlation between major sym and chromosomal genotypes and the distribution of genotypes across host plant species and sampling location were determined using χ² analysis. In contrast to findings of previous studies, a strict association existed between major sym plasmid and chromosomal genetic groups, suggesting a lack of successful sym plasmid transfer between major Rhizobium chromosomal types. These data indicate that previous observations of sym plasmid transfer in agricultural settings may seriously overestimate the rates of successful conjugation in systems not impacted by human activities. In addition, a nonrandom distribution of Rhizobium genotypes across host plant species and sampling site demonstrates the importance of both factors in shaping Rhizobium population dynamics.

Transmissible plasmids are considered important in the divergence and adaptation of bacterial populations because they have several unique features that contribute to genomic plasticity and may affect the evolution of the loci they encode (1–3). They might: be lost and regained in populations, rapidly change in copy number, and undergo higher mutation rates because of the common occurrence of reiterated DNA (4, 5). In addition, transmissible plasmids are thought to undergo lateral transfer at rates higher than chromosomal genes, which may be particularly important in adaptation to variable environments (5, 6). To the extent that lateral transfer disrupts the linkage of plasmid and chromosomally encoded loci, strong selection at plasmid loci may not affect genetic divergence throughout the bacterial genome (5). Just as sexual recombination dilutes the effects of selection on particular loci in eukaryotic populations, plasmid transfer may mitigate the effects of selection for plasmid-encoded phenotypes.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: sym plasmid, symbiotic plasmid; nodAB, nodulation AB loci; nodCIJT, nodulation CIJT loci; 16S rRNA, 16S ribosomal RNA gene; RFLP, restriction fragment length polymorphism. Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. U72626 and U72627).

†To whom reprint requests should be addressed. e-mail: werjen@minerva.cis.yale.edu.
vival in the soil are thought to be chromosomally encoded, as evidenced by loss of the sym plasmid in *Rhizobium* soil populations (23–25) and the significant main effect of chromosomal but not sym plasmid variation on competitive growth in the soil (ref. 26 but see ref. 27 for catabolic loss upon curing sym in the soil). Evidenced by loss of the sym plasmid in the soil, these populations are thought to be chromosomally encoded, as suggested by the two replicons may be affected by distinct suites of selective pressures.

In agricultural legume populations, lateral transfer of sym plasmids among *Rhizobium* strains is evidenced by disjunctions between genetic groups at sym and chromosomal loci (23, 28–32). In addition, strong correlations between agricultural legume species and sym genotypes but not nonsymbiotic genotypes (23, 28) argues that lateral transfer of sym genes may decouple the adaptation of loci mediating the interaction with the host plant from those loci involved in competitive growth in the soil (33). Establishing a monoculture of introduced host plants was shown recently to select for transfer of competitive sym genotypes into symbiotic and nonsymbiotic rhizobia in the field (34). That the symbiotic genes in this particular *Rhizobium* population were chromosomally encoded argues for a strong differential effect of host legume on symbiotic loci, relative to the effect at nonsymbiotic loci.

However, selective pressures in fields of introduced or agricultural legumes may differ considerably from those in native legume communities. Introduction of plants in monoculture, inoculation of *Rhizobium* at high densities, and lack of selection acting on host plants for symbiotic compatibility with indigenous *Rhizobium* may each affect the dynamics of the *Rhizobium*–legume symbiosis and alter rates of successful plasmid transfer across bacterial chromosomal groups. Patterns of high transfer rates of sym plasmids and apparent decoupling of sym and nonsymbiotic loci observed in human-impacted environments have shaped our view of sym plasmids, but these patterns may seriously misrepresent plasmid dynamics in populations of indigenous *Rhizobium* nodulating native legume species.

Given the potential importance of plasmid transfer in bacterial diversification and our limited understanding of plasmid dynamics in native populations, the goal of this study was to estimate levels of sym plasmid transfer within a *Rhizobium* population nodulating native legume species. Similar to previous studies of agricultural systems, sym plasmid transfer was determined from the correlation between major genotypes at nodulation and nonsymbiotic loci. Both host plant species (23, 28, 35) and sampling location (36, 37) are considered important in shaping genetic divergence of *Rhizobium*, so we used the unique strategy of sampling nodules of the same legume species across several sites to distinguish the effects of host plant and sampling location on the divergence of *Rhizobium* plasmid and chromosomal loci. In contrast to previous studies, we observed a strict correlation between genetic groups at symbiotic plasmid and chromosomal loci across several sampling locations and host plant species, which strongly argues for a surprising stability of sym plasmids within chromosomal lineages of native *Rhizobium* populations.

### MATERIALS AND METHODS

**Sampling Location and Strategy.** *Rhizobium* were sampled from nodules of four species of clover native to mountain meadows in the Sierra Nevada: *Trifolium bolanderi*, *T. longipes*, *T. monanthum*, and *T. wormskjoldii*. *Trifolium* plants were sampled at two meadows, Fresno Dome and House meadows, located 66 km from each other. Relative to the California State University Fresno campus, House meadow is 64 km north of west, and Fresno Dome meadow is 74 km west of north. The meadows are ~2130 m in elevation and are in separate watersheds. At both meadows, three *Trifolium* species were sampled (*T. bolanderi*, *T. longipes*, and *T. monanthum*), and at Fresno Dome meadow the fourth species, *T. wormskjoldii*, was also sampled. All samples were taken in June and July, 1994.

Within each meadow, three 15 x 30-m plots were selected based on the presence of at least three of the four *Trifolium* species. Within each of the six plots, 10–12 plant individuals were sampled for each *Trifolium* species included in a given plot (~30–48 total plant individuals per plot). Plant individuals were sampled randomly and were removed with ~10 cm diameter x 10 cm depth of surrounding soil. A portion of soil core was removed for soil acidity and moisture analyses, and samples were returned to the laboratory on ice. Plants were stored in intact soil cores for up to 2 days at 4°C. Soil and other plant roots were gently removed, and the root system was rinsed in deionized water and stored at 4°C.

**Isolation of Bacterial Strains.** Five to eight arbitrarily selected nodules were removed from plant roots, treated with 1% hypochlorite for 2 minutes, rinsed three times in sterile deionized water, and then crushed and streaked on yeast manitol agar (38). Plates were incubated for 5–days at 30°C and restreaked as necessary to obtain pure cultures. Isolates were stored frozen at ~70°C in yeast manitol broth plus 7% dimethyl sulfoxide.

**Restriction Fragment Length Polymorphism (RFLP) Analysis.** A subset of the *Rhizobium* strains was selected for genetic analysis such that isolates from each of the four *Trifolium* host plants were represented across several sampling plots. Total genomic DNA of 69 isolates was prepared as described (39). DNA samples (8–10 μg) were digested to completion (16 h) with HindIII and EcoRI separately and electrophoresed on a 0.7% agarose gel in TBE (89 mM Tris/89 mM boric acid/2 mM EDTA, pH 8.0). Gels were transferred to nylon membranes (Boehringer Mannheim). Labeled DNA probes were prepared by random priming, sequentially hybridized to the nylon membranes, and detected colorimetrically (Boehringer Mannheim Genius system). Only strongly hybridizing bands were scored. Lengths of hybridizing fragments were determined by probing nylon with labeled DNA of the size marker on the gel, a 1-kb ladder. Membranes were stripped of probe between hybridizations.

The probes used in this study were derived from ANU843, a reference strain of *R. leguminosarum* bv. *trifoli* (*R. leguminosarum* isolated from *Trifolium*), and include three regions: nodulation AB loci (*nodAB*), nodulation CJT loci (*nodCJT*), and a 1-kb repeated chromosomal sequence (Table 1).

**Analysis of RFLP Data.** Isolates were grouped according to their hybridization pattern at each of the three regions sampled (*nodAB*, *nodCJT*, and the 1-kb reiterated chromosomal sequence). Nonrandom distribution of RFLP types across the two meadows and four host plants species was tested using \( \chi^2 \) analysis (IMPUTE 3.1 program, SAS Institute, Cary, NC).

**DNA Sequencing.** Genealogies of the 16S rRNA locus are considered representative of bacterial species phylogenies (41). To position *Rhizobium* isolates on a 16S rRNA gene
RESULTS

Identification of Genetic Groups. Genetic groups of \textit{Rhizobium} were distinguished based on patterns of hybridizing restriction fragments. At the chromosomal locus (probe \emph{pRt1013}), two distinct RFLP groups (groups 1 and 2) were detected, which share only one fragment (Table 2). Groups 1 and 2 are each comprised of four subtypes, which have several fragments in common. Hybridization patterns at \emph{nodAB} and \emph{nodCIJT} reveal three main RFLP groups. Group A is comprised of subtypes FA1 to FA3 and HA1 to HA3, group B includes subtypes B1 to B4, and group C includes a single genotype. The lengths of hybridizing fragments suggest that rearrangements or duplications have occurred at each region probed. These patterns were not unexpected nor do they affect our ability to identify major genetic groups (see Discussion).

Stable Associations Between Symbiotic Plasmids and Major Chromosomal Groups. To estimate the level of \emph{sym} plasmid transfer among different chromosomal lineages, the correlation between \emph{nod} and chromosomal genetic groups was determined (Table 3). \emph{Nod} group A occurred only in chromosomal group 1, and \emph{nod} group B occurred in chromosomal group 2. The four strains with \emph{nod} type C were also chromosomal group 2. This strict association between chromosomal groups and the two dominant \emph{nod} groups (A and B) indicates a lack of successful transfer of nodulation genes between chromosomal groups 1 and 2. Plasmid transfer within chromosomal groups 1 and 2 was not tested because of the small sample of rare subtypes.

Associations Between \textit{Rhizobium} Strains and \textit{Trifolium} Host Plant Species. At all sampling sites, nodule occupants of the clovers \emph{T. bolanderi} and \emph{T. longipes} were genetically distinct at \emph{nod} and chromosomal loci from strains nodulating \emph{T. monanthum} and \emph{T. wormskioldii} (Table 4a). The single case of \textit{Rhizobium} type 1/A (chromosomal group 1, \emph{nod} group A) nodulating \emph{T. monanthum} and the four cases of type 2/B nodulating \emph{T. bolanderi} and \emph{T. longipes} demonstrate rare instances of natural cross-infection. Within the \emph{T. bolanderi} and \emph{T. longipes} pair, there was no evidence for host-strain associations. However, host specificity within the \emph{T. monanthum}/\emph{T. wormskioldii} pair is suggested by the occurrence of \emph{nod} group C only in nodules of \emph{T. wormskioldii} collected from Fresno Dome site 1.

Genetic Subdivision of \textit{Rhizobium} Between Sampling Sites. Distinct genotypes at \emph{nod} and chromosomal loci were distributed nonrandomly across the two meadows sampled. This subdivision between meadows is most apparent at \emph{nodCIJT} because Fresno Dome and House meadows had unique sets of genotypes at this locus within \emph{nod} group A (Table 4b). To a lesser extent, genotypes within chromosomal type 2 also were distributed nonrandomly between meadows (Table 4c).

Phylogenetic Position of Isolates. Three isolates representing both chromosomal groups 1 and 2 were identical for the 296-bp segment of the \emph{16S rRNA} gene (GenBank database accession nos. U72626 and U72627), indicating that the two chromosomal groups detected are considered the same species by conventional taxonomic methods. Based on a comparison with all known prokaryotic \emph{16S rRNA} sequences, these native isolates cluster with the type strain \textit{R. leguminosarum} LMG 5485.
within House and Fresno Dome meadows had characteristic subtypes of meadows (c, pRt1013 genotype 2 were distributed nonrandomly across populations. Relative to chromosomal regions, plasmids are thought to be important in the diversification of bacterial populations. Relative to chromosomal regions, plasmids are easily lost and regained in populations, may change in copy number regulation as a mechanism for gene amplification, and potentially undergo elevated mutation rates (4, 5). The feature of plasmids most often emphasized as evolutionarily important is their lateral transfer among distinct bacterial chromosomal lineages, which has been demonstrated in several human-impacted environments (5, 6, 28). The significance of translineages, which has been demonstrated in several human-

Table 3. Number of Rhizobium isolates of each chromosomal genotype that carry a sym plasmid of a given nod genotype

<table>
<thead>
<tr>
<th>Chromosomal genotype</th>
<th>FA1</th>
<th>FA2</th>
<th>FA3</th>
<th>HA1</th>
<th>HA2</th>
<th>HA3</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>B4</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>15</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>5</td>
<td>5</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>1b</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>1c</td>
<td>1</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>1</td>
<td>1</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>1d</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>2a</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>1</td>
<td>1</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>2b</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>7</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>2c</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>2d</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

 indicates zero isolates. nod genotypes were distributed nonrandomly across chromosomal genotypes ($\chi^2 = 87.4, df = 12, P < 0.000$). Chi-square analysis was performed after omitting cells with expected values < 1.

Table 4. Number of Rhizobium isolates in genetic groups, as distributed across (a) each of four Trifolium host plant species and (b and c) each of two sampling meadows

<table>
<thead>
<tr>
<th>Rhizobium genetic group (pRt1013/ nod)</th>
<th>T. bolanderi</th>
<th>T. longipes</th>
<th>T. monanthum</th>
<th>T. wormskoidii</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/A</td>
<td>16</td>
<td>17</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2/B</td>
<td>1</td>
<td>3</td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td>2/C</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sampling meadow</th>
<th>FA1</th>
<th>FA2</th>
<th>FA3</th>
<th>HA1</th>
<th>HA2</th>
<th>HA3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresno Dome</td>
<td>16</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fresno House</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pRt1013 genotype</th>
<th>Fresno Dome</th>
<th>Fresno House</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2b</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>2c</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>2d</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Chromosomal and nodulation genotypes were distributed nonrandomly across host plant species (a, $\chi^2 = 85.0, df = 6, P < 0.000$). Each of House and Fresno Dome meadows had characteristic subtypes within nod type A (b, $\chi^2 = 39.9, df = 2, P < 0.000$). Likewise, subtypes within pRt1013 genotype 2 were distributed nonrandomly across meadows (c, $\chi^2 = 23.9, df = 2, P < 0.0005$). Chi-square analysis was performed after omitting cells with expected values < 1.

9518 (45) with a sequence similarity of 97.3% and are identical to R. leguminosarum strain 8002 (46).

**DISCUSSION**

The genetic plasticity conferred by transmissible plasmids is thought to be important in the diversification of bacterial populations. Relative to chromosomal regions, plasmids are easily lost and regained in populations, may change in copy number regulation as a mechanism for gene amplification, and potentially undergo elevated mutation rates (4, 5). The feature of plasmids most often emphasized as evolutionarily important is their lateral transfer among distinct bacterial chromosomal lineages, which has been demonstrated in several human-impacted environments (5, 6, 28). The significance of transmissible plasmids in adaptation to sporadic, or “local,” selection pressures is suggested by the tendency for these replicons to encode locally adaptive traits such as virulence, antibiotic resistance, toxin production, and symbiotic phenotypes (2, 5). Divergence in prokaryotes is driven largely by ecological rather than sexual isolation (47, 48); therefore, the frequent transfer of these ecologically important traits may have profound implications for bacterial diversification and speciation. Conjugation of virulence, resistance, and symbiotic plasmids may mitigate the effect of plasmid level selection on chromosomal divergence and thus may allow genetic cohesion of chromosomal populations despite ecological and genetic differentiation of plasmid populations.

Given the potential importance of plasmids in bacterial evolution and the emphasis of previous studies on human-impacted populations, our objective was to explore the dynamics of an ecologically important plasmid within a native bacterial population. This study provides evidence for restricted successful transfer of plasmids among chromosomal groups within a population of R. leguminosarum isolated from nodules of four native, cooccurring species of Trifolium. The strict association of nod genotypes with distinct chromosomal genotypes strongly argues against any successful plasmid transfer between major chromosomal groups. This observed correlation stands in sharp contrast with previous field and laboratory studies of Rhizobium, which demonstrate sym plasmid transfer among divergent chromosomal lineages (23, 28–32). The novelty of our results is likely due to sampling Rhizobium indigenous to native legume species rather than strains associated with agricultural or introduced host plant species.

Although successful sym plasmid transfer clearly does not occur frequently between the major chromosomal lineages we detected, we cannot rule out the possibility that sym plasmids do transfer within those chromosomal groups. Such transfer would only be revealed by higher levels of genetic resolution. Probing with longer regions of DNA effectively samples more nucleotide sites and increases the likelihood of detecting closely related genotypes. However, for two reasons, we are confident that the 1-kb probe used here adequately distinguishes major chromosomal groups. First, this probe of a reiterated chromosomal locus samples a region greater than 1 kb; the lengths of hybridizing fragments indicate that the restriction site variation we detected occurs outside of the region probed. It is therefore not surprising that our relatively short chromosomal probe detected a similar number of genetic groups per isolate sampled as was found in previous studies (8 genetic groups/69 isolates sampled in this study compared with 10/56 (29) and 18/176 (39)). Second, previous studies show that genetic groups distinguished by this 1-kb probe agree with genetic groups identified by much longer probes (26 kb) and multi-locus enzyme electrophoresis analysis (39, 49). Therefore, we are confident that the major chromosomal types distinguished with the 1-kb probe are genetically different groups within this population.
The observed stability of \textit{sym} plasmids and these major chromosomal groups has several possible explanations, including physiological, genetic, and ecological constraints on conjugation in native bacterial populations. First, limited opportunities for lateral transfer may constrain conjugation in native settings. Plasmid transfer in soil environments requires high cell densities of both donor and recipient cells (50), a condition thought to exist only in the immediate vicinity of the root and inside the nodule. The amplified growth caused by specific cell densities of both donor and recipient cells (50), a condition setting the stage. Plasmid transfer in soil environments requires high jugation in native bacterial populations. First, limited opportunities for lateral transfer exist because the expression of the recipient plasmid is regulated by specific chromosomal groups (31, 39, 49). This increased variability in the \textit{sym} genome has been attributed to recombination within the \textit{sym} plasmid, facilitated by the high frequency of reiterated sequences around the \textit{sym} plasmid (18, 25, 31). In addition to higher mutation rates, increased population subdivision is known to elevate overall genetic divergence at a particular locus (54). Although our data do not allow direct comparisons of chromosomal and \textit{sym} plasmid diversity levels, we found many more distinct genotypes at the \textit{nod} region than at the chromosomal locus. Our results argue that subdivision between meadows contributes significantly to the observed variation within \textit{nod} type A. Likewise, the presence of \textit{nod} type C at Fresno Dome site 1 but not Fresno Dome site 2, sites within 100 m of each other, suggests a limited distribution of this \textit{nod} group. Limited migration of \textit{sym} plasmids between sites due to loss in soil populations (23–25), genetic drift of one variant into each group (55), or site-specific differences in soil profiles that select for alternative \textit{nod} phenotypes (56) may contribute to population subdivision at symbiotic loci.

In summary, the plasmid stability observed in this study suggests that constraints on plasmid transfer, such as limited opportunities for lateral transfer, chromosomal divergence, or the coupling of selective pressures shaping plasmid and chromosomal variation, may be especially important in indigenous microbial populations relative to agricultural systems. The maintenance of phenotypically important loci on plasmids may result from unique properties of plasmid genes other than transfer, including the ability to be lost and regained depending on the presence of a certain selective pressure or the potential to undergo higher rates of evolution due to relatively high frequency of reiterated DNA. These alternative forms of genetic plasticity may be important in the adaptation of native \textit{Rhizobium} populations and may account for the maintenance of symbiotic genes on transmissible plasmids. The ability to lose nodule and nitrogen-fixation genes may be adaptive to \textit{Rhizobium} in the soil, where these loci are apparently detrimental and where \textit{sym} plasmids are lost at high frequencies. In addition, the occurrence of \textit{sym} genes on plasmids also may provide a mechanism for amplifying these loci by regulation of plasmid copy number. The stability of bacterial genomes depends on the strength of selection for transconjugants and opportunities for lateral transfer, both of which may be diminished in native microbial populations. A complete picture of the role of transmissible plasmids in bacterial diversification and genome plasticity will therefore require additional sampling of populations that do not experience human-mediated selection on plasmid phenotypes.

We thank Amber Buhler, Tony Rubio, Renee Denton, and Kristina Schierenbeck for their essential help in sampling and isolating bacterial strains. This work was supported by a National Institutes of Health First Award (GM 47471) and a National Science Foundation Young Investigator Award (DEB-9458247) to M.A.R.; a grant from the Forest Service U.S. Department of Agriculture (PSW-91-0021CA) to E.E.H.; and a Howard Hughes Medical Institute Predoctoral Fellowship and a National Science Foundation Doctoral Dissertation Improvement Grant (DEB-9623537) to J.J.W.
