INTRODUCTION

Speciation in higher organisms usually occurs in genetic isolation. Successive rounds of gene duplication and divergence, followed by individual gene loss, is thought to have contributed to morphological diversification [1]. Speciation has not been as extensively studied in bacteria. Small gene duplication events have been noted in many sequenced bacterial genomes [2] but lateral gene transfer (LGT) also contributes to genome diversification. Bacteria exchange genes by conjugation, transformation, and transduction which are widespread in nature and can occur between distantly related organisms [3]. Genome sequencing has revealed substantial rates of foreign gene acquisition [4]. Analysis of all gene phylogenies in the sequenced members of the γ Proteobacteria indicated that LGT rather than gene duplication provided most of the diversity in genomic repertoires [5]. LGT also has the potential to drive metabolic innovation. The dissemination of antibiotic resistance genes on a global scale is the paradigm, but examples of disseminated gene clusters for metabolic pathways and pathogenicity determinants also exist [6,7]. These results provide compelling evidence that LGT can influence genotype evolution at the level of sub-speciation and by extension LGT may aid bacterial speciation.

While gene duplication in M. xanthus led to the production of several large multi-gene families [8], the role of LGT in M. xanthus genome evolution has not been evaluated. LGT is generally supported if a phylogenetic tree for a gene is in disagreement with that of the 16S rDNA gene, although incongruent trees can also arise by gene loss from related lineages. The other method to detect LGT relies on differences in the nucleotide sequence composition of a gene relative to average of that particular host. Given enough time acquired genes converge in codon usage with the bulk of the genome by amelioration [9], which make ancient transfers difficult to detect with compositional algorithms. Furthermore, LGT may be most successful when foreign genes and recipient genomes have similar codon usage [10]. Nevertheless, genes with codon usage that differs from that of the host are excellent candidates for LGT [11,12].

Prokaryotic evolution is the product of environmental pressures combined with changes in the gene repertoire over time. The myxobacteria provide an opportunity to examine genome evolution within the context of speciation because fruiting body development is a unique trait that defines a phylogenetic order. In this work we examined the evolutionary history of M. xanthus genes required for fruiting body development using compositional and BLAST algorithms. Our results suggest that genes for metabolic enzymes, particularly those involved in polysaccharide biosynthesis, were more likely to be acquired by LGT and genes for sensory systems were more likely to be vertically inherited with numerous examples of duplication and divergence.

RESULTS

M. xanthus and other myxobacteria are members of the δ Proteobacteria, one of the most diverse groups of Bacteria in terms of habitat distribution and respiration strategies. Hundreds
of δ Proteobacteria species are known, but for the sake of brevity, only those genera with a complete genome sequence will be mentioned here. With the exception of the myxobacteria and Bdellovibrio most of the genera, including Anaeromyxobacter, Desulfotalea, Desulfovibrio, Geobacter, Lauenonia, Pelobacter, and Syntrophus are anaerobic (use terminal electron acceptors other than O2). Many are also chemolithothrophs (use inorganic compounds as energy sources) and autotrophs (derive all their carbon from CO2). While the modus operandi for most genera in the δ Proteobacteria is metabolic diversity, the myxobacteria are neither chemolithothrophs nor autotrophs and instead devote their resources to social cooperativity directed toward predation and the construction of a unique multicellular structure, the asexual fruiting body.

A phylogenetic approach was initially used to identify possible cases of LGT in M. xanthus. Similarity searches using the BLASTP algorithm, with a 10⁻¹⁰ cutoff, were performed with each putative M. xanthus protein. The top four BLASTP hits were used because the top hit does not always represent the closest homolog and more distant hits can be widely diverged from the M. xanthus query sequence. One or more of the top four hits was a δ Proteobacteria for 55.0% of the genes suggesting a vertical descent as the inheritance paradigm for over half of the M. xanthus genes. Genomes also contain unique genes, in this case 22.2%, that have not been found in any other organism (so-called ORFans). The remaining gene products (22.8%) have no homologs among δ Proteobacteria in the top 4 BLASTP hits, but often striking homology with gene products from other organisms suggestive of an alien origin.

The same phylogenetic analysis was applied to other members of the δ Proteobacteria. With the exception of Bdellovibrio, which contains 43.0% δ Proteobacteria genes, other genera in the δ Proteobacteria contained a higher proportion of δ Proteobacteria genes, including Anaeromyxobacter (60.7%), Desulfovibrio (84.1%), Geobacter (90.2%), Lauenonia (83.6%), and Pelobacter (74.7%). Bdellovibrio is thought to have acquired an unusually large number of genes via LGT [13]. These results suggest that M. xanthus has received more alien genes by LGT than most other δ Proteobacteria.

**Fruiting body development**

The myxobacteria represent an evolutionary branch that culminated in a unique and striking form of multicellularity. The evolutionary history of genes known to be required for fruiting body development was examined in order to glean some insight into principles guiding the evolution of this group of organisms. Only genes in which mutations diminish the capacity to form fruiting bodies or cause at least a 10-fold decrease in sporulation are included here. Many genes that affect the timing of fruiting body development were examined in order to glean some insight into the mechanism of fruiting body development. This group of genes was supplemented with developmental mutants identified using the mariner-based transposable element magellan-4 [14], which was used previously to identify motility genes in M. xanthus [15–17]. 40,000 magellan-4 insertions were screened for loss of fruiting body development. Mutant genes were backcrossed into the wild type DK1622 to confirm that the transposon causes the mutant phenotype. These genes, together with previously identified developmental genes, are given in table 1. The phylogeny of each gene was examined using the BLASTP algorithm. Genes with a codon usage that differed from that of the majority of the host genes were identified with software developed by Karlin and Mrazek [11,12].

Among the genes required for development very few exhibited a codon bias suggestive of LGT (Table 1). The exceptions include a unique gene, MXAN4406, and MXAN0501, which encodes the essential translation initiation factor InfC that is found in other δ Proteobacteria. However, the M. xanthus InfC protein is unique in that it contains a 66 amino acid C-terminal extension that is essential for development but not translation [10–20]. The precise function of this extension remains unknown, but is thought to aid in generating an essential developmental signal, the D-signal. We were unable to identify another InfC protein with a similar extension in the database.

While the remaining genes had a normal codon bias, the tBLASTn algorithm suggested that some were acquired by LGT because their closest relatives in the database were not members of the δ Proteobacteria (Table 1). The contributions of LGT and vertical inheritance to the pool of genes for fruiting body development can be approximated from these data. 57 genes (73%) have a δ Proteobacteria phylogeny and a normal codon bias suggestive of vertical inheritance. 17 genes (22%) have either abnormal codon bias or produce protein products whose closest relatives are from another bacterial group. The remaining 2 genes (5%) have a normal codon bias but are unique and their phylogenetic source could not be identified. These results suggest that M. xanthus fruiting body development is not possible without alien genes.

When the genes were examined within the context of their function two striking correlations were observed. Genes encoding essential developmental enzymes were often acquired by LGT (Table 1, 7/14, 50%). Most notably this includes genes required for exopolysaccharide (eps) and lipopolysaccharide (gfb) biosynthesis. On the other hand most of the genes required for signal production and sensory transduction have a phylogeny rooted in the δ Proteobacteria and a normal codon bias (Table 1, 37/41, 90%). Many of these genes have several paralogs in M. xanthus and seem to be products of duplication and divergence. These include two component systems, serine/threonine protein kinase systems, sigma 54-dependent response regulators, and chemoreceptor pathways [8].

The phylogenetic sources of the alien developmental genes can suggest commonalities about the pool of donors. In some cases a clear gene source does not exist. For example, the MXAN4621 and MXAN5125 proteins have similar amino acid identities with proteins from widely different phyla (Table 1). Nevertheless the phylogenetic origin of each alien gene in the genome was explored systematically to determine whether any trends exist. The phyla containing the top four homologs of each alien gene are plotted in figure 1 for Myxococcus, Anaeromyxobacter and Bdellovibrio. Relative to the latter two organisms, Myxococcus has an unusually high abundance of genes whose closest relatives are found in the Actinobacteria and Cyanobacteria. Most soil Actinobacteria (like Streptomyces) are obligate aerobes. Cyanobacteria also live in aerobic habitats since they generate O2 during photosynthesis. These results suggest that M. xanthus acquired genes from organisms in aerobic habitats.

**Aerobic respiration**

How alien genes are functionally integrated into existing pathways was examined with a less complex pathway, the electron transport chain. The nearly unique presence of aerobic respiration among the δ Proteobacteria, suggested that myxobacterial electron transport genes were derived by LGT. Examination of electron transport is also expected to reveal how recently acquired genes are integrated into functional networks. Was the entire pathway captured as a functional unit or were individual components acquired and used to replace existing components?
**Table 1.** Genes required for *M. xanthus* fruiting body development.

| MXAN Locus Tag | Protein Product | Function | Codon Bias | Phylogeny | Code |
|----------------|-----------------|----------|------------|-----------|------|
| 0581           | InfC            | Translation initiation factor | 0.038 | Delta Proteobacteria | S |
| 0733           | RodK            | Sensor histidine kinase |          | Delta Proteobacteria | S |
| 1014           | SdeK            | Sensor histidine kinase |          | Delta Proteobacteria | S |
| 1020           | Hypothetical    |          |           | Delta Proteobacteria |     |
| 1078           | Nla19           | Sigma 54-dependent response regulator |          | Delta Proteobacteria | S |
| 1167           | Nla28           | Sigma 54 dependent response regulator |          | Delta Proteobacteria | S |
| 1294           | CsgA            | C-signal |          | Delta Proteobacteria | S |
| 1402           | LadA            | Transcription factor |          | Actinobacteria | S |
| 1450           | Oar             | TonB-dependent receptor |          | Delta Proteobacteria | S |
| 2044           | Pph1            | Ser/Thr protein phosphatase |          | Delta Proteobacteria | S |
| 2670           | AsgA            | A-signal production |          | Alpha Proteobacteria | S |
| 2778           | PhoP2           | DNA binding response regulator |          | Delta Proteobacteria | S |
| 2779           | PhoR2           | Sensor histidine kinase |          | Delta Proteobacteria | S |
| 2905           | DofA            | Hypothetical |          | Unique | |
| 2913           | AsgB            | DNA binding protein |          | Delta Proteobacteria | S |
| 3117           | FruA            | CsgA-dependent response regulator |          | Delta Proteobacteria | S |
| 3204           | RelA            | Stringent response |          | Delta Proteobacteria | S |
| 3213           | ActA            | CsgA regulator |          | Delta Proteobacteria | S |
| 3214           | ActB            | Sigma 54-dependent response regulator |          | Delta Proteobacteria | S |
| 3225           | FrgA            | Polysaccharide export |          | Delta Proteobacteria | S |
| 3692           | Nla18           | Sigma 54-dependent response regulator |          | Delta Proteobacteria | S |
| 3993           | BsgA/Lon        | ATP-dependent protease |          | Delta Proteobacteria | S |
| 4016           | Pfk1            | 6-phosphofructokinase |          | Delta Proteobacteria | E |
| 4017           | Pkn4            | Ser/Thr protein kinase |          | Delta Proteobacteria | S |
| 4042           | Nla6            | Sigma 54 dependent response regulator |          | Delta Proteobacteria | S |
| 4138           | FrzF            | Protein methyl transferase |          | Delta Proteobacteria | S |
| 4139           | FrzG            | Protein methyl esterase |          | Delta Proteobacteria | S |
| 4140           | FrzE            | Chemotaxis histidine kinase |          | Delta Proteobacteria | S |
| 4141           | FrzCD           | Chemotaxis receptor |          | Delta Proteobacteria | S |
| 4142           | FrzB            | Hypothetical |          | Unique | |
| 4143           | FrzA            | Hypothetical |          | Delta Proteobacteria | |
| 4144           | FrzZ            | Response regulator |          | Delta Proteobacteria | S |
| 4146           | AldA            | Alanine dehydrogenase |          | Firmicutes | E |
| 4149           | FrzS            | Response regulator |          | Delta Proteobacteria | S |
| 4486           | FruE            | Hypothetical | 0.064 | Unique | |
| 4564           | Esg             | Branched chain keto acid dehydrogenase |          | Delta Proteobacteria | E |
| 4565           | Esg             | Branched chain keto acid dehydrogenase |          | Delta Proteobacteria | E |
| 4621           | RfbC/SasA       | Glycosyl transferase |          | Actinobacteria | E |
| 4778           | PhoR1           | Sensor histidine kinase |          | Delta Proteobacteria | S |
| 4787           | PhoP4           | DNA-binding response regulator |          | Unclassified Proteobacteria | S |
| 5123           | MrpA            | Sensor histidine kinase |          | Delta Proteobacteria | S |
| 5124           | MrpB            | Sigma 54-dependent response regulator |          | Delta Proteobacteria | S |
| 5125           | MrpC            | Transcription factor |          | Planctomyces | S |
| 5766           | TPR domain protein |          |          | Delta Proteobacteria | |
| 5772           | PiIQ             | Type IV pilus |          | Delta Proteobacteria | |
| 5775           | PiIN             | Type IV pilus |          | Delta Proteobacteria | |
The protein components of the *M. xanthus* electron transport system were identified through comparative phylogeny. The upstream elements, NADH dehydrogenase (complex I) and succinate dehydrogenase (complex II), are common to many electron transport pathways (figure 2). The principal *Myxococcus* quinone is a menaquinone with 8 isoprenoid units [21,22]. The Proteobacteria along with several other phyla lack the traditional bc1-complex III and contain a newly described complex III containing a five-heme cytochrome *c*, a one-heme cytochrome *c*, and a three-subunit molybdopterin oxidoreductase [23]. Genes encoding three copies of this complex, potentially one for each cytochrome *c* oxidase complex (denoted MF1cc in figure 3) are found in *M. xanthus* [23]. There are two broad classes of cytochrome oxidase, which reduces O2 to H2O (complex IV). Cytochrome *c* oxidase is coupled to the quinol:cytochrome *c* oxidoreductase (MF1cc) complex III. *M. xanthus* contains genes for three cytochrome *c* oxidases of this type, two coxBAC operons, and a cbb3 operon (table 2). The quinol oxidases derive electrons directly from the quinone pool and *M. xanthus* contains genes for both subunits of cytochrome *d* quinol oxidase (cydAB, table 2).

The origin of each *M. xanthus* electron transport chain gene was examined using both phylogenetic and compositional approaches. In all cases the codon usage was consistent with other highly

| MXAN Locus Tag | Protein Product | Function | Codon Bias1 | Phylogeny2 | Code3 |
|----------------|----------------|----------|-------------|------------|-------|
| 5776           | PilM           | Type IV pilus | Delta Proteobacteria |            |       |
| 5780           | PilI           | Efflux ABC permease | Delta Proteobacteria |            |       |
| 5786           | PilC           | Type IV pilus assembly protein | Delta Proteobacteria |            |       |
| 5788           | PilB           | Type IV pilus assembly ATPase | Delta Proteobacteria |            |       |
| 5870           | SigE           | Sigma factor | Delta Proteobacteria |            |       |
| 6307           | FbdB           | Chloride channel | Delta Proteobacteria |            |       |
| 6413           | PhoP3          | DNA binding response regulator | Delta Proteobacteria | S         |       |
| 6414           | PhoR3          | Sensor histidine kinase | Delta Proteobacteria | S         |       |
| 6692           | DiFe           | Chemosensory histidine kinase | Delta Proteobacteria | S         |       |
| 6694           | DiFc           | CheW-like coupling protein | Delta Proteobacteria | S         |       |
| 6696           | DiFa           | Chemotaxis receptor | Delta Proteobacteria | S         |       |
| 6699           | FbdA           | Thiol oxidoreductase | Gamma Proteobacteria | E         |       |
| 6704           | Acetyl transferase | Delta Proteobacteria | E             |           |       |
| 6855           | MokA           | Hybrid sensor kinase/response regulator | Delta Proteobacteria | S         |       |
| 6889           | HthA           | LuxR family transcriptional regulator | Delta Proteobacteria | S         |       |
| 6890           | HthB           | DNA-binding protein | Delta Proteobacteria | S         |       |
| 6996           | AsgD           | Sensor histidine kinase/response regulator | Delta Proteobacteria | S         |       |
| 7261           | DevS           | Hypothetical | Cyanobacteria |            |       |
| 7262           | DevR           | Hypothetical | Spirocheta |            |       |
| 7263           | DevT           | Hypothetical | Cyanobacteria |            |       |
| 7324           | FapA           | Hypothetical | Unique |            |       |
| 7415           | EpsZ           | Glycolys transferase | Delta Proteobacteria | E         |       |
| 7421           | EpsV           | Chain length determinant family protein | Delta Proteobacteria |            |       |
| 7422           | EpsU           | Glycolys transferase | Chloroflexi | E         |       |
| 7433           | EpsO           | Von Willebrand factor type A domain protein | Alpha Proteobacteria |            |       |
| 7438           | EpsK           | Metal resistance protein | Gamma Proteobacteria |            |       |
| 7440           | EpsL/Na24      | Sigma 54-dependent response regulator | Delta Proteobacteria | S         |       |
| 7441           | EpsH           | Glycolys transferase | Alpha Proteobacteria | E         |       |
| 7445           | EpsE           | Glycosyl transferase | Alpha Proteobacteria | E         |       |
| 7448           | EpsD           | Glycosyl transferase | Delta Proteobacteria | E         |       |
| 7450           | EpsB           | Glycosyl hydrolase | Firmicutes | E         |       |
| 7451           | EpsA           | Glycosyl transferase | Delta Proteobacteria | E         |       |

1Codon bias was determined using the software available at the Computational Microbiology Laboratory http://www.cmbl.uga.edu/software.html. The larger the number the greater the deviation from normal *M. xanthus* codon usage. Genes with no entries have a codon bias consistent with other *M. xanthus* genes.

1BLASTn algorithm was used to identify the four most closely related homologs. Each gene was classified as δ Proteobacteria if at least one of the four top homologs belongs to an organism in that group. Gene was classified as unique based on a 1e-10 cutoff. Gene was classified as ‘other’ if the closest four homologs belonged to another phylogenetic group.

3Code refers to the classification category. Proteins known to be involved in signal production or sensory transduction are denoted with an ‘S’. The remaining genes were examined for a putative enzymatic function in the annotation, which is indicated by an ‘E’.

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expressed *M. xanthus* genes which is not surprising given that these are essential genes. However, the phylogenetic approach indicated that the succinate dehydrogenase proteins SdhA (MXAN3539), SdhB (MXAN3540), and SdhC (MXAN1072), which together form complex II, are most similar to their counterparts in aerobic Firmicutes and Actinobacteria and are not present in any other δ Proteobacteria (not shown). Furthermore, the cytochrome oxidase genes (complex IV) were acquired from diverse phylogenetic sources. In contrast, NADH dehydrogenase (complex I) and the MF1cc quinol:cytochrome c oxidoreductase (complex III) are most similar to their counterparts in other δ Proteobacteria. These results argue that the electron transport pathway is encoded by a patchwork of genes, some inherited vertically and others acquired from diverse phylogenetic sources.

Figure 1. Taxonomic distribution of best normalized BLASTP matches outside the δ Proteobacteria. Bars indicate the number of best matches in a phylum or domain for *Myxococcus xanthus* (ivory bars), *Anaeromyxobacter dehalogenans* (magenta bars), and *Bdellovibrio bacteriovorus* (blue bars). Competitive matching was used for the detection of best hits using an E-value threshold of $10^{-10}$ and a minimum BLASTP score of $10^{-10}$.

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Figure 2. The *Myxococcus xanthus* electron transport chain. Phylogenetic trees were generated from individual Complex IV proteins (gray boxes).

See text for detailed description.

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Cytochrome oxidase genes have been subject to intense scrutiny because of their dissemination by LGT over vast phylogenetic distances [7]. The distribution of one subunit from each cytochrome oxidase was examined (bold subunits in table 2). Among the top twenty BLASTP hits to the M. xanthus cytochrome oxidase $c$ subunit, $Bdellovibrio$ is the only $\delta$ Proteobacteria member (not shown) suggesting that this $c$ subunit was acquired by the only two aerobic members of this group.

A neighbor-joining tree of the CydA subunit of the cytochrome $d$ quinone oxidase revealed two versions of this operon within the $\delta$ Proteobacteria (figure 3). One is found in all $\delta$ Proteobacteria with the exception of $M$. xanthus and is presumed to be ancient and vertically transmitted. $M$. xanthus appears to have lost this operon sometime after its divergence from $\delta$ dehalogenans. It is surprising to find this oxidase in anaerobic organisms where $O_2$ is highly toxic [24]. A distinctly different cyd$AB$ operon is found in $\delta$ dehalogenans and $M$. xanthus and was likely incorporated by LGT prior to the divergence of the two organisms (figure 3). The $M$. xanthus cyd$AB$ operon falls in the same clade as diverse phylogenetic groups, most notably Actinobacteria and Planctomycetes.

The $cyr$BAC operon is found in many members of the $\delta$ Proteobacteria (figure 4). Again, the presence of this operon is surprising for anaerobic species where the function is not known and suggests an ancient origin in the $\delta$ Proteobacteria. In $M$. xanthus and $\delta$ dehalogenans the $cyr$BAC operon has a different phylogenetic history from that of the other $\delta$ Proteobacteria and falls in the same clade as an eclectic mixture of organisms from diverse phyla. The results are consistent with the idea that $\text{Myxococcus}$ and $\text{Anaeromyxobacter}$ have lost the ancient version of this operon and acquired a different one. In addition, the $cyr$BAC operon has been duplicated in $M$. xanthus.

In summary, the $M$. xanthus electron transport chain is a chimeric pathway containing components from diverse phylogenetic sources. $M$. xanthus has lost ancestral cytochrome oxidase genes common to other $\delta$ Proteobacteria and acquired other cytochrome oxidase genes. Acquisition of novel cytochrome oxidase and succinate dehydrogenase genes is coincident with the appearance of aerobic growth. Curiously, the only other aerobic organism in the $\delta$ Proteobacteria, $Bdellovibrio$, has notable differences from $\text{Myxococcus}$ suggesting that it acquired aerobic metabolism using different gene sources.

| Cytochrome c Oxidase | Gene Name | CoxB | CoxD (CoxA) | CoxC | NA |
|----------------------|-----------|------|-------------|------|----|
| Cytochrome c Oxidase Function | Subunit | Subunit | Subunit | Subunit |  |
| MXAN number | 3869 | 3868 | 3867 | 3866 |  |
| Number of AA | 346 | 545 | 207 | 121 |  |
| MXAN number | 6086 | 6087 | 6088 | 6089 |  |
| Number of AA | 348 | 556 | 222 | 151 |  |

| Cytochrome bd Quinol Oxidase | Gene Name | CydA | CydB |
|-------------------------------|-----------|------|------|
| Cytochrome c Oxidase Function | Subunit I | Subunit II |
| MXAN number | 6912 | 6913 |  |
| Number of AA | 342 | 445 |  |

| Cytochrome cbb$_3$ Oxidase | Gene Name | FixG | CcoP | CcoQ | CcoNO | CcoS |
|-------------------------------|-----------|------|------|------|-------|------|
| Cytochrome c Oxidase Function | Fe-S | Ferredoxin | Subunit III | Subunit II | Maturation Protein |
| MXAN number | 5538 | 5539 | 5540 | 5541 | 5542 |  |
| Number of AA | 479 | 176 | 60 | 793 | 63 |  |

Trees are shown for enzyme subunits in bold in figures 3 and 4. doi:10.1371/journal.pone.0001329.t002

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circles are next to members of the
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DISCUSSION
The genome of virtually every free-living bacterial species contains
a contingent of genes acquired by LGT [4]. Genes acquired by
LGT can provide selective advantages with regard to antibiotic
resistance, carbon utilization, and habitat range [4,6,7]. Indeed,
most recent changes to the metabolic network of E. coli are due
to LGT rather than gene duplication [25]. We estimate that roughly
a quarter of the Myxococcus genome has been acquired by LGT
based on phylogeny and/or codon usage. Some alien genes came
from pathways that are found in diverse phyla, for example
succinate dehydrogenase and cytochrome oxidase, and their
import would be expected to provide a strong selective advantage
by allowing aerobic growth. Other alien genes were fashioned into
a unique developmental cycle and presumably played a different
role in their previous host.

Developmental innovation is correlated with a
habitat shift
What event(s) facilitated the innovation of this unique developmental
cycle and the divergent evolution of Myxococcales from other δ
Proteobacteria? We propose that the ancient myxobacterium gained
access to a novel gene pool via LGT. Whether this habitat shift predated
the emergence of fruiting body development is not clear but this issue
could potentially be resolved when the genome sequences of more δ
Proteobacteria are completed.

Myxococcus electron transport is a chimeric
pathway
Alien genes are generally integrated at the periphery of metabolic
networks [23] where they could provide an immediate selective
advantage. Multiple terminal electron acceptors provide flexibility
in the face of ever changing environmental stresses. Of the four
new M. xanthus terminal oxidase complexes, one involved the
addition of a new oxidase (αco, cbb3-type cytochrome oxidase), one
involved replacement of an ancient oxidase (αco, cytochrome bd
quinol oxidase), and one involved replacement of an ancient
oxidase followed by duplication of the imported operon (αco; heme-
copper cytochrome c oxidase). O2 has one of the most positive
reduction potentials of any terminal electron acceptor (O2/
H2O = 0.82 V) so these cytochrome oxidases are located at the
periphery of the electron transport chain. Succinate dehydroge-

nase (complex II) is located at the other end of the electron
transport chain where it, along with NADH dehydrogenase (complex I), provide reducing equivalents. Succinate dehydrogenase catalyzes the interconversion of succinate and fumarate with the reduction of FAD allowing an organism to harvest the full
potential of the tricarboxylic acid (TCA) cycle. Succinate dehydrogenase transfers reducing equivalents derived from the TCA cycle to the electron transport chain. In organisms with mixed respiration strategies like
E. coli succinate dehydrogenase is produced only during aerobic growth [27]. The phylogenetic
sources for the M. xanthus dehydrogenase and oxidases are
different suggesting a piecemeal integration into the electron
transport pathway.

Predation as a means of acquiring genes
In a microcosm containing 13C labeled E. coli added to agricultural
soil, the heavy carbon isotope was enriched in wild myxobacteria
indicating that myxobacteria are predators [28]. Does predation

Figure 4. Neighbor-Joining Tree of Subunit I (CtaD or CoxA) of the
predicted cytochrome c oxidase. The tree was generated by
identifying the twenty most similar homologs in the non-redundant
database at NCBI using the BLASTP algorithm as of Sept. 2006. Proteins
were aligned using the Muscle algorithm. Bootstrap values and
visualization were generated by the Mega 3.1 program. Tree branches
were condensed for those with a bootstrap value >50%. Small filled
circles are next to members of the δ Proteobacteria. Acidobacteria-
Acidobacteria bacterium Ellin345; Anaeromyxobacter1-Anaeromyxobacter
dehalenogens 2CP-C YP_465481.1; Anaeromyxobacter2-Anaeromyxobacter
dehalenogens 2CP-C YP_465481.1; Bdellovibrio-Bdellovibrio bacteriovorus
HD100; Blastopirellula-Blastopirellula marina DSM 3645; Chloro-
flexus-Chloroflexus auranticus J-10-ff; D_desulfuricans-Desulfovibrio
desulfuricans G20; D_vulgaris1-Desulfovibrio vulgaris; D_vulgaris2-Desul-
fovibrio vulgaris subsp. vulgaris str. Hildenborough; G_metalldendecus-
Geobacter metalldendecus GS-15; G_sulfurreducens-Geobacter uraniredu-
cens PCA; G_uranireduccens-Geobacter uranireduccens RH4; Gloe-
bacter-Gloeobacter violaceus PCC 7421; L_interrogans1-Leptospira inter-
rogans serovar Copenhagenii str. Fiocruz L1-130; L_interrogans2-Leptos-
pora interrogans serovar Lai str. 56607; Myxococcus1-Myxococcus xanthus
xanthus DK 1622 YP_6320481.1; Myxococcus2-Myxococcus xanthus
DK 1622 YP_6320481.1; Nitrosothermus-Rhodothermus hamburgensis X14; Nitroso-
coccus-Nitrosococcus oceani ATCC 19707; P_carbinolicus-Pelobacter
carbinolicus DSM 2388; Ralstonia-Ralstonia eutropha JMP134; Rhodopir-
eillula-Rhodopirellula baltica SH 1; Rhodothermus-Rhodothermus marinus;
Salinibacter-Salinibacter ruber DSM 13855; Solfibacter-Solibacter usitatus
Ellin6076; Synechococcus-Synechococcus sp. PCC 7002; Synechococcus_e-
longatus-Synechococcus elongatus PCC 7942; T_thermophilus1-Thermus
thermophilus HB827; T_thermophilus2-Thermus thermophilus HB8

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Evolution of Myxobacteria
enhance the acquisition of new genes by the predator? The LGT observed with *Bdellovibrio* has been proposed to arise, at least in part, through the predatory capabilities of the organism [13]. Indeed, the *Bdellovibrio* genome is enriched in genes whose closest homolog is in the γ-Proteobacteria, the group containing the principal prey species [13]. Like *Bdellovibrio*, the *M. xanthus* genome is enriched in genes whose closest homolog is in the γ-Proteobacteria, the group containing the commonly used prey species *E. coli* [28]. Myxobacteria have a much broader prey range than *Bdellovibrio* and, significantly, a broader distribution of close gene homologs (figure 1). Some notable differences from *Bdellovibrio* include genes from Actinobacteria, Acidobacteria, and Cyanobacteria (i.e., >8-fold increase), which tend to be residents of same type of soil habitats [29]. Acidobacteria are thought, on the basis of 16S gene abundance, to be one of the most prominent members of soil, though few species have been cultivated [30]. It has been recently proposed that Acidobacteria form a sister clade with the δ-Proteobacteria [31]. While vertical inheritance from a common ancestor could result in the high representation of Acidobacteria genes, this hypothesis does not explain why *Bdellovibrio* has so few Acidobacteria genes (figure 1). Organisms in the other highly represented bacterial groups are known to be excellent food sources for *M. xanthus* in the laboratory [32]. *M. xanthus* is known to acquire genes from *E. coli* by conjugation [33] and transduction [34] under laboratory conditions but gene transfer in the natural environment has not been experimentally demonstrated.

**Conclusions and prospectus**

Our results argue that genes acquired from community members influence bacterial evolution. Successful community members may foster the evolution of successful communities by sharing genetic and phenotypic innovations that promote fitness. Cooperative evolution can have strong selective advantages in nature as demonstrated by the widespread emergence of antibiotic resistance or LGT of terminal oxidases in electron transport chains. It follows then that biologically diverse habitats aid the evolution of new bacterial species by providing a larger pool of prospective genes. While studies of the biogeography of microorganisms is in its infancy, bacterial communities can be endemic to certain areas or associated with unique species of higher organisms [35]. Habitat destruction and loss of species diversity could restrict the evolution of new bacterial groups by limiting combinations of LGT.

**MATERIALS AND METHODS**

**Isolation of fruiting body deficient mutants**

*Mycococcus xanthus* LS2208 was grown to a density of 5 x 10^8 cells/ml in CYE broth [10 g/L Difco Casitone, 5 g/L yeast extract, 10 mM 3-(N-morpholino)propanesulfonic acid (MOPS; pH 7.6), and 4 mM MgSO_4]. A 5 ml aliquot was harvested by centrifugation, washed twice with 1 ml of sterile distilled water, and resuspended in 30 μl sterile distilled water. Washed cells were mixed with plasmid pMycoMar [14], and electroporated at 0.65 kV, 400 Ω, and 25 μF [36]. The electroporation cuvette was flushed immediately with 1 ml of CYE broth to recover cells, which were then incubated with shaking at 32°C for 4 h before plating on CF agar [10 mM Tris HCl, pH 7.6, 1 mM KH_2PO_4, pH 7.6, 8 mM MgSO_4, 0.2 mg/ml Na-pyruvate, 2 mg/ml Na-citrate, 150 μg/ml casitone, 1 mg/ml Na-tryptophan, 5 mg/ml Na-tryptophan, 1.5% Difco agar] supplemented with kanamycin (50 μg/ml) and incubated at 32°C. After 9–10 days, plates were screened under the microscope to identify colonies that were defective in making fruiting bodies. Potential mutants were screened again on CF Km plates and once on TPM agar [10 mM Tris HCl, pH 7.6, 1 mM KH_2PO_4, pH 7.6, 10 mM MgSO_4, 1.5% Difco agar]. Approximately 40,000 colonies were screened. Strains containing a *magellan-4* insertion were backcrossed to *M. xanthus* DK1622 (wild type) by electroporation of 1 μg genomic DNA [37] or generalized transduction with phage Mx4 [38].

**Cloning of M. xanthus genomic DNA flanking magellan-4 insertions**

To clone *magellan-4* insertions, genomic DNA was isolated from vegetative cultures grown in CYE medium containing 50 μg/ml kanamycin. A 1 ml cell culture was harvested by centrifugation and resuspended in 0.2 ml of 1X PBS buffer [8 g NaCl, 0.2 g KCl, 1.44 g Na_2PO_4 and 0.24 g KH_2PO_4 in 1000 ml distilled H_2O; pH 7.4]. Genomic DNA was isolated by using Invitrogen Easy-DNA kit. Genomic DNA (0.5 μg) was digested with BstHII (New England Biolabs) in a total volume of 20 μl and digestions were dialyzed on a 0.025 μm pore size filter (Millipore) against distilled water for 30 min (drop dialysis). 8 μl of this DNA was treated with T4 DNA ligase (Promega) and drop-dialyzed before electroporation into *E. coli* host CC118 [15]. Electroporants were recovered on LB agar containing kanamycin (50 μg/ml) after incubation at 37°C for 24 h. Plasmid DNA was sequenced with primers M1 and M2 [15].

**Fruiting body formation and sporulation assay**

*Mycococcus xanthus* cells were grown in CYE broth to about 5 x 10^8 cells/ml. Cells were resuspended in TPM buffer [10 mM Tris HCl, pH 7.6, 1 mM KH(H_2)PO_4, pH 7.6, 10 mM MgSO_4] to a final density of 1 x 10^10 cells/ml. 10 μl of each suspension was spotted onto TPM agar plates and incubated at 32°C. Digital images of fruiting body formation were taken every 24 h for a total of 72 h. Cells were resuspended in 1 ml of TPM buffer, heated at 50°C for 2 hours, and sonicated to kill vegetative cells. Spore production was determined by direct counts using a Petroff-Hauser chamber. Spore viability was determined by plating serial dilutions on CYE agar plates. Plates were incubated at 32°C for 5 days before counting colonies.

**Genomic analyses**

Phylogenetic and comparative genomic analyses were conducted with data from >300 genomes. Predicted peptides from each of eight completed δ-Proteobacteria genomes were obtained from NCBI in March and April 2006 including *Anaeromyxobacter dehalogenans*, *Bdellovibrio bacteriovorus*, *Desulfofaba psychrophila*, *Desulfovibrio desulfuricans*, *Geobacter sulfurreducens*, *Lawsonia intracellularis*, *Mycococcus xanthus*, and *Pelobacter carbinolicus*. Comparison of peptides was determined using the BLASTP algorithm without filtering against the non-redundant peptide database at NCBI during this time period [39]. The top ten hits for each predicted peptide were identified with an E-value <10^-10. The species of each top hit and the corresponding bacterial group were identified from the BLAST algorithm output. The identity of each bacterial group used the taxonomic data provided by NCBI. Alignments of predicted peptides were generated using the Muscle algorithm [40]. Neighbor-joining trees and tree visualization were performed using MEGA version 3.1 [41]. Tree branches with a bootstrap value <50% were condensed.

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Author Contributions
Conceived and designed the experiments: LS. Performed the experiments: SB. Analyzed the data: BG LS SB. Contributed reagents/materials/analysis tools: LS. Wrote the paper: LS. Other: Performed bioinformatics: BG. Generated several figures: BG.

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