Lateral Gene Transfer from the Dead

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Abstract.—In phylogenetic studies, the evolution of molecular sequences is assumed to have taken place along the phylogeny traced by the ancestors of extant species. In the presence of lateral gene transfer, however, this may not be the case, because the species lineage from which a gene was transferred may have gone extinct or not have been sampled. Because it is not feasible to specify or reconstruct the complete phylogeny of all species, we must describe the evolution of genes outside the represented phylogeny by modeling the speciation dynamics that gave rise to the complete phylogeny. We demonstrate that if the number of sampled species is small compared with the total number of existing species, the overwhelming majority of gene transfers involve speciation to and evolution along extinct or unsampled lineages. We show that the evolution of genes along extinct or unsampled lineages cannot be approximated as those of independently evolving lineages described by a few global parameters. Using this result, we derive an algorithm to calculate the probability of a gene tree and recover the maximum-likelihood reconciliation given the phylogeny of the sampled species. Examining 473 near-universal gene families from 36 cyanobacteria, we find that nearly a third of transfer events (28%) appear to have topological signatures of evolution along extinct species, but only approximately 6% of transfers trace their ancestry to the common ancestor of the sampled cyanobacteria. [Gene tree reconciliation; lateral gene transfer; macroevolution; phylogeny.]

From the first growth of the tree, many a limb and branch has decayed and dropped off; and these lost branches of various sizes may represent those whole orders, families, and genera which have now no living representatives, and which are known to us only from having been found in a fossil state. Charles Darwin, On the Origins of Species. London, 1859

Most of the diversity of life that ever existed on earth has gone extinct and can only be glimpsed from the fossil record. Although the comparative approach allows the reconstruction of some morphological and genetical characteristics of ancestral species, it is only informative for species that have founded extant lineages. Yet, the information enclosed in genome sequences is abundant and particularly meaningful for the reconstruction of the descent and evolution of their carriers (Zuckerkandl and Pauling 1965; Boussau and Daubin 2010; David and Alm 2011), so much so that it may have recorded accounts of extinct lineages. This possibility exists because the success of lateral gene transfer (LGT) as an evolutionary process implies that each gene possesses its own, unique history, which is not necessarily confined to the history of those species that have survived (Maddison 1997; Galtier and Daubin 2008; Fournier et al. 2009; Abby et al. 2012).

Several models have recently been developed to reconcile seemingly contradictory gene phylogenies with the species phylogeny by tracing the path on the species phylogeny along which they evolved as a result of a series of speciations, gene duplications, LGT, and losses (Tofigh 2009; Doyon et al. 2010; David and Alm 2011; Szöllősi and Daubin 2012; Szöllősi et al. 2012). None of these models, however, take into consideration the fact that, in the presence of LGT, gene trees record evolutionary paths along the complete species tree, including extinct and unsampled branches, and not only along the phylogeny of the species in which they reside today. This is the case because, as first noted by Maddison (1997) and later elaborated by Gogarten and coworkers (Zhaxybayeva and Gogarten 2004; Fournier et al. 2009), while LGT events imply that the donor and receiver lineages existed at the same time, the donor lineage might have subsequently become extinct, or more generally, might not have been sampled.

Here, we demonstrate that, if the number of species considered in the species phylogeny is small compared with the total number of species, the overwhelming majority of gene transfers involve speciation to and evolution along extinct or unsampled species. Furthermore, we show that, if this condition is met, the evolution of genes along the unrepresented parts of the species phylogeny can to good approximation be treated as those of independently evolving lineages, the behavior of which depends only on the global parameters of the speciation dynamics. This in turn allows us to derive the probability of observing a gene phylogeny by extending the ODT model introduced previously (Szöllősi et al. 2012). Applying our model to a data set derived from 36 cyanobacterial species, we perform a preliminary assessment of the phylogenetic signal for the evolution of transferred genes along extinct species.
To describe the evolution of genes within the genomes of species, we assume genes to evolve independently according to a birth-and-death process that consists of gene duplication, transfer, and loss (Tofigh 2009; Szöllösí and Daubin 2012; Szöllösí et al. 2012). As shown in Figure 1b, a gene in the genome of any of the N species can: (i) be duplicated at rate \( d \); (ii) be transferred from a donor species to any of the other N−1 possible host species at a rate \( t/(N−1) \); or (iii) be lost at a rate \( l \). Gene copies can also be born and be lost as a result of the speciation dynamics: (iv) at the species level, lineages experience speciation at a rate \( s \), in which case they are replaced by two copies in the two new species or; (v) suffer extinction at an identical rate \( e \). A branch \( c \) of the represented tree \( S \) in general corresponds to a series of speciation events, however, as shown in Figure 1c, only the last one of these, the speciation event that gave rise to two represented lineages, is explicitly present for internal branches as the (green online) speciation node terminating the branch.

**Almost All Transfers Involve Speciation**

To understand what fraction of transfers involves evolution along unrepresented species, we must compare the relative rate of transfers that are direct transfers between branches of the represented phylogeny \( S \) and indirect transfers that result in a gene returning to \( S \) after exiting it via speciation or transfer to unrepresented species.

To compare the contribution of indirect transfers and direct transfers to observed gene histories, we consider first only direct transfers and indirect transfers that involve a speciation to an unrepresented species. To describe the shape of the species tree generated by the Moran process introduced above, we can use the coalescent approach. Here, under Kingman’s coalescent, the time to the most recent common ancestor of the \( n \) sampled species is of the order of \( 2N/n(1−1/n) ≈ 2N/n \) (Kingman 1982). This implies that the expected number of unrepresented speciation events per branch of the species tree is much larger than one, being of the order of \( (α×2N/n(2n−2))≈N/n \geq 1 \), as there are \((2n−2)\) branches of \( S \). This suggests that for any pair of coexisting branches of the represented tree, a gene that descends from one of the branches and is transferred to the other, is likely to have experienced a speciation event “away” from the represented phylogeny spanned by the \( n \) sampled species before being transferred back to it.

To quantify the above argument, we can compare the expected number of transfers from branch \( b \) to branch \( c \) of the represented phylogeny, resulting from either a direct transfer or a more complex history involving a speciation event. Clearly, if the branches do not overlap in time, the expected number of direct transfers is zero. To consider overlapping branches, let us consider for simplicity that both \( b \) and \( f \) are terminal branches—similar results can be derived for any other pair of overlapping branches. The expected branch lengths are then \( E(b) = E(f) = N/n \).
and (iii) the rate at which it coalesces with branch $f$. 

$$t = t_f' \text{ with rate } \frac{\sigma}{N}$$

evolution outside

$$e^{-\sigma (t_f'-t_f)}$$

Transfer back at $t = t_f' \text{ with rate } \frac{\tau}{N-1}$

Figure 2. The overwhelming majority of transfers involve evolution along unrepresented species. A direct transfer (dark gray, blue online) between two terminal branches of the represented phylogeny occurs with rate $\tau/(N-1)$ and involves a single transfer event. An indirect transfer (light gray, red online) that leaves an indistinguishable record in the gene tree topology. To count indirect transfers, we trace their history backwards in time: transfer back to the host branch on the represented tree (branch $c$) occurs with a rate $\tau/(N-1)$ from each of the $N-n$ unrepresented species, of these we are only concerned with ones which descend from the relevant donor branch (branch $f$), the number of these can be calculated using the exponential coalescence probability and the rate of unrepresented speciations $\sigma/N$ from the donor branch (branch $f$).

with overlap $\min(t_c, t_f) \leq N/\sigma$. Integrating over possible transfer times, the expected number of direct transfers is then

$$T_{\text{direct}} \approx \int_0^{\frac{N}{\sigma}} \int_0^{\frac{1}{N-1}} \frac{\tau}{2n(N-1)} \left(2N\right)^n d\tau' d\tau'$$

To estimate the expected number of indirect transfers that are topologically indistinguishable from the above direct transfers, we can reason backwards in time as illustrated in Figure 2: (i) the rate at which a transfer occurs from each of the $(N-n)$ unrepresented species to branch $c$ is $\tau/(N-1)$, (ii) the probability of this gene lineage not coalescing back to any of the $n$ branches of the represented tree during a time interval $t$ is $\exp(-n\sigma/\sigma t)$, and (iii) the rate at which it coalesces with branch $f$ is $\sigma/N$. Integrating over possible speciation and transfer times gives:

$$T_{\text{indirect}} \approx \int_0^{\frac{N}{\sigma}} \int_0^{\frac{1}{N-1}} \frac{\tau}{2n(N-1)} \left(2N\right)^n \left(e^{-\sigma \tau} \frac{(N-n)\tau}{(N-1)} - \frac{(N-n)\tau}{(N-1)} + \frac{\tau}{2n(N-1)} \left(2N\right)^n \right) d\tau' d\tau'$$

Equations (1) and (2) show that if the number of sampled species is small compared with the total number of species ($n \ll N$), then the expected number of direct transfers is small compared with indistinguishable indirect ones ($T_{\text{direct}} \ll T_{\text{indirect}}$), that is, the contribution of direct transfers to observed gene histories is negligible.

To compare the two types of possible indirect transfers back to $S$—those exiting via speciation and those via transfer—we must contrast the rate $\sigma$ at which gene copies exit branch $f$ as a result of speciation and the rate $\tau/(N-1) \times (N-1-n) \approx \tau$ that gene copies exit as a result of transfer. Estimates of $\tau$, and more generally gene birth and death rates, are available from several sources, all of which agree that the expected number of gene birth and death events per branch is below unity. Models that consider the dynamics of the number of homologous gene copies along a species phylogeny (referred to as phylogenetic profiles) (Csíkos and Miklós 2009) have consistently found that birth and death rate is of the same order, with an excess of loss compensated by origination of new families, in agreement with phenomenological models of gene family size distribution (Karev et al. 2002; Szöllősi and Daubin 2012). In a detailed study, Csíkos et al. found for 28 archaea that the expected number of birth events (duplication and gain) is 0.12 and that the expected number of losses is 0.36 (Csíkos and Miklós 2009) per branch per gene. More recently, the ODT model that attempts to explicitly explain the evolution of multicopy gene trees (representative of complete genomes) along an ultrametric species tree has arrived at similar results (Szöllősi et al. 2012), finding for 36 cyanobacterial genomes $\tilde{b} \approx 0.2$, $\tilde{l} \approx 1$, in units corresponding to a tree with unit height. Assuming, as above, that the time to the most recent common ancestor of the sampled species is of the order of $2N/\sigma$, that is, the expected number of gene copies (per gene) exiting a branch of $S$ is proportional to $N/n$, while the number exiting as a result of transfer is less than one. Since the rate at which a gene that has exited the represented phylogeny returns to $S$ as a result of transfer at some point in the future is independent of the mode of exit from $S$, we can conclude that indirect transfers are dominated by paths that include a speciation. In summary, if the number of sampled species is small compared with the total number of species, transfers in observed gene histories are dominated by paths that include a speciation to an unrepresented species and subsequent transfer back to the represented tree.

The Probability of Observing a Gene Tree

Reconciling gene trees with the species tree requires iterating over possible paths along which a gene tree may have been generated by a series of speciations, duplications, transfers, and losses (Fig. 3). In existing methods (Tofigh 2009; Doyon et al. 2010; Szöllősi and Daubin 2012; Szöllősi et al. 2012), this is accomplished by only considering paths along the represented phylogeny and using a dynamic programming approach exploiting the independence of gene birth and death events and by extension gene lineages. Although gene duplication, transfer, and loss can reasonably be modeled as independent birth and death events, speciation and extinction necessarily involve the simultaneous birth and death of many genes. Along the represented phylogeny, speciation events are fully specified and can be explicitly taken into account (Szöllősi et al. 2012). This is not the case, however, for speciation and extinction events that occur
in the unrepresented part of the phylogeny, or do not correspond to speciation nodes of the represented phylogeny. Therefore, unrepresented speciations result in nonindependence of gene lineages.

Consider for instance the probability $E_k(t)$ that $k$ genes present at time $t$ in a species not ancestral to the sample of $n$ extant species leave no observed descendant. Conditional on the complete phylogeny, $\phi$ including all extant species lineages, gene lineages are independent, and therefore $E_k(t)(\phi) = (E(t)(\phi))^k$. Averaging over all complete phylogenies compatible with the phylogeny reconstructed based on the $n$ species, however, results in $E_k(t)(\phi) = (E(t)(\phi))^k \neq (E_k(t)(\phi))^k$, which is not a product of $k$ independent factors.

On the other hand, $n \ll N$ implies that $E(t) = 1$. Introducing the notation $\tilde{E}(t)(\phi) = 1 - E(t)(\phi)$ and $\tilde{E}(t) = 1 - \epsilon(t)$, and neglecting second- and higher order terms in $\epsilon(t)$ and $\epsilon(t)$, we have

$$
\tilde{E}_k(t) = (\tilde{E}_k(t)(\phi))_b = [1 - \epsilon(t(\phi))]_b = \left(1 - k\epsilon(t(\phi))_b\right) = 1 - k\epsilon(t)
$$

$$
\simeq (1 - \epsilon(t))^k = \tilde{E}(t)^k .
$$

A similar argument can be derived for $k$-gene propagator $G_k(s, t)$ (see the Appendix). Therefore, if $n \ll N$, then to good approximation, the evolution of two genes observed in the same unrepresented species can be treated as independent without specifying the full phylogeny.

Under the above assumption that unrepresented speciation and extinction events can be considered in a genewise independent manner, we can describe the evolution of gene copies that appear as single gene lineages when observed from the present. We can calculate: (i) the extinction probability $E(t)$ that a gene seen at time $t$ on branch $e$ of $S$ leaves no observed descendant, that is, no descendant exists at time $t = 0$ in the genome of any of the $n$ sampled species; (ii) the extinction probability $\tilde{E}(t)$ that a gene seen at time $t$ in an unrepresented species leaves no observed descendant; (iii) the single gene propagation probabilities $G_k(s, t)$ that all observed descendants of a gene seen at time $s$ on branch $e$ descend from a descendant seen at a later time $t < s$ on branch $e$; and (iv) $G(s, t)$ the probability that all observed descendants of a gene seen at time $s$ in an unrepresented species descend from a descendant seen at time $t < s$ in an unrepresented species. Each of the above functions can be expressed as differential
equations describing evolution backwards in time by considering the set of possible events that change the relevant probability. These can be derived analogously to (Tofigh 2009; Stadler 2011; Szöllősi et al. 2012) and can be found in the Appendix.

Given a rooted gene tree topology G, we can now calculate the probability \( P(G|S, M) \) of observing G, where M denotes the parameters of the model, by summing over all possible paths along S and over all complete phylogenies compatible with \( S \) spanning the \( n \) species of the sample. We can sum over all paths by recursively mapping the branches of G onto branches of S generalizing the ODT models algorithm (Szöllősi et al. 2012) to include evolution along unrepresented species (cf. Figs 3 and A1 in the Appendix).

A branch of G represents the evolution of a gene copy for which (i) if the branch is nonterminal, all observed descendants descend from one of the two daughter gene lineages which emerge from the gene tree node in which the branch terminates or (ii) if the branch is terminal, a gene is observed in one of the genomes mapping to a leaf of S. To describe possible paths along S that this gene copy may take before arriving at the gene tree node in which it terminates, we must consider five events: (i) single-copy evolution along branch \( e \) of S described by \( G_{e} \), (ii) single-copy evolution outside S described by G, (iii) speciation from a branch of S to an unrepresented species such that only descendants of this copy are observed, (iv) transfer such that only descendants of the transferred copy are observed, and (v) speciation represented in S such that only one of the descending copies leaves an observed descendant.

To derive the recursion expressing the probability of G as the sum over possible paths along S, we discretize time along S keeping track of speciation times \( t_{i} \) along S. Speciations represented in S define the time intervals \([0, t_{1}], ..., [t_{i-1}, t_{i}], ..., [t_{n-1}, t_{n}]\) referred to as time slices (Tofigh 2009; Doyon et al. 2010) with indices 0, ..., \( i \), ..., \( n \). We further divide each time slice into \( D \) equal time intervals of height \( \Delta t_{i} = (t_{i+1} - t_{i}) / D \).

The probability of the gene lineage leading to node u of G being seen on branch \( e \) of S at time \( t = t_{i} + \Delta t_{i} \) given the probabilities at time \( t = t_{i} \) is

\[
P_{e}(u, t + \Delta t_{i}) = G_{e}(t + \Delta t_{i}, t)P_{e}(u, t)
\]

where \( P_{e}(u, t) \) denotes the probability of the gene lineage leading to node u of G being seen in an unrepresented species at time t, \( v \) and \( w \) descend from u in G. As shown in Figure A1a in the Appendix, the terms correspond to (i) no event with an observed descendant; (ii) birth of two gene lineages by duplication, such that both leave observed descendants; (iii) and (iv) birth of two gene lineages with observed descendants as a result of an unrepresented speciation; and finally, (v) unrepresented speciation followed by the loss of the copy in branch \( e \) such that only the copy in the unrepresented phylogeny leaves an observed descendant. In the above expression, we only consider indirect transfers that involve a speciation, see the Appendix for the full expression.

The probability of being seen in such an unrepresented species is

\[
\tilde{P}(u, t + \Delta t_{i}) = \tilde{G}(t + \Delta t_{i}, t)\tilde{P}(u, t)
\]

where \( \tilde{P}(u, t) \) denotes the probability of the gene lineage leading to node u of G being seen in an unrepresented species at time t, \( v \) and \( w \) descend from u in G. As shown in Figure A1a in the Appendix, the terms correspond to (i) no event with an observed descendant; (ii) birth of two gene lineages by duplication, such that both leave observed descendants; (iii) and (iv) birth of two gene lineages with observed descendants as a result of an unrepresented speciation; and finally, (v) unrepresented speciation followed by the loss of the copy in branch e such that only the copy in the unrepresented phylogeny leaves an observed descendant. In the above expression, we only consider indirect transfers that involve a speciation, see the Appendix for the full expression.

The terms (cf. Fig. A1b) correspond to (i) no event with an observed descendant; (ii) birth of two gene lineages by duplication, or transfer, such that both leave observed descendants; (iii) and (iv) birth of two gene lineages with observed descendants as a result of transfer back to the represented phylogeny; and finally, (v) transfer back to the represented phylogeny following which the copy in the unrepresented donor lineage does not leave an observed descendant. Terms involving gene lineages \( v, w \) are zero if u is a leaf of G in both the above expressions.

At speciation times \( t = t_{i} \) where branches \( f \) and \( g \) descend from \( e \) in S, a represented speciation takes place that may be followed by a loss:

\[
P_{e}(u, t) = P_{e}(v, t)P_{g}(w, t) + P_{f}(w, t)P_{g}(v, t)
\]

where \( E_{f}(S) \) denotes the set of branches of S in time slice \( i \). As shown in Figure A1b, the terms correspond to (i) no event with an observed descendant; (ii) birth of two gene lineages by speciation, duplication, or transfer, such that both leave observed descendants; (iii) and (iv) birth of two gene lineages with observed descendants as a result of transfer back to the represented phylogeny; and finally, (v) transfer back to the represented phylogeny following which the copy in the unrepresented donor lineage does not leave an observed descendant. Terms involving gene lineages \( v, w \) are zero if u is a leaf of G in both the above expressions.

The terms (cf. Fig. A1c) correspond to (i) and (ii) represented speciation such that both resulting gene lineages lead to observed descendants; and (iii) and (iv) represented speciation such that only one of them do. Finally, at time \( t = 0 \) on each terminal branch \( e \) of S, the presence of observed genes is expressed as:

\[
P_{e}(u, 0) = \begin{cases} 1 & \text{if } u \text{ is a leaf of } G \text{ found in } e \\ 0 & \text{otherwise} \end{cases}
\]
As illustrated in Figures 3b and A1, each term in Equations (4)–(7) above corresponds to a series of speciation, duplication, and transfer events that recursively draw the gene phylogeny into the species tree. The recursion calculates the probability of a gene tree with m genes in O(Dn²m) steps, as there are fewer than n branches in each time slice and n time slices. Summing over roots of G can be accomplished with identical complexity using double recursion. The most likely reconciliation can be recovered by tracing back along the sum choosing at each step the event with the highest probability.

Calculating the probability of a gene tree requires knowledge of the ultrametric species tree S, with branch lengths corresponding to time, the rate of duplication \( \delta \), transfer \( \gamma \), and loss \( \lambda \), as well as the parameters of the speciation dynamics, the species replacement rate \( \sigma \), and the total number of species \( N \). The number of parameters is reduced, if we assume the time to the common ancestor of the sampled species to correspond to its expected value under speciation dynamics. Choosing units such that \( S \) is of unit height, this corresponds to the choice \( \sigma = 2N \). Furthermore, under the present choice of parameters and time scale, the probability of a gene tree and its maximum-likelihood reconciliation depends only very weakly on \( N \), as long as the condition \( n \ll N \) is satisfied. This is the case because the expected number of transfers between branches of \( S \) is nearly independent of \( N \). In particular, if we assume that a gene lineage returns at most once to \( S \) and arrive at the result derived in Equation (2) according to which the number of transfers is independent of \( N \).

**Routes to Cyanobacterial Genomes**

To carry out a preliminary analysis of the signal for evolution outside the represented phylogeny in real data, we considered a set of 473 single-copy gene families present in the genome of at least 34 of 36 cyanobacteria and use the dated species tree reconstructed in Szöllösi et al. (2012). We choose single-copy near-universal gene families as they are expected to be (i) relatively slowly evolving and hence to harbor a strong signal of homology and yield high-quality alignments, and (ii) they can be assumed to be well described by a single set of uniform duplication, transfer, and loss rates, at least in contrast to more complex data sets composed of multicity families. For each family, gene tree topologies and duplication, transfer, and loss rates that maximize the joint likelihood (Maddison 1997; Szöllösi and Daubin 2012) were inferred as described in the Appendix. Using these results, 1000 reconciliations per family were sampled by stochastic backtracking along the sum over reconciliations.

On average, we found 0 duplication, 2.15 transfers, and 2.56 losses per family. The distribution in time of transfer events and the preceding speciations to unrepresented species are shown in Figure 4a. The majority of transfers occur between branches of \( S \) that overlap in time, hence the resulting gene tree carries no topological signature of the length of time spent evolving along unrepresented lineages. Transfers between branches that do not overlap in time, for which the gene tree topologies explicitly record evolution outside the represented tree, correspond to 27.8% of all transfers. About a fifth of these (5.9% of all transfers) branch above the root indicating transfer from outside the sampled diversity of cyanobacteria. The median interval of time spent evolving in unrepresented lineages is 0.085 (or 222 million years, henceforth myr) for transfers between overlapping branches and 0.39 (or 1000 myr) for transfers between nonoverlapping branches. Similar values are obtained if we consider only the maximum-likelihood reconciliations, except for the median interval of time spent evolving in unrepresented lineage for transfers between overlapping branches which is only 0.0028 (or 8.1 myr corresponding to the minimum length allowed by time discretization). The corresponding value for transfers between nonoverlapping branches, 0.36 (or 990 myr), is nearly identical to the value above.

We emphasize that an important caveat of these results is that the accuracy of our method to infer correct reconciliations and gene topologies has not been assessed. This could be accomplished by explicit simulations of gene family evolution along the complete phylogeny. Such simulations are, however, outside the scope of the current publication, as they are technically challenging due to the large number of species in the complete phylogeny, and because they must address a potentially long list of possible questions. In lieu of simulation, it is possible to examine the posterior support of individual transfer events, which, as described in the Appendix, can be calculated as the fraction of times we find a given transfer event among the sampled reconciliations for each family. Using this measure, we find that transfers are well supported with 66.8% of transfer events having support over 0.95.

It is also important to discuss to what extent we can expect observed transfers between nonoverlapping branches to be robust to increasing the number of sampled species. Consider the extreme case that all \( N \) extant species are sampled. It is clear that transfers between overlapping branches of \( S \) (red in Fig. 4b) may correspond to transfer between nonoverlapping branches of the full phylogeny spanned by all \( N \) extant species. To ascertain how often we expect the opposite to occur, to have a transfer between nonoverlapping branches of \( S \) correspond to transfers between overlapping branches of the full phylogeny spanned by all \( N \) extant species, we need to estimate how often we expect to sample an extant descendant of the unrepresented donor lineage involved in a transfer between nonoverlapping branches of \( S \) (light bars, yellow online, in Fig. 4b). Assuming a tree with unit height, the total branch length of the full phylogeny under Kingman’s coalescent is of the order of \( \log(N) \), while the total branch length including extinct species is of the order \( N \). Thus, we expect...
that only a vanishing fraction of the order log(N)/N of donor lineages have left extant descendants. This implies that not only do most transfers involve speciation to, and evolution along branches of the complete phylogeny, but also the majority of these donor lineages have gone extinct. Consequently, most transfers between nonoverlapping branches of S correspond to transfers between nonoverlapping branches of the full phylogeny where the donor lineage has gone extinct.

In summary, we find that nearly a third (27.8%) of transfers evolve on average a billion years along lineages unrepresented in the phylogeny—most often, in fact, along extinct lineages, and only a moderate fraction of transfers originate from outside the cyanobacteria. Furthermore, both of these estimates are conservative, as increasing the number of sampled species is expected to lead to an increase in the ratio of transfers between nonoverlapping branches, and to a decrease in the fraction of transfers from outside of cyanobacteria. The first of the above results, however, apply only to transfers between branches of S, that is, transfers observed for the n = 36 cyanobacteria considered. For the complete set of transfers between branches of the full phylogeny, the fraction of transfers evolving along extinct lineages is potentially different, for example, a macroscopic fraction of transfers are expected to correspond to direct transfers between its branches.

**DISCUSSION**

The results developed above are conditional on two crucial assumptions: (i) that the number of sampled species is small compared with the total number of species, and (ii) the evolution of gene lineages can be treated as independent, both in the represented and in the unrepresented part of the phylogeny. As we argue above, if genes are duplicated, transferred, and lost independently, the former assumption (i.e., n ≪ N) implies that the evolution of genes outside the represented phylogeny can also be treated as
in the deep part of the genealogy. LGTs, on the stochasticity of Kingman's coalescent, in particular data. Single-locus inference is limited by the intrinsic to inferring past demography based on single-locus Drummond 2008), and as such, have the potential biodiversity.

The observation that LGT events record evolutionary paths in Figure 3, we do not know the exact evolutionary scenario we must sum over all reconciliations. This process can be represented as

APPENDIX

The dynamic programming algorithm described in Equations (4)–(7) calculates the likelihood of a gene tree given the species tree and the rates of speciation, duplication, transfer, and loss. As illustrated in Figure 3, the likelihood is calculated in a piecewise independent manner, from the evolution of gene copies that appear as single gene lineages when observed from the present. Because in contrast to Figure 3, we do not know the exact evolutionary scenario we must sum over all reconciliations. This process can be represented as

Below we provide the expressions for each of these functions that can be derived using the theory of birth- and-death processes. We also discuss the independence assumption in relation to the single gene propagators, write down the complete form of Equation (4) and describe the details of the data analysis presented in the main text.

SUPPLEMENTARY MATERIAL

Data files related to this paper have been deposited at Dryad under doi:10.5061/dryad.27d0g.

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independent, even if the complete phylogeny is not specified.

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More importantly, as long as these conditions are met, it is possible to extend the above results to more general models of speciation. Modeling variation in \( N \), the total number of species, over geological times, could be of particular interest. Indeed, a corollary of the observation that LGT events record evolutionary paths along the complete species tree is that the phylogenies of genes from a limited sample of extant species carry information about extinct lineages, and therefore about the size and dynamics of ancient biodiversity. In fact, patterns of gene transfer may be even more informative about past biodiversity than the species tree itself. Drawing an analogy with population genetics, inferring biodiversity dynamics based on species trees (Nee 2001; Morlon et al. 2010; Stadler 2011) is similar to inferring past demography based on single-locus data. Single-locus inference is limited by the intrinsic stochasticity of Kingman’s coalescent, in particular in the deep part of the genealogy. LGTs, on the other hand, are analogous to multiple loci (Heled and Drummond 2008), and as such, have the potential to increase the statistical power for inferring past biodiversity.
Evolution of Single Genes

The forward Kolmogorov equations describing single gene extinction and propagation can be derived analogously to Tofigh (2009), Stadler (2011), and Szöllösi et al. (2012). The main differences is that here we also consider the speciation dynamics.

The extinction probability for branch $e$ of $S$:

$$
\frac{d}{dt} E_e = + (\lambda - 1 - E_e) E_e - \frac{\sum f \in \mathcal{E}_i(S)}{N-1} (1-E_f) E_e - \left( \frac{\sum f \in \mathcal{E}_i(S)}{N-1} (1-E_f) \right) E_e
$$

where $\mathcal{E}_i(S)$ denotes the set of branches of $S$ in time slice $i$, and $n_i$ their number. The terms correspond to (i) loss (ii) the rate duplications, and (iii) transfers to represented hosts, both conditional on survival, and finally, (iv) the rate of unrepresented speciations and transfers to unrepresented hosts, again conditional on survival. The initial conditions specify that at the end of branch $e$ the probability of extinction is 0 if we are at time $t=0$, that is, $e$ is a terminal branch of $S$, and the product of the extinction probability of the descendants of $e$ in $S$ otherwise.

The extinction probability in an unrepresented species:

$$
\frac{d}{dt} \bar{E} = + (\lambda + \kappa - 1 - \bar{E}) \bar{E} - \frac{\sum f \in \mathcal{E}_i(S)}{N-1} (1-\bar{E}) \bar{E}_e - \left( \frac{\sum f \in \mathcal{E}_i(S)}{N-1} (1-\bar{E}) \right) \bar{E}_e
$$

where $\mathcal{E}_i(S)$ denotes the set of branches of $S$ in time slice $i$, and $n_i$ their number. The terms correspond to (i) loss (ii) the rate duplications, and (iii) transfers to represented hosts, both conditional on survival, and finally, (iv) the rate of unrepresented speciations and transfers to unrepresented hosts, again conditional on survival. The initial conditions specify that at the end of branch $e$ the probability of extinction is 0 if we are at time $t=0$, that is, $e$ is a terminal branch of $S$, and the product of the extinction probability of the descendants of $e$ in $S$ otherwise.
Note that the term corresponding to transfer back to \( S \) acts as an inhomogeneity in the absence of which the only solution is \( E(t) = 1 \).

The single observed lineage propagator along branch \( e \) of \( S \):

\[
\frac{d}{dt} G_e = -\left(\lambda + \delta(1 - 2E_e)\right) G_e (A.3)
\]

\[
- \left\{ \sum_{f \in \mathcal{E}(S)} \frac{\tau}{N - 1}(1 - E_f) \right\} G_e
\]

\[
- \left\{ \frac{N - n_i}{N - 1}(1 - E) \right\} G_e
\]

\[
- \left\{ \alpha(1 - E) \right\} G_e
\]

\[ G_e(t, t_e) = 1. \]

The single observed lineage propagator in an unrepresented species:

\[
\frac{d}{dt} \tilde{G} = -[\alpha + \lambda] G (A.4)
\]

\[
- \left\{ \frac{\alpha + \delta + \frac{N - n_i}{N - 1} \tau}{1 - 2E} \right\} \tilde{G}
\]

\[
- \left\{ \sum_{f \in \mathcal{E}(S)} \frac{\tau}{N - 1}(1 - E_f) \right\} \tilde{G}
\]

\[
\tilde{G}(t, t_e) = 1.
\]

Note that if we set \( E(t) = 1 \) and neglect gene birth and death, which is much slower than the speciation dynamics, that is, \( \delta + \tau + \lambda < \alpha \), we recover the exponential probability of coalescence with the represented tree assumed in Equation (2).

The propagator describing the evolution of \( k \) gene copies can be expressed using the single gene copy propagator. Consider the expression for \( \tilde{G}_k(s, t) \), the probability that \( k \) genes seen at time \( s \) in an unrepresented species all leave a single descendant descending from the copy seen at time \( t < s \) in an unrepresented species:

\[
\frac{d}{dt} \tilde{G}_k = -[\alpha + \lambda] \tilde{G}_k (A.5)
\]

\[
- \left\{ \alpha(1 - 2E_k) + k \left( \frac{\alpha + \delta + \frac{N - n_i}{N - 1} \tau}{1 - 2E} \right) \right\} \tilde{G}_k
\]

\[
- \left\{ \sum_{f \in \mathcal{E}(S)} \frac{\tau}{N - 1}(1 - E_f) \right\} \tilde{G}_k
\]

\[ \tilde{G}(t, t_e) = 1. \]

Since Equation (3) implies \(-\sigma \tilde{G}_k - \sigma(1 - 2E) \tilde{G}_k = \sigma(1 - (E^2) \tilde{G}_k \) and neglecting second- and higher order terms in \( 1 - E \) gives \( 2(1 - (E^2)) \geq 2(1 - E) \) and the above can be written as

\[
\frac{d}{dt} \tilde{G}_k = -k(\alpha + \lambda) \tilde{G}_k (A.6)
\]

\[
-k \left\{ \alpha(1 - 2E) + \left( \delta + \frac{N - n_i}{N - 1} \tau \right)(1 - 2E) \right\} \tilde{G}_k
\]

\[
-k \left\{ \sum_{f \in \mathcal{E}(S)} \frac{\tau}{N - 1}(1 - E_f) \right\} \tilde{G}_k,
\]

which has the solution \( \tilde{G}_k = (\tilde{G})^k \). Analogous reasoning can be used to show that \( E_k(t) \) and \( G_k(s, t) \) can be used to factor the respective functions describing the evolution of multiple gene copies.

The Probability of a Gene Tree

The expressions for \( P_e(u, t) \) and \( P_f(u, t) \) are derived under the approximation that unrepresented speciation and extinction is independent per gene, as discussed above. The full expression for \( P_e(u, t + \Delta t) \) including terms corresponding to direct transfers and indirect transfers that depart \( S \) via a preceding transfer events, which are neglected in Equation (4), is:

\[
P_e(u, t + \Delta t) = G_e(t + \Delta t, t)P_e(u, t)
\]

\[
+ \left\{ \beta \Delta t \right\} P_e(u, t)P_e(v, t)
\]

\[
+ \sum_{f \in \mathcal{E}} \frac{\tau \Delta t}{N - 1} P_e(v, t)P_f(w, t)
\]

\[
+ \sum_{f \in \mathcal{E}} \frac{\tau \Delta t}{N - 1} P_f(v, t)P_e(w, t)
\]

\[
+ \sum_{f \in \mathcal{E}} \frac{\tau \Delta t}{N - 1} P_f(v, t)P_f(w, t)
\]

\[
+ \left\{ \alpha + \frac{N - n_i}{N - 1} \right\} \Delta t \tilde{P}(v, t)P_e(w, t)
\]

\[
+ \left\{ \alpha + \frac{N - n_i}{N - 1} \right\} \Delta t \tilde{P}(v, t)P_f(w, t)
\]

\[
+ \left\{ \alpha + \frac{N - n_i}{N - 1} \right\} \Delta t \tilde{P}(v, t)P_f(w, t)
\]

Routes to Cyanobacterial Genomes

The data set was constructed for near universal single-copy genes from all 36 cyanobacterial genomes found in Version 5 the HOGENOM database (Penel et al. 2009). Amino acid sequences were extracted for each family that had a single copy in at least 34 of the 36 cyanobacterial genomes. For each family, sequences were aligned using MUSCLE (Edgar 2004) with default parameters. The multiple alignment was subsequently cleaned using GBlocks (Talavera and Castresana 2007).
with the options:
\[ -t=p \ -b1 50 \ -b2 50 \ -b5=a \ -t=p \]. (A.8)

Subsequently, we inferred a gene topology \( G \) that maximizes the joint likelihood
\[
P_{\text{exODT}}(G|S, h, t, \lambda, \sigma, N) \times P_{\text{Felsenstein}}(\text{alignment}|G),
\]
(A.9)

where the first term corresponds to the likelihood of observing the unrooted gene tree topology \( G \) according to the exODT model developed above [Eqs. (4) and (5)], while the second term corresponds to the classic Felsenstein likelihood (Felsenstein 1981) of the alignment. For the exODT model, we fixed the parameter values \( N = 10^9 \) and \( \sigma = 2N \), used the dated phylogeny from Szöllősi et al. (2012) and estimated global gene birth and death rates as described below. To calculate the Felsenstein likelihood, we used the Bio++ library (David L.A., Alm E.J. 2011. Rapid evolutionary innovation during an archaea genealogy. Proc. Natl Acad. Sci. USA 109: 4962–4967).

Gene trees inference was performed in a two-step approach:

**Initial estimate**

1. Using the DTL rates \( \delta = 1.0 \times 10^{-2}, \tau = 1.0 \times 10^{-3}, \lambda = 2.0 \times 10^{-2} \) for each family, the joint likelihood was calculated for all nearest neighbor interchanges (NNIs) (Felsenstein 2004) and a move was accepted if it improved the joint likelihood.

2. For the set of trees obtained global DTL parameters were estimated that maximize the product of the joint likelihood of all 473 gene families.

**Final estimate**

1. Using the obtained DTL rates for each family, the joint likelihood was calculated for all NNIs (Felsenstein 2004) and a move was accepted if it improved the joint likelihood.

2. For the set of trees obtained global DTL parameters were again estimated with the results: \( \delta = 1.010 \times 10^{-5}, \tau = 4.438 \times 10^{-3}, \text{ and } \lambda = 1.015 \times 10^{-1} \).

Before performing NNIs starting gene tree topologies were estimated using an amalgamation approach (David and Alm 2011) wherein the Felsenstein likelihood was approximated using conditional clade probabilities (Höhna and Drummond 2012) based on posterior sample of 10,000 tree topologies obtained using PhyloBayes using an LG+F4+I substitution model.

The support of transfer events was measured based on a posterior sample of 1000 reconciliations per family. For each family, we assessed the support of all transfer events in the reconciliation that was seen the largest number of times. Two transfers were considered equivalent if they involved the transfer of the same gene lineage between identical branches of the species tree.

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