**Gene Duplication and Evolution**

Lynch and Conery (1) presented one of the first serious efforts to study the evolutionary fate of gene duplication using genomic sequence data. Their analysis led to several interesting observations, particularly with respect to the rate of gene duplication in eukaryotic genomes and the subsequent half-life of duplicates. These two parameters are of particular importance in studying the evolutionary processes of gene duplication and subsequent functional divergence. The most frequent class of duplications appeared to be similar in all six species, which suggests some silencing process for old duplicates. Several additional considerations in the analysis and interpretation, however, might have led to some different conclusions.

First, Lynch and Conery (1) used the number of substitutions per silent site, $S$, and the silent sites, $L$, to measure the age of a duplicate-gene pair [figure 2 of (1)]. It is unclear, however, that silent divergence is a suitable proxy for a molecular clock involving different genes or gene duplicates. For example, Zeng et al. (2) reported 9- to 15-fold differences in $S$ values and a flat distribution of $S$ for 24 single-copy genes in *Drosophila*. Two points are important in this context: (i) this large variation in $S$ is expected when the divergence time is low, and (ii) the divergence time for each comparison made by Zeng et al. (2) was fixed. Thus, for different genes, $S$ may vary by more than an order of magnitude given a fixed divergence time. This situation differs from description of divergence time using $S$ values from homologous genes across a group of organisms, in which a dependable molecular clock may exist. The same $S$ values may represent duplicates of very different ages, and the different $S$ values may be from duplicates of the same or similar ages. Thus, figure 2 of (1) should be viewed with caution as a description of the age distribution of gene duplications. A related issue is the reliability of estimates of $S$, because many of the values presented by Lynch and Conery (1) were larger than 1. Estimates larger than 1 are associated with a large variance due to saturation of substitutions and should generally be considered unreliable (3).

Second, the calculation of the half-life of gene duplicates was based on the untested, hidden assumption that the rate of gene duplication is constant over evolutionary time—an assumption implicit in both figure 3 and equation 3 of (1). Unfortunately, there are insufficient data with which to estimate the variation in the rate of gene duplication on a short time scale; nevertheless, there is some evidence that the duplication rate for some families may indeed not be stationary over a short evolutionary time. For example, in the mouse Sp100-rs family, a short lineage of *Mus musculus* has created at least 60 gene duplicates within 1.7 million years; other lineages such as the sibling taxa *Mus caroli*, a group that diverged 2.5 million years ago, contain few duplicates (4). If the duplication rate over the time during which divergence is observed is much lower than the recent rate of duplication, the half-life calculated by Lynch and Conery would represent a serious underestimate.

Finally, an alternative interpretation for the short half-life of duplicate genes before silencing may deserve consideration. Assuming that small values of $S$ may more reliably reflect a short evolutionary time, the authors chose to estimate the half-life of duplicate genes only from gene pairs with $S$ values in the range of 0 to 0.25. They estimated a mean half-life of 4 million years, concluding that “the fate awaiting most gene duplications appears to be silencing rather than preservation,” and, hence, that “duplicate genes may only rarely evolve new functions.” Yet their analysis appears to have ignored several important features of the data [figure 2 of (1)]. (i) Notwithstanding their model of “young” duplicates, the tails of the distribution are long and flat, which suggests that the data are actually heterogeneous. (ii) The proportions of the duplications that reside in the tails are high—85% for *Drosophila melanogaster*, 66% for *Caenorhabditis elegans*, and 65% for *Saccharomyces cerevisiae*. (iii) The tails include old and ancient duplications. The heterogeneity of the age distribution in figure 2 of (1) suggests that the short half-life calculated from young duplicate-gene pairs cannot be extended to most pairs. After all, a large proportion of these older duplicates may be much older than 4 million years, with real ages of tens or hundreds of million years. It is likely that these genes have been functional since their origin; otherwise, the duplicate sequences would have been deleted from the genome (5).

In addition, the absolute number of old or ancient gene duplicates is relatively large. For example, 40% of the approximately 13,600 coding sequences in the *D. melanogaster* genome appear to have arisen by gene duplication (6). Thus, some 34% of the fly genome, or 4624 genes [40% × 85% × 13,600, with the 85% from item (ii), above], comprise old or ancient duplicates. It is therefore misleading to assert that the vast majority of gene duplicates are quickly silenced, even if the calculation of the half-life is correct. Rather, it appears that the accumulation of “survivors” of the silencing process constitutes a large fraction of modern eukaryotic genomes.

Lynch and Conery (1) have proposed a number of provocative hypotheses regarding the evolution of duplicate genes, using data from nine eukaryotic species. One hypothesis is that the ratio of replacement ($R$) to silent ($S$) nucleotide substitutions among recently duplicated genes is near 1.0, the neutral expectation. Their analysis indicates that this phase of relaxed selection is confined to recently duplicated gene pairs. Another hypothesis is that many duplicate-gene pairs are short-lived, with half-lives of 3 to 7 million years, depending on the organism. Unfortunately, their conclusions are compromised by the fact that their data, obtained through GenBank taxon searches, included many redundant records. For example, 43.3% of the gene pairs in their *Arabidopsis* data set had no synonymous differences ($S = 0$). We randomly examined 50 of these gene pairs and found that 86% were derived from the same genomic sequence, mostly because of the presence of a single gene on two overlapping clones. These redundant sequences were used to estimate the rate at which duplicate-gene pairs reverted to single copies, a procedure that tended to overestimate the rate of gene loss. Such problems were not limited to

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**References**


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An analogy for the application of half-lives is the mortality of newborns centuries ago: At that time the infant mortality rate was very high, because medical science was underdeveloped—but just because the “half-life” of newborns is short, it does not follow that half of all adults will die shortly. We suggest that figure 2 of (1) supports a conclusion opposite to the one that Lynch and Conery drew: A large proportion of duplicate genes either have evolved new functions (7) or have been maintained by subfunctionalization (8, 9) or other mechanisms.
Arabidopsis; 58.3% of human gene pairs and 67.7% of mouse gene pairs had \( R = S = 0 \). Because Lynch and Conery recognized the potential problem of redundancy, human and mouse gene pairs with \( S < 0.01 \) were not used in their analyses. In many cases, however, both gene sequences from an \( S < 0.01 \) pair were compared with a more distant gene family member, which did result in the use of redundant data entries.

Also problematic are the mammalian gene pairs in the \( 0.01 < S < 0.05 \) class, which were crucial to the conclusion by Lynch and Conery (1) that selective constraint is temporarily relaxed after gene duplication. We manually inspected 20 pairs from each species and found that 50% in human and 80% in mouse are actually allelic or alternatively spliced forms of the same locus. Allelism was determined primarily by GenBank annotation, which provided the same gene name for different sequence entries. These observations suggest that several data sets were problematic and cast doubt on the value of the analyses presented by Lynch and Conery.

There are additional problems with the approach used by Lynch and Conery (1). First, the authors applied an exponential-decay model to estimate the rate of gene turnover, assuming a steady state between the origin and loss of duplicated pairs. In yeast and Arabidopsis, however, this assumption has clearly been violated by episodic, large-scale genomic duplication events (2, 3). Second, their model failed to account for the fact that the number of pairwise comparisons within gene families can be substantially larger than the number of actual duplication events. To estimate the rate of gene loss, one needs to know the distribution of the latter, not the former. Finally, the authors proposed a curvilinear model for the relationship of \( \frac{R}{S} \) to \( S \), which failed to test that model against the null hypothesis that \( R \) is a simple linear function of \( S \). In our reanalyses, we found that the curvilinear model fits significantly better than the linear model for all nine species, but we obtained substantially different parameter estimates, with smaller sums of squares than those reported. Our results, which appear to support the curvilinear model, will require independent verification in light of the problems with several data sets.

Although they have not succeeded in demonstrating empirical support for all of their hypotheses, Lynch and Conery nonetheless have offered a variety of stimulating ideas—the apparently high rate of gene duplication, the role of duplication in chromosomal repatterning, and the role of gene duplication in reproductive isolation between species—that call for further investigation.
by processes substantially different from the
incremental single-gene duplications
that we focused on in (1). Most notable is
the process of complete genome duplica-
tion, the ancient remnants of which have
been implicated in yeast (3) and Arabidop-
sis (4). Although the genomic extent is not
yet understood, a massive amount of gene
duplication occurred early in the vertebrate
lineage (5), and we cannot rule out the
possibility of similar large-scale events pri-
or to the radiation of the animal phyla. The
probability of duplicate-gene preservation
following polyploidization may be substan-
tially elevated relative to that for single-
copy duplicates for two reasons. First, as
we noted previously, polyploidization
maintains the dosage ratios of all pairs of
genes relative to the situation in the diploid
state, and selection may favor the mainte-
nance of the ancestral stoichiometric ratios.
Second, when whole chromosomes are
duplicated, the constituent genes are guaran-
teed to initiate with all essential regulatory
regions intact, and this may further reduce
the likelihood of negative selection against
new copies.

Zhang et al. argue that three of the data
sets that we worked with in (1) contained
flaws that may have influenced the outcome
of our analyses. We agree that this issue
merits close scrutiny, and at the close of this
response, we will present some reanalyses for
both the Arabidopsis and human genomes
that take into consideration the concerns
raised by Zhang et al. First, however, we
respond to three technical issues raised by
these authors:

1) As noted in (1), the inability to easily
distinguish allelic sequences or alternative
spliced forms from duplicate genes raises
potential complications with some databases.
This is unlikely to be a serious problem
with inbred species such as C. elegans or
haploid species such as S. cerevisiae,
whose genomic sequences are well annotat-
ed and curated. For outbred species, it is
difficult to see how one can unambiguously
resolve this issue with data sets constructed
from random sequences (contrary to the
suggestion by Zhang et al.), and 5% se-
quence divergence seems rather high for
allelic variants. Nevertheless, this problem
remains a serious consideration for data
sets that are not highly refined. If nondup-
licate sequences are inadvertently includ-
ed in a survivorship analysis for duplicate
loci, the estimated half-life will be unaf-
lected so long as the incidence of such

2) As noted above, the ancient genome
duplications known to have occurred in Ara-
bidopsis and yeast may have no bearing on our
conclusions, because the duplicate pairs as-
sociated with these events were not included
in our demographic analyses.

3) The distinction raised by Zhang et al.
between numbers of extant duplicate pairs
and number of actual duplication events is
correct and important. However, multigene
families were excluded from our analyses
and the vast majority of the young gene
duplicates that we identified were simple
pairs (in which case, there is no ambiguity
with respect to event counting), so this
distinction has little effect on our estimates.
Nevertheless, the reanalyses presented be-
low are based on estimates of duplication
events rather than on observed numbers of
duplicate pairs.

After publication of (1), a well-curated
version of the Arabidopsis genome became
available (6) that has eliminated most of the
redundancies and ambiguities noted by
Zhang et al. A complete reanalysis of the data
is beyond the scope of this response and will
be reported elsewhere (7); to summarize,
however, using our prior methods for demo-
graphic analysis, we have estimated the rate
of origin of new duplicates in Arabidopsis,
based on the new data set, to be 0.0022 per
gene per million years, which is of the same
order of magnitude as that observed for D.
melanogaster (0.0023) and yeast (0.0083),
but lower than that for C. elegans (0.0208).
Because the incidence of putative duplicates
in the nearly identical class is greatly reduced
in this newly available data set (consistent
with the arguments of Zhang et al.), the
half-life estimate increases from our previous
value of 3.2 million years to 23.4 million
years, which exceeds our previous estimates
for invertebrates by a factor of seven and for
mammals by a factor of three.

We are also now able to provide an esti-
mate of the rate of origin of new duplicates
in the human genome, using the database of
the publicly funded project (8, 9). Our estimate,
0.0071 per gene per million years, falls in the
middle of the range for other species. Our
revised estimate of the half-life for human
duplicate genes, 16 million years, is about
double our previous estimate. However,
because assembly problems probably result in
the exclusion of substantial numbers of
young gene duplicates from the “complete”
human genomic sequence (9, 10), our esti-
mated rate of origin of new duplicates in
humans is probably downwardly biased,
whereas our estimated half-life is likely up-
wardly biased. A recent comparison of chro-
omosomal contents in mice and humans
strongly supports our contention that a high
rate of duplicate-gene turnover occurs in
mammals (11).

One must be cautious to avoid overinter-
preting the degree of precision associated
with all of these estimates; most large-scale
genome projects are still in a stage of mat-
uration, with updated annotations being re-
leased regularly. At this point, however, we
see no reason to alter our basic conclusions
that the rate of origin of new duplicates in
eukaryotes is quite high, often in the range
of 0.002 to 0.020 per gene per million
years, and that most gene duplicates have a
relatively short life-span, the average being
in the neighborhood of 1 to 10 million
years (with a possible exception in Arabidopsis).
Functional studies will be required to de-
termine the fraction of duplicates identifi-
able from coding-region identity that are
actually biologically active.

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