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The evolutionary dynamics of plant duplicate genes

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Given the prevalence of duplicate genes and genomes in plant species, the study of their evolutionary dynamics has been a focus of study in plant evolutionary genetics over the past two decades. The past few years have been a particularly exciting time because recent theoretical and experimental investigations have led to a rethinking of the classic paradigm of duplicate gene evolution. By combining recent advances in genomic analysis with a new conceptual framework, researchers are determining the contributions of single-gene and whole-genome duplications to the diversification of plant species. This research provides insights into the roles that gene and genome duplications play in plant evolution.

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Introduction

The evolutionary diversification of genomes and genetic systems is driven in part by duplication events [1•,2]. Gene duplications contribute to the establishment of new gene functions [3] and underlie the origins of evolutionary novelty [4]. Plants are exceptional among eukaryotic organisms in that duplicate loci compose a large fraction of their genomes, partly because of the frequent occurrence of genomic segmental duplications and polyploidization events in plants. For example, in the *Arabidopsis thaliana* and rice genomes up to 90% and 62% of loci are duplicated, respectively, and it is estimated that 70–80% of angiosperm species have undergone polyploidization at some point in their evolutionary history [5–7,8••,9•].

The high proportion of duplicate genes in plant genomes reflects the rate of retention of duplicate copies among plant species [10,11]. The birth rate of genes in *A. thaliana* (0.002 duplicates per gene per million years) is in the same order of magnitude as that observed for yeast and *Drosophila*, although ten-fold slower than that found in *Caenorhabditis elegans*. The half-life to silencing and loss of a gene duplicate in *A. thaliana*, however, is estimated at 23.4 million years, which is 3–7-fold higher than for animal genomes [11]. It is not clear why duplicate genes persist for longer periods in *A. thaliana* than in animal genomes, nor is it known whether this is a general characteristic of plant genomes. Understanding the mechanisms underlying the retention of duplicate genes in plant genomes continues to remain a topic of intense interest [8••,9•,12,13••,14,15].

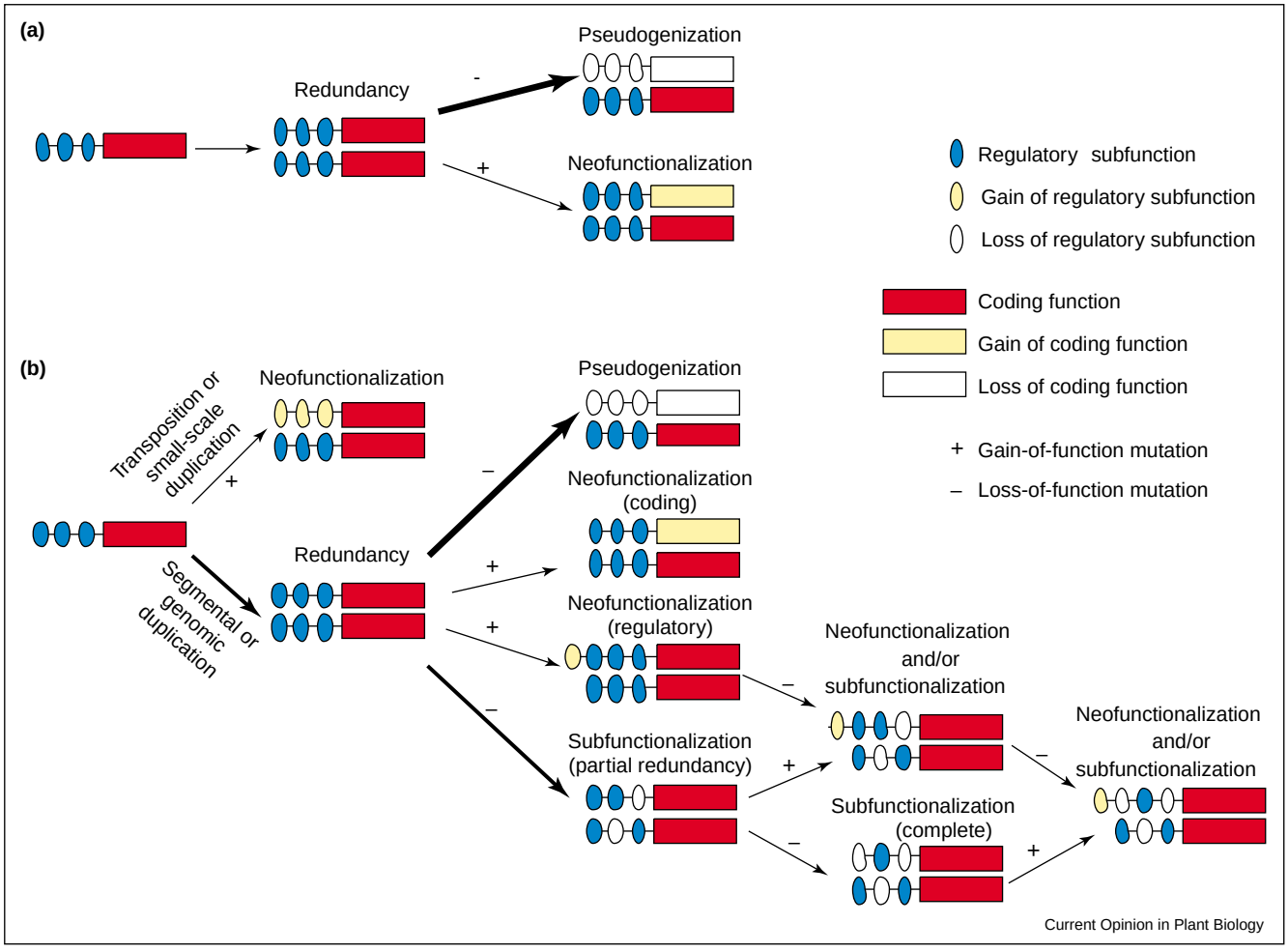
Theoretical and experimental studies in the past few years have advanced our understanding of the evolutionary dynamics of duplicate loci in plant genomes, and have led to a rethinking of the paradigm that provided the conceptual foundations of studies on duplicate gene evolution [10,13••,16,17••]. The confluence of a new conceptual framework for gene duplication with new genomic technologies has allowed investigators to study gene duplications on a genome-wide scale [8••,9•,12, 14,18••]. This, in turn, has reshaped our understanding of the evolution of duplicate genes and genomes.

The fates of duplicate genes: a new paradigm emerges

In the thirty years since the publication of Ohno's landmark book entitled '*Evolution by Gene Duplication*' [2], the reigning paradigm regarding the fate of duplicated genes predicts that one of the duplicates is either lost (pseudogenization) or gains a new function (neofunctionalization) (Figure 1a). Because deleterious mutations are more probable than advantageous mutations, it has been presumed that most duplicate copies are lost and only a few neofunctional loci are maintained by selection.

It has since become apparent that positive selection does play a key role in preserving some gene copies, and indeed can act at a very early stage of the gene duplication process. For example, a population genetic analysis of three unlinked duplicate gene pairs in *A. thaliana* that originated less than 1.2 million years ago (mya) revealed significantly reduced levels of nucleotide polymorphism in the progenitor locus, the duplicate locus or both. This reduced nucleotide variation, which is associated with a recent selective sweep, is evidence that positive selection plays a prominent role in the establishment of duplicate loci [17••]. Although the action of positive selection is consistent with the process of neofunctionalization, the precise targets of selection in these duplicate genes are unclear in the absence of functional data [19]. It will also be of interest to determine whether selection acts to

Figure 1



(a) Classic Ohno model of duplicate gene fates. Mechanisms of duplication and fates of genes are indicated. Thickness of arrows indicate relative frequency of possible fates. **(b)** Recent theoretical work supports a much more complex model for the fates of duplicate genes.

preserve tandem-linked duplicate loci, which are prevalent in plant genomes [14,17••] and for which neofunctionalization is theoretically unlikely [20].

Although Ohno's classic paradigm provides key insights into duplicate gene evolution, it fails to account for the preponderance of retained duplicates in whole genomes [21]. The duplication-degeneration-complementation (DDC) model [16,22], which harkens to the 'gene sharing' concept proposed by Hughes [23], suggests an additional evolutionary fate for duplicate loci. This model, also referred to as subfunctionalization, suggests that duplicate genes acquire debilitating yet complementary mutations that alter one or more subfunctions of the single gene progenitor (Figure 1b). The strength of this model is that it does not rely on the sparse occurrence of beneficial mutations, but on more frequently occurring loss-of-function mutations in regulatory regions [16]. Subsequent empirical studies on expression divergence between duplicate genes suggest that changes in expression regimes occur both frequently and rapidly, consistent with the predictions of this model [13••,24,25].

The plant MADS-box gene family: a case study of the fate of duplicate genes

The diversification of the MADS-box transcription factor family, whose members control key aspects of plant vegetative and reproductive development, is a clear example of the role that subfunctionalization plays in shaping genetic systems. Indeed, one of the earliest-documented examples of subfunctionalization in plants is the lineage-specific duplication of an *AGAMOUS* (*AG*)-like MADS-box gene in maize [26]. In maize, the ancestral function of *AG* (which is expressed in stamens and carpels) is partitioned between the duplicated genes *ZMM2* (expressed primarily in stamens) and *ZAG1* (expressed primarily in carpels) [26]. A more recent example of subfunctionalization involving MADS genes

comes from the dioecious plant *Silene latifolia*, in which the male sex is determined by the presence of a morphologically distinct Y chromosome. In this species, an autosomal homolog of the MADS gene *APETALA3* (*AP3*), which is involved in petal and stamen identity, was duplicatively transferred to the Y chromosome, and subsequently underwent divergence in gene expression [27].

On a larger evolutionary time-scale, the evolutionary forces that govern the fates of duplicate genes are responsible for the incredible diversity of MADS-box genes found in present-day flowering plants. MADS-box genes are found ubiquitously in eukaryotes as type I and II gene subfamilies [28]. The proliferation of type II MADS-box genes, known as the MIKC-class genes, is unique to plants [29]. A comparative genomic survey indicates that although the birth rate of type II MADS-box genes in plants is lower than that of type I genes, the former are preferentially retained in the genome [30•]. This pattern suggests that the loss of type II MADS genes is more deleterious than the loss of type I loci, a prediction supported by the observation that type II genes tend to be subject to stronger purifying selection. The lower death rate of type II MADS genes might be due to subfunctionalization if the divergence in function that is observed across this subfamily necessitates the retention of family members in the genome [31•,32]. The selective retention of type II MADS genes also creates a certain degree of redundancy between closely related paralogs [33], however, suggesting that only partial subfunctionalization occurs in certain clades.

An examination of the *AG* subfamily of MADS-box genes, which controls key aspects of inner whorl floral development in flowering plants [34•], reveals evidence of both subfunctionalization and overlapping redundancy. The *AG* subfamily is subdivided via an ancient duplication at the base of the angiosperms into the C-class and D-class lineages. The D-class genes are almost all expressed in the ovules, where they specify ovule identity, whereas the C-class lineage is primarily involved in carpel and stamen identity [35]. By contrast, an *AG*-like gene in gymnosperms is expressed in both the female reproductive structure (megasporophyll) and the ovule [36–39], suggesting that the genetic function of the ancestral gene in this group was partitioned into two distinct lineages within the angiosperms.

Another issue raised by this study is whether the partitioning of subfunctions is equally shared between lineages. The *Antirhinnum majus PLENA* (*PLE*) gene was presumed to be an ortholog of the *A. thaliana AG* gene because mutant analysis indicated that both genes provide the primary C-class function in their respective species [40,41]. However, the true ortholog of *AG* in *A. majus* is *FARINELLI* (*FAR*), which functions redundantly in specifying stamen identity [34•,42]. By contrast, the orthologs of *PLE* in *A. thaliana* are the *SHATTERPROOF* (*SHP*) genes, which primarily control differentiation of the seed valve margin while sharing overlapping redundancy with *AG* [33,43]. Thus, subfunctions of the progenitor gene were asymmetrically partitioned between *AG/SHP* paralogs in the *A. thaliana* lineage relative to the *PLE/FAR* paralogs in the *A. majus* lineage. This functional asymmetry also underscores the possible difficulties in using functional similarity as evidence for evolutionary orthology because the latter does not necessarily lead to the former.

All is not lost: redundancy remains

One of the predictions of the subfunctionalization model is that duplicate genes will share overlapping redundant functions early in the process of functional divergence, and phylogenetic and functional analyses clearly support this prediction [16,33,34•,43,44]. Within *A. thaliana*, the C-class genes *AG* and *SHP1/SHP2*, along with the D-class gene *SEEDSTICK* (*STK*), share overlapping functions in carpel identity, a remnant of their shared ancestral role [33]. Given that these paralogs diverged at the base of the angiosperm lineage, it is clear that duplicate genes can exist stably in at a partially redundant state over a protracted evolutionary period. Although subfunctionalization can occur rapidly, it is not clear when the transition to complete functional partitioning occurs. Indeed, theoretical considerations of redundancy predict that duplicate genes will reach an evolutionarily stable equilibrium of partial redundancy, potentially delaying the transition to complete subfunctionalization [45].

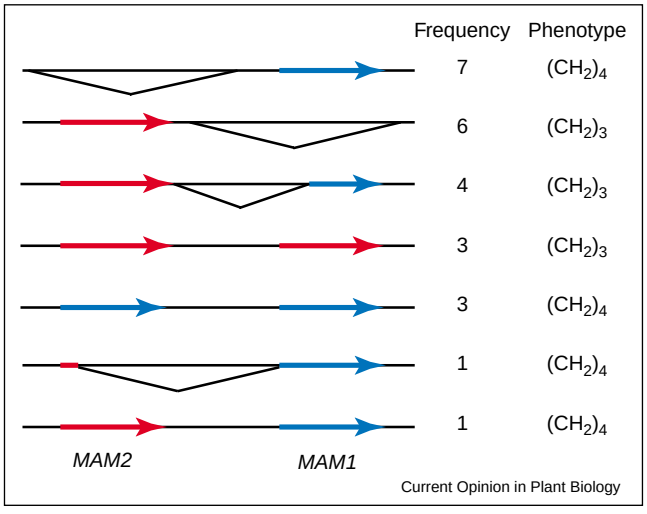
Although complete functional redundancy is difficult to confirm, numerous examples of paralogous genes in plants for which the deletion of one copy yields no observable phenotype do exist [46–52], including the three *SEPALLATA1/2/3* (*SEP1/2/3*) and two *SHP1/SHP2* MADS-box paralogs [43,44,53]. The protein changes in these paralogous redundant genes show no evidence of functional divergence, suggesting that amino-acid replacements are functionally constrained [54]. Although purifying selection apparently constrains divergence between paralogs, population genetic analyses indicate that significant heterogeneity in nucleotide diversity, suggestive of positive selection, occurs within the transcriptional unit of *SHP1* and the promoter of *SHP2*. By contrast, *SEP2* and *SEP1* appear to be evolving neutrally [55]. These differences in evolutionary dynamics between paralogs might be a consequence of the position of these genes in the floral developmental pathway. The *SEP* loci are involved in the highly conserved function of floral patterning, whereas the *SHP* loci control the more variable trait of seed shattering.

Duplicate and defend

The duplication of genes within or between closely related species might lead to phenotypic variation in

specific traits, and manifest itself as functionally relevant, ecologically significant polymorphisms. Gene duplication, for example, contributes to the ability of plants to mount a defense response against disease and herbivory by allowing the functional diversifcation of genes that are involved in pathogen recognition and herbivory defense. This diversification is most evident in the large family of disease resistance (*R*) genes that encode the nucleotide-binding site plus leucine-rich repeat (NBS–LRR) proteins. The *Arabidopsis* genome contains more than 150 NBS–LRR genes found as isolated genes or in tandemly arrayed clusters that are dispersed throughout the genome. Gene duplication of NBS–LRRs followed by positive selection for diverse amino acids in the LRR protein-recognition domain might provide the means by which the plant's recognition of novel pathogens evolves [56]. Interestingly, a genomic analysis of the history and chromosomal locations of NBS–LRR gene duplications revealed that tandem duplications have contributed to the birth of the majority of NBS–LRRs in the *Arabidopsis* genome, whereas the duplicative transfer of NBS–LRRs to dispersed chromosomal locations is largely the result of segmental duplication [55]. Because tandemly arrayed NBS–LRRs are subject to frequent intergenic exchange, it is believed that those genes found in different chromosomal regions have a greater chance of evolving new pathogen recognition functions [56]. Moreover, a recent study of the NBS–LRR *Cf-2* pathogen-resistance gene family in the wild species *Solanum pimpinellifolium* suggests that complex evolutionary dynamics surround duplicate *Cf-2* genes, including the differential selection of gene copies [57].

Figure 2



The genotypic frequencies and structural organization of the *MAM* duplicate loci from a survey of 25 *A. thaliana* ecotypes. The *MAM* loci modify the carbon length of the glucosinolate basic side chain; *MAM2* adds three methylene groups ($[CH_2]_3$), whereas *MAM1* adds four methylene groups ($[CH_2]_4$). Slanted lines indicate the loss of particular duplicate copies. (Based on Figure 1 of [59•].)

Gene duplication also plays a significant role in the evolutionary dynamics of anti-herbivory genes. In *A. thaliana*, resistance to generalist insect herbivores is conferred in part by glucosinolates, a class of plant secondary metabolites. The composition and quality of glucosinlate production varies between *Arabidopsis* accessions. This variation is due, in part, to the tandemly duplicated methylthioalkylmalate synthase (*MAM*) genes, *MAM1* and *MAM2*, that are involved in glucosinolate biosynthesis [58]. *MAM1* and *MAM2* are functionally divergent duplicates: the *MAM2* locus is a stronger deterrent against generalist herbivores than the *MAM1* locus [59•]. A population genetic analysis of the *MAM* genes found evidence of differential gene loss and differential selection between the *MAM* loci within *A. thaliana* accessions (Figure 2; [59•]). These data support the action of balancing selection on the *MAM2* locus but not on *MAM1*. Because glucosinolates can also stimulate feeding in specialist herbivores, selection at the *MAM2* locus might be caused by the ecological trade-off between protection against generalist insect herbivores and increased susceptibility to specialists.

Variable patterns of evolution exist in another class of anti-herbivory genes, the tandemly arrayed family of six *A. thaliana* trypsin inhibitor (*ATTI*) genes. The expression of these six genes varies significantly between loci, among allelic classes within *A. thaliana*, and in response to herbivory [60]. These data suggest that subfunctionalization is acting to maintain divergent functions between duplicate loci. Despite their close proximity to each other (within 10 kb), population genetic analyses suggest that evolutionary history also varies between *ATTI* loci, although the flanking loci, *ATTI1* and *ATTI6*, which are separated from the core loci by recombination, have the greatest opportunity to be acted upon by natural selection [60,61].

Polyploids: ancient and new

Polyploidization is a major mechanism by which duplicate genes are introduced into plant genomes, and this is reviewed elsewhere in this issue (see review by Adams and Wendel). Several aspects of polyploidization do, however, highlight several interesting facets that surround the evolutionary dynamics of duplicate genes in plants. In a recent genomic analysis of 14 model plant species, nine species were documented to have one or more whole-genome duplication events in their evolutionary past [18••]. Up to four whole-genome duplication events appear to have occurred in *A. thaliana* [6,7,8••,62,63]. Genomics technologies might now provide a systematic explanation of the fates of duplicate genes on a whole-genome level.

As predicted by theory [20,64,65], the majority of duplicated genes resulting from polyploidization events are lost in the transition to diploidy, although the percentage of retained duplicates varies between species [10,11]. The type of duplicate genes lost or retained after

polyploidization is correlated with function. Among the retained duplicates from the last whole-genome duplication event in *A. thaliana*, genes involved in signal transduction and transcriptional regulation are over represented, whereas DNA-repair genes have been preferentially lost [13••,15]. The majority of duplicates retained (57%) tend to have divergent expression patterns as predicted by the DDC model, whereas over 20% have asymmetric rates of protein evolution; both are suggestive of functional divergence [13••].

Functional divergence can occur rapidly after polyploidization, perhaps even within a generation, and this phenomenon has been studied in both naturally occurring recent polyploids and in synthetic polyploids. For example, among 40 homeologous gene pairs in natural tetraploid cotton (which was the result of a whole-genome duplication event that occurred 1–2 mya), nine gene pairs exhibited biased expression, with the homeolog from one parental genome contributing more to the transcriptome than the other [66••]. Interestingly, 11 of these 18 homeologs showed tissue-specific silencing or biased expression in at least one of 10 organs tested, indicating that they were at an early phase of subfunctionalization. Similar patterns of biased expression were observed in synthetic *Gossypium* polyploids, demonstrating that functional divergence could occur within one generation of polyploidization [66••]. Similar conclusions were reached in a study of synthetic *Arabidopsis* allotetraploids produced in a cross between *A. thaliana* and *Arabidopsis arenosa* [67,68•].

Conclusions

A new conceptual framework for gene duplication combined with emerging genomic data and technologies has advanced our understanding of the contributions of gene and genome duplication to the evolution of plants. Care must be taken, however, in interpreting the influx of genomic data in a proper functional and phylogenetic context. For instance, although gene expression can diverge rapidly between duplicate genes, expression data alone cannot reveal whether the combined expression domains of the duplicates reflect that of the progenitor gene or whether one or both of the duplicates have gained a novel expression regime. Indeed, in a comparative study of lineage-specific duplicates in humans and mice, changes in expression were more often associated with the gain of novel expression domains, consistent with neofunctionalization [69•]. Similarly, inferring patterns of plant genome evolution requires a 'phlyogenomics' approach; genomic data on the structural arrangements of extant genes must be compared to that from related taxa to determine the extent and timing of genome duplication events [9•]. Only when such comparative approaches are used will we be able to truly understand the contribution of single-gene and whole-genome duplications to the emergence and diversification of plant species.

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