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New Genes in Drosophila Quickly Become Essential

Sidi Chen, Yong E. Zhang, Manyuan Long*

To investigate the origin and evolution of essential genes, we identified and phenotyped 195 young protein-coding genes, which originated 3 to 35 million years ago in Drosophila. Knocking down expression with RNA interference showed that 30% of newly arisen genes are essential for viability. The proportion of genes that are essential is similar in every evolutionary age group that we examined. Under constitutive silencing of these young essential genes, lethality was high in the pupal stage and also found in the larval stages. Lethality was attributed to diverse cellular and developmental defects, such as organ formation and patterning defects. These data suggest that new genes frequently and rapidly evolve essential functions and participate in development.

Essential genes are often portrayed as conserved and ancient (1, 2), whereas younger genes, which exist in only one or a few species, have been considered to be more dispensable and to perform relatively minor organismal functions (1–4). It is unclear how essential genes arise and how new genes accumulate essential functions. New genes arise continuously through various mechanisms, such as DNA-based duplication, retroposition, and de novo origination (5, 6). When they first arose, new genes were expected to be nonessential because their immediate ancestral species were able to survive without them (Fig. 1A). However, little is known about their phenotypes and degrees of essentiality.

By comparative genomic analysis of 12 closely related Drosophila species (7), we identified 566 new genes in the D. melanogaster genome and dated their evolutionary ages through phylogenetic distributions (8) (fig. S1). All these genes originated less than 35 million years (My) after the divergence from D. willistoni (9), so we called them young genes. To assay their phenotypic effects in viability, we obtained Drosophila RNA interference (RNAi) lines targeting these genes (10, 11) and excluded RNAi lines with predicted off-target effects and lines with detectable phenotypes by P-element insertion, resulting in a set of lines targeting 195 young genes. Crosses resulting in constitutive silencing of these genes allowed us to assay the phenotypic effects on viability in the F1 generation (8) (fig. S2).

Unexpectedly, 59 of these genes were lethal under constitutive RNAi knockdown (Table 1 and tables S1, S3, and S4). We confirmed lethality in most of the genes (93%) with different driver constructs (table S6, part II), ruling out positional effects or construct effects. Furthermore, in deficiency libraries, lines deleting these genes are homozygous lethal, although a deletion block can be large and can contain other genes (12). Furthermore, several genes in the list (table S1)—HP6 (CG15636), CG12842, and spin2 (CG8137)—were found to be lethal using various gene disruption methods, including P-element disruption, RNAi with independent constructs, and misexpression assays (13–15). Therefore, we found 59 young genes that are essential for viability (Table 1 and table S1), a conservative number due to false negatives because RNAi insert into different chromosomal locations, we repeated the crosses with these lines and found that 45 of 47 (96%) genes showed similar viability phenotypes between lines (table S6, part II), ruling out positional effects or construct effects. Furthermore, in deficiency libraries, lines deleting these genes are homozygous lethal, although a deletion block can be large and can contain other genes (12). Furthermore, several genes in the list (table S1)—HP6 (CG15636), CG12842, and spin2 (CG8137)—were found to be lethal using various gene disruption methods, including P-element disruption, RNAi with independent constructs, and misexpression assays (13–15). Therefore, we found 59 young genes that are essential for viability (Table 1 and table S1), a conservative number due to false negatives because RNAi does not reduce the mRNA level to zero (10).

These data suggest that young genes are as essential as old genes in terms of viability.

We analyzed the age distribution of young essential genes by mapping the origin events of these 59 genes onto the Drosophila phylogenetic tree (8). We found that essential genes emerged throughout the evolutionary period examined (Fig. 1B and table S2). The youngest, p24-related-2 (CG33105), arose within the last 3 My and is thus D. melanogaster–specific (table S2). In each age group, the proportion of genes that are essential was around 30% (Table 1), suggesting that whether or not a gene is essential is independent of its age. These data reveal that the proportion of newly arisen essential genes reaches a plateau within a few million years. Reminiscent of the

Table 1. Summary statistics of lethal phenotypes of young genes. (I) Gene age was described in (8); age groups 0 to ~3 My and 3 to ~6 My were pooled to increase sample size. A gene was considered essential for viability if it was constitutive RNAi lethal (9); fertility is not a subject in this study. (II) Lethality stage of “pupal” includes all substage categories, such as prepupal, early pupal, late pupal, and pharate. Before pupal” includes multiple larval stages, including early larvae and late larvae. “Other” includes mixed-stage lethal, stage undefined, or stage unknown.

<table>
<thead>
<tr>
<th>Age (My)</th>
<th>Essential genes</th>
<th>Nonessential genes</th>
<th>Subtotal</th>
<th>Proportion of essential</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–6</td>
<td>4</td>
<td>9</td>
<td>13</td>
<td>31%</td>
<td>1.00</td>
</tr>
<tr>
<td>6–11</td>
<td>25</td>
<td>51</td>
<td>76</td>
<td>33%</td>
<td>0.78</td>
</tr>
<tr>
<td>11–25</td>
<td>13</td>
<td>30</td>
<td>43</td>
<td>30%</td>
<td>0.60</td>
</tr>
<tr>
<td>25–35</td>
<td>17</td>
<td>46</td>
<td>63</td>
<td>27%</td>
<td>0.24</td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td>136</td>
<td>195</td>
<td>30%</td>
<td>0.31</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (My)</th>
<th>Essential genes</th>
<th>Nonessential genes</th>
<th>Subtotal</th>
<th>Proportion of essential</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;40</td>
<td>86</td>
<td>159</td>
<td>245</td>
<td>35%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lethality stage</th>
<th>Young genes</th>
<th>Old genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pupal</td>
<td>43</td>
<td>50%</td>
</tr>
<tr>
<td>Before pupal</td>
<td>38</td>
<td>44%</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
<td>6%</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>100%</td>
</tr>
</tbody>
</table>

p* = Two-tailed Fisher’s exact test P for essential/nonessential young genes in each age group compared with those for old genes; p† = Two-tailed Fisher’s exact test P for pupal/non-pupal lethals for young genes compared with those for old genes.

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Walsh model, a new duplicate gene can quickly evolve a novel and important function by accumulating advantageous mutations (17), especially in the species with large effective population sizes, such as Drosophila (18). These observations may explain why duplicate genes are as essential as singletons (19–22), although most genes examined in these mammalian studies are relatively ancient.

We investigated the native gene expression patterns of these genes with D. melanogaster life-cycle time-course expression profiling (23). Interestingly, most of the 59 genes we identified are highly expressed at the late larval stages (L2 and L3) or during metamorphosis; some genes are also expressed during the embryonic and L1 stages (fig. S4), which suggests that their gene products are subject to transcriptional regulation during the life cycle.

We examined the developmental stages in which lethality occurs under constitutive silencing and found that lethality occurs at various developmental stages (Fig. 2). The vast majority (47 of 59, 80%) of the young essential genes consistently showed lethality during pupation; four new genes (CG11466, CG33459, CG6289, and CG8358) showed lethality at larval stage, whereas a few other genes show lethality at both larval and pupal stages, which we termed mixed-stage lethality (Table 1 and tables S1 and S5). About 50% of old genes are lethal during pupation, and the other half are lethal at earlier stages, because many early-stage developmental genes are conserved (10) (Table 1). In comparison, young genes are highly enriched in pupal lethals (Table 1; Fisher’s exact test, two-tailed, \( P = 9 \times 10^{-4} \)). These data suggest that new genes have evolved essential functions in larval and pupal development, and frequently regulate development in the pupal stage, with 10% or more regulating the development in the larval or even embryonic stages (table S1) (13).

Examination of metamorphosis failures of pupal lethals demonstrated several distinct classes. The majorities (37 of 47, 79%) of pupal lethals were classified as class I (i.e., pharate lethal; complete pharate formed but failed in the final steps of pupal development and/or eclosion), with only a few falling into class II (pupa development aborted at the prepupal or early pupal stage, without proper formation of rudimentary heads or early leg structures) or class III (development failed over multiple stages, including prepupal, early pupal, late pupal and/or complete pharate stages) (tables S1 and S5 and fig. S3). These data suggested that young essential genes tend to play vital roles in middle or late stages of development, with a few cases in early stages.

We applied a tissue-specific loss-of-function (LOF) analysis to wing and notum development to investigate specific underlying defects (8). Under tissue-specific RNAi, almost every young essential gene we examined showed visible morphological abnormalities that were distinct in range, position, affected cell type, severity, and penetrance (Fig. 3 and table S7). These new duplicates often show novel expression patterns of these new genes have drastically diverged from the parental species is (13).

The vast majority (56 of 59, 95%) of young essential genes were generated through gene duplication, including DNA-based duplication and RNA-based retroposition (Fig. 1, B to D, and table S2). These new duplicates often show novel chimeric gene structures, including new coding regions and untranslated regions (Fig. 1, C and D, fig. S1, and table S2). The protein sequences of these genes have drastically diverged from those of their parental copies, with a median divergence of 47.3% (table S2). A few (3 of 59)
young essential genes originated de novo (Fig. 1, B and E, and table S2). In general, the proportions of new genes that are essential do not differ significantly among the three types of origination mechanisms: 32% (50 of 156) for DNA-based duplication, 26% (6 of 23) for RNA-based retroposition, and 19% (3 of 16) for de novo origination (table S9, \( P > 0.4 \)).

Young essential genes appeared predominantly autosomal (57 of 59), with only two X-linked genes (table S2). Only 15% (2 of 13) X-linked genes were essential for viability, compared with the ~30 to 35% observed for both young and old autosomal genes (fig. S5), which suggests that X-linked genes are less likely to be essential for viability (two-tailed Fisher's exact test, \( P = 0.047 \)).

Sequence evolution shows that young essential genes have higher protein substitution rates (fig. S7A; two-tailed Fisher's exact test, \( P = 5 \times 10^{-8} \)) and higher Ka/Ks ratios (ratios of the rate of amino acid substitution to silent substitution) than their parental genes (fig. S7B; Wilcoxon rank test, \( P = 0.03 \)), likely caused by either relaxation of functional constraint or positive selection. We measured the proportion of substitution under positive selection by comparing between- and within-species variation (fig. S8). Old essential genes were highly constrained with a highly negative \( \alpha \) (−1.48) (fig. S8). The essential genes aged 11 to ~35 My have a slightly negative \( \alpha \) (−0.32), significantly higher than the previous group (likelihood ratio test, \( P < 0.01 \)) (fig. S8). The youngest essential genes (<11 My) have a positive \( \alpha \) (+0.25) (fig. S8), significantly higher than the two previous groups and their parental genes (likelihood ratio tests, \( P < 0.01 \)). These analyses reveal adaptive evolution with young genes and increased purifying selection as genes become older, similar to the pattern of Adh-duplicated new genes (25).

**Fig. 2.** Staging lethality of gene silencing by fluorescence tracking. Living flies with RNAi–green fluorescent protein dual constructs are shown for six major *D. melanogaster* developmental stages: L1, first instar larva; L2, second instar larva; L3, third instar larva; EP, early pupa; PH, pharate (late pupa); A, adult. (Right) Genotypes of the flies. (Left) Stage of lethality. N.S., no flies of this genotype survived to this stage.

**Fig. 3.** Representative cellular and developmental defects. Representative tissue-specific LOF of young essential genes leading to (A) defects in notum scutellar morphologies, (B) irregular bristle patterning and loss of bristles, (C) necrosis and tissue death at multiple places in the notum, (D) tumor formation at the junction between scutum and scutellum, (E) loss of asymmetric patterning with mirror-like wings and ectopic bristles, and (F) possible signaling defect with wing notches. Genotypes of flies are shown above each image, with scale bars in the lower right corners. Yellow arrowheads point to particular phenotypic defects.

**Developmental Stage**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>L1</th>
<th>L2</th>
<th>L3</th>
<th>EP</th>
<th>PH</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>L2 lethal</td>
<td>N. S.</td>
<td>N. S.</td>
<td>N. S.</td>
<td>N. S.</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td>L3 lethal</td>
<td>N. S.</td>
<td>N. S.</td>
<td>N. S.</td>
<td>N. S.</td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
</tr>
<tr>
<td>EP lethal</td>
<td>N. S.</td>
<td>N. S.</td>
<td>N. S.</td>
<td>N. S.</td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
<tr>
<td>PH lethal</td>
<td>N. S.</td>
<td>N. S.</td>
<td>N. S.</td>
<td>N. S.</td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
</tr>
</tbody>
</table>

![Image](image15.png)
We finally investigated the viability phenotype of the parental genes with available RNAi lines (table S10) and retrieved the phenotypic information of several additional genes from previous studies (10). We summarized the essentiality relationship between parental gene–new gene pairs and found that the parental gene of a young essential gene can be either essential or nonessential, and vice versa (tables S10 and S11). These data suggested that a new essential gene can rise from either an essential or a nonessential parent (given that it represents the ancestral state of essentiality) and that either essential genes or nonessential genes can give rise to each type of gene. These processes appeared to be relatively independent (table S11, Fisher’s Exact test, two-tailed, $P = 0.296$).

A previous case study of the sterile phenotype of a paternal-effect gene suggested that genes essential for fertility could arise in 10 My (26). Our observation of lethal phenotypes caused by the knockdown of young genes suggested that essential vital genes have been frequently generated in recent evolutionary periods. A new gene might not have become essential immediately after its origination. It, however, can integrate into a vital pathway by interacting with existing genes, and such interaction would be optimized by mutation and selection. This coevolution may lead to the new gene becoming indispensable. This observation is supported by our modeling (8) with large-scale interaction data (27, 28), revealing genome-wide interactions of young essential genes with many previously unrelated genes (fig. S6).

The mechanism for the evolution of essentiality would change with the types of new genes. A de novo gene has to evolve essentiality through neofunctionalization because it has no ancestral template. A duplicated gene, generated from an ancestral copy of its parental gene, could become essential from the loss of parents, or from the switch of essentiality from paralogs, or through subfunctionalization (29). However, in our data set, the vast majority of the young essential genes have detectable older and conserved paralogs (table S2) and experienced rapid sequence evolution (table S2 and Fig. S7). The prevalent gene structure renovation (table S2), together with the independence between parental gene essentiality and new gene essentiality (table S11), support the neofunctionalization origin of essentiality for most new protein-coding genes, many of which may contribute to the lineage-specific developmental program.

References and Notes

8. Materials and methods are available as supporting material on Science Online.
11. K. Kelemen, T. Micheler, VDRC project members, Personal communication to FlyBase, FBrf0208510: RNAi-philC31 construct and insertion data submitted by the Vienna Drosophila RNAi Center (2009); http://flybase.org/0208510.html.
30. We thank C. H. Langley and D. Begun for providing polymorphism data; W. Du, J. Gavin-Smyth, Q. Guo, and M. Guffey for technical assistance and discussion; J. Coyne, M. Kreitman, and X. Ni for critically reading and/or revising the manuscript; and the members of the manyuan long laboratory, C. Ferguson, R. Hudson, C. I. Wu, and T. Nagylaki, for valuable discussion. S.C. was supported by University of Chicago Biological Sciences Division Fellowships. This research was supported by National Institutes of Health (RO1GM065429-01A1 and RO1GM078070-01A1) and National Science Foundation (CAREER Award MCB 0238168 to M.L. Y.E.Z. was also supported by the Searle Funds from Chicago Biomedical Consortium (2009, Spark).

Supporting Online Material

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Materials and Methods
Figs. S1 to S8
Tables S1 to S11
References

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Cytoplasmic Partitioning of P Granule Components Is Not Required to Specify the Germline in C. elegans

Christopher M. Gallo,* Jennifer T. Wang,* Fumio Motegi, Geraldine Seydoux†

Asymmetric segregation of P granules during the first four divisions of the Caenorhabditis elegans embryo is a classic example of cytoplasmic partitioning of germline determinants. It is thought that asymmetric partitioning of P granule components during mitosis is essential to distinguish germline from soma. We have identified a mutant (pptr-1) in which P granules become unstable during mitosis and P granule proteins and RNAs are distributed equally to somatic and germline blastomeres. Despite symmetric partitioning of P granule components, pptr-1 mutants segregate a germline that uniquely expresses P granules during postembryonic development.

pptr-1 mutants are fertile, except at high temperatures. Hence, asymmetric partitioning of maternal P granules is not essential to specify germ cell fate. Instead, it may serve to protect the nascent germline from stress.

A general characteristic of germ cells is the presence of cytoplasmic RNA-rich granules called germ granules (I). In Caenorhabditis elegans, germ (P) granules are present in all germ cells except mature sperm, and they segregate asymmetrically with the germline-precursors (P blastomeres) during the first embryonic divisions (Fig. 1A)(2). Like embryonic germ granules of other organisms, P granules have been hypothesized to harbor the determinants that specify the germline. However, their function and segregation mechanisms are not fully understood (2, 3).

To monitor P granule dynamics, we used confocal microscopy to image live embryos expressing the P granule protein PGL-1 fused to green fluorescence protein (GFP)(4). We obtained similar results with GFP fusions to two other P granule proteins PGL-3 and GLH-1 (5, 6). In the live movies, we analyzed granule dynamics (number, size, and movement) and the overall distribution of each protein by quantifying total (granular + diffuse cyto-