Selfish genetic elements subvert the laws of Mendelian segregation to promote their own transmission (1–5). In what is perhaps the most extreme scenario, selfish elements can kill individuals that do not inherit them, leading to genetic incompatibilities between carriers and noncarriers (5–9). Selfish elements are predicted to spread in natural populations (5, 6), and consequently, there is interest in using synthetic forms of such elements to drive population replacement of pathogen vectors in the wild (10, II). However, despite the prominent role of selfish elements in genome evolution and their promise in pathogen control, their underlying genetic mechanisms have been resolved in only a few cases (5). Our laboratory previously identified a paternal-effect selfish element in the nematode C. elegans (12, 13). This element is composed of two tightly linked genes: peel-1, a sperm-delivered toxin, and zeel-1, a zygotically expressed antidote. In crosses between isolates that carry the element and ones that do not, the peel-1 toxin is delivered by the sperm to all progeny, so that only embryos that inherit the element and the zeel-1 antidote survive. An analogous element, Medea (maternal-effect dominant embryonic arrest), has been described in the beetle Tribolium; however, the underlying genes remain unknown (6, 9).

A maternal-effect selfish genetic incompatibility in C. elegans

As part of ongoing efforts to study natural genetic variation in C. elegans, we introgressed a genetic marker located on the right arm of Chr. V from the standard laboratory strain N2 into the strain DL238 by performing eight rounds of backcrossing and selection. DL238 is a wild strain isolated in the Manuka National Reserve, Hawaii, USA, and is one of the most highly divergent C. elegans isolates identified to date (14). To confirm the success of the introgression, we genotyped the resulting strain at single-nucleotide variants (SNVs) between DL238 and N2 by whole-genome sequencing. As expected, with the exception of a small region on the right arm of Chr. V where the marker is located, most of the genome was homozygous for the DL238 alleles (Fig. 1A). However, to our surprise, we observed sequence reads supporting the N2 allele at many SNVs on Chr. III, including two large regions that were homozygous for the N2 allele despite the eight rounds of backcrossing (Fig. 1A and fig. S1). This observation suggested that N2 variants located on this chromosome were strongly selected during the backcrossing.

To investigate the nature of the selection, we performed a series of crosses between the N2 and DL238 strains and examined their progeny. To avoid effects of the peel-1/zeel-1 element, which is present in N2 and absent in DL238, we performed a cross between DL238 males and a near-homozygous N2 strain (Fig. 1A and fig. S1). This region contains 10 genes and two pseudogenes in N2. We hypothesized that the incompatibility could stem from a cytoplasmically inherited toxin that kills embryos if they lack a zygotically expressed antidote, analogous to the mechanism of the peel-1/zeel-1 element (12, 13). To test this model and to discriminate between maternal and paternal effects, we crossed heterozygous F1 DL238 × N2 peel-1/zeel-1 males and hermaphrodites with DL238 hermaphrodites or males, respectively (Fig. 1B and fig. S2). We observed 48.59% (N = 389) lethality when F1 hermaphrodites were crossed to DL238 males, but only baseline lethality (1.17%, N = 171) in the reciprocal cross of F1 males to DL238 hermaphrodites. A finding of 50% lethality when the F1 parent is the mother and no lethality when the F1 parent is the father indicates that the incompatibility is caused by maternal-effect toxicity that is rescued by a linked zygotic antidote (fig. S2). We tested whether the new incompatibility was independent from the paternal-effect peel-1/zeel-1 element by crossing DL238 and N2 worms and setting the F1 progeny. We observed 41.37% (N = 307) embryonic lethality among the F2 progeny, consistent with the expectation of Mendelian segregation of two independent incompatibilities (43.75%) (Fig. 1D).

**pha-1 and sup-35 constitute a selfish element that underlies the incompatibility between DL238 and N2**

To identify the genes underlying the maternal-effect incompatibility between N2 and DL238, we sequenced the genome of DL238 using Illumina short reads and aligned those reads to the N2 reference genome. We focused our attention on the two regions on Chr. III that were completely homozygous for the N2 allele in the introgressed strain (Fig. 1A and fig. S1). Inspection of short-read coverage revealed a large ~50-kb region on the right arm of the chromosome with very poor and sparse alignment to the N2 reference (Chr III: 11,086,500–11,145,000) (Fig. 2A). This region contains 10 genes and two pseudogenes in N2. We noticed that pha-1, annotated as an essential gene in the reference genome, appeared to be completely missing in DL238 (Fig. 2A) (15). pha-1 was originally identified as an essential gene required for differentiation and morphogenesis of the pharynx, the C. elegans feeding organ (15). But if pha-1 is essential for embryonic development and missing in DL238, then how are DL238 worms able to live? pha-1 lethality can be fully suppressed by mutations in three other genes: sup-35, sup-36, and sup-37 (16). We found no coding variants in sup-36 and sup-37, which reside on chromosomes IV and V, respectively (16) (fig. S3). However, sup-35, which is located 12.5 kb upstream of pha-1, also appeared to be missing or highly divergent in DL238 (Fig. 2A and fig. S3).

We hypothesized that sup-35 and pha-1 could constitute a selfish element responsible for the observed incompatibility between the N2 and DL238 isolates. In our model, sup-35 encodes a maternally deposited toxin that kills embryos and pha-1 is an expressed antidote. The observed pattern of embryonic lethality (no lethality in the parents nor in the F1; 25% lethality in the F2) is consistent with an interaction between the genotype of the zygote and a maternal or paternal effect (Fig. 1C) (17). We hypothesized that the incompatibility could stem from a cytoplasmically inherited toxin that kills embryos if they lack a zygotically expressed antidote, analogous to the mechanism of the peel-1/zeel-1 element (12, 13).

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Fig. 1. A maternal-effect genetic incompatibility on Chr. III. (A) A marker on Chr. V was introgressed from the reference strain N2 into the DL238 wild isolate. Short-read sequencing of the introgression strain revealed homozygous N2 variants on Chr. III, indicating strong selection in favor of N2 variants during the generation of this strain. (B) DL238 males were crossed to hermaphrodites carrying a null allele of the peel-1/zeel-1 element (niDf9) in an otherwise N2 background (N2 peel-1/zeel-1). F1 hermaphrodites were allowed to self-fertilize (top). Alternatively, F1 hermaphrodites (middle) or males (bottom) were backcrossed to the DL238 parental strain. Embryonic lethality was scored in the F2 progeny as percentage of unhatched eggs. Dashed gray lines indicate expected embryonic lethality under the maternal-effect toxin and zygotic antidote activities. This major reinterpretation of the roles of pha-1 and sup-35 is strongly supported by multiple lines of evidence from previous studies: (i) sup-35 overexpression phenocopies pha-1 mutations, showing that sup-35 is sufficient to cause embryonic lethality (19); (ii) All defects associated with pha-1 mutations are suppressed by mutations in sup-35 (18–21). (iii) When N2 hermaphrodites heterozygous for a deletion that includes both sup-35 and pha-1 (DF2/+ mother) reproduce by selfing, the 25% of their progeny that are homozygous for this deletion arrest as embryos with pharyngeal defects (16, 19). The lethality and pharyngeal defects of these homozygous embryos can be rescued by growing the heterozygous DF2/+ mothers in sup-35 RNA interference, which depletes sup-35 transcripts from the germ line (20). These results indicate that maternally deposited sup-35 is sufficient to kill embryos that lack pha-1, which is consistent with the role of sup-35 as a selfish element in the C. elegans genome.
We predicted that these isolates should be in-
compatible with N2. Because QX1211 and ECA36
were predicted to be maternal-effect toxins, we
examined the sequences of sup-35/pha-1
element. Global variation in the activity of the
sup-35/pha-1 element

We examined the sequences of sup-35 and pha-1 in
152 C. elegans wild isolates that represented
unique isotypes (22) in the Caenorhabditis elegans
Natural Diversity Resource (23). Two isolates,
QX1211 (California, USA) and ECA36 (Auckland,
New Zealand), harbored a highly mutated copy
of pha-1, with multiple nonsynonymous SNVs as
well as frameshifts expected to completely disrupt
the protein (figs. S3 and S4). Both of these isolates
also appeared to be missing sup-35 (fig. S3).
We predicted that these isolates should be in-
compatible with N2. Because QX1211 and ECA36
carry the same haplotype in the sup-35/pha-1 region,
we focused on further characterizing QX1211. We
crossed QX1211 to N2 peel-1(+) worms and
observed 23.9% (N = 355) lethality in the F2
progeny, consistent with QX1211 carrying a de-
generate copy of pha-1. Lethality was abolished
when we crossed QX1211 with N2 sup-35; peel-1(+) (0%,
N = 290). Furthermore, we observed
background levels of embryonic lethality (1.0%,
N = 294) in the F2 progeny of a DL238 × QX1211
cross, as expected because both strains lack func-
tional sup-35. Our analysis also revealed that
the highly divergent Hawaiian isolate CB4856
carried a functional sup-35/pha-1 element, which
explains why previous studies did not detect it
(12). Consistent with this observation, crossing
the CB4856 and DL238 isolates led to the
expected embryonic lethality in the F2 (22.3%,
N = 349).
We looked for additional variation in sup-35,
sup-36, and sup-37 across the 152 isolates, which
could potentially affect the activity of the sup-35/
pha-1 element (figs. S5 to S7). We found eight
nonsynonymous variants (three in sup-35, three
in sup-36, and two in sup-37) and one premature
stop codon in sup-35 that removed 47% of the
protein. We also identified potential deletions by
visually inspecting read alignments in each of
the 152 isolates. Whereas sup-36 and sup-37 had
consistent coverage in all isolates, we identified
two structural variants in sup-35: a 530-base
pair deletion in the third intron, and a large
12.1-kb deletion that removed part of the last
exon and the 3′ untranslated region and fused
sup-35 to Y48A6C1, a pseudogene that has partial
homology with sup-35, creating a chimeric tran-
script (figs. S8 and S9). We tested strains carrying
each of these variants for a maternal-effect lethality
in crosses with DL238 (table S2 and fig. S10) and
found that the lethality was completely abolished
in strains carrying the chimeric sup-35/Y48A6C1
gene and in the strain carrying the premature
stop codon in sup-35, indicating that these var-
iants disrupt sup-35 function. Thus, loss of
maternal-effect toxin. Finally, whole-genome se-
quence alignment across 26 nematode species indi-
cates a lack of pha-1 conservation (Fig. 2A). This
observation is more consistent with its recent
evolution as part of a selfish element in
C. elegans.
**Fig. 3.** The sup-35/pha-1 N2 haplotype is derived and is marked by an inversion. Left: A gene tree built using Bayesian inference from the coding region of Y48A6C.4 in 152 C. elegans isolates and four other Caenorhabditis species. DL238, QX1211, and ECA36 cluster together in a separate branch from all other C. elegans isolates. Right: Schematic representation of the synteny in the region containing the sup-35/pha-1 element, as well as three highly conserved genes in the close vicinity (hmt-1, Y48A6C.4, and Y47D3A.29); w denotes alleles that are pseudogenized. The genes sup-35 and Y48A6C.4 are inverted in DL238, QX1211, and ECA36 relative to the other 149 C. elegans isolates. The gene order and orientation of hmt-1, Y48A6C.4, and Y47D3A.29 in other Caenorhabditis species suggest that the inverted haplotype is the ancestral, and that the haplotype found in 149 isolates is the derived one.

Sup-35 activity has occurred independently at least twice in carriers of N2-like alleles of the element.

**DL238 and QX1211 carry an ancestral sup-35/pha-1 haplotype**

The alignment of DL238 and QX1211 short reads to the N2 reference genome was very sparse throughout the sup-35/pha-1 region and at nearby genes, with some genes aligning only in exons and others not aligning at all (Fig. 2A). Moreover, several attempts to define the boundaries of the pha-1 deletion in DL238 with diverse combinations of polymerase chain reaction primer pairs were unsuccessful. This suggested that the DL238 and QX1211 haplotypes were highly divergent from the N2 reference and that major genomic rearrangements may have occurred.

To resolve the genomic structure of the sup-35/pha-1 element in these isolates, we de novo assembled the genomes of DL238 and QX1211 using a combination of our own and previously published Illumina short reads (23, 24), followed by targeted Sanger sequencing to resolve repetitive regions and confirm scaffolds. The de novo assemblies confirmed that pha-1 is absent from DL238 and is highly pseudogenized in QX1211, and that sup-35 is pseudogenized in both (Fig. 3 and fig. S11). DL238 and QX1211 share a very similar haplotype, with the exception of a large deletion in DL238 that encompasses pha-1, fhxa-128, and several exons of Y47D3A.1 (fig. S11). We also identified other large structural variants in both DL238 and QX1211 at the sup-35/pha-1 locus. First, relative to the N2 reference genome, nearly 20 kb of sequence is missing completely from both isolates (fig. S11). Second, the region spanning the pseudogenized sup-35 and Y48A6C.4 is inverted relative to the N2 reference (Fig. 3 and fig. S11). This inversion was confirmed by single-molecule Oxford Nanopore long-read sequencing (fig. S12). As a consequence of the inversion, the pseudogenized sup-35 and pha-1 are located next to each other in QX1211, rather than flanking Y48A6C.4 as in the N2 reference genome (Fig. 3 and fig. S11).

To gain further insights into the evolution of the sup-35/pha-1 element, we aligned the N2, DL238, and QX1211 haplotypes to the homologous regions of diverse Caenorhabditis species, using the highly conserved genes (hmt-1, Y48A6C.4, and Y47D3A.29) that delineated the region (Fig. 3 and fig. S11). Unexpectedly, our analysis revealed that the order and orientation of these three genes in the other Caenorhabditis species matched that in DL238 and QX1211 rather than the order and orientation in N2. This observation suggests that the sup-35/pha-1 haplotype in DL238 and QX1211 derives from an early stage in the evolution of the selfish element, which was followed by a major inversion that now defines the N2 haplotype, and subsequently by degeneration of the element in DL238 and QX1211. In further support of this model, a gene tree built using the coding region of Y48A6C.4 from all the C. elegans isolates and the other Caenorhabditis species showed that DL238, QX1211, and ECA36 cluster in a separate branch from all other C. elegans isolates (Fig. 3).

**Discussion**

We discovered a selfish genetic element in C. elegans that is composed of a maternally deposited toxin, sup-35, and a zygotically expressed antidote, pha-1. The antidote, pha-1, was originally thought to be a developmental gene, in large part due to the specific pharyngeal defects observed in mutants (15, 19, 25–27). However, the precise role of pha-1 in embryonic development remained elusive and controversial (20, 21, 28). Our results indicate (i) that pha-1 pharyngeal defects are a direct consequence of sup-35 toxicity and (ii) that sup-35 and pha-1 act as a selfish element, instead of being integral components of C. elegans embryonic development as originally suggested.

One important insight emanating from previous work in light of our results is that the sup-35/pha-1 element exerts its toxicity by recruiting genes that are directly involved in C. elegans development (16, 18–20, 26). The other two known suppressors of pha-1 lethality, sup-36 and sup-37, are essential for sup-35 toxicity and are conserved in other nematodes (18, 20). Interestingly, sup-37 is required for normal pharyngeal pumping and promotes ovulation in the somatic gonad independently of pha-1 function (20). Null sup-37 mutants are inviable and undergo early larval arrest. However, a single missense and viable mutation in sup-37 is sufficient to abolish sup-35 toxicity (18, 20). Together with the finding that SUP-37 physically interacts with SUP-35 (18), this suggests that the sup-35/pha-1 selfish element is hijacking a developmental pathway to kill those embryos that do not inherit it. The specificity in the activity and expression of sup-36 and sup-37 may explain the pharyngeal phenotypes of pha-1 mutants. We hypothesize that PHA-1 could act as an antidote by directly inhibiting the interaction between SUP-35 and SUP-37. The transcription factor lin-35/Rb and the E2 ubiquitin conjugation enzyme ube-15 down-regulate sup-35 (19). An attractive possibility is that this regulation evolved as an additional mechanism to cope with sup-35 toxicity, as part of an arms race between the selfish element and its host. Future studies may further resolve the mechanism of sup-35 toxicity and its regulation.

One of the most intriguing aspects of toxin-antidote systems is their origin. The study of the pha-1/sup-35 element provides some clues. pha-1 has no known orthologs, and only a few highly divergent protein sequence matches are found in closely related Caenorhabditis species.
On the other hand, sup-35 is a homolog of another C. elegans gene, rmd-2, which is conserved in other nematodes (19). A phylogenetic analysis shows that sup-35 is more closely related to C. elegans rmd-2 than to rmd-2 genes from other species and is likely a paralog of rmd-2 (figs. S13 and S14). These results suggest that the origin of the sup-35/pha-1 element involved the duplication of a preexisting gene (rmd-2) and the recruitment of a novel gene of unknown origin in the lineage leading to C. elegans.

Among 152 C. elegans wild isolates examined, only DL208, QX1211, and ECA36 do not carry the derived inversion in the sup-35/pha-1 element, and in all three of them, the selfish element is highly pseudogenized. Similar inversions have been described in the Drosophila segregation distortion reporter locus and in the mouse t haplotypes (6, 29, 30); they are thought to stabilize two-component driver systems by preventing recombination from decoupling the components (6). Has the inversion facilitated the spread of sup-35/pha-1 through the C. elegans population to all but a few isolates? Ongoing efforts to identify more divergent isolates, as well as nematode species that are more closely related to C. elegans, may fill in the gaps in our understanding of the evolution of this element.

Lastly, our work highlights the importance of studying natural genetic variation for understanding gene function. Despite the indisputable value of a common reference strain, it has proved extremely difficult in the context of the N2 background alone to either confirm or rule out pha-1 as an essential component of C. elegans embryonic development. The study of other wild isolates has made possible our characterization of sup-35/pha-1 as a selfish element. Our results show that some essential genes may, in fact, turn out to be antidotes to unknown toxins. Selfish elements conferring genetic incompatibilities may be more common than previously thought, and some of them may be hiding in plain sight.

REFERENCES AND NOTES


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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/356/6342/1051/suppl/DC1 Materials and Methods Figs. S1 to S14 Tables S1 and S2 References (33–54)

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A maternal-effect selfish genetic element in *Caenorhabditis elegans*

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**Selfish genetic interactions in nematodes**

Identifying the effects and evolution of selfish genetic elements can be difficult because of their biased inheritance. Ben-David *et al.* identified a selfish genetic element that drives maternal-effect lethality in the nematode *Caenorhabditis elegans* (see the Perspective by Phadnis). This incompatibility stems from the interaction between a maternally deposited toxin and a zygotically expressed antidote. Interestingly, the antidote is encoded by the gene *pha-1*, which has been described as an essential gene in embryonic development.

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