Supporting online material

Recombinant Inbred Lines

We reciprocally crossed N2 (Bristol) and CB4856 (Hawaii), yielding two classes of male and hermaphrodite F1s differing in their mitochondrial and X chromosome complements. We performed each of the four possible crosses among these, yielding four classes of F2 hermaphrodites and a single class of F2 males, ignoring the male mitochondrial genome, which is not transmitted. We performed all four classes of crosses among the F2s, with each contributing 64 male and 64 hermaphrodite worms to the 512-worm F3 population, at which point random pair mating was initiated. Each generation a single male and a single hermaphrodite were taken from each cross to contribute to the random pair mating pool of the subsequent generation. From the F10 generation, two hermaphrodites were taken from each successful cross and individually selfed. After ten generations of single-worm descent, we isolated DNA from each of the 239 surviving recombinant inbred lines and genotyped them at 1450 SNP markers using Illumina's GoldenGate assay (Illumina, San Diego).

To estimate the expected allele frequency skew due to segregation drift in the construction of the RILs, we simulated the passage of autosomes and X chromosomes along the RIL pedigree with a perl script (available on request). We modeled *C. elegans* chromsomes with complete interference, i.e., 50 cM chromosomes with exactly zero or one cross-overs resolved as recombinants in each meiosis. For each chromosome, we found the marker whose frequency in the resulting RILs departed most from 50%. While the highest frequency observed in 10,000 simulated autosomes is 0.78, the actual frequency of the most skewed marker in our RIL population is 0.98, excluding the

possibility that the observed allele frequency skew on chromosome 1 could have arisen without selection The distribution for simulated X-chromosomes was very similar to that found for autosomes, shifted slightly toward less extreme skew, and the observed skew on the actual RIL X-chromosome is consistent with neutrality.

Linkage of lethality to *bli-3*

Bristol and Hawaii males were crossed to MT1344 hermaphrodites, which carry *bli-3(e767)* and *lin-17(n677)* in a Bristol genetic background, and all embryos laid by two Bristol/MT1344 and two Hawaii/MT1344 hermaphrodites were collected on individual plates. Adult F2 progeny were scored as Blister or Non-Blister. Non-Blister F2s were classified as +/*bli-3* or +/+ by scoring the presence or absence of Blister worms in F3 broods. *bli-3* is located 10 cM from the most skewed RIL marker.

Worm maintenance and embryonic lethality scoring

Worms were maintained as described (S*1*). Lethality from selfing hermaphrodites was scored by transferring L4 hermaphrodites to fresh plates on day one, then singling them to fresh plates on day two. After approximately eight hours, hermaphrodites were removed and embryos counted. Unhatched embryos were counted 18-24 hours later. Lethality from mated hermaphrodites was scored by mating 3-4 L4 hermaphrodites to 8-10 males on day one, then singling mated hermaphrodites to fresh plates on day two and counting embryos as for the selfing hermaphrodites. Once the surviving progeny had reached adulthood, broods from mated hermaphrodites were scored for the presence of male progeny. Broods having significantly less than 50% male progeny were discarded to avoid the possibility of self-progeny. In general, broods exhibited 50% male progeny or no male progeny, indicating that mating was either complete or did not occur at all.

In most experiments, worms were kept at room temperature, 20-24˚C. At this temperature, worms typically laid 50-100 embryos over an eight-hour period. When more embryos per hermaphrodite were required, worms were placed at 17˚C and transferred every 12 hours to fresh plates. Lethality was not temperature sensitive and was not affected by the temperature shift. Sex ratios within broods affected by the incompatibility were unaltered.

Mapping *zeel-1* and *peel-1* using the Recombinant Inbred Lines (RILs)

We crossed RILs to each of Bristol and Hawaii and scored lethality from self-fertilizing F1 hermaphrodites (self-cross) and Hawaii hermaphrodites mated to F1 males (male backcross). These crosses distinguished two classes of RILs. When crossed to Bristol, lines in the first class showed 25% and 50% lethality in the self-cross and male backcross, respectively, indicating that they carried $zeel\text{-}1_{Hawaii}$. When crossed to Hawaii, lines in the first class showed no lethality, indicating they carried $peel\text{-}1_{Hawaii}$. Lines in the second class showed the opposite pattern of lethality. When crossed to Bristol, they showed no lethality, indicating they carried $zeel\text{-}1_{Bristol}$, and when crossed to Hawaii, they showed 25% and 50% lethality in the self-cross and male backcross, respectively, indicating that they carried $peel\text{-}1_{Bristol}$.

According to the Illumina genotypes, in only one genomic interval did all lines of the first class carry the Hawaii haplotype and all lines of the second class carry the Bristol haplotype. After sequencing the breakpoints of informative RILs, we narrowed this interval to positions 2,317,234 to 2,379,249 on chromosome I.

Incomplete penetrance

To evaluate penetrance among progeny from self-fertilizing F1 hermaphrodites,

Hawaii males were crossed to Bristol hermaphrodites, and all embryos laid by three F1 hermaphrodites were collected on individual plates. F2 progeny were allowed to self-fertilized, and adult F3 progeny were collected for genotyping. Groups of 20-50 F3 adults were digested for 1.5 hours at 65ºC then 15 minutes at 95ºC in 50ul of 50 mM KCl, 10 mM Tris-HCl pH 8.3, 2.5 mM $MgCl_2$, 0.45% NP-40, 0.45% Tween-20, 0.01% gelatin, 0.1 mg/ml Proteinase K. Digests were genotyped at two PCR length polymorphisms spanned by primers 5'-ctgaagcatgccggatttat-3' (forward) and 5'-tccgtccaatattcaatcgac-3' (reverse); and 5'-aaaatgaacaaattttggtgaaaaa-3' (forward) and 5'-cgacgctactccagaaaaca-3' (reverse) (95˚C 30 seconds, 59˚C 30 seconds, 72˚C 1.5 minutes). These polymorphisms are located 12.0 kb to the left and 54.2 kb to the right of the *zeel-1* start codon, respectively.

Of 865 embryos collected, 660 survived to hatching, and of the surviving progeny 24 were homozygous for $zeel\text{-}1_{Hawaii}$. Assuming that all 205 unhatched embryos were homozygous for $zeel\text{-}1_{Hawaii}$, we estimate the penetrance of lethality to be roughly 90%. Sixteen of the surviving $zeel\text{-}1_{Hawaii}$ homozygotes showed severely deformed morphologies, yet their progeny were entirely wildtype.

We used the same procedure to evaluate penetrance among progeny from F1 hermaphrodites mated to F1 males, except that embryos laid on the first day of mating were discarded to avoid contamination by self-progeny, and digests were genotyped at a length polymorphism less than 1 kb from the *zeel-1* deletion, spanned by primers 5'-tggatacgattcgagcttcc-3' (forward) and 5'-ccccctaatttccaagtggt-3' (reverse). Of 794 embryos collected, 549 survived to hatching, and none were homozygous for $zeel\text{-}1_{Hawaii}$.

Wild Isolates

C. elegans isolates were acquired from the CGC and as gifts from Marie-Anne Felix, Antoine Barriere, and Elie Dolgin. Because strains collected from a single locality may be genetically identical relatives, we included from within localities only strains that are known to be genetically distinct, on the basis of data from publications (S*2-6*) and from our own genotyping and sequencing. For each wild isolate, we scored embryonic lethality as above in the broods of selfing wild isolate (WI) hermaphrodites, WI/Hawaii hermaphrodites, WI/Bristol hermaphrodites, WI/Hawaii male x Hawaii backcrosses, WI/Bristol male x Hawaii backcrosses, and WI/Bristol male x WI backcrosses. The dataset of phenotypes is presented in Table S7. To confirm that strains of different compatibility types from within a single compost sample were incompatible with one another and not merely with either Bristol or Hawaii, we also performed the appropriate crosses between MY14 and MY16 (Mecklenbeck, Germany).

Sequencing polymorphic interval in Hawaii

Within the polymorphic interval, primers designed against the Bristol sequence generally do not amplify product in Hawaii. We obtained three PCR products within the interval, and then used the GenomeWalker Universal Kit (Clontech, Mountain View, CA) to obtain sequence adjacent to these fragments. Genome Walking was repeated until contigs could be connected by PCR amplification. All sequence assembled by GenomeWalker was confirmed by amplifying and sequencing directly from Hawaii genomic DNA. The deletion spanning *zeel-1* was confirmed using primers 5'-ggatccatatattcggaaattga-3' (forward) and 5'-ggaagctcgaatcgtatcca-3' (reverse), which span a 19 kb interval in Bristol but give a 3kb product in Hawaii (98°C 10 seconds, 60°C

14 seconds, 72˚C 1.5 minutes; Phusion, New England Biolabs, Ipswich, MA). Sequence was obtained for > 500 bp on either end of this product. Sequencing of the central section could not be completed because it is apparently composed of tandem repeats of an 11 base pair motif.

Genotyping wild isolates for the *zeel-1* deletion

Wild isolates were genotyped for the *zeel-1* deletion with a three-primer PCR with primers 5'-ggatccatatattcggaaattga-3' (forward), 5'-ggaagctcgaatcgtatcca-3' (reverse), and 5'-agatgcttaggggcaattt-3' (decoy forward). The forward and reverse primers lie 19 kb apart in Bristol but only 3 kb apart in Hawaii. The decoy forward primer lies within the *zeel-1* deletion, 300 bp upstream of the reverse primer. Thus, strains with an intact *zeel-1* give a 300 bp product, while those carrying the deletion give a larger sized product. Using this assay, all Bristol-compatible wild isolates gave a 300 bp product, and all Hawaii-compatible wild isolates gave a 1.5 to 7 kb product. The variability in product size among Hawaii-compatible strains probably reflects variation in copy number of the tandemly repeated 11 base pair motif.

***srbc-64* sequencing and molecular population genetics**

The first coding segment of *srbc-64* was amplified with primers 5'-gtacgtccgcactgatgct-3' (forward) and 5'-cgggaatatctggcgaatta-3' (reverse) and sequenced with the forward primer. Because of the high polymorphism, primers amplifying the second and third coding segments in both Bristol and Hawaii could not be chosen. Instead, these segments were amplified in a reaction containing three primers, one common to both strains and the other two specific for either Bristol or Hawaii. Products were sequenced with the shared primer. Primers for the second coding segment were 5'-

gctgagtggccgcaaaag-3' (forward), 5'-gagtctgcgcctaagcctaa-3' (reverse Bristol), and 5'-cccaagcctaaatagcgtca-3' (reverse Hawaii). Primers for the third coding segment were 5'-ccctttatctacgtggccta-3' (forward Bristol), 5'-aggccaagaaaaactcttcc-3' (forward Hawaii), and 5'-tttactaaagttgcttgtggaaaa-3' (reverse).

srbc-64 alleles were aligned with T-Coffee (S*7*) and adjusted manually. The alignment contained 1226 positions; gapped positions were excluded from subsequent analysis. Because non-coding sequences are largely unalignable between Bristol- and Hawaii-like haplotypes, our alignment is primarily (71%) coding sequence. The use of coding sequence and the inclusion only of strains known to be genetically distinct both introduce a bias toward rare alleles. Of the 80 polymorphisms (excluding insertion/deletion variants) observed in the dataset, 75 distinguish the incompatible haplotypes. The remainder occur only on one or the other haplotype class.

Summary statistics were estimated with the *compute* program from libsequence software (S*8*). P-values were estimated within *compute* from 10,000 simulations of the standard neutral equilibrium coalescent.

Number of sequences	45
Sites (excluding gaps)	1193
Segregating sites	80
Theta (per site)	0.0153
Pi (per site)	0.0306
Tajima's D	3.5561 ($p < 0.0001$)
Number of haplotypes	6 ($p < 0.0001$)

Variation at *srbc-64* within each incompatibility class conforms to neutral equilibrium expectation, with non-significantly negative Tajima's D (S*9*) (-1.38 for Hawaii compatible strains, -0.56 for Bristol compatible strains) in keeping with the known sources of bias described above.

We estimated d_N, d_S, and ω in the pairwise runmode of the CODEML program in PAML version 3.15 (S*10*). We aligned sequences corresponding to the Bristol gene models from WormBase (ws177) and parameterized CODEML with estimated base frequencies for each codon position (CodonFreq model 2). Both *Y39G10AR.16* and *nekl-1* have sequences in Hawaii that would result in frameshifts if the ws177 Bristol annotations were applicable. *Y39G10AR.16* shows ω substantially less than 1 when the putatively frameshifted codons are excluded, as described in the caption to Table 2, suggesting that the gene is probably intact in Hawaii.

For *nekl-1*, cDNA clones indicated that the predicted gene annotation was incorrect. We cloned *nekl-1* cDNA from Bristol and Hawaii by reverse transcription with primer 5'- tctgtattctccctttctgga-3' followed by amplification with primers 5'- tggatacgattcgagcttcc -3' (forward) and 5'- acattccgtcgatcataggc-3' (reverse). cDNAs were then TOPO cloned into pCR-TOPO (Invitrogen, Carlsbad, CA). Multiple splice variants were obtained from both Bristol and Hawaii, but all clones had an additional intron encompassing the putative frameshift in Hawaii (a 1 bp deletion in predicted exon 13). For analysis of dN and dS, we chose the longest open reading frame present among the cDNA clones.

To estimate the divergence time of the incompatible haplotypes, we used the relationship $d = \mu t$, where d is the evolutionary distance between the haplotypes in

substitutions per site, μ is the mutation rate per site per generation, and t is the number of generations separating the haplotypes. We estimated d_S as above from a concatenated alignment of the 1038 codons of the three alignable genes in the interval, *srbc-64, Y39G10AR.16*, and *ugt-31,* yielding a point estimate of $d_S = 0.145$ synonymous substitutions per synonymous site. To estimate a confidence interval for d_S taking sampling error into account, we generated 1000 datasets of 1038 codons by bootstrap resampling of the concatenated dataset. The SNP mutation rate, μ, was estimated from direct sequencing of *C. elegans* mutation accumulation lines as $\mu = 0.9 \times 10^{-8}$ per base pair per generation (S*11*). Because divergence between the haplotypes represents the sum of divergences of each haplotype from their last common ancestor, the age of the last common ancestor of the haplotypes is $t/2$. The estimated time to the ancestor of the haplotypes 8.0×10^6 generations, with a 95% confidence interval spanning 6.5×10^6 to 9.7×10^6 generations. The age of the common ancestor (coalescence time) of typical *C. elegans* genes is a few tens of thousands of generations by this measure.

The generation time in *C. elegans* is very poorly constrained. In ideal conditions the generation time may average as little as four days, but in nature many generations are likely to be dominated by a long period of reproductive dormancy, measured in months, during which the worms occur as dauer larvae. Therefore, we have not attempted to convert divergence time from generations to years.

Transgenic rescue

Fosmids and subclones were bombarded into QX1001 or QX1015, strains carrying *unc-119(ed3)* in a Hawaii genetic background. These strains were created by introgressing *unc-119(ed3)* into Hawaii for more than eight generations. Bombardment

was performed as described (S*12*), using an equimolar ratio of the *unc-119(+)* rescue vector, pDP#MM016B (S*13*), and the fosmid or subclone.

Subclones were generated by ligating fragments containing each gene and its upstream intergenic region into pPD61.125 (Andy Fire vector kit, Addgene, Cambridge, MA) cut with the appropriate restriction enzyme. For *srbc-64*, a fragment containing the gene was amplified from genomic Bristol DNA using primers 5'-gtcccactcaccaaactcgt-3' (forward) and 5'-gagttacgacgcccatttgt-3' (reverse) and then cut with NgoMIV and XbaI. *Y39G10AR.16* was cloned by amplifying from genomic Bristol DNA with primers 5'-ttaggcttaggcgcagactc-3' (forward) and 5'-ggtgagcttcacaccgattt-3' (reverse) and then cutting with XbaI. *ugt-31* and *Y39G10AR.5* were excised from fosmid *WRM0633bE09* using restriction enzymes NgoMIV and SacII, and SacI and XmaI, respectively. To generate the frameshift, the *Y39G10AR.5* subclone was cut with StyI, treated with Klenow, and re-ligated.

Most transgenic lines carried their transgenes on extrachromosomal arrays, which are not transmitted to all progeny. Progeny not inheriting the array are Unc, while those inheriting it are Non-Unc. To test positive transformants for rescue of embryonic lethality, Non-Unc, transgenic males were crossed to DB38, which carries *unc-119(ed3)* in a Bristol genetic background. Embryonic lethality was scored from Non-Unc F1 hermaphrodites (self-cross), Non-Unc F1 males x Hawaii hermaphrodites (male backcross, paternal transgene inheritance), and Non-Unc, transgenic hermaphrodites mated to Bristol/Hawaii F1 males (male backcross, maternal transgene inheritance). An one-sided chi square test was used to test for significance, with the expectation of 25% lethality in the self-cross and 50% lethality in the male backcrosses.

To test whether transgenic rescue required inheritance of the *Y39G10AR.5* transgene, males from a transgenic line carrying the *Y39G10AR.5* subcone were crossed to DP38. Embryos were collected from selfing, Non-Unc F1 hermaphrodites (self-cross) and from Non-Unc F1 males crossed to hermaphrodites carrying *unc-119(ed3)* in a Hawaii genetic background (male backcross). Adult progeny were digested for 1.5 hours at 65ºC then 15 minutes at 95ºC in 10ul of 50 mM KCl, 10 mM Tris-HCl pH 8.3, 2.5 mM $MgCl_2$, 0.45% NP-40, 0.45% Tween-20, 0.01% gelatin, 0.1 mg/ml Proteinase K. Digests were genotyped at a PCR length polymorphism spanned by primers 5'-tggatacgattcgagcttcc-3' and 5'- ccccctaatttccaagtggt-3'. This polymorphism is located less than 1 kb from the *zeel-1* deletion.

Cloning *zeel-1* cDNA

cDNA clones from Bristol confirmed the predicted exon structure of *zeel-1*. First strand synthesis of cDNA was generated by reverse transcription of Bristol RNA using primer 5'-cgttcacccattttcttgga-3'. cDNA was amplified by PCR using primers 5'gctgtgcaaagtttcaacaaa-3' and 5'-tgtttattggcttcgtgagc-3' and then TOPO cloned into pCR-TOPO (Invitrogen, Carlsbad, CA).

Testing transgenes for the paternal effect

Fosmids and subclones carrying *zeel-1* were tested for the paternal effect. If a transgenic line carries *zeel-1* and *peel-1* on an extra-chromosomal array, and if the transgenic copy of *peel-1* can induce the paternal effect, then embryos not inheriting the array (i.e. not inheriting *zeel-1*) are not expected to survive. Thus, such a line should segregate dead embryos and Non-Unc progeny, but <u>not</u> Unc progeny. In contrast to this prediction, all transgenic lines carrying *WRM0633bE09* or the *Y39G10AR.5* subclone on

extra-chromosomal array produced ≥ 20% Unc progeny and < 2% dead embryos. If a transgenic line carries *zeel-1* and *peel-1* as integrated transgenes, and if the transgenic copy of *peel-1* can induce the paternal effect, then a cross between Hawaii and the transgenic line is expected to produce 25% lethality in the F2 generation. For the single transgenic line carrying an integrated copy of fosmid *WRM0633bE09*, this cross produced < 2% dead embryos.

Transgenic lines carrying fosmids and subclones that did not cover *zeel-1* (fosmid *WRM0614dC06* and subclones *srbc-64*, *Y39G10AR.16*, and *ugt-31*) could not be tested for the paternal effect. Because these transgenes do not contain *zeel-1*, they would never have been recovered if the paternal effect had been active.

RNAi

RNAi was performed by feeding as described (S*14*). For all genes but *mcm-4*, which served as a positive control for the effectiveness of RNAi, Hawaii male x Bristol hermaphrodite crosses were set up on RNAi-expressing bacteria such that F1 individuals were conceived and reared in the presence of RNAi. Hawaii hermaphrodites used in the male backcross were also conceived and reared on RNAi-expressing bacteria. For *mcm-4*, which is maternal effect lethal in Bristol hermaphrodites, crosses were set up on OP50 and F1 individuals were transferred to RNAi-expressing bacteria at the L4 stage. RNAi could not be used to evaluate genes *Y39G10AR.7* and *mcm-4* for the paternal effect because these genes have a maternal effect lethal RNAi phenotype.

For the following genes, RNAi clones from the Ahringer library (S*15*) were used.

Gene	Ahringer library RNAi clone
mcm-4	sjj_Y39G10A_246.e

Y39G10AR.16	sjj_Y39G10A_243.a
ugt-31	sjj_Y39G10A_243.c
zeel-1	sjj_Y39G10A_229.a

For *Y39G10AR.15*, an RNAi clone was generated by amplifying a gene fragment from Bristol DNA with primers 5'-cagctaccgtaccgtggagt-3' (forward) and 5'-ggggtttagcaatcaaacca-3' (reverse), cutting the fragment with HindIII and AccI, and cloning it into RNAi feeding vector L4440 (Andy Fire vector kit; Addgene, Cambridge, MA). The clone was then transferred into HT115 cells for feeding.

Mapping absense of paternal effect in MY19

MY19 was crossed to MT1344, which carries *bli-3(e767)* and *lin-17(n677)* in a Bristol genetic background. *bli-3* is located 10 cM from the *zeel-1/peel-1* interval. F1 individuals were crossed to Hawaii, and hermaphrodite progeny were allowed to self-fertilize. The resulting self-cross broods were scored for embryonic lethality and the presence of Blister worms. Seven broods were excluded for having fewer than 30 embryos. In the remaining broods, 37 to 113 (median = 77) embryos were collected. The paternal effect was classified as present in worms segregating 14-27% dead embryos (median = 21%) and absent in worms segregating 0-7% dead embryos (median = 0%).

Figure S1 *C. elegans* incompatibility

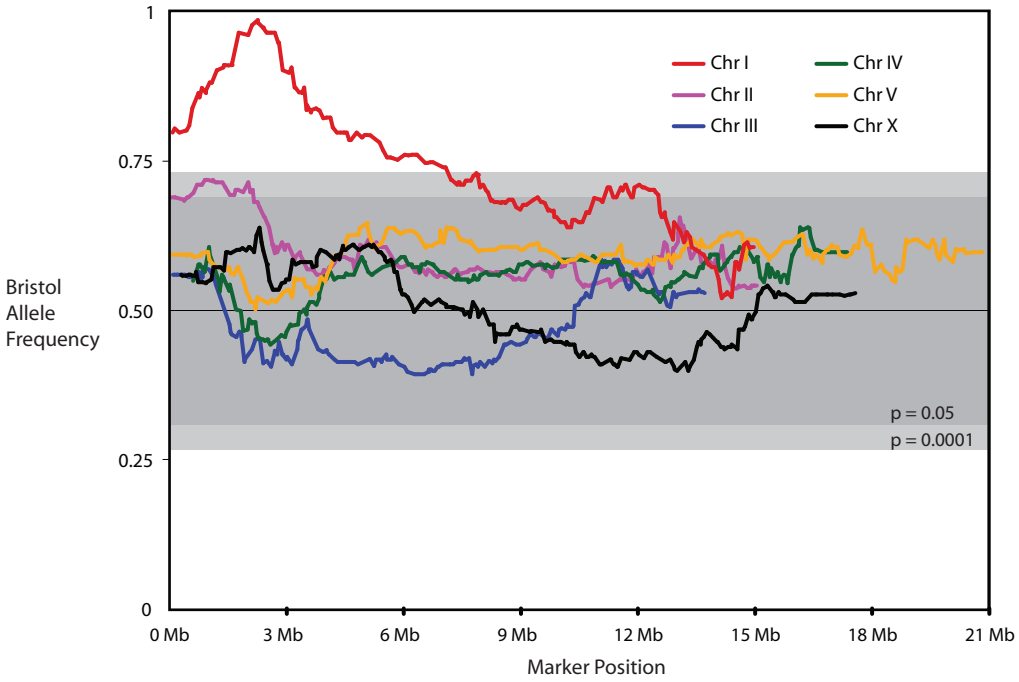

Fig. S1. Recombinant inbred lines exhibit a deficit of Hawaii alleles on the left side of chromosome I. The observed frequency of the Bristol allele among 239 advanced intercross RILs is plotted for 1450 markers at their positions along each of the chromosomes. The shaded boxes define the region within which 95% or 99.99% of neutral autosomes are expected to reside, based on simulations of *C. elegans* chromosomes transmitted along the twenty-generation pedigree employed in generating the RILs. The extreme value observed on chromosome I is far outside the bounds of neutral drift.

Table S1. *zeel-1* and *peel-1* are tightly linked. Hermaphrodite progeny were collected from crosses between F1 hermaphrodites and Hawaii males (hermaphrodite backcross) and Hawaii hermaphrodites and F1 males (male backcross) and allowed to self-fertilize. Lethality was scored in the resulting self-cross broods. The observed pattern matches that expected under complete linkage between *zeel-1* and *peel-1*.

Under a model of complete linkage, all gametes produced by an F1 individual will carry either $zeel\text{-}1_{Bristol}$ $peel\text{-}1_{Bristol}$ or $zeel\text{-}1_{Hawaii}$ $peel\text{-}1_{Hawaii}$. All surviving progeny from the male backcross will have genotype $zeel\text{-}1_{Bristol}$ $peel\text{-}1_{Bristol}/zeel\text{-}1_{Hawaii}$ $peel\text{-}1_{Hawaii}$. Thus, all should segregate 25% dead embryos in their own broods. Progeny from hermaphrodite backcross will occur in a 1:1 ratio of $zeel\text{-}1_{Bristol}$ $peel\text{-}1_{Bristol}/zeel\text{-}1_{Hawaii}$ $peel\text{-}1_{Hawaii}$ to $zeel\text{-}1_{Hawaii}$ $peel\text{-}1_{Hawaii}/zeel\text{-}1_{Hawaii}$ $peel\text{-}1_{Hawaii}$. The former class will segregate 25% dead embryos, while the latter will segregate less than 1%.

Under an unlinked model, gametes from an F1 individual will contain all four allelic combinations. Surviving progeny from the male backcross will occur in a 1:1 ratio of $zeel\text{-}1_{Bristol}/zeel\text{-}1_{Hawaii}$ $peel\text{-}1_{Bristol}/peel\text{-}1_{Hawaii}$ and $zeel\text{-}1_{Bristol}/zeel\text{-}1_{Hawaii}$ $peel\text{-}1_{Hawaii}/peel\text{-}1_{Hawaii}$. These classes will segregate 25% and less than 1% dead embryos, respectively. Progeny from the hermaphrodite backcross will occur four equal classes:

$zeel\text{-}1_{Bristol}/zeel\text{-}1_{Hawaii}$ $peel\text{-}1_{Bristol}/peel\text{-}1_{Hawaii}$

$zeel\text{-}1_{Hawaii}/zeel\text{-}1_{Hawaii}$ $peel\text{-}1_{Bristol}/peel\text{-}1_{Hawaii}$

$zeel\text{-}1_{Bristol}/zeel\text{-}1_{Hawaii}$ $peel\text{-}1_{Hawaii}/peel$-1Hawaii

zeel-$1_{Hawaii}/zeel\text{-}1_{Hawaii}$ $peel\text{-}1_{Bristol}/peel\text{-}1_{Hawaii}$.

The first two classes will segregate 25% and 100% dead embryos, respectively, while the remaining classes will segregate less than 1%.

	Hermaphrodite backcross			Male backcross		
	Ratio expected			Ratio expected		
Embryonic lethality (%)	Complete linkage	No linkage	Number observed	Complete linkage	No linkage	Number observed
~25	1/2	1/4	81	1	1/2	64
<1	1/2	1/2	58	0	1/2	0
100	0	1/4	0			

Table S2. Wild isolate genotypes and phenotypes. blue, non-Bristol allele; n/d, not determined.

	forward primer	reverse primer	assay	5'-agagggggccaattgaaaagt-3'	5'-gccggaaattctgaaaacc-3'	sequenced or Illumina genotyped	5'-agggagaggtttcattcctgc-3'	5'-ttgagcttcatcttggcatct-3'	cut with HhaI	5'-cagctaccgtaccgtggagt-3'	5'-gggggtttagcaatcaaacca-3'	sequenced		5'-ggatcgcatggaaatctgaa-3'	5'-cgggaatatctggcgaatta-3'	length polymorphism	*srbc-64 exons* see Supporting Online Material	5'-ctgaagcatgccggatttat-3'	5'-tcccgtccaaatattcaatcgac-3'	length polymorphism	5'-tcagtgtcgaatttaatgtttct-3'	5'-tggtaagtggtccgaaat-3'	length polymorphism	*zeel-1* deletion see Supporting Online Material	5'-tggatacgattcgagcttcc-3'	5'-cccctaatttccaagtggt-3'	length polymorphism	5'-tggaatatgctgatggtgga-3'	5'-tcagtgcccatatttggga-3'	cut with MslI	5'-tcaacttttgaaggggagcac-3'	5'-cgcctctttcatctgaagc-3'	cut with DraI	5'-cacgaaaatagcgcagtgaa-3'	5'-ccacgtgtccttctcatttg-3'	cut with BanI	5'-ttttggctcgctttcaaaat-3'	5'-ccctcttcacaaagttttcg-3'	cut with BslI
	position			1563141			2316401			2325346	2325382	2325838	2325382	2328096			*2327990-2330155*	2330042			2337504			2338614-2357688	2357924			2362109			2370979			2376392			2439662		
strain	phenotype																																						
ED3072	Bristol-compatible		T	not cut					T	T	A	T	long			Bristol-like	long			short			300 bp	short			cut			not cut			cut			not cut		
ED3073	Bristol-compatible		T	not cut					T	T	A	T	long			Bristol-like	long			short			300 bp	short			cut			not cut			cut			not cut		
JU258	Bristol-compatible		T (Illumina)	not cut					T	T	A	T	long			Bristol-like	long			short			300 bp	short			cut			not cut			cut			not cut		
JU362	Bristol-compatible		T (Illumina)	not cut					T	T	A	T	long			Bristol-like	long			short			300 bp	short			cut			not cut			cut			not cut		
JU642	Bristol-compatible		T (Illumina)	not cut					T	T	A	T	long			Bristol-like	long			short			300 bp	short			cut			not cut			cut			not cut		
MY18	Bristol-compatible		A (illumina)	not cut					T	T	A	T	long			Bristol-like	long			short			300 bp	short			cut			not cut			cut			not cut		
MY19	Doubly-compatible		A (illumina)	not cut					T	T	A	T	long			Bristol-like	long			short			300 bp	short			cut			not cut			cut			not cut		
JU312	Bristol-compatible		A (illumina)	not cut					T	T	A	T	long			Bristol-like	long			short			300 bp	short			cut			not cut			cut			cut		
JU346	Bristol-compatible		A (illumina)	not cut					T	T	A	T	long			Bristol-like	long			short			300 bp	short			cut			not cut			cut			cut		
JU402	Bristol-compatible		A (illumina)	not cut					T	T	A	T	long			Bristol-like	long			short			300 bp	short			cut			not cut			cut			cut		
JU407	Bristol-compatible		A (illumina)	not cut					T	T	A	T	long			Bristol-like	long			short			300 bp	short			cut			not cut			cut			cut		
MY16	Bristol-compatible		A (illumina)	not cut					T	T	A	T	long			Bristol-like	long			short			300 bp	short			cut			not cut			cut			cut		
PB303	Bristol-compatible		A (illumina)	not cut					T	G	A	T	long			Bristol-like	long			short			300 bp	short			cut			not cut			not cut			not cut		
PX174	Bristol-compatible		A (illumina)	not cut					T	G	A	T	long			Bristol-like	long			short			300 bp	short			cut			not cut			not cut			not cut		
JU694	Bristol-compatible		A (illumina)	not cut					T	G	A	T	long			Bristol-like	long			short			300 bp	short			cut			cut			not cut			not cut		
AB1	Bristol-compatible		T (Illumina)	not cut					T	G	A	T	long			Bristol-like	long			short			300 bp	short			cut			cut			not cut			not cut		
CB4852	Bristol-compatible		T (Illumina)	not cut					T	G	A	T	long			Bristol-like	long			short			300 bp	short			cut			cut			not cut			not cut		
CB4932	Bristol-compatible		T (Illumina)	not cut					T	G	A	T	long			Bristol-like	long			short			300 bp	short			cut			cut			not cut			not cut		
DR1344	Bristol-compatible		T (Illumina)	not cut					T	G	A	T	long			Bristol-like	long			short			300 bp	short			cut			cut			not cut			not cut		
DR1349	Bristol-compatible		T (Illumina)	not cut					T	G	A	T	long			Bristol-like	long			short			300 bp	short			cut			cut			not cut			not cut		
JU399	Bristol-compatible		T (Illumina)	not cut					T	G	A	T	long			Bristol-like	long			short			300 bp	short			cut			cut			not cut			not cut		
JU440	Bristol-compatible		T (Illumina)	not cut					T	G	A	T	long			Bristol-like	long			short			300 bp	short			cut			cut			not cut			not cut		
JU561	Bristol-compatible		T (Illumina)	not cut					T	G	A	T	long			Bristol-like	long			short			300 bp	short			cut			cut			not cut			not cut		
LSJ1	Bristol-compatible		T (Illumina)	not cut					T	G	A	T	long			Bristol-like	long			short			300 bp	short			cut			cut			not cut			not cut		
TR403	Bristol-compatible		T (Illumina)	not cut					T	G	A	T	long			Bristol-like	long			short			300 bp	short			cut			cut			not cut			not cut		
N2	Bristol-compatible		T (Illumina)	not cut					T	G	A	T	long			Bristol-like	long			short			300 bp	short			cut			cut			not cut			not cut		
ED3017	Bristol-compatible		T	n/d					T	G	A	T	n/d			Bristol-like	n/d			n/d			300 bp	n/d			n/d			n/d			n/d			not cut		
ED3028	Bristol-compatible		T	n/d					T	G	A	T	n/d			Bristol-like	n/d			n/d			300 bp	n/d			n/d			n/d			n/d			not cut		
ED3040	Hawaii-compatible		A	n/d					C	T	T	A	n/d			Hawaii-like	n/d			n/d			~1.5 kb	n/d			n/d			n/d			n/d			not cut		
ED3042	Hawaii-compatible		A	n/d					C	T	T	A	n/d			Hawaii-like	n/d			n/d			~1.5 kb	n/d			n/d			n/d			n/d			not cut		
ED3043	Hawaii-compatible		A	n/d					C	T	T	A	n/d			Hawaii-like	n/d			n/d			~1.5 kb	n/d			n/d			n/d			n/d			not cut		
ED3077	Hawaii-compatible		A	n/d					C	T	T	A	n/d			Hawaii-like	n/d			n/d			~1.5 kb	n/d			n/d			n/d			n/d			not cut		
JU1172	Hawaii-compatible		A	n/d					C	T	T	A	n/d			Hawaii-like	n/d			n/d			~1.5 kb	n/d			n/d			n/d			n/d			not cut		
CB4853	Hawaii-compatible		A (illumina)	cut					C	T	T	A	short			Hawaii-like	short			long			~1.5 kb	long			not cut			not cut			cut			not cut		
PB306	Hawaii-compatible		A (illumina)	cut					C	T	T	A	short			Hawaii-like	short			long			~1.5 kb	long			not cut			not cut			cut			not cut		
DR1345	Hawaii-compatible		A (illumina)	cut					C	T	T	A	short			Hawaii-like	short			long			~1.5 kb	long			not cut			not cut			cut			not cut		
JU323	Hawaii-compatible		A (illumina)	cut					C	T	T	A	short			Hawaii-like	short			long			~1.5 kb	long			not cut			not cut			cut			not cut		
ED3005	Hawaii-compatible		A	cut					C	T	T	A	short			Hawaii-like	short			long			~1.5 kb	long			not cut			not cut			cut			not cut		
ED3048	Hawaii-compatible		A	cut					C	T	T	A	short			Hawaii-like	short			long			~1.5 kb	long			not cut			not cut			cut			not cut		
PX179	Hawaii-compatible		T (Illumina)	cut					C	T	T	A	short			Hawaii-like	short			long			~1.5 kb	long			not cut			not cut			cut			not cut		
KR314	Hawaii-compatible		T (Illumina)	cut					T	T	A	T	short			Hawaii-like	short			long			~ 3 kb	long			not cut			not cut			cut			not cut		
JU1171	Hawaii-compatible		T	cut					T	T	A	T	short			Hawaii-like	short			long			~ 2 kb	long			not cut			not cut			cut			cut		
MY14	Hawaii-compatible		T (Illumina)	cut					T	T	A	T	short			Hawaii-like	short			long			~ 2 kb	long			not cut			not cut			cut			cut		
CB4856	Hawaii-compatible		A (illumina)	cut					T	T	A	T	short			Hawaii-like	short			long			~ 3 kb	long			not cut			not cut			cut			cut		
JU1088	Hawaii-compatible		A	not cut					T	T	A	A	short			Hawaii-like	short			long			~ 5 kb	long			not cut			not cut			cut			cut		
MY1	Hawaii-compatible		A (illumina)	not cut					T	T	A	A	short			Hawaii-like	short			long			~ 7 kb	long			not cut			not cut			cut			cut		

Table S3. Transgenic rescue requires inheritance of the transgene. Males from a transgenic line carrying the *Y39G10AR.5* subclone were crossed to DP38, which carries *unc-119(ed3)* in a Bristol genetic background. *unc-119(+)* was the visible marker used to select positive transformants and is co-transmitted with the transgene on an extra-chromosomal array. Embryos were collected from selfing, Non-Unc F1 hermaphrodites (self-cross) and Non-Unc F1 males crossed to hermaphrodites carrying *unc-119(ed3)* in a Hawaii genetic background (male backcross). Surviving progeny were genotyped at a marker adjacent to the *zeel-1* deletion in Hawaii. Progeny inheriting the transgene are Non-Unc, while those not inheriting it are Unc. The single Hawaii/Hawaii, Unc progeny from the self-cross probably reflects the incomplete penetrance of lethality in this cross.

	Self-cross		Male Backcross	
Marker genotype	Unc	Non-Unc	Unc	Non-Unc
Bristol/Bristol	11	27	n/a	n/a
Bristol/Hawaii	16	52	57	22
Hawaii/Hawaii	1	29	0	35
dead embryo	16		43	

Table S4. Deletion alleles for *srbc-64* and *Y39G10AR.17* do not abolish the paternal effect.

gene	cross	% embryonic lethality (total)
srbc-64	Hawaii/*srbc-64(tm1946)* hermaphrodite	23%(269)
	Hawaii hermaphrodite x Hawaii/*srbc-64(tm1946)* male	46%(167)
	srbc-64(tm1946) hermaphrodite	1%(82)
Y39G10AR.17	Hawaii/*Y39G10AR.17(ju541)* hermaphrodite	27%(215)
	Hawaii hermaphrodite x Hawaii/*Y39G10AR.17(ju541)* male	54%(225)
	Y39G10AR.17(ju541) hermaphrodite	3%(138)
nekl-1	Hawaii/*nekl-1(ttTi14271)* hermaphrodite	24%(170)
	Hawaii hermaphrodite x Hawaii/ *nekl-1(ttTi14271)* male	60%(91)
	nekl-1(ttTi14271) hermaphrodite	1%(79)

Table S5. RNAi against genes in the *peel-1* interval does not abolish the paternal effect. Percent embryonic lethality (total) is given for the self-cross and male backcross in the presence of RNAi against genes in the *peel-1* interval. As expected, RNAi against *mcm-4* was maternal effect lethal in Bristol and F1 hermaphrodites, which are sensitive to germline RNAi, but not in Hawaii hermaphrodites, which are germline resistant. The fact that RNAi against *Y39G10AR.5* did not increase lethality in the self-cross and male backcross suggests that knockdown of this gene may be ineffective, since expression of *Y39G10AR.5* is required for viability in these crosses.

	RNAi clone				
cross	*zeel-1*	*ugt-31*	*Y39G10AR.16*	*Y39G10AR.15*	*mcm-4*
F1 hermaphrodite	26%(501)	25%(341)	23%(534)	24%(557)	100%(425)
Hawaii hermaphrodite x F1 male	55%(315)	50%(347)	51%(411)	44%(144)	not determined
Bristol hermaphrodite	0%(49)	0%(49)	0%(47)	0%(155)	100%(62)
Hawaii hermaphrodite	0%(77)	0%(74)	0%(71)	1%(138)	0%(22)

Table S6. Absence of paternal effect in wild isolate MY19 mapped 10 cM from *bli-3*. MY19 was crossed to MT1344, which carries *bli-3(e767)* and *lin-17(n677)* in a Bristol genetic background. F1 individuals were crossed to Hawaii, and hermaphrodite progeny were genotyped at *bli-3* (via the presence of absence of Blister progeny) and were scored for present or absence of the paternal effect.

		bli-3 genotype	
		bli-3/+	+/+
Paternal effect	present	47	5
	absent	6	49

Table S7. Wild isolate localities and incompatibility phenotypes.

Strain	Locality	Compatibility
AB1	Adelaide	Bristol
AB3	Adelaide	Hawaii
CB3192	Altadena	Bristol
CB3197	Altadena	Bristol
CB4507	Palm Canyon	Bristol
CB4851	Bergerac	Bristol
CB4852	England	Bristol
CB4853	Altadena	Hawaii
CB4855	Stanford	Hawaii
CB4856	Hawaii	Hawaii
CB4932	Taunton	Bristol
DH424	El Prieto Canyon	Bristol
DR1344	Bergerac	Bristol
DR1345	Claremont	Hawaii
DR1349	Pasadena	Bristol
DR1350	Pasadena	Hawaii
ED3005	Edinburgh	Hawaii
ED3017	Edinburgh	Bristol
ED3028	Edinburgh	Bristol
ED3040	Johannesburg	Hawaii
ED3042	Western Cape	Hawaii
ED3043	Western Cape	Hawaii
ED3072	Limuru	Bristol
ED3073	Limuru	Bristol
ED3077	Nairobi	Hawaii
JU1088	Japan	Hawaii
JU1171	Chile1	Hawaii
JU1172	Chile2	Hawaii
JU258	Madeira	Bristol
JU301	Le Blanc	Bristol
JU312	Merlet	Bristol
JU314	Merlet	Bristol
JU323	Merlet	Hawaii
JU346	Merlet	Bristol
JU360	Franconville	Bristol
JU362	Franconville	Bristol
JU395	Hermanville	Bristol
JU399	Hermanville	Bristol
JU402	Hermanville	Bristol
JU407	Hermanville	Bristol
JU440	Beauchene	Bristol
JU531	Primel	Bristol
JU561	Sainte Barbe	Bristol
JU642	Le Perreux	Bristol
JU694	Franconville	Bristol
KR314	Vancouver	Hawaii
LSJ1	Berkeley	Bristol
MY1	Lingen	Hawaii
MY14	Mecklenbeck	Hawaii
MY16	Mecklenbeck	Bristol
MY18	Roxel	Bristol
MY19	Roxel	Doubly Compatible
MY2	Roxel	Hawaii
N2	Bristol	Bristol
PB303	Isopod1	Bristol
PB306	Isopod2	Hawaii
PS2025	Altadena	Hawaii
PX174	Lincoln City Oregon	Bristol
PX176	Eugene	Bristol
PX179	Eugene	Hawaii
RC301	Freiburg	Bristol
TR403	Madison	Bristol

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