Supporting Information

Remnant et al. 10.1073/pnas.1311341110

SI Text

Frequency of the Duplication in the DGRP and Australian Populations

The *Drosophila* Genomic Reference Panel (DGRP) dataset of 168 sequenced lines contained three strains with the 113-kb duplication surrounding *Resistance to Dieldrin* (*Rdl*; Ral-317, Ral-318, and Ral-378). Subsequent to the release of this public resource, Ral-378 has been removed from the list of available lines and was not included in genomewide analysis studies (1). Estimates of Ser^{301} frequency in the DGRP were based on 4 lines (Ral-317, -318, -378, and -491) of 168 lines (2.4%).

Our Australian survey consisted of 20 males collected at 19 locations ranging from Cooktown to Tasmania along the Australian East coast. Duplication primers were tested without yielding positive products. However, the *nwk-Roo* product was amplified from 9 of the 19 populations, indicating its presence in intron 7 of *nwk* in some individuals in that pool. There was a strong correlation between the presence of *nwk-Roo* and the Ser^{301} mutation, which was present in 3% of the populations tested. No populations showed amplification of the *glutamate receptor IB* (*Glu-RIB*) product.

1. Mackay TFC, et al. (2012) The *Drosophila melanogaster* genetic reference panel. *Nature* 482(7384):173–178.

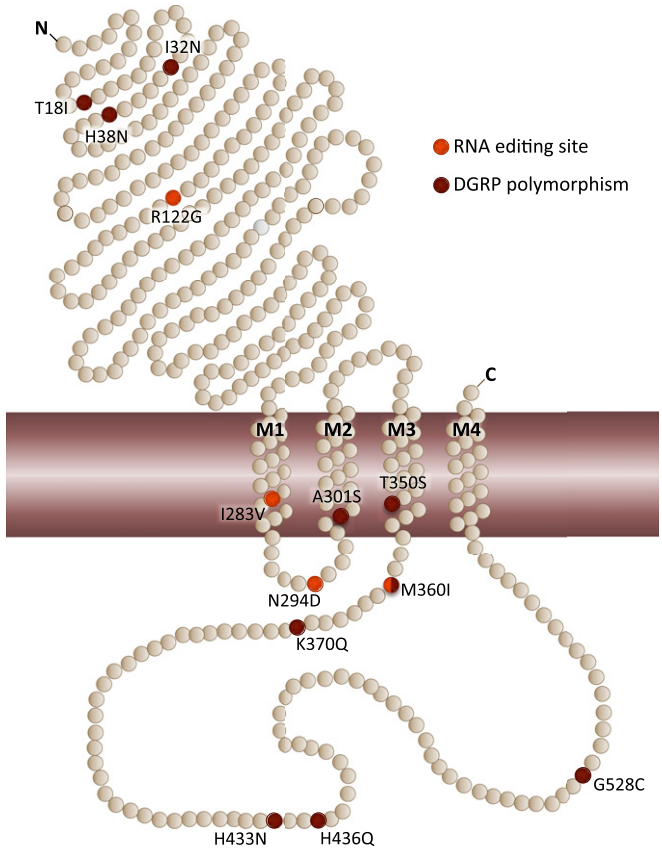

Fig. S1. Schematic representation of the RDL subunit monomer. Each subunit consists of a large, extracellular N-terminal ligand-binding region, followed by four transmembrane domains. Subunits pentamerize to form a chloride channel, which is lined by the second transmembrane domain. Localization of nonsynonymous polymorphisms identified in the DGRP genome sequences and RNA editing sites are indicated (adapted from ref. 1).

1. Wotring VE, Miller TS, Weiss DS (2003) Mutations at the GABA receptor selectivity filter: A possible role for effective charges. *J Physiol* 548(Pt 2):527–540.

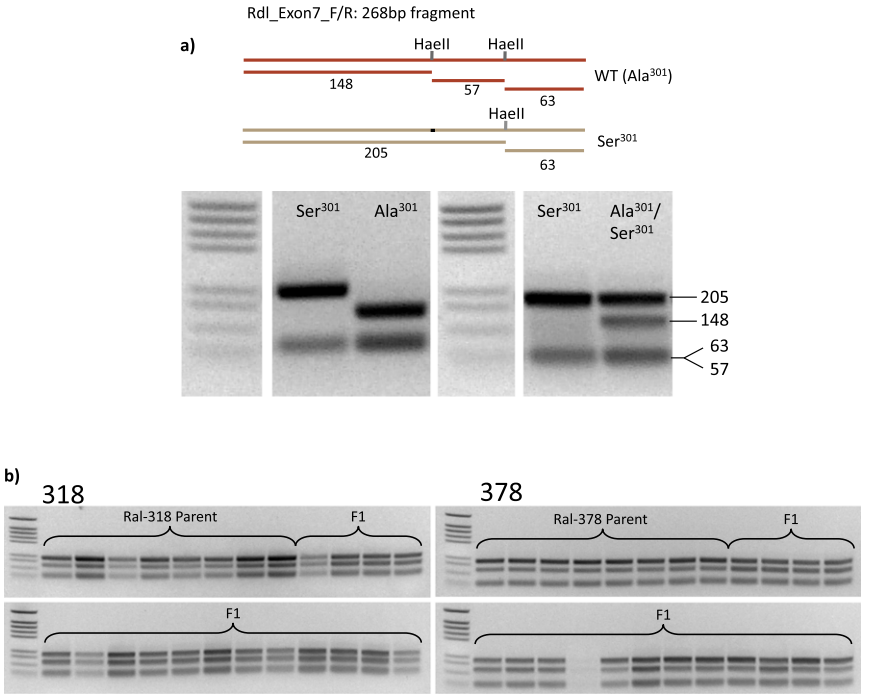

Fig. S2. HaeII diagnostic restriction digest assay to detect Ala^{301} and Ser^{301} alleles in *Rdl* exon 7. (*A*) Of the 268-bp PCR fragment, HaeII digest yields three bands for WT Ala^{301} alleles (148, 63, and 57 bp). Ser^{301} removes a HaeII restriction site yielding two bands (205 and 63 bp). (*B*) Testing of Ral-318 and Ral-378 parents and F_1 offspring from a cross to WT w^{1118} lines. w^{1118} exhibit the WT Ala^{301} banding pattern as seen in *A*. Male and female parents used in reciprocal crosses to w^{1118} are indicated and show all four bands (205, 148, 63, and 57 bp), indicating heterozygous genotype. F_1 offspring retain the same banding pattern, showing that the Ser^{301} allele is inherited from the Ral- parents 100% of the time, supporting the duplication hypothesis.

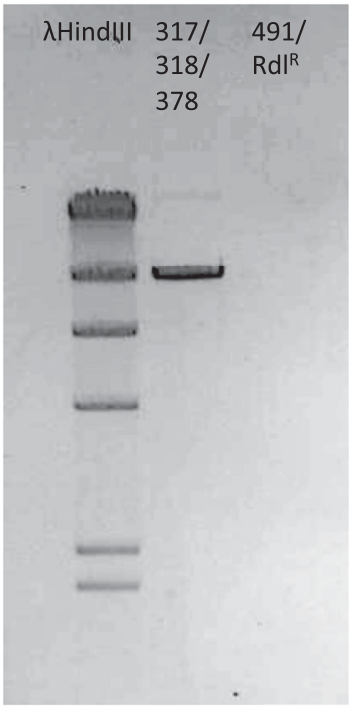

Fig. S3. PCR product obtained from DGRP Ral-317 spanning the *Roo* transposable element (TE) using the primers *Glu_int1_F* and *nwk_int7_R*. The same product was present in Ral-318 and -378 but was not present in Ral-491 or Rdl^R-*MDRR*.

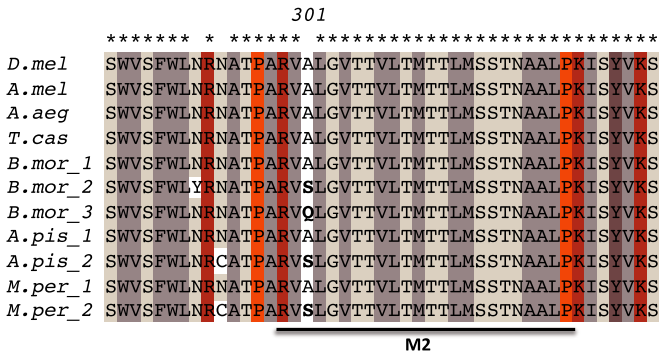

Fig. S4. Alignment of the portion of *Rdl* surrounding the equivalent 301 residue in insect species, showing copy number variation and the relative amino acid variation at the 301 site. *Bombyx mori* contains three *Rdl* orthologs, with a different residue at 301 (1 = Ala, 2 = Ser, 3 = Gln). Both aphid species contain two *Rdl* orthologs: *Acyrthosiphon pisum* and *Myzus persicae* (1 = Ala, 2 = Ser). In sequences taken from resistant and susceptible *Myzus persicae* strains, copy 1 of *Rdl* contains either Ala or Gly alleles, and copy 2 has either of two different Ser alleles. *D.mel, D. melanogaster; A.mel, Apis mellifera; A.aeg, Aedes aegypti; T.cas, Tribolium castaneum; B.mor, Bombyx mori; A.pis, Acyrthosiphon pisum*; *M.per, Myzus persicae* (1–3).

1. Yuan G, Gao W, Yang Y, Wu Y (2010) Molecular cloning, genomic structure, and genetic mapping of two Rdl-orthologous genes of GABA receptors in the diamondback moth, Plutella xylostella. *Arch Insect Biochem Physiol* 74(2):81–90.
2. Dale RP, et al. (2010) Identification of ion channel genes in the Acyrthosiphon pisum genome. *Insect Mol Biol* 19(Suppl 2):141–153.
3. Anthony N, Unruh T, Ganser D, ffrench-Constant R (1998) Duplication of the *Rdl* GABA receptor subunit gene in an insecticide-resistant aphid, *Myzus persicae*. *Mol Gen Genet* 260(2-3): 165–175.

Table S1. Polymorphisms in *Rdl* found in the 168 sequenced DGRP lines

Polymorphism	No. of lines	Frequency	Location in protein
T18I	2	0.012	Signal peptide
I32N	1	0.006	Signal peptide
H38N	32	0.190	Extracellular N-terminal domain
A301S	4	0.024	M2 domain; site of $Rdl^{MD\text{-}RR}$ mutation
T350S	1	0.006	M3 domain; site of *D. simulans* mutation ($Thr^{350}$to Met) (1)
M360I	3	0.018	M3-M4 loop; RNA-editing site
K370Q	1	0.006	M3-M4 loop
H433N	1	0.006	M3-M4 loop
H436Q	1	0.006	M3-M4 loop
G528C	1	0.006	M3-M4 loop

1. Le Goff G, Hamon A, Bergé J-B, Amichot M (2005) Resistance to fipronil in *Drosophila simulans*: Influence of two point mutations in the RDL GABA receptor subunit. *J Neurochem* 92(6): 1295–1305.

Table S2. Genes partially or fully duplicated in the 113-kb region surrounding *Rdl*, showing nonsynonymous heterozygous substitutions identified in duplicated lines

Gene No.	Name	Function	NS polymorphisms
CG4684	*nwk*	Protein binding; negative regulation of synaptic growth; neuromuscular synaptic transmission	—
CG10537	*Rdl*	$GABA_A$ receptor activity	A301S, M360I
CG4484	*Slc45-1*	Sucrose transmembrane transporter activity	P352A
CG4476		Neurotransmitter transporter activity; potassium: amino acid symporter activity	P187T, A252V, V520I
CG4483		Src homology-3 domain; Zinc finger, palmitoyltransferase	I13M, N112D, D274E, R161Q
CG4477		Trypsin-like serine protease	F7L, L16F, S295C, A297V, M309I
CG4481	*Glu-RIB*	Ionotropic glutamate receptor activity	—
CG42509		Unknown; part of discistronic transcript with Glu-RIB	—

Genes in bold are fully duplicated. *Glu-RIB*, *glutamate receptor IB*; NS, nonsynonymous; *nwk*, *nervous wreck*; *Rdl*, *resistance to dieldrin*.

Table S3. Primers used to confirm putative duplication topology, exon 7 HaeII digest, exon 7–8 sequencing, and cloning of *Rdl* left and right arms for recombineering

Name	Sequence (5′-3′)	Location
Glu_int1_F	TTGCTTTGGTCAATGTTTGC	Intron 1 of *Glu-RIB*, upstream of *Roo* insertion
Glu_int1_R	TCGTTCTCGTTAGTTCGACAGA	Intron 1 of *Glu_RIB*, downstream of *Roo* insertion
Roo_LTR_F	GAACGGAGCCCAAAATTGTA	3′ end of *Roo* element LTR
Roo_LTR_R	CTCTGCGTAGGCCATTTAC	5′ end of *Roo* element LTR
nwk_ex7_F	GAGTATTCCTCCGCCAGGTA	Exon 7 of *nwk*, upstream of *Roo* insertion
nwk_int7_R	AATCGTAGGGCATTTCATCG	Intron 7 of *nwk*, downstream of *Roo* insertion
Rdl_LA-AscI_F	GGCGCGCCGTCTAAACAGCGACACC	5′ of the *Rdl* locus, to generate the left arm for P[acman] recombineering
Rdl_LA-BamHI_R	CCAAGAAAATAGTTAGGATCCGAGGAGACG	
Rdl_RA-BamHI_*F*	CAGGATCCCACGTTACGCATACGCCGTG	3′ of the *Rdl* locus, to generate the left arm for P[acman] recombineering
Rdl_RA-PacI_R	TTAATTAACAGCTGCACATGCCACG	
Rdl_Ex7_F	CAACTATTCGCGTTTAGCCTGC	Exon 7 of *Rdl*; used for *HaeII* digests and sequencing
Rdl_Ex7_R	GACCATAACGAAGCATGTTCCC	Exon 7 of *Rdl*; used for *HaeII* digests
Rdl_Ex8_R	CACCGTATTCTCCAGCGTTCC	Exon 8 of *Rdl*; used for sequencing

Table S4. Frequency of WT and mutant *Rdl* cDNA

Genotype	No. of clones	Frequency
WT: $Ala^{301}Met^{360}$	21	0.47
Mutant: $Ser^{301}Ile^{360}$	24	0.53

Frequency of changes identified in individual clones obtained by sequencing the *Rdl* exon 7–8 PCR product of cDNA generated from adult heads from DGRP lines Ral-318 and Ral-378.

Table S5. Frequency of RNA editing in WT and mutant *Rdl* cDNA

	No. of clones			Frequency		
Change	Total	In WT	In mutant	Total	In WT	In mutant
I283V	37	17	20	0.82	0.81	0.83
N294D	13	12	1	0.28	0.57*	0.04*
M/I360V	7	1	6	0.15	0.05	0.25

Frequency of changes identified in individual clones obtained by sequencing the *Rdl* exon 7–8 PCR product of cDNA generated from adult heads from DGRP lines Ral-318 and Ral-378.
*Significant difference in RNA editing frequency between WT and mutant (Fisher exact test, $P = 0.00013$).

Table S6. LC50 values for dieldrin (lethal concentration for 50% of the population) with 95% CIs and fold resistance with 95% CIs observed in natural populations and transgenic lines with *Rdl* duplication and resistance mutations

Line	Genotype	LC50 (μg/μL) (95% CI)	Fold (95% CI)
Ral-882	$\frac{Rdl^{WT}}{Rdl^{WT}}$	0.3 (0.25–0.33)	1 (0.84–1.19)
Ral-378	$\frac{Rdl^{WT}/\,Rdl^{S301}}{Rdl^{WT}/\,Rdl^{S301}}$	8.2 (7.9–8.6)	27.8 (24.4–31.7)
Rdl^{R} *MD-RR*	$\frac{Rdl^{S301}}{Rdl^{S301}}$	13,750 (12,550-14,600)	45,800 (40,430-53,360)
Φ-86Fb-*empty*	$\frac{P[acman]^{Empty}}{P[acman]^{Empty}};\ \frac{Rdl^{WT}}{Rdl^{WT}}$	0.22 (0.19–0.24)	1 (0.87–1.15)
Φ-86Fb-P[ac]-Rdl^{WT}	$\frac{P[acman]\text{-}Rdl^{WT}}{P[acman]\text{-}Rdl^{WT}};\ \frac{Rdl^{WT}}{Rdl^{WT}}$	0.23 (0.21–0.25)	1 (0.93–1.22)
Φ-86Fb-P[ac]-Rdl^{A301S}	$\frac{P[acman]\text{-}Rdl^{S301}}{P[acman]\text{-}Rdl^{S301}};\ \frac{Rdl^{WT}}{Rdl^{WT}}$	1.3 (1.2–1.4)	6 (5.2–6.6)