Supporting Information

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SI Text

Genetic Screening for Anthelmintics Resistance Mutants. For the genetic screen, solutions of albendazole (Sigma A4673), ivermectin (Sigma 18898), and levamisole were prepared at 500 μM, 110 nM, and 10 mM in 1% DMSO, respectively. A large population of synchronized L4 worms was mutagenized in a 30 mM ethyl methane sulfonate (EMS) and then propagated up until synchronized F2 generation L1 larvae as described (1). A total of 30,000 mutagenized F1 animals were used to generate 130,000 mutagenized F2 animals, from which 10,000 (per anthelmintic) were pipetted into 48-well plates at a density of 20–30 worms/well along with 50 μM albendazole, 1 mM levamisole, or 11 nM ivermectin (final concentrations). That only 8% of the mutagenized F2 were used per anthelmintic makes it very likely that the mutants are all independently derived. All wells included 20 μL OP50 (OD600 = 3.0) and a final volume of 200 μL. For Cry5B and Cry21A, the L1 larvae were pipetted onto ENG-IC plates spread with *E. coli*–expressed Cry5B or Cry21A (2). Anthelmintic-exposed worms were then incubated at 20 ° for 3 days. Any nematodes that matured and were mobile were then transferred out of the wells or plates and propagated. Progeny from these putative candidates were then reexposed to anthelmintic to confirm their resistance.

Calculation of CI Values. CI values can be calculated for mutually nonexclusive drugs using the following equation (3):

$$\mathrm{CI} = \frac{(\mathrm{D})_1}{(\mathrm{Dx})_1} + \frac{(\mathrm{D})_2}{(\mathrm{Dx})_2} + \frac{(\mathrm{D})_1 * (\mathrm{D})_2}{(\mathrm{Dx})_1 * (\mathrm{Dx})_2}$$

where $(D_x)_1$ is the dose of $Drug_1$ alone that inhibits x%, $(D_x)_2$ is the dose of $Drug_2$ alone that inhibits x%, $(D)_1$ is the dose of $Drug_1$ in the combination that inhibits x%, and $(D)_2$ is the dose of $Drug_2$ in the combination that inhibits x%. The third fraction is the correction for mutually nonexclusive drugs that is omitted if the drugs are assumed to be mutually exclusive.

1. Hu Y, Xiao SH, Aroian RV (2009) The new anthelmintic tribendimidine is an L-type (levamisole and pyrantel) nicotinic acetylcholine receptor agonist. *PLoS Negl Trop Dis* 3:e499.
2. Wei JZ, et al. (2003) *Bacillus thuringiensis* crystal proteins that target nematodes. *Proc Natl Acad Sci USA* 100:2760–2765.
3. Chou TC, Talalay P (1984) Quantitative analysis of dose-effect relationships: The combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme Regul* 22:27–55.

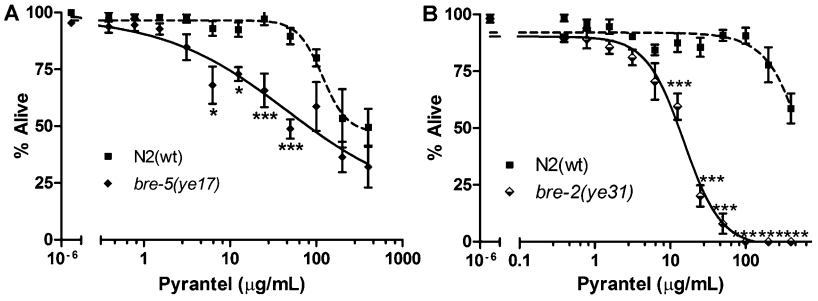

Fig. S1. Cry5B-resistant mutants are hypersusceptible to pyrantel. (*A*) Dose-dependent mortality response of wild-type N2 animals and *bre-5(ye17)* mutant animals to pyrantel. (*B*) Dose-dependent mortality response of wild-type N2 animals and *bre-2(ye31)* mutant animals to pyrantel.

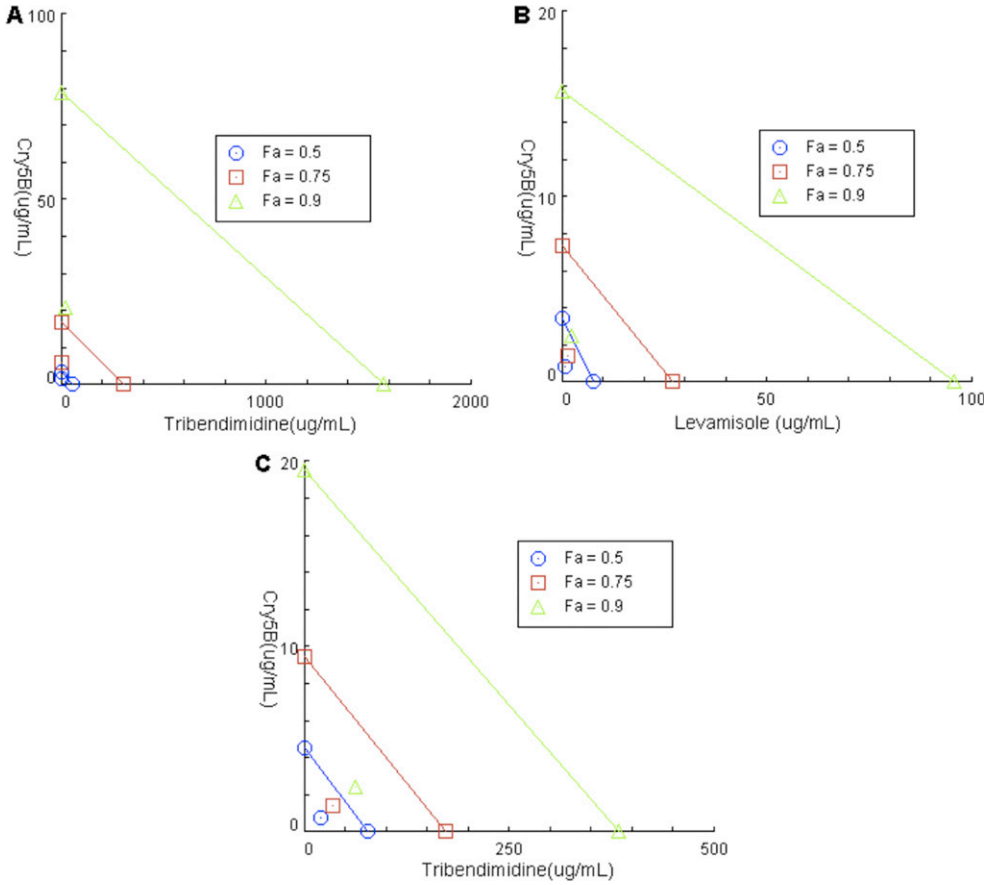

Fig. S2. Analysis of combinations of Cry proteins and nAChR agonists using isobolograms. Shown are isobolograms at different effect levels (Fa; Fa = 0.5, 0.75, and 0.9 refers to 50%, 75%, and 90% mortality respectively) for the following: (*A*) Cry5B and tribendimidine (1:1 mass mixture); (*B*) Cry5B and levamisole (1:1 mass mixture); and (*C*) Cry5B and tribendimidine (1:1 LC_{50} mixture). Numeric values are derived from data presented in Fig. 4 and plotted using CompuSyn. The dose required to achieve different Fa of each individual drug is plotted on the *y* axis (Cry protein) or *x* axis (nAChR agonist) and connected by a straight line for each specific Fa. The Fa of the combination are shown as individual points not connected by a line. If the specific Fa of the combination falls to the left of (below) the corresponding isobologram line, the combination is synergistic. All combinations at all Fa appear to be synergistic.

Table S1. LC_{50} values along with 95% confidence intervals associated with experimental results

Fig. no.	Genotype	Drug	LC_{50} or IC_{50}*	LC50 (μM)	95% CI*	95% CI overlaps vs. wt 95% CI?‡	Results
1*A*	N2 (wt)	Cry5B	7.16	0.05	6.56–7.82		
	lev-8 (ye493)		5.02	0.04	4.62–5.46	No	H
	ben-1(e1880)		12.64	0.09	11.36–14.08	No	R
1*B*	N2 (wt)	Cry5B	8.61	0.06	7.76–9.54		
	unc-50 (ye494)		5.61	0.04	5.01–6.26	No	H
1*C*	N2 (wt)	Cry5B	8.71%	NA	7.92–9.57%		
	lev-8 (ye493)		4.00%	NA	3.63–4.39%	No	H
	ben-1(e1880)		7.72%	NA	7.01–8.51%	Yes§	H
1*D*	N2 (wt)	Cry5B	9.39%	NA	8.60–10.24%		
	unc-63(ye492)		5.96%	NA	5.35–6.60%	No	H
	unc-50(ye493)		4.13%	NA	3.73–4.56%	No	H
2*B*	N2 (wt)	Cry21A†	2.72	0.020	2.47–3.01		
	lev-8(ye493)		1.87	0.014	1.69–2.07	No	H
3*A*	N2 (wt)	Trib	51.76	114.4	45.70–58.97		
	bre-5 (ye17)		9.39	20.7	8.28–10.64	No	H
	ben-1(e1880)		31.51	69.6	27.59–35.69	No	H
3*B*	N2 (wt)	Lev	18.07	75.1	15.10–21.32		
	bre-5 (ye17)		9.19	38.2	7.57–10.81	No	H
	ben-1(e1880)		29.06	120.8	24.81–33.23	No	R
3*C*	N2 (wt)	Trib	50.45	111.4	44.65–56.64		
	bre-2 (ye31)		6.67	14.7	5.87–7.46	No	H
3*D*	N2 (wt)	Lev	11.37	47.2	10.00–12.80		
	bre-2 (ye31)		1.39	5.8	1.27–1.52	No	H
3*E*	N2 (wt)	Trib	22.72	50.2	20.77–24.80		
	bre-5 (ye17)		13.52	29.9	12.01–15.05	No	H
	ben-1(e1880)		16.83	37.2	15.16–18.58	No	H
	lev-8(ye493)		59.59	131.7	54.80–64.46	No	R
S1*A*	N2 (wt)	Pyr	ND	ND	ND		
	bre-5 (ye17)		81.14	229.0	61.20–112.17		H
S1*B*	N2 (wt)	Pyr	ND	ND	ND		
	bre-2 (ye31)		10.67	30.1	9.28–12.12		H

H, hypersusceptible; Lev, levamisole; ND, not determined (highest dose used did not result in >50% lethality); Pyr, pyrantel; R, resistant; Trib, tribendimidine.

*Units are μg/mL unless otherwise specified as % (representing % *E. coli*–expressing Cry5B).

†LC_{50} values between Cry21A and Cry5B cannot be directly compared because the former assays are carried out in the presence of Bt spore crystal lysates and the latter with purified Cry protein.

‡Lack of overlap of 95% confidence interval between mutant and wild-type is taken to be indicative of a statistically significant difference with $P < 0.01$ (1, 2).

§In this case, CI arms do overlap but the overlap is less than one full arm, indicates $P < 0.05$ (1).

1. Cumming G, Fidler F, Vaux DL (2007) Error bars in experimental biology. *J Cell Biol* 177:7–11.

2. Wirth MC, Federici BA, Walton WE (2000) Cyt1A from *Bacillus thuringiensis* synergizes activity of *Bacillus sphaericus* against *Aedes aegypti* (Diptera: Culicidae). *Appl Environ Microbiol* 66:1093–1097.

Table S2. Genetic screen for *C. elegans* mutants resistant to different classes of anthelmintics

Genetic screening	No. of F2 Screened	No. of F2-resistant worms found
Cry5B	10,000	8
Cry21A	10,000	0
Albendazole	10,000	22
Levamisole	10,000	31
Ivermectin	10,000	8

Table S3. Dose-reduction index (DRI) values for anthelmintics in different combinations at specific effect levels

Combination	ED_{50}	ED_{75}	ED_{90}	ED_{95}
Cry5B	1.96	3.55	6.42	9.62
Trib (1:1 mass)	252	955	3608	8909
Cry5B	4.14	5.13	6.35	7.34
Lev	9.11	18.83	38.9	63.7
Cry5B	5.76	6.78	7.98	8.91
Trib (1:1 LC_{50})	3.87	4.87	6.14	7.19

All values calculated by CompuSyn. Lev = levamisole;Trib = tribendimidine.