

Table S1. Frequency of the Y215H mutation in the samples collected from several U.S. states.

Sample	State	Project	n	Last treatment with amitraz	Data collection	% Y215H mutation
NJM-001	New Jersey		24	08/2018	10/2018	50
NJM-002	New Jersey		19	09/2018	10/2018	89
NJM-008	New Jersey		24	07/2018	10/2018	96
EP-2	New Jersey		24	04/2018	10/2018	54
NJ-03	New Jersey	NHBDS	24		06/2016	0
NJ-08	New Jersey	NHBDS	24		09/2016	0
NJ-11	New Jersey	NHBDS	20		04/2016	0
HI-OR-AV01	Oregon	BIP	24	09/2018	10/2018	88
HI-OR-AV02	Oregon	BIP	23	09/2018	09/2018	96
HI-OR-AL38	Oregon	BIP	16		09/2018	100
HI-OR-AL51	Oregon	BIP	16		09/2018	96
HI-OR-1681	Oregon	BIP	8		07/2017	60
HI-OR-1685	Oregon	BIP	16		07/2017	31

OR-24	Oregon	NHBDS	13		06/2017	0
KP-MI-22	Michigan	BIP	17	09/2018	10/2018	94
KP-MI-33	Michigan	BIP	17	09/2018	10/2018	90
KP-MI-56	Michigan	BIP	24		10/2018	100
KP-MI-58	Michigan	BIP	23		10/2018	96
MI-19	Michigan	NHBDS	16	09/2017	10/2017	63
MI-04	Michigan	NHBDS	16		10/2016	0
MI-09	Michigan	NHBDS	24		06/2016	10
MA-10	Massachusetts	NHBDS	32		2020	19
MA-13	Massachusetts	NHBDS	15		08/2017	33
MA-22	Massachusetts	NHBDS	16		08/2016	0
MA-21	Massachusetts	NHBDS	16		07/2016	0
MT-13	Montana	NHBDS	28		2020	93

MT-20	Montana	NHBDS	16	09/2017	81
MT-03	Montana	NHBDS	16	07/2017	0
DE-22	Delaware	NHBDS	17	2020	6
DE-13	Delaware	NHBDS	14	11/2016	0
DE-19	Delaware	NHBDS	15	11/2016	0
PA-11	Pennsylvania	NHBDS	20	2020	5
PA-13	Pennsylvania	NHBDS	14	12/2016	0
PA-07	Pennsylvania	NHBDS	15	11/2016	0

Table S2. Octopamine and tyramine receptor sequences in arthropod species.

Species	Protein description	Accession	Length	Abbreviation
<i>Apis mellifera</i>	Octopamine receptor	NP_001011565	587 aa	Am_OctAR
<i>Bombyx mori</i>	Octopamine receptor	NP_001091748	507 aa	Bm_OctAR
<i>Galendromus occidentalis</i>	Probable G-protein coupled receptor No9	XP_028966904	507 aa	Go_OctAR
<i>Ixodes scapularis</i>	Probable G-protein coupled receptor No9	XP_029851527	533 aa	Is_OctAR
<i>Rhipicephalus microplus</i>	Alpha 2 adrenergic-like octopamine receptor	AFC88977	480 aa	Rm_OctAR
<i>Tribolium castaneum</i>	Probable G-protein coupled receptor No9	NP_001280520	586 aa	Tc_OctAR
<i>Tropilaelaps mercedesae</i>	octopamine receptor 1-like	OQR70152	584 aa	Tm_OctAR
<i>Varroa destructor</i>	Probable G-protein coupled receptor No9	XP_022669321	532 aa	Vd_OctAR
<i>Apis mellifera</i>	Octopamine receptor beta-1R	XP_397139	427 aa	Am_Octβ1R
<i>Aethina tumida</i>	Octopamine receptor beta-1R-like	XP_019864623	447 aa	At_Octβ1R
<i>Bombus impatiens</i>	Octopamine receptor beta-1R-like	XP_003489997	427 aa	Bi_Octβ1R

<i>Bombyx mori</i>	Octopamine receptor beta-1R	XP_004922133	444 aa	Bm_Octβ1R
<i>Drosophila melanogaster</i>	Octopamine beta1 receptor, isoform A	NP_651057	508 aa	Dm_Octβ1R
<i>Helicoverpa armigera</i>	Octopamine receptor beta-1R-like	XP_021187626	442 aa	Ha_Octβ1R
<i>Manduca sexta</i>	Octopamine receptor beta-1R-like	XP_030039476	444 aa	Ms_Octβ1R
<i>Tribolium castaneum</i>	Octopamine receptor beta-1R	NP_001280514	527 aa	Tc_Octβ1R
<i>Apis dorsata</i>	Octopamine receptor beta-2R-like isoform X3	XP_006621349	412 aa	Ad_Octβ2R
<i>Apis mellifera</i>	Octopamine receptor beta-2R isoform X1	XP_396348	438 aa	Am_Octβ2R
<i>Acyrtosiphon pisum</i>	Octopamine receptor beta-2R	XP_001944827	443 aa	Ap_Octβ2R
<i>Bombus impatiens</i>	Octopamine receptor beta-2R-like isoform X1	XP_003488300	437 aa	Bi_Octβ2R
<i>Drosophila melanogaster</i>	Octopamine beta2 receptor, isoform F	NP_001163596	630 aa	Dm_Octβ2R
<i>Galendromus occidentalis</i>	Octopamine receptor beta-2R-like	XP_028966328	398 aa	Go_Octβ2R
<i>Ixodes scapularis</i>	Octopamine receptor beta-2R isoform X1	XP_029841166	384 aa	Is_Octβ2R

<i>Megachile rotundata</i>	Octopamine receptor beta-2R-like isoform X1	XP_003701983	435 aa	Mr_Octβ2R
<i>Rhipicephalus microplus</i>	Beta 2 adrenergic-like octopamine receptor	AFC88978	389 aa	Rm_Octβ2R
<i>Tribolium castaneum</i>	Octopamine receptor beta-2R isoform X2	XP_974214	392 aa	Tc_Octβ2R
<i>Tropilaelaps mercedesae</i>	Octopamine receptor beta-3R-like	OQR67197	439 aa	Tm_Octβ?R
<i>Varroa destructor</i>	Octopamine receptor beta-2R-like isoform X6	XP_022664702	366 aa	Vd_Octβ2R
<i>Aethina tumida</i>	Octopamine receptor beta-3R-like	XP_019864626	397 aa	At_Octβ3R
<i>Apis mellifera</i>	Octopamine receptor beta-3R isoform X1	XP_003249152	413 aa	Am_Octβ3R
<i>Bombus impatiens</i>	Octopamine receptor beta-3R-like isoform X1	XP_003489998	419 aa	Bi_Octβ3R
<i>Drosophila melanogaster</i>	Octopamine beta3 receptor, isoform J	NP_001034043	445 aa	Dm_Octβ3R
<i>Diabrotica virgifera virgifera</i>	Octopamine receptor beta-3R-like	XP_028130986	435 aa	Dv_Octβ3R
<i>Nicrophorus vespilloides</i>	Octopamine receptor beta-3R-like	NP_001317479	446 aa	Nv_Octβ3R
<i>Ostrinia furnacalis</i>	Octopamine receptor beta-3R-like	XP_028179439	445 aa	Of_Octβ3R

<i>Tribolium castaneum</i>	Octopamine receptor beta-3R-like	NP_001280505	398 aa	Tc_Octβ3R
<i>Apis mellifera</i>	Tyramine receptor	NP_001011594	399 aa	Am_TyrR
<i>Centruroides sculpturatus</i>	Probable G-protein coupled receptor No18	XP_023231574	405 aa	Cs_TyrR
<i>Galendromus occidentalis</i>	Tyramine receptor 1-like	XP_003741345	425 aa	Go_TyrR
<i>Ixodes scapularis</i>	Tyramine receptor 1	XP_002415939	400 aa	Is_TyrR
<i>Limulus polyphemus</i>	Probable G-protein coupled receptor No18	XP_013784920	439 aa	Lp_TyrR
<i>Periplaneta americana</i>	Tyramine receptor 1	CAQ48240	441 aa	Pa_TyrR
<i>Rhipicephalus microplus</i>	G-protein coupled receptor	CAA09335	419 aa	Rm_TyrR
<i>Sitophilus oryzae</i>	Octopamine receptor	XP_030755585	455 aa	So_TyrR
<i>Tribolium castaneum</i>	Tyramine/octopamine receptor	NP_001164311	453 aa	Tc_TyrR
<i>Tropilaelaps mercedesae</i>	G-protein coupled receptor-like	OQR78833	369 aa	Tm_TyrR
<i>Varroa destructor</i>	Octopamine receptor-like isoform X1	XP_022670329	369 aa	Vd_TyrR

Table S3. Primers used for amplification and sequencing of *Vd_oct α r*, *Vd_oct β r* and *Vd_tar1* genes.

Gene		Name	Sequence 5'→3'	Localization
<i>Vd_octαr</i>	Forward primers	Vd_OctAR_5UTR	CAACATCGGTCGTTTCACTG	5'UTR
		Vd_OctAR_344F	GCCACTTCTGCAAGATCTGG	Exon 6
		Vd_OctAR_881F	TGGCCAAGAACAAAGGTGGA	Exon 7
	Reverse primers	Vd_OctAR_938R	CGCAGGGTCATTTGTTGCAT	Exon 7
		Vd_OctAR_3UTR	GGACTAAACACTGCGGGGTA	3'UTR
<i>Vd_octβr</i>	Forward primers	Vd_OctBR_5UTR1	CACGAACGACAAAACCGGTC	5'UTR
		Vd_OctBR_5UTR3	GCGTCGGTTGAAATCGGAAG	5'UTR
		Vd_OctBR_246F	CGCGATGACATTCAATGCGT	Exon 1
		Vd_OctBR_476F	GCTCATATCCTTCGTGCCCA	Exon 1
		Vd_OctBR_iF	CGTTGATGTCTGTTTGCTGTTTG	Intron
	Reverse primers	Vd_OctBR_563R	GACCATCCGAACGAGTGTGT	Exon 1

<i>Vd_tar1</i>		Vd_OctBR_437iR	AGCTACGCATGCTGATCGAT	Intron
		Vd_OctBR_1103R	GATTTGTGAAAGTAAAGTGTGACG	Exon 2
		Vd_OctBR_3UTR	TAATCACGCCCACGGATACG	3'UTR
	Forward primers	Vd_TAR1_5F	GGTCTATCTCATTTTCTTTTCACCG	5'UTR
		Vd_TAR1_E3F	CCATAAACGCACTTTACGGCGGGTG	Exon 3
		Vd_TAR1_i3F	GAGGAGGCTAGTCAGGCCAAGATGGCC	Intron 3
	Reverse primers	Vd_TAR1_E3R	CTTTCTCGGAGCCGTTTTCGCGTTG	Exon 3
		Vd_TAR1_i3R	GGCCATCTTGGCCTGACTAGCCTCCTC	Intron 3
		Vd_TAR1_E4R	GCCACCGAATCTTCACGTTCAGCG	Exon 4
		Vd_TAR1_3R	GAGATTAGGGATGCATCAGACGAACGC	Exon 4-3'UTR
