

Electronic Supplementary Material to:

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New ways to acquire resistance - Imperfect convergence in insect adaptations to a potent plant toxin.

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Table S1. RT-qPCR primers designed for *P. aegrotus*. NKA - Na,K-ATPase, NS - nervous system.

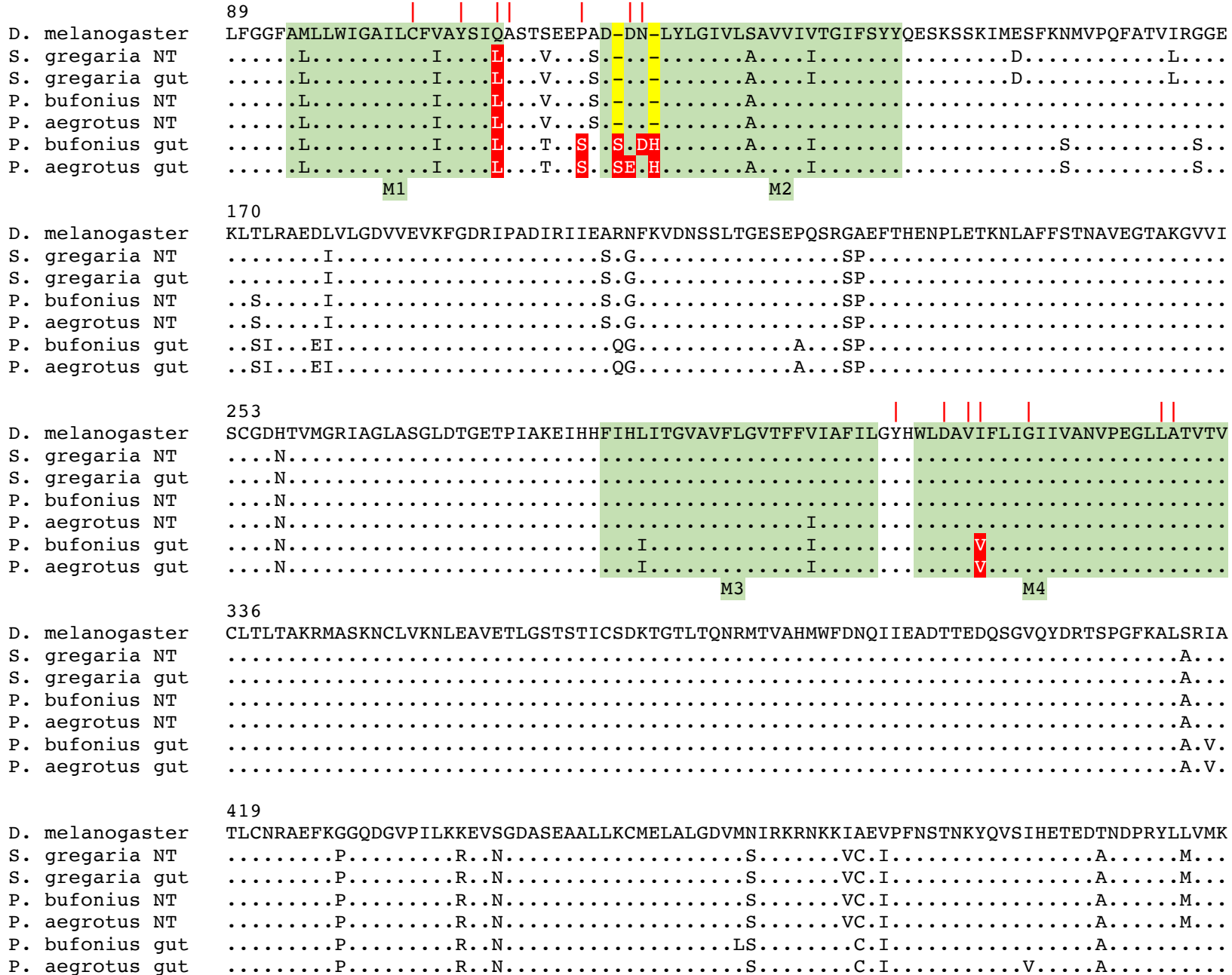
gene	direction	sequence
NKA gut	forward	5' TCAGAGAACCATTTGTACCTGGGTATTGTG
	reverse	5' CGTATAACTGTTGCGAACTGAGGAACCAT
NKA NS	forward	5' AGAACCTTCTGATGACAATTTGTACCTGGG
	reverse	5' ACTGAGGAACCATGTTTTTGAATGACTCCA
actin	forward	5' TGACAGGATGCAGAAGGAAATCACCGCC
	reverse	5' TGCTTGGAGATCCACATCTGCTGGAAGG
18S	forward	5' TTTCTTGATTCCGGTGGGTGGTGGTGCATGG
	reverse	5' TAATCGCTGACAGCACGAAGGATGTCACGC
β -tubulin	forward	5' TTTCCCTCGTCTGCACTTCTTCATGCCC
	reverse	5' TCACATGCAGCCATCATGTTTTTGGCATCG

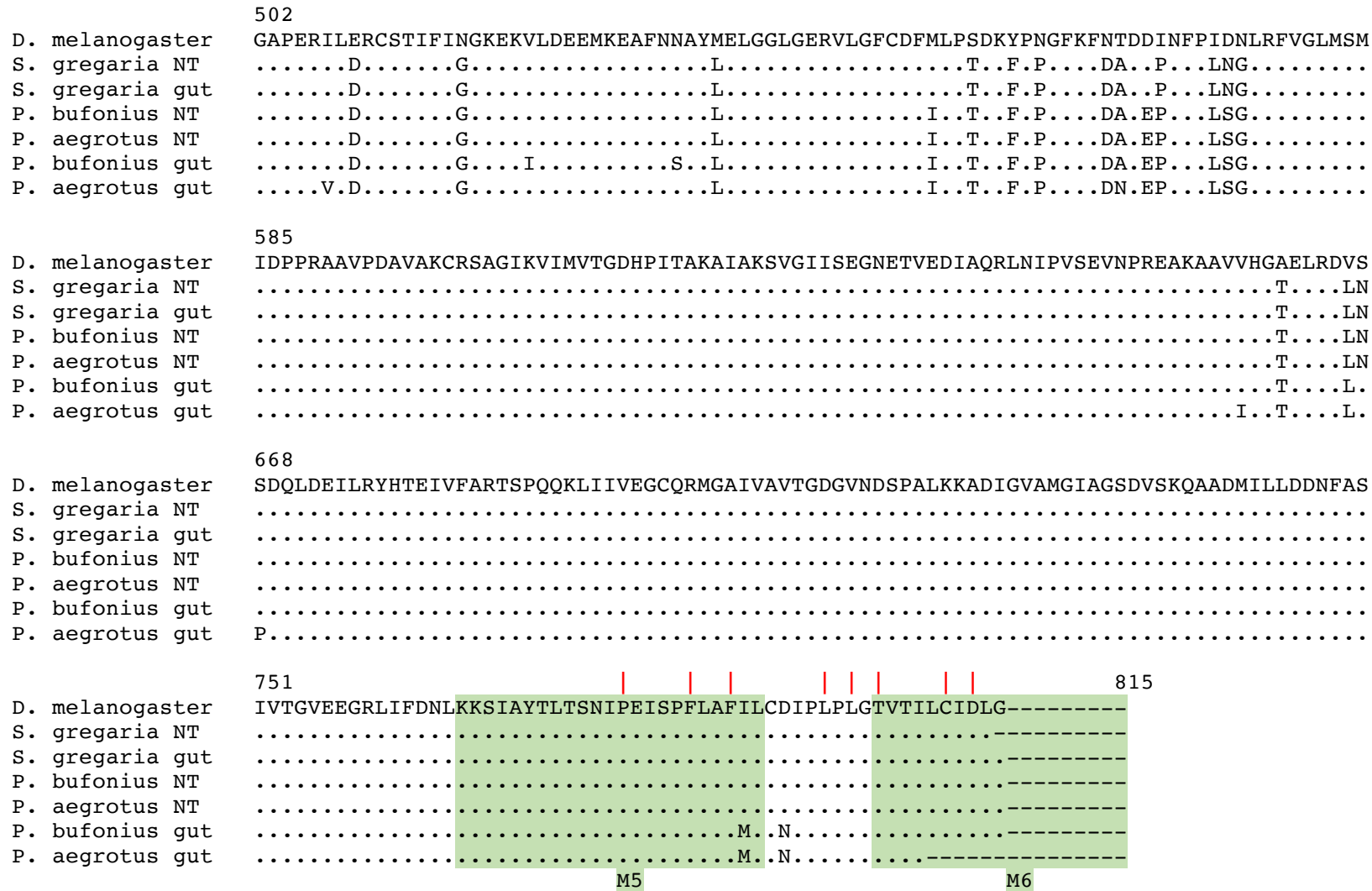
Table S2. Mutagenesis primers to reconstruct the two versions of the *P. aegrotus* (Paeg) first extracellular loop starting from a *D. melanogaster* construct. NT nervous system.

mutation introduced	step	gene copy	forward primer sequence*
Q111L	1	both	5' GGCCTATTCTATCCTGGCCAGCACCAGCGAGG
S115V-A119S	2	Paeg_NT	5' CTGGCCAGCACCGTCGAGGAGCCGTCCGACGATAATT
S115T-P118S	2	Paeg_gut	5' GGCCAGCACCACCGAGGAGTCGGCCG
insS120a-D121E	3	Paeg_gut	5' CGAGGAGTCGGCCGACTCTGAAAATTTGTATCTGGG
insH122a	4	Paeg_gut	5' GTCGGCCGACTCTGAAAATCATTGTATCTGGGTATTG

* reverse primers are reverted complements of forward primers

Fig. S1





Supplementary Figure 1. Translated amino acid alignment of the Na,K-ATPase alpha gene sequences of the pyrgomorphid grasshopper genes, *S. gregaria* and *D. melanogaster*. The alignment shown assumes that two independent amino acid insertion occurred in the gut copy of the pyrgomorphid species (yellow gaps in the other species). This hypothesis on the position of insertions maintains the conserved asparagine (N) at position 122 in the *P. aegrotus* gut copy and leads to the insertion of a histidine (H) after position 122. Numbering indicates the homologous positions in the pig Na,K-ATPase, transmembrane domains are colored in light green and numbered M1 - M6 (annotation of the crystal structure of the pig Na,K-ATPase 4HYT Protein Data Bank, [1]). Vertical red lines on top of the amino acids indicate that mutations at these sites have been

functionally tested to increase resistance to cardiac glycosides in the vertebrate or insect enzyme ([2-15]), residues in red highlight exchanges at these positions and the two assumed amino acids insertions. NT - nervous tissue.

References

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