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Homem, R. A., Buttery, B., Richardson, E., Tan, Y., Field, L. M., Williamson, M. S. and Davies, T. G. E. 2020. Evolutionary trade-offs of insecticide resistance – the fitness costs associated with target-site mutations in the nAChR of *Drosophila melanogaster*. *Molecular Ecology*. pp. 1-15.

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Supplementary Table 1: List of primers used in the study

Primer name	Purpose	Sequence (5'-3')
A6_Seq_5' _gRNA_F	Sequencing gRNA target site on 5' end of the nAChR_α6 gene	AATTTTCTTGACTTTCTT
A6_Seq_5' _gRNA_R		TTGAGTCATAATTGTGGAT
A6_Seq_3' _gRNA_F	Sequencing gRNA target site on 3' end of the nAChR_α6 gene	AAGAGTGTAGAAAATGGTT
A6_Seq_3' _gRNA_R		AGATTGATATGAGACGATT
A6_DsRed_int_5`_F	Genotyping nAChR_α6 knock-out/ DsRed knock-in	ATACGAAGTTATCTACGC
A6_DsRed_int_5`_R		TGATAAAAATGATAAGTGTG
A6_DsRed_int_3`_F	Genotyping nAChR_α6 knock-out/ DsRed knock-in	TTTGCTTTTGACTTA
A6_DsRed_int_3`_R		GAGCACTAGTATAACTTCGT
A6_RT-PCR_F	Validating nAChR_α6 knock-out	CCCGGCCGTAATAAATTGCA
A6_RT-PCR_R		GCATCATGTTCATGGTCGCC
R81T_RT-PCR_F	Validating R81T knock-in	CCCGGCCGTAATAAATTGCA
R81T_RT-PCR_R		GCATCATGTTCATGGTCGCC
B1_Seq_HA_Left_F	Sequencing nAChR_β1 target region and genotyping R81T integration	GGCGCATTCAACGTTTCAGT
B1_Seq_HA_Left_R		ATGGTGTTGGTTGGTGGTT
B1_Seq_HA_Right_F	Sequencing nAChR_β1 target region	CGTACCCGTACTTCGTACCG
B1_Seq_HA_Right_R		AGGTCACATCGATGGTGCAG
attP2_BglII_F	Restriction ligation cloning of second attP site into pDsRed-attP to generate pDsRed-2attP	TGAAGATCTGTAGTGCCCCAAGTGGG
attP2_SpeI_R		GCCACTAGTATAACTTCGTATAGCATAACATTATACG
A6_5'_HA_SphI_F	Restriction ligation cloning nAChR_α6 5' homology arm into pDsRed-2attP	GCATGCCCAAGGCAGGCGAATGGC
A6_5'_HA_EcoRI_R		GAATTCCATCGCTGCAGCTGTCGCTGTCTC

A6_3' _HA_XhoI_F	Restriction ligation cloning nAChR_α6 3' homology arm into pDsRed-2attP	CTCGAGAACGGTTAATAATTATGCAATAAG
A6_3' _HA_BglII_R		AGATCTCGGAGGGTGTGCATTTT
A6_3' _gRNA_pCDF4_F	Gibson assembly of nAChR_α6 3' and 5' gRNA sequences into pCDF4	TATATAGGAAAGATATCCGGGTGAACTCGCTAACGGTACAC ACACGCCAGTTTAGAGCTAGAAATAGCAAG
A6_5' _gRNA_pCDF4_R		ATTTTAACTTGCTATTTCTAGCTCTAAAACCCGAGCTCTCG CTCTCTATCGACGTTAAATTGAAAATAGGTC
B1_left_gRNA_pCDF4_F	Gibson assembly of nAChR_β1 left and right gRNA sequences into pCDF4	TATATAGGAAAGATATCCGGGTGAACTCGTGATTGTGATCA GATGGTGGTTTAGAGCTAGAAATAGCAAG
B1_right_gRNA_pCDF4_R		ATTTTAACTTGCTATTTCTAGCTCTAAAACCCGTAGTCGGCC TCATCCCACGACGTTAAATTGAAAATAGGTC
RpL32_F	Housekeeping genes used as control in RT-PCRs	GCGCTTGTCGATCCGTAAC
RpL32_R		GCCCAAGGGTATCGACAACA