

Supplementary Information for:

## **Evolutionary shifts in pheromone receptors contribute to speciation in four *Helicoverpa* species**

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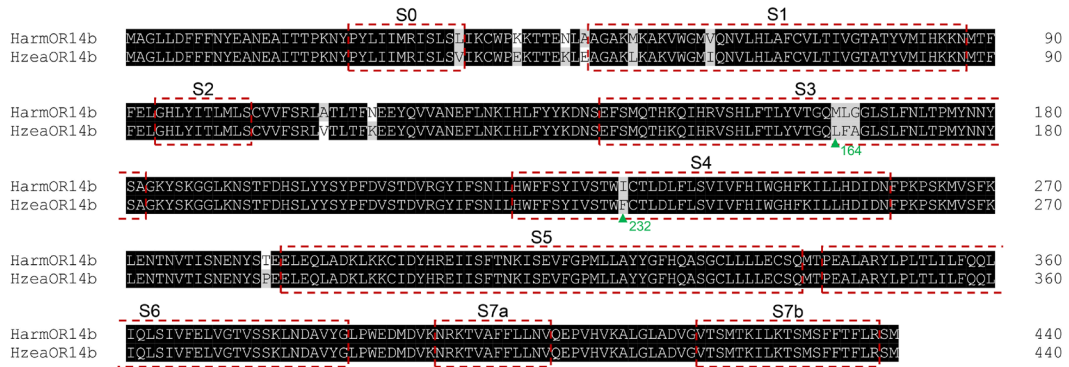
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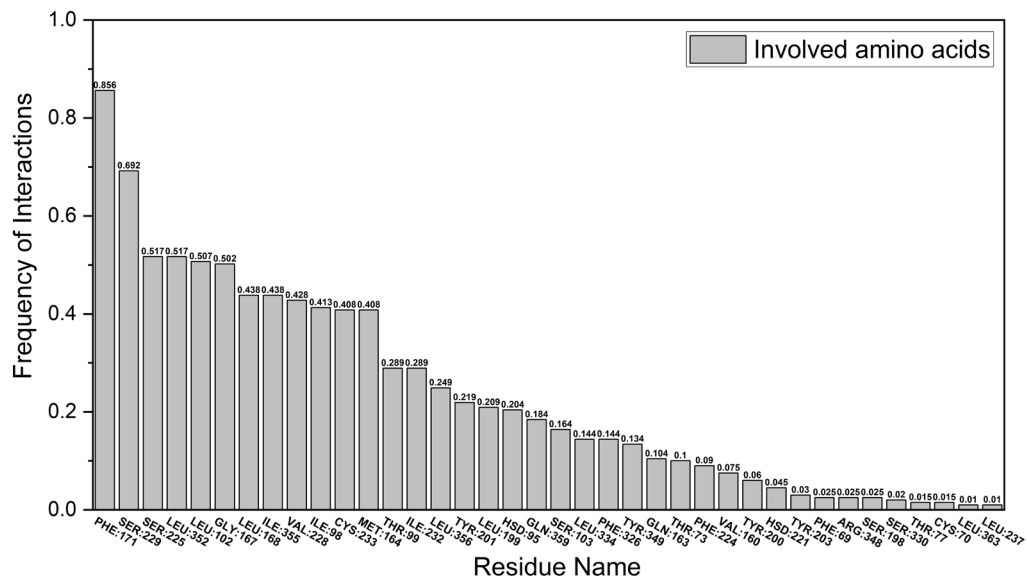
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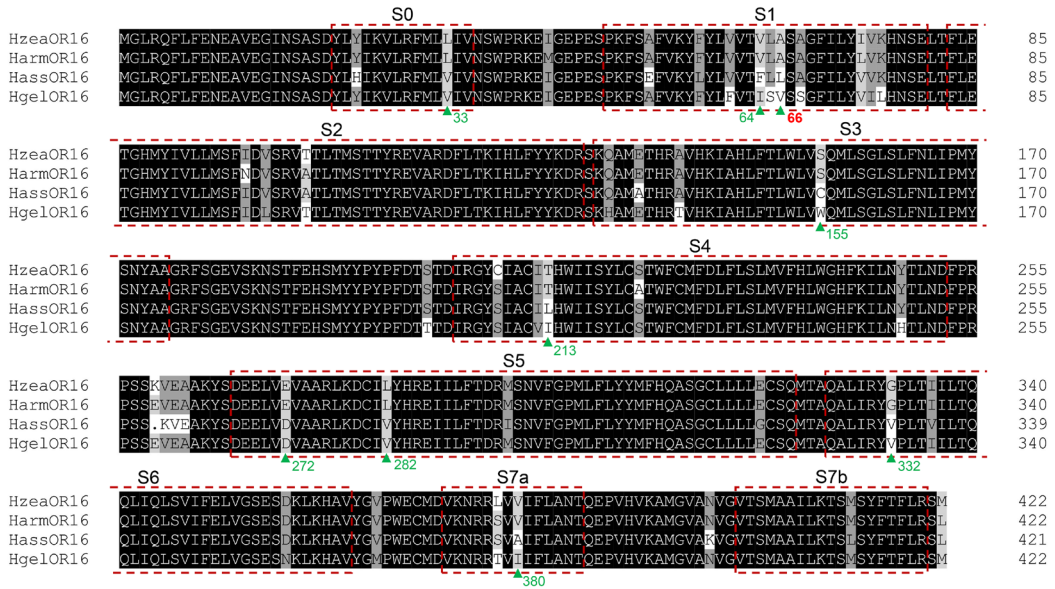
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**Fig. S1** Multiple sequence alignment of HarmOR14b and HzeaOR14b. Residues 164, 232 are two possible specific sites that contribute to the binding process and marked in green.



**Fig. S2** Substrate interaction frequency of HarmOR14b with Z9-14:Ald obtained from the 1 $\mu$ s MD simulation of the Z9-14:Ald bound HarmOR14b.



**Fig. S3** Multiple sequence alignment of homologous OR16. The divergent amino acids between group HarmOR16, HzeaOR16 and group HassOR16, HgelOR16 were marked in green triangles. Residue 66 is the only possible specific site that indirectly contributes to the binding process.

Table S1 Primers used in this study. The Kozak sequence (GCCACC) and the candidate mutated sites are marked in green and red, respectively.

| Primer                                       | Forward (5' to 3')                   | Reverse (5' to 3')              |
|--|--------------------------------------|---------------------------------|
| <b>Primers for gene clone</b>                |                                      |                                 |
| HgelOrco                                     | ATGATGACCAAAGTGAAGGC                 | TTACTTGAGTTGTACCAACACC          |
| HgelOR6                                      | ATGAGCTTTAAAAAATTTCTTTTC             | TCACATACTGCGTAAAAAGGTG          |
| HgelOR11                                     | ATGTATGCCGGGAATGCTG                  | TAAAACGTGCGCAGAAAG              |
| HgelOR13                                     | ATGAAAATTCTATCAGATGGTTC              | TCACTGTTCTTCTTCTGCAAC           |
| HgelOR14b                                    | ATGGCAGGGTTGCTTGATT                  | TCACATACTGCGTAGGAAGGTG          |
| HgelOR16                                     | ATGGGTCTTCGACAATTTCTAT               | TTACATACTCCTTAGAAACGTGAAG       |
| HzeaOrco                                     | ATGATGACCAAAGTGAAGG                  | TTACTTGAGTTGTACCAACACC          |
| HzeaOR6                                      | ATGAGCTTCAGAAAATTTCTTTTC             | TCACATACTGCGTAGAAAGGTG          |
| HzeaOR11                                     | ATGTATGCCGGCAATGCTG                  | TTAGAACGTGCGTAGAAAGGC           |
| HzeaOR13                                     | ATGAAAATTCTATCAGACGGTTC              | TCACTGTTCTTCTTCTGCAACT          |
| HzeaOR14                                     | ATGAGAGGCATACGTGACTTTAT              | TCACTTACTGCGAAGAAAGGT           |
| HzeaOR14b                                    | ATGGCAGGATTGCTTGATT                  | TCACATACTCCGTAAGAAGGTG          |
| HzeaOR15                                     | ATGACTGGTTTTTTGTGACATTATC            | TTACATGCTGCGTAGAAAAGC           |
| HzeaOR16                                     | ATGGGACTTCGTCAATTTCT                 | TTACATACTCCTTAGAAACGTGA         |
| <b>Primers for pT7Ts vector construction</b> |                                      |                                 |
| HgelOrco                                     | TCAGGGCCCCGCCACCATGATGACCAAAGTGAAGGC | TCACTCGAGTTACTTGAGTTGTACCAACAC  |
| HgelOR6                                      | TCAACTAGTGCCACCATGAGCTTTAAAAAATTTTC  | TCACTCGAGTCACATACTGCGTAAAAAGG   |
| HgelOR11                                     | TCAGATATCGCCACCATGTATGCCGGGAATGCTG   | TCAGCATGCTTAAAACGTGCGCAGAAAGG   |
| HgelOR13                                     | TCAACTAGTGCCACCATGAAAATTCTATCAGATGG  | TCAGCGGCCGCTCACTGTTCTTCTTCTGCAA |
| HgelOR14b                                    | TCAAGATCTGCCACCATGGCAGGGTTGCTTGATT   | TCAGCATGCTCACATACTGCGTAGGAAG    |
| HgelOR16                                     | TCAACTAGTGCCACCATGGGTCTTCGACAATTTTC  | TCACTCGAGTTACATACTCCTTAGAAACGTG |
| HzeaOrco                                     | TCAGGGCCCCGCCACCATGATGACCAAAGTGAAGG  | TCACTCGAGTTACTTGAGTTGTACCAACAC  |
| HzeaOR6                                      | TCAACTAGTGCCACCATGAGCTTCAGAAAATTTCT  | TCACTCGAGTCACATACTGCGTAGAAAG    |
| HzeaOR11                                     | TCAGATATCGCCACCATGTATGCCGGCAATGCTG   | TCAGCATGCTTAGAACGTGCGTAGAAAG    |
| HzeaOR13                                     | TCAACTAGTGCCACCATGAAAATTCTATCAGACGG  | TCACTCGAGTCACTGTTCTTCTTCTGCAAC  |
| HzeaOR14                                     | TCAAGATCTGCCACCATGAGAGGCATACGTGAC    | TCACTCGAGTCACTTACTGCGAAGAAAG    |
| HzeaOR14b                                    | TCAAGATCTGCCACCATGGCAGGATTGCTTGAT    | TCAGCATGCTCACATACTCCGTAAGAAGG   |

| Primer                                   | Forward (5' to 3')                  | Reverse (5' to 3')            |
|--|-------------------------------------|-------------------------------|
| HzeaOR15                                 | TCAAGATCTGCCACCATGACTGGTTTTTGTGACAT | TCACTCGAGTTACATGCTGCGTAGAAAAG |
| HzeaOR16                                 | TCAACTAGTGCCACCATGGGACTTCGTCAATTTC  | TCACTCGAGTTACATACTCCTTAGAAACG |
| <b>Mutation (HarmOR14b to HzeaOR14b)</b> |                                     |                               |
| HarmOR14b-M164L                          | CTGGTCAACTGCTTGGTGGTC               | AGTGTAAGTGGTAAATAGCGGGC       |
| HarmOR14b-I232F                          | TGTCAACCTGGTCTGTACTCTCG             | AACATCCGCTTGCCTGGTG           |
| <b>Mutation (HzeaOR14b to HarmOR14b)</b> |                                     |                               |
| HzeaOR14b-L164M                          | TACTGGTCAAATGTTTGCTGGTC             | TGGAAACCATAGTAAGCCAACAG       |
| HzeaOR14b-F232I                          | GTATCAACCTGGATCTGTACGCTCG           | TTAATGTGAGTGGCAAATAACGGGC     |
| <b>Mutation (HarmOR16 to HgelOR16)</b>   |                                     |                               |
| HarmOR16-L33V                            | TACTGCGATTCATGCTAGTTATCG            | GTTAGCGTAGCAACTCTGGACAC       |
| HarmOR16-V64I                            | GACTATTTTGGCCTCTGCTG                | AACTTCTCCTGAAAATCTGCC         |
| HarmOR16-A66V                            | GACTGTTTTGTTCTGCTG                  | AACTTCTCCTGAAAATCTGCC         |
| HarmOR16-L74V                            | TGGATTCATTTATATGTCGTTAAGC           | GTGGACAGCACGGTGAGTCTC         |
| HarmOR16-S155W                           | CATTATGGCTTGATGGCAGATG              | AGTAATAGACAGCCGCTTGC          |
| HarmOR16-T213I                           | CTATTGCGTGTATTATACATTGG             | ATCTGCGAACATTCGAGAAG          |
| HarmOR16-E272D                           | GTTGATGTGGCTGCAAGGC                 | GTCACTCTCGCTCCCAACC           |
| HarmOR16-L282V                           | GGATTGTATTGTTATCATCGTGAG            | CCAACCAATTCGAAAATAACTG        |
| HarmOR16-G332V                           | GCTCTTATACGTTATGTACCACTAAC          | CTGGTTCTTGAGTATTAGCGAGG       |
| HarmOR16-V380I                           | AACAGAAGGTCGGTGATCATC               | AAACGTGAAGTAGGACATAGAGGTC     |
| <b>Mutation (HarmOR16 to HassOR16)</b>   |                                     |                               |
| HarmOR16-A66L                            | GACTGTTTTGCTCTCTGCTG                | AACTTCTCCTGAAAATCTGCC         |
| <b>Mutation (HgelOR16 to HarmOR16)</b>   |                                     |                               |
| HgelOR16-V66A                            | TGACTATTTGCGCCTCTTCTGG              | AACTTCTCCTGAAAACCTGCC         |
| <b>Mutation (HassOR16 to HarmOR16)</b>   |                                     |                               |
| HassOR16-L66A                            | GACTTTTTTGGCCTCAGCTGG               | AACTTCTCCTGAAAACCTGCC         |

Table S2 Information for MD stimulations in this study.

| <b>MD simulations</b>   |                  |                   |                        |
|---|------------------|-------------------|------------------------|
| <b>OR Complex</b>   | <b>Substrate</b> | <b>Repeat No.</b> | <b>Simulation time</b> |
| (OR14b) <sub>2</sub> : (Orco) <sub>2</sub>                      | Z9-14:Ald        | 2                 | 1 $\mu$ s              |
| (OR14b) <sub>2</sub> : (Orco) <sub>2</sub>                      | Z9-16:Ald        | 2                 | 1 $\mu$ s              |
| (OR16 <sup>Wild Type</sup> ) <sub>2</sub> : (Orco) <sub>2</sub> | Z11-16:OH        | 2                 | 500 ns                 |
| (OR16 <sup>A66L</sup> ) <sub>2</sub> : (Orco) <sub>2</sub>      | Z11-16:OH        | 2                 | 500 ns                 |