# Title: Odorant-binding proteins expression patterns in recently diverged species of *Anastrepha* fruit flies

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## Supplementary Information

**Supplementary Table S1.** Pairwise amino acid similarities between *Drosophila melanogaster* OBPs and their *Anastrepha fraterculus* and *A. obliqua* putative orthologs.

**Supplementary Table S2.** *A. fraterculus* and *A. obliqua* OBP sequences used as reference for the RNA-seq gene expression analyses.

**Supplementary Table S3.** Primer pairs selected for qPCR analyses and results for efficiency tests.

**Supplementary Table S1.** Pairwise amino acid similarities between *Drosophila melanogaster* OBPs and their *Anastrepha fraterculus* and *A. obliqua* putative orthologs.

| ***D. melanogaster* OBP (accession number)** | ***Anastrepha* OBP** | **Pairwise amino acid similarity (%) *A. fraterculus*** | **Pairwise amino acid similarity (%) *A. obliqua*** |
|---|---|---|---|
| *OBP56a* (NP_611442.1) | *OBP56a* | 31.42 | 36.97 |
| *OBP56d* (NP_611444.2) | *OBP56d* | 58.33 | 60.14 |
| *OBP56h* (NP_611448.2) | *OBP56h_1* | 64.28 | 64.28 |
| | *OBP56h_2* | 54.35 | 52.17 |
| *OBP57c* (NP_611481.1) | *OBP57c* | 42.93 | 44.26 |
| *OBP99c* (NP_651711.1) | *OBP99c* | 75.16 | 74.51 |
| *OBP50a* (NP_725385.1) | *OBP50a* | 34.76 | 31.27 |
| *OBP83cd* (NP_649612.1) | *OBP83cd* | 64.37 | 63.56 |

**Supplementary Table S2.** *A. fraterculus* and *A. obliqua* OBP sequences used as reference for the RNA-seq gene expression analyses.

| ***A. fraterculus* gene** | **Accession number** | ***A. obliqua* gene** | **Accession number** | **Pairwise amino acid identity (%)** |
|---|---|---|---|---|
| *AfraOBP8a* | KU317957 | *AoblOBP8a* | KU317933 | 97.42 |
| *AfraOBP19a* | KU317958 | *AoblOBP19a* | KU317934 | 100 |
| *AfraOBP19b* | KU317976 | *AoblOBP19b* | KU317935 | 97.67 |
| *AfraOBP19c* | KU317959 | *AoblOBP19c* | KU317936 | 93.94 |
| *AfraOBP19d* | KU317960 | *AoblOBP19d* | KU317937 | 99.36 |
| *AfraOBP47b* | KU317961 | *AoblOBP47b* | KU317938 | 96.37 |
| *AfraOBP49a* | KU317962 | *AoblOBP49a-1* | KU317939 | 95.79 |
| *AfraOBP50a-1* | KU317977 | *AoblOBP50a* | KU317941 | 92.75 |
| *AfraOBP50e* | KU317963 | *AoblOBP50e* | KU317942 | 99.13 |
| *AfraOBP56a* | KP939314 | *AoblOBP56a* | KX018812 | 95.87 |
| *AfraOBP56d-1* | KU317979 | *AoblOBP56d-1* | KU317943 | 88.46 |
| *AfraOBP56h-1* | KU317965 | *AoblOBP56h-1* | KU317945 | 93.55 |
| *AfraOBP56h-2* | KU317966 | *AoblOBP56h-2* | KU317946 | 96.30 |
| *AfraOBP57c* | KU317967 | *AoblOBP57c* | KU317947 | 95.48 |
| *AfraOBP59a* | KU317968 | *AoblOBP59a* | KU317948 | 99.02 |
| *AfraOBP83cd* | KU317969 | *AoblOBP83cd* | KU317949 | 97.92 |
| *AfraOBP83ef* | KU317970 | *AoblOBP83ef* | KU317950 | 99.61 |
| *AfraOBP83g* | KU317971 | *AoblOBP83g* | KU317951 | 95.07 |
| *AfraOBP99a* | KU317972 | *AoblOBP99a* | KU317952 | 100 |
| *AfraOBP99b* | KU317973 | *AoblOBP99b* | KU317953 | 98.03 |
| *AfraOBP99c* | KU317974 | *AoblOBP99c* | KU317954 | 94.67 |
| *AfraOBP99d* | KU317975 | *AoblOBP99d-1* | KU317955 | 97.35 |

**Supplementary Table S3.** Primer pairs selected for qPCR analyses and results for efficiency tests.

| Gene | Positive selection | *In silico* differential expression | Sequences accession number | Primers | Sequences | E (%) | $R^2$ |
|---|---|---|---|---|---|---|---|
| *OBP50a* | yes[26] | no | Af = KU317977<br>Ao = KU317941 | Foward<br>Reverse | CATAGCATCGACGAAAACGG<br>CCGCCTTCTCTAATTGCTCG | 97.5 | 0.997 |
| *OBP56a* | no | females | Af = KP939314<br>Ao = KX018812 | Foward<br>Reverse | GAGGCCAAATTCGATGTGTT<br>ACACTTTTCCCGATCGTTTG | 101.6 | 0.990 |
| *OBP56d* | no | females and males | Af = KU317979<br>Ao = KU317943 | Foward<br>Reverse | AAGTCGTTAGGTCCCTTGG<br>TAGCACTTGTACCTCTCGAG | 98.1 | 0.998 |
| *OBP56h-1* | yes[26] | no | Af = KU317965<br>Ao = KU317945 | Foward<br>Reverse | TGTCACCGTTCTAATGTGTCA<br>ATTATCCTTGGCATCGCTGG | 98.5 | 0.991 |
| *OBP56h-2* | yes[26] | no | Af = KU317966<br>Ao = KU317946 | Foward<br>Reverse | CAATGCGCCATGGTCAAAC<br>CGCTAGCCAACTTTGCATC | 102.6 | 0.995 |
| *OBP57c* | yes[26] | no | Af = KU317967<br>Ao = KU317947 | Foward<br>Reverse | GGCTTGTCTAGAGCAGCACA<br>TTCTCGTCCATGAAGCCCAG | 99.1 | 0.991 |
| *OBP83cd* | no | females | Af = KU317969<br>Ao = KU317949 | Foward<br>Reverse | GTGCGTTACAAGGAGTGGTC<br>TCGCAATTATCCGCCAATGG | 101 | 0.973 |
| *OBP99c* | yes[27] | no | Af = KU317974<br>Ao = KU317954 | Foward<br>Reverse | TGAAGCTGAGGTATTGGCCAT<br>AGACACTTGTGACCGCGATA | 99.4 | 0.998 |

Af = *A. fraterculus*; Ao = *A. obliqua*; E = efficiency; $R^2$ = standard curve correlation coefficient.