# Female-Specific Odorant Receptors Expressed in the Adult Antennae of the Silkworm, *Bombyx mori*


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## Introduction

The Lepidoptera are an important insect pest group globally, and olfaction plays an important role in their pest behavior. Chemical cues that mediate critical adult behaviors such as mating (Figure 1) and host selection for egg laying are first detected by the peripheral sensory system comprised of antennae, mouthparts and tarsi that house neurons containing odorant and gustatory receptors (Ors and Grs). The larval stage causes feeding damage to host plants, and a limited number of olfactory and gustatory neurons (~ 64) in the sensory organs (Figure 2) mediate feeding behavior.

The silkworm *Bombyx mori* (F. Bombycidae) is one emerging model organism that represents the Lepidoptera and its genome has been sequenced. We annotated 40 new *Or* genes from the silkworm genome and characterized their sex-biased expression pattern in adult moth antennae. A preliminary analysis of expression in the larval stage has also been completed. Next, the silkworm gustatory receptors will be annotated and their expression patterns characterized. Our goal is to identify silkworm Ors and Grs that mediate host plant selection and feeding behavior, and to apply this research to economically important lepidopteran pest species.

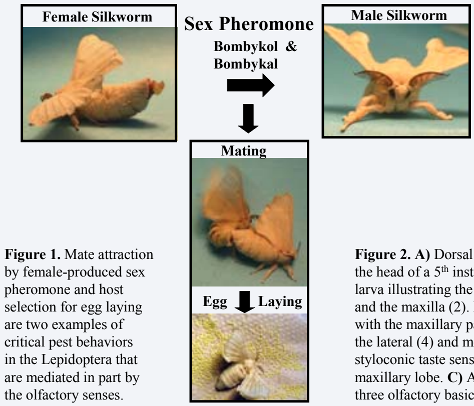



**Figure 1.** Mate attraction by female-produced sex pheromone and host selection for egg laying are two examples of critical pest behaviors in the Lepidoptera that are mediated in part by the olfactory senses.

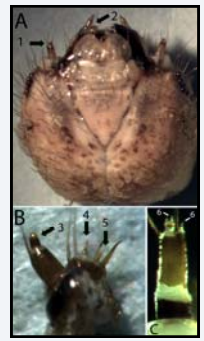



**Figure 2. A)** Dorsal view of the head of a 5[th] instar silkworm larva illustrating the antenna (1) and the maxilla (2). **B)** Maxilla, with the maxillary palp (3) and the lateral (4) and medial (5) styloconic taste sensilla on the maxillary lobe. **C)** Antenna with three olfactory basiconic sensilla (6).

## Silkworm *Or* Phylogenetics and Expression

We annotated 40 new Ors from the silkworm genome (Figure 3) and characterized their expression levels in adult female and male antennae relative to abdomens by quantitative real-time PCR using SYBR green dye (Table 1). BmOrs 1-7 have been previously published and include the bombykol and bombykal pheromone receptors (Sakurai et al., 2004; Krieger et al., 2005; Nakagawa et al., 2005 ). A neighbor-joining (corrected distance) phylogenetic tree was constructed from all known lepidopteran Ors (Figure 3). Three Ors are particularly interesting due to their expression patterns (indicated by an ➡). **BmOr19** and **BmOr30** are specifically expressed in female but not male antennae, and **BmOr10** is highly abundant in caterpillar mouth-parts.

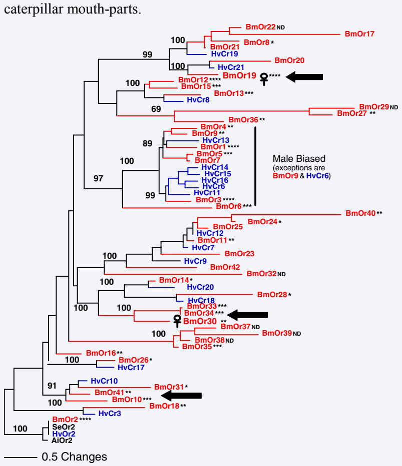



**Figure 3.** Neighbor-joining (corrected distance) phylogenetic tree of the ***B. mori*** and ***Heliothis virescens*** (F. Noctuidae) Ors identified to date, rooted using lepidopteran orthologs of DmOr83b. The ***H. virescens*** Ors are reported in Krieger et al. (2004). Bootstrap support is reported as a % of 1000 replicates. Gene expression levels relative to *BmOr2* are depicted using asterisks: **** = expression levels approximately equal to BmOr2; *** = $10^{-1}$ X lower; ** = $10^{-2}$ X lower; and * = $10^{-3}$ X lower. Lack of an asterisk indicates that expression was not detected. ND = no data (not tested).

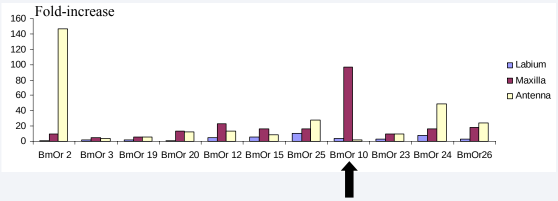
**Table 1.** Silkworm *Or* gene expression levels in female and male adult antennae determined by quantitative real-time PCR. All values were normalized using expression levels of *BmRPS3* and reported as relative levels (fold-increase) above background values measured in 5th instar larval abdomens. Relative expression was calculated using the $2^{-\Delta\Delta C_T}$ equation (Livak and Schmittgen, 2001).
NE = No expression. ND = No data (not tested).

| BmOr no. | ♀ | ♂ | ♀:♂ | BmOr no. | ♀ | ♂ | ♀:♂ |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 2701 | 0.0004 | 26 | 64 | 37 | 1.7 |
| 2 | 16384 | 17560 | 0.93 | 27 | 294 | 111 | 2.6 |
| 3 | 37 | 198668 | 0.0002 | 28 | 169 | 169 | 1.0 |
| 4 | 1911 | 30574 | 0.06 | 29 | ND | ND | |
| 5&7 | 39 | 1783 | 0.02 | **30** | **776** | **8.6** | **90** ⬅ |
| 6 | 169 | 10086 | 0.02 | 31 | 512 | 549 | 0.93 |
| 8 | 74 | 79 | 0.9 | 32 | ND | ND | |
| 9 | 338 | 239 | 1.4 | 33-4 | 52 | 223 | 0.23 |
| 10 | 832 | 478 | 1.7 | 35 | 388 | 223 | 1.7 |
| 11 | 1218 | 1261 | 1.0 | 36 | 169 | 97 | 1.7 |
| 12 | 15287 | 4705 | 3.2 | 37 | ND | ND | |
| 13 | 3566 | 10086 | 0.4 | 38 | ND | ND | |
| 14 | 891 | 1024 | 0.9 | 39 | ND | ND | |
| 15 | 274 | 104 | 2.6 | 40 | 60 | 84 | 0.71 |
| 16 | 1552 | 4390 | 0.4 | 41 | 84 | 60 | 1.4 |
| 17 | NE | NE | | 42 | NE | NE | |
| 18 | 5793 | 5404 | 1.1 | | | | |
| ➡ **19** | **7132** | **9** | **792** | 43 | 256 | 208 | 1.2 |
| 20 | NE | NE | | 44 | 1097 | 891 | 1.2 |
| 21 | NE | NE | | 45 | 10809 | 1552 | 7.0 |
| 22 | ND | ND | | 46 | 1552 | 256 | 6.0 |
| 23 | NE | NE | | 47 | 2702 | 315 | 8.6 |
| 24 | 20 | 60 | 0.3 | | | | |
| 25 | NE | NE | | | | | |

## *Or* Expression in Larvae

Expression of 11 *Ors* was measured in 5[th] instar larval sensory organs (Figure 2); the antennae (olfactory neurons) and the maxilla (olfactory and gustatory neurons). The labium (lip) was included as a tissue that lacks known chemosensilla. *BmOr2* (DmOr83b ortholog) and *BmOr3* (Bombykal pheromone receptor) were included as control genes.

**Figure 4**. *Or* gene expression in larval sensory organs relative to abdomens (fold-increase) after normalization to *BmRPS3*, determined by quantitative real-time PCR. Olfactory neurons on the antennae mediate short-range attraction while gustatory and olfactory neurons on the maxilla mediate feeding behavior. The labium is a mouth-part tissue that lacks known chemosensilla.




## Discussion

- The phylogenetic analysis (Figure 3) indicates that many Or lineages diverged prior to the formation of the Bombycidae and Noctuidae families. As a result, Or lineages contain representatives from both species as opposed to forming species specific groups. In some cases function may be conserved between similar Ors. For example, 11 of 13 Bm/Hv Ors that group together in a lineage with the characterized silkworm pheromone receptors are expressed at higher levels in male versus female antennae ( Table 1; Krieger et al., 2004; Sakurai et al., 2004; Krieger et al., 2005; Nakagawa et al., 2005 ). Therefore, these receptors may detect additional components of the female sex pheromone.

- Our expression analysis was validated by including several Ors whose function has been characterized. *BmOr2*, a member of the DmOr83b lineage that is ubiquitously expressed in olfactory neurons, was expressed at high levels equally between female and male antennae (Table 1) (and in larval antennae, Figure 4). Similarly, the silkworm pheromone receptors *BmOr1 & 3* were expressed selectively in male antennae at high levels (Table 1) (but not in larval antennae, Figure 4) as was expected based on their specific function.

- The expression levels of different *Or* genes in the antennae varied greatly and is likely related in general to the abundance of olfactory neurons on the antennae expressing each *Or* gene. For example, the pheromone-sensitive olfactory neurons are the most common on adult male silkworm antennae, and *BmOr 1* and *3* are expressed at very high levels. *BmOrs 12*, *13 & 15* cluster together on the phylogenetic tree, and all are highly expressed in both male and female antennae (Table 1; Figure 3). Therefore, they likely represent an abundant olfactory neuron that may be tuned to common odors such as green leaf volatiles.

- ***BmOrs 19*** and ***30*** are expressed exclusively in female antennae (Table 1), and therefore may detect odors that mediate female-specific behaviors (i.e. opposite to the pheromone receptors *BmOr1 & 3* that are exclusive to male antennae). ***BmOr19*** is highly abundant, while ***BmOr30*** is moderately abundant. The most common sensilla on adult female silkworm antennae respond to either benzoic acid or terpenoids such as linalool (Priesner, 1979; Heinbockel and Kaissling, 1996), therefore these are candidate ligands. In contrast, several *Ors* are expressed in both sexes but at ratios modestly biased towards either female or male antennae (Table 1). In these cases, one sex may have a greater sensitivity to the particular odorant.

- Preliminary results detected ***BmOr10*** at very high levels in the caterpillar maxilla, a gustatory organ that mediates caterpillar feeding behavior. The maxilla has both gustatory and olfactory neurons.

- Based upon expression patterns, we have identified three candidate Ors (**BmOr10, 19 & 30**) that may contribute to olfactory pathways that mediate host detection, selection and feeding behaviors. Efforts to characterize the ligand-binding specificity of these Ors is currently underway. Future goals include characterizing the expression patterns of silkworm *Or* and *Gr* genes in adult and larval sensory organs to identify additional candidates for functional characterization. Advances in our understanding of the peripheral sensory system of the silkworm can be applied to economically important pest species, where host detection and selection for egg laying by female moths, and feeding by larval caterpillars, are key pest behaviors.

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