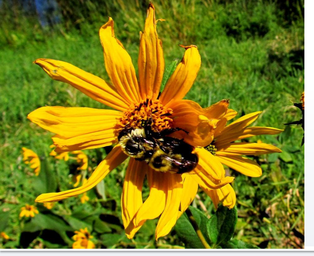

# Molecular Evolution of the Chemoreceptor Gene Families in the Common Eastern Bumblebee, *Bombus impatiens*


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## Introduction

Bees are well known as important pollinating insects. Bumblebees are known as one of the most important wild pollinators. They are also commonly used in commercial green house pollination. The common eastern bumblebee, *Bombus impatiens*, have been widely used as a managed pollinator in protected and open field crops in North America.

Chemical communication plays an important role in the communication and social behavior of bees. In the past few years, a great number of chemoreceptor genes have been assembled and analyzed. Major components of chemoreceptor genes including:

1. **Odorant receptors (Ors)**: serve vital roles in communication and environmental signal recognition.
   Fly (*Drosophila melanogaster): 62 Ors* [1]
   Honey bee (*Apis mellifera): 170 Ors* [1]
   European bumblebee (*Bombus terrestris): 165 Ors* [2]
2. **Gustatory receptors (Grs)**: have important function in colleting nectar and pollen.
   Fly: 68 Grs [1]
   Honey bee: 12 Grs [1]
   European bumblebee: 25 Grs [2]

Prediction: If **ecological selection** has lead to complex social insect behavior, the **chemoreceptor genes** should show an evolutionary signature of **positive selection**.

Annotated Ors and Grs → Tested **positive selection** sites on Ors and Grs → Explored the roles of Ors and Grs in ecological adaptation

## Methods

**1.Identification of *B. impatiens* Ors and Grs by Manual Bioinformatics**

Manually annotated Ors in B. impatiens by doing TBLASTN searches with B. terrestris Ors as queries for related genes at NCBI [2].

| | |
|---|---|
| "BiOr" | *B. impatiens* Ors |
| "BiGr" | *B. impatiens* Grs |
| "PSE" | Pseudogenes |
| "NTE" | N-terminus loss |
| "CTE" | C-terminus loss |

**2.Phylogenetic Analysis**

**ClustalX**: multiple protein alignments to identify irregularities and modify gene structures [3]

↓

**TrimAl**: removing poorly aligned regions and large gaps [4]

↓

**PHYML**: using maximum likelihood analysis to do phylogenetic analysis [5]

**3. Molecular Evolution Analysis**

Analyses were implemented with the codeml program in the **PAML** package [6].

The **selective pressure** (ω; the ratio of the normalized nonsynonymous substitution rate [$d_N$] to the normalized synonymous substitution rate [$d_S$]) was estimated using maximum likelihood [7].

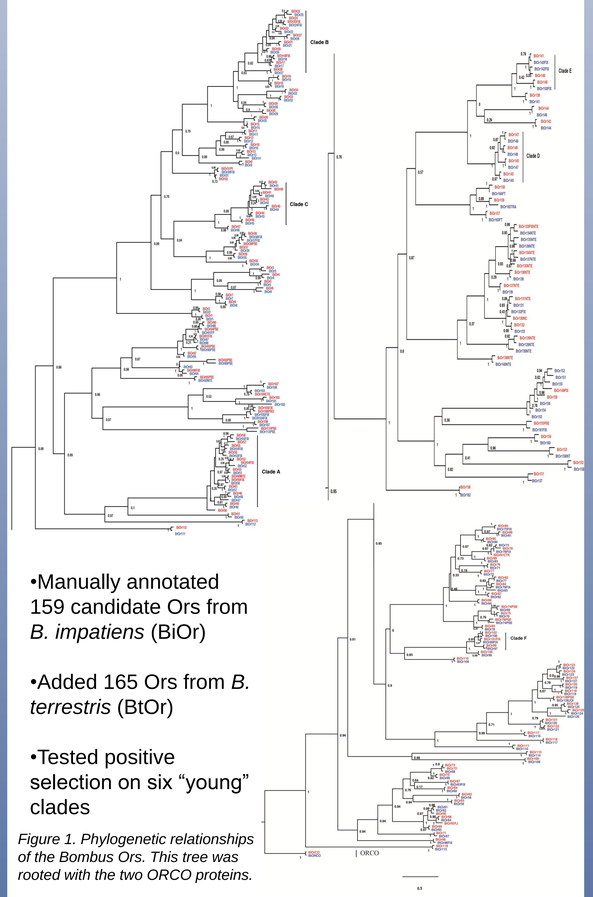
| Model | Meaning |
|---|---|
| M0 | one ratio |
| M1a | nearly neutral |
| M2a | positive selection |
| M3 | discrete |
| M7 | beta |
| M8 | beta and ω |
| M8a | null model for M8 |

| ω value | Meaning |
|---|---|
| less than one | purifying selection |
| equal to one | neutral evolution |
| more than one | positive selection |

1. **branch-specific model**: (compared M0 and M1a) identify any lineage with possible positive selection sites (Table 1)
2. **site-specific model**: (compared M8a and M8) detect specific sites with positive selection (Table 2)

## Result

**The Odorant Receptors**




- Manually annotated 159 candidate Ors from *B. impatiens* (BiOr)
- Added 165 Ors from *B. terrestris* (BtOr)
- Tested positive selection on six "young" clades

*Figure 1. Phylogenetic relationships of the Bombus Ors. This tree was rooted with the two ORCO proteins.*

**Pattern of Positive Selection**

- No clear evidence of positive selection in any of the tested Or clades
- All three Gr clades show evidence of strong positive selection

*Table 1. Likelihood ratio tests for selective pressures on branches within the Bombus Grs.*

| Clades | n | Likelihood Ratios: One Ratio | Likelihood Ratios: Free Ratio | 2Δl[a] 1 versus Free | df | *P* Value |
|---|---|---|---|---|---|---|
| Clade 1 | 15 | -4430.79 | -4422.82 | 15.94 | 14 | 3.17E-01 |
| Clade 2 | 7 | -3028.16 | -3027.21 | 1.9 | 6 | 9.29E-01 |
| Clade 3 | 15 | -5244.76 | -5236.03 | 17.46 | 14 | 2.33E-01 |

[a] Twice the logarithm of likelihood ratio.

*Table 2. Likelihood ration tests of positive selection on sites in the Bombus Grs.*

**Clade 1**: 9 sites (PP>95%) + 1 site (PP>99%)
**Clade 2**: 2 sites (PP>95%)
**Clade 3**: 8 sites (PP>95%) + 9 sites (PP>99%)
(PP means posterior probability)

| Clades | n | 2Δl[a] M1a versus M2a | 2Δl[a] M8 versus M8a | Parameters Estimated under M8 | Positively Selected Sites[b] |
|---|---|---|---|---|---|
| Clade 1 | 15 | 30.36 | 30.32 | $P_0$= 0.90; $P$=0.03; $q$=0.02; ($P_1$ = 0.10); ω=3.67 | 6T 10R 80V 88L 115R 154L **160Q** 281F 287M 330V |
| Clade 2 | 7 | 13.83 | 13.83 | $P_0$= 0.81; $P$=38.60; $q$=99 ($P_1$= 0.19); ω=2.75 | 4A 56Q |
| Clade 3 | 15 | 92.31 | 91.72 | $P_0$=0.92 ; $P$=0.29; $q$=0.24; ($P_1$= 0.08); ω=5.26 | 52I **55S** 108I 139I **151P 153L 155T** 156T 157T 162I **305F 309Q 313P 314S 316S** 320I 334A |

[a] Twice the logarithm of likelihood ratio.
[b] Positive selection sites estimated under M8 model by BEB approach with PPs > 95% are listed with PP > 99% under M8 model in bold.

The putatively positively selected sites were distributed clearly heterogeneous between different protein regions when plotted onto the chemoreceptor topology under M8 (Fig 3).

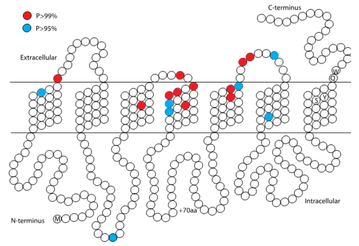



*Figure 3. Locations of positively selected amino acid residues for Clade 3 of the Bombus Grs.*

**The Odorant Receptors**

- Dr. Robertson manually annotated 24 candidate Grs from *B. impatiens* (BiGr)
- Added 24 Grs from *B.terrestris* (BtOr) and 15 Grs from *A. mellifera* (AmGr)
- Tested for positive selection on three clades

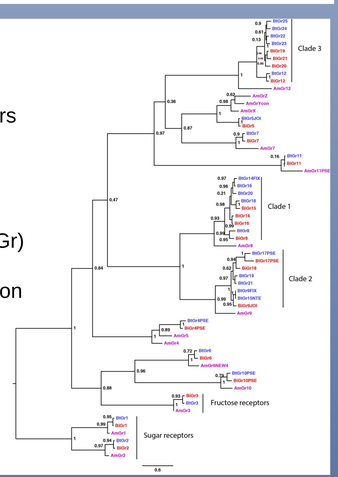



*Figure 1. Phylogenetic relationships of the Bombus and Apis mellifera Grs. The tree was rooted with the sugar receptors.*

## Discussion

1. In 159 annotated **odorant receptors**, there is no strong positive selection sites.
   Possibility: Bees do not require the complex function of Ors and have less Ors compared with other insects.
   - Ants: large number of Ors to detect odorant stimuli [8]
   - Aphids: developed sensory system on environment [9]
   - Plants: evolved the mechanism to attract and reward bees for pollination [1]
2. In 24 annotated **gustatory receptors**, there are 27 sites showing positive selection.
   Possibility: The number of Grs is quite divergent among species.

| Species | Number of Grs |
|---|---|
| *Apis mellifera*[1] | 12 |
| *Nasonia*[10] | 58 |
| *Bombus terrestris*[2] | 25 |
| *Bombus impatiens* | 24 |

**Future Study**

There are other ways to test for positive selection besides PAML. Besides odorant receptor and gustatory receptors, future investigations should include ionotropic receptors (Irs), another major group of chemoreceptor gene families. Irs have more broad function in detecting environmental chemicals. Before delivery to odorant receptors, most airborne molecules, like hydrophobic odorants and pheromones, need odorant binding proteins (OBPs) to facilitate the delivery process. I am currently working on the Ir and OBP gene families in *B. impatiens.*

## Significance

There are some previous studies about Or and Gr molecular evolution with orthologous comparisons, however it is not obvious why positive selection should be exhibited in these comparisons as the orthologous receptors have not been shown to detect different ligands. There are only two published studies have explored positive selection with the chemoreceptor gene families of a single species. Molecular information on bumblebee chemoreceptor can help us understand mechanisms of speciation and diversity. Moreover, the identification of Ors and Grs has important function for evolutionary biology, functional genetics, comparative genetics and agriculture development. Gaining deeper understanding about the molecular level of Ors and Grs could help explain the complex social behaviors of bees.

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11. Left image: "Common Eastern Bumble Bees on Jerusalem Artichoke" by D. Gordon E. Robertson - Own work by September 17, 2013. http://commons.wikimedia.org/wiki/File:Common_Eastern_Bumble_Bees_on_Jerusalem_Artichoke.jpg#/media/File:Common_Eastern_Bumble_Bees_on_Jerusalem_Artichoke.jpg.
12. Right image: "Bumble Bee Queen - *Bombus impatiens*" by Bruce Marlin- Own work by May 10, 2009. http://bugguide.net/node/view/274387.