

## Supplemental Materials

### Tissue, developmental, and caste-specific expression of odorant binding proteins in a eusocial insect, the red imported fire ant, *Solenopsis invicta*

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Keyhani

#### Supplemental Tables

**Table S1. List of *S. invicta* OBP genes**

| Name    | AA Length | GenBank accession number | Cysteine | Signal Peptide |
|---------|-----------|--------------------------|----------|----------------|
| SiOBP1  | 139       | HQ853350                 | 6        | 1-19           |
| SiOBP2  | 152       | HQ853351                 | 4        | 1-17           |
| SiOBP3  | 153       | AF427898.1               | 6        | 1-19           |
| SiOBP4  | 153       | HQ853352                 | 6        | 1-19           |
| SiOBP5  | 144       | HQ853353                 | 6        | 1-22           |
| SiOBP6  | 146       | HQ853354                 | 6        | 1-23           |
| SiOBP7  | 133       | HQ853355                 | 6        | 1-17           |
| SiOBP8  | 153       | HQ853356                 | 4        | No SP          |
| SiOBP9  | 129       | HQ853357                 | 6        | 1-20           |
| SiOBP10 | 147       | HQ853358                 | 6        | 1-19           |
| SiOBP11 | 149       | HQ853359                 | 6        | 1-24           |
| SiOBP12 | 174       | HQ853360                 | 6        | 1-20           |
| SiOBP13 | 160       | HQ853361                 | 6        | 1-18           |
| SiOBP14 | 162       | HQ853362                 | 6        | 1-19           |
| SiOBP15 | 162       | HQ853363                 | 6        | 1-22           |
| SiOBP16 | 171       | HQ853364                 | 6        | 1-20           |

**Table S2. List of primers used for quantitative RT-PCR primers**

|        | Forward                  | Reverse                 |
|--------|--------------------------|-------------------------|
| SiOBP1 | TGACCGGTCTATCACGTGCT     | GACGCATTTCCGCCACATTGT   |
| SiOBP2 | TCATTGAAAGAGTGGGCCGAAT   | GCAGTCAGGCATTCCGTTAGA   |
| SiOBP3 | TGAAGATAACCGAACTACACAA   | AGCACCTGTTTCCTTGAT      |
| SiOBP4 | AGCTCTTCTCGGTTGGTGAA     | GCCCAAGCTTTTGTCACATT    |
| SiOBP5 | ACGCATAGATGACGGTAT       | TGATAAGTGGTTGAGCAATC    |
| SiOBP6 | ATATTTGCCAGTTGTCGTAAA    | ATCGTTGGATTAACATCATAGAG |
| SiOBP7 | AAAGGAGAGGTAGCTGAAAGTGAC | TGCCTCCTTTTTCACAACCTGT  |
| SiOBP8 | TTAAGAGGCTACAGAACATTATG  | AGAGTTACCTTCCAGTCAAT    |
| SiOBP9 | AGGCTGGAACATTGAACAAA     | TATCGCATGCATTCTCCGCT    |

|               |                        |                        |
|---------------|------------------------|------------------------|
| SiOBP10       | TTGGTCTGGTCGACGACAAG   | GGAGTAGCTGCGCGTAACA    |
| SiOBP11       | TTCTCGTCTGTGTCTCTG     | TCTGCTCCTTCTAACTTATCA  |
| SiOBP12       | ATGGAAGGAACCAACATTAAG  | CCTGCATACATTTTCTTTCTTC |
| SiOBP13       | GCTGTGCGCATGTGTTTTGA   | ACACGCCATAAAGCAGCCAT   |
| SiOBP14       | CCCACCAGCCGAAATAATAGCT | CGCAGCCGCTTCTTTTACG    |
| SiOBP15       | GCTGACTTTGTCAAACCGATGG | AATTTGGTGCATTGGTCCGC   |
| SiOBP16       | AAAAGTCTCGTGCTTTGCGC   | TGCAACAAAGCAACCAACT    |
| SiOBP17       | TGCTTTGTGCCTGTGTTTTA   | TTTCTGCATTACAGATTCA    |
| EF1- $\alpha$ | AAGAGAACCCGAAAGCCATT   | GCCTCAACGCACATAGGTTT   |
| GAPDH         | AAGCTGTGGCGTGATGGCCG   | AGGAGGCAGGCTTGCGGAGT   |

**Table S3. Validation of RT-PCR Primer sets.**

| Gene           | Amplicon size (bp) | Exon Location | Approximate size on gel (bp) <sup>1</sup> | Optimal ratio ( $\mu$ M: $\mu$ M) | T <sub>M</sub> (°C) | Efficiency (%) |
|----------------|--------------------|---------------|---|-----------------------------------|---------------------|----------------|
| <i>SiOBP1</i>  | 202                | 183-384       | 200                                       | 2:2                               | 59                  | 106.32         |
| <i>SiOBP2</i>  | 218                | 154-371       | 200                                       | 2:2                               | 60                  | 93.54          |
| <i>SiOBP3</i>  | 195                | 189-315       | 200                                       | 2:2                               | 59                  | 105.57         |
| <i>SiOBP4</i>  | 263                | 140-402       | 250                                       | 2:2                               | 60                  | 90.16          |
| <i>SiOBP5</i>  | 135                | 249-383       | 150                                       | 2:2                               | 60                  | 102.03         |
| <i>SiOBP6</i>  | 120                | 306-425       | 120                                       | 2:2                               | 60                  | 110.41         |
| <i>SiOBP7</i>  | 205                | 148-352       | 200                                       | 2:2                               | 58                  | 100.51         |
| <i>SiOBP8</i>  | 185                | 142-326       | 200                                       | 1.5:1.5                           | 58                  | 97.76          |
| <i>SiOBP9</i>  | 198                | 122-319       | 200                                       | 2:2                               | 60                  | 105.80         |
| <i>SiOBP10</i> | 190                | 233-422       | 190                                       | 2:2                               | 59                  | 93.08          |
| <i>SiOBP11</i> | 175                | 20-194        | 180                                       | <b>2:2</b>                        | 60                  | 95.75          |
| <i>SiOBP12</i> | 222                | 268-469       | 220                                       | 2:2                               | 60                  | 95.06          |
| <i>SiOBP13</i> | 220                | 15-234        | 220                                       | 2:2                               | 60                  | 94.30          |

|                                |     |         |     |         |    |        |
|--------------------------------|-----|---------|-----|---------|----|--------|
| <i>SiOBP14</i>                 | 289 | 159-447 | 300 | 1.5:1.5 | 60 | 100.32 |
| <i>SiOBP15</i>                 | 207 | 157-363 | 200 | 2:2     | 60 | 101.08 |
| <i>SiOBP16</i>                 | 237 | 4-240   | 250 | 1.5:1.5 | 60 | 103.00 |
| <i>SiOBP17</i>                 | 198 | 14-211  |     |         |    |        |
| <i>EF1-<math>\alpha</math></i> | 79  |         | 100 | 1.5:1.5 | 60 | 108.24 |
| <i>GAPDH</i>                   | 184 |         | 200 | 2:2     | 60 | 100.35 |

<sup>1</sup>Determined by agarose gel electrophoresis.

<sup>2</sup>Primers failed to prime against RNA samples and did not produce usable E-values (less than 90% efficiency).

**Table S4. List of proteins used in phylogenetic analyses (Fig. 1)**

| Species                      | Name                         | NCBI Reference Sequence |              |
|------------------------------|------------------------------|-------------------------|--------------|
| <i>Harpegnathos saltator</i> | HsOBP1-X1                    | XP_011144088            |              |
|                              | HsOBP1-X2                    | EFN89245                |              |
|                              | HsOBP2                       | EFN79108                |              |
|                              | HsOBP3                       | XP_011139553            |              |
|                              | HsOBP4                       | XP_011147605            |              |
|                              | HsOBP5-X1                    | EFN77138                |              |
|                              | HsOBP5-X2                    | XP_011150623            |              |
|                              | HsOBP6                       | EFN84434                |              |
|                              | HsOBP7                       | XP_011150624            |              |
|                              | HsOBP8-X1                    | XP_011145649            |              |
|                              | HsOBP8-X2                    | EFN80433                |              |
|                              | <i>Camponotus floridanus</i> | CfOBP1                  | XP_011251750 |
|                              |                              | CfOBP2                  | XP_011254774 |
| CfOBP3                       |                              | EFN68669                |              |
| CfOBP4                       |                              | XP_011268025            |              |

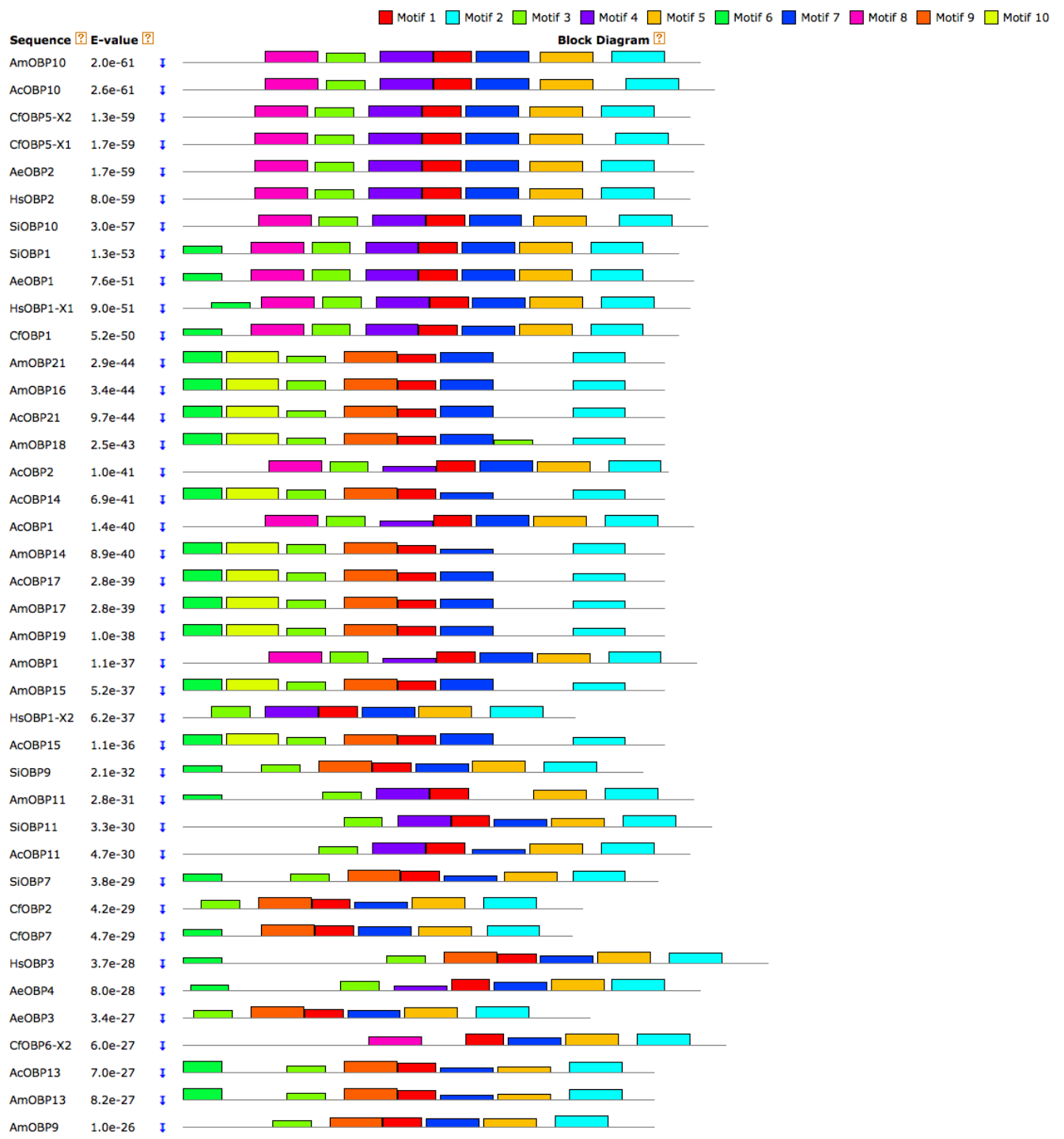
|                             |           |              |
|-----------------------------|-----------|--------------|
|                             | CfOBP5-X1 | XP_011253322 |
|                             | CfOBP5-X2 | XP_011253323 |
|                             | CfOBP6    | XP_011253326 |
|                             | CfOBP7    | XP_011255852 |
| <i>Acromyrmex echinator</i> | AeOBP1    | EGI67501     |
|                             | AeOBP2    | EGI61877     |
|                             | AeOBP3    | EGI63313     |
|                             | AeOBP4    | EGI61878     |
|                             | AeOBP4    | EGI61878     |
|                             | AeOBP5    | EGI63909     |
|                             | AeOBP6    | XP_011056723 |
|                             | AeOBP7    | EGI57538     |
|                             | AeOBP8    | EGI63317     |
|                             | AeOBP9    | XP_011069000 |
|                             | AeOBP10   | XP_011069001 |
|                             | AeOBP11   | XP_011068667 |
|                             | AeOBP4    | EGI61878     |
|                             | AeOBP5    | EGI63909     |
|                             | AeOBP6    | XP_011056723 |
|                             | AeOBP7    | EGI57538     |
|                             | AeOBP8    | EGI63317     |
|                             | AeOBP9    | XP_011069000 |
|                             | AeOBP10   | XP_011069001 |
|                             | AeOBP11   | XP_011068667 |
| <i>Solenopsis invicta</i>   | SiOBP1    | NP_001291522 |
|                             | SiOBP2    | ADX94399     |
|                             | SiOBP3    | XP_011157711 |
|                             | SiOBP4    | XP_011157725 |
|                             | SiOBP5    | XP_011156042 |

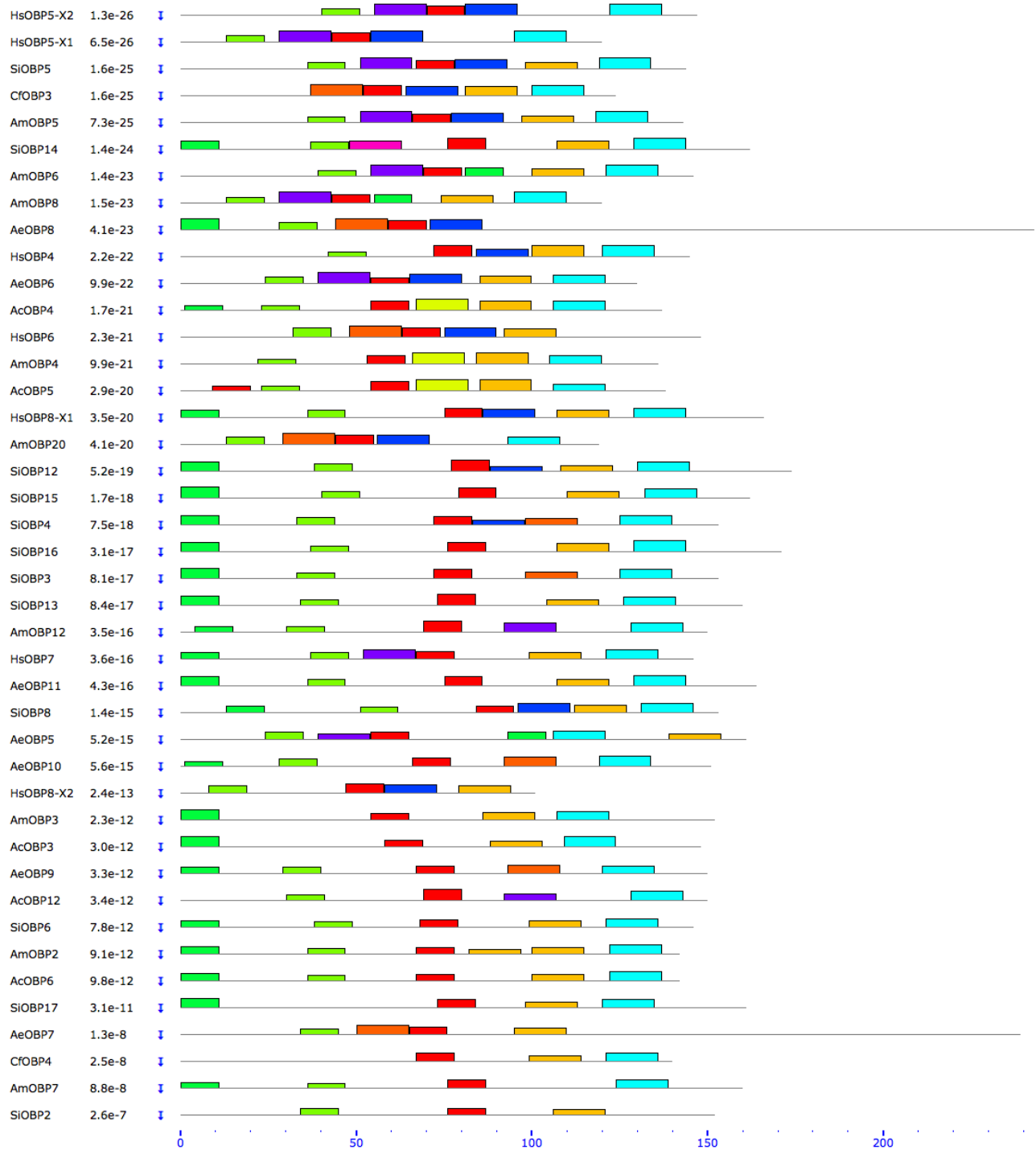
|                       |         |              |
|-----------------------|---------|--------------|
|                       | SiOBP6  | XP_011165204 |
|                       | SiOBP7  | XP_011167532 |
|                       | SiOBP8  | XP_011170254 |
|                       | SiOBP9  | XP_011173007 |
|                       | SiOBP10 | XP_011171270 |
|                       | SiOBP11 | XP_011171271 |
|                       | SiOBP12 | ADX94408     |
|                       | SiOBP13 | XP_011157738 |
|                       | SiOBP14 | ADX94410     |
|                       | SiOBP15 | ADX94411     |
|                       | SiOBP16 | ADX94412     |
| <i>Apis cerana</i>    | AcOBP1  | AEZ65021     |
|                       | AcOBP2  | ABD97847     |
|                       | AcOBP3  | AHN15444     |
|                       | AcOBP4  | AKQ98505     |
|                       | AcOBP5  | AAR83081     |
|                       | AcOBP6  | ABD97844     |
|                       | AcOBP10 | AKQ98506     |
|                       | AcOBP11 | AGQ03796     |
|                       | AcOBP12 | ALR87119     |
|                       | AcOBP13 | ALR87120     |
|                       | AcOBP14 | AKQ98508     |
|                       | AcOBP15 | ALR87121     |
|                       | AcOBP17 | AJA33390     |
|                       | AcOBP21 | AKQ98509     |
| <i>Apis mellifera</i> | AmOBP1  | NP_001011590 |
|                       | AmOBP2  | NP_001011591 |
|                       | AmOBP3  | NP_001035311 |
|                       | AmOBP4  | NP_001011589 |

|         |              |
|---------|--------------|
| AmOBP5  | NP_001011588 |
| AmOBP6  | NP_001011593 |
| AmOBP7  | NP_001035310 |
| AmOBP8  | NP_001164515 |
| AmOBP9  | NP_001035315 |
| AmOBP6  | NP_001011593 |
| AmOBP7  | NP_001035310 |
| AmOBP8  | NP_001164515 |
| AmOBP9  | NP_001035315 |
| AmOBP10 | NP_001035294 |
| AmOBP11 | NP_001035316 |
| AmOBP12 | NP_001035319 |
| AmOBP13 | NP_001035314 |
| AmOBP14 | NP_001035313 |
| AmOBP15 | NP_001035298 |
| AmOBP16 | NP_001035295 |
| AmOBP17 | NP_001035297 |
| AmOBP18 | NP_001035317 |
| AmOBP19 | NP_001035299 |
| AmOBP20 | NP_001035312 |
| AmOBP21 | NP_001035296 |

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# Supplemental Fig. S1







**Supplemental Figure S1 7. Motif analyses of OBPs.** Analyses of the 82 OBPs used in the phylogenetic tree (Figure S1 and Supplemental Table S4) using MEME (version 4.11.2, <http://meme-suite.org/tools/meme>). The size (height) of the amino acid represents the degree of conservation of that amino acid in the consensus sequence.