**Primary supplemental PDF**

**Supplemental tables**

| Chemical name | Example ant taxa using the compound as alarm pheromone | Major behavior in *O. biroi* (Fig. 1E) | Extracted from *O. biroi*? [S1] |
|---|---|---|---|
| 4-methyl-3-heptanone | *Atta texana* [S2]; *Pogonomyrmex* sp. [S3]; *Neoponera villosa* [S4]; Myrmicinae subfamily [S5]; *Atta* sp. [S6]; *Eciton burchellii* & *E. hamatum* [S7] | Immediate panic alarm | Yes |
| 4-methyl-3-heptanol | *Pogonomyrmex barbatus* [S8]; *Atta sexdens* [S9]; Dolichoderinae subfamily [S10]; *Harpegnathos saltator* [S11] | Immediate panic alarm | Yes |
| 4-methyl-3-hexanol | *Tetramorium impurum* [S12-14] | Immediate panic alarm | No |
| 6-methyl-5-hepten-2-one | *Formica* sp. [S15]; *Iridomyrmex purpureus* [S16]; *Aenictus rotundatus* [S17]; *Eciton burchellii* [S18]; *Pogonomyrmex barbatus* [S8]; *Lasius fuliginosus* [S19] | Ants leave nest | No |

**Table S1. Background information on the four ant alarm pheromones used in this study, related to Fig. 1.**

| Šidák's multiple comparisons test | Mean diff. | 95% CI of diff. | Adjusted P value |
|---|---|---|---|
| 4-methyl-3-hexanol (n=10) vs. 4-methyl-3-heptanone (n=17) | -2.049 | -22.87 to 18.78 | >0.99 (ns) |
| 4-methyl-3-hexanol (n=10) vs. 4-methyl-3-heptanol (n=11) | 7.805 | -15.03 to 30.64 | 0.97 (ns) |
| 4-methyl-3-hexanol (n=10) vs. blend (n=9) | 8.724 | -15.29 to 32.73 | 0.96 (ns) |
| 6-methyl-5-hepten-2-one (n=10) vs. 4-methyl-3-heptanone (n=17) | 20.92 | 0.096 to 41.75 | 0.048 (*) |
| 6-methyl-5-hepten-2-one (n=10) vs. 4-methyl-3-heptanol (n=11) | 30.77 | 7.94 to 53.61 | 0.0025 (**) |
| 6-methyl-5-hepten-2-one (n=10) vs. blend (n=9) | 31.69 | 7.69 to 55.70 | 0.0032 (**) |
| vehicle control (n=8) vs. 4-methyl-3-heptanone (n=17) | 78.87 | 56.47 to 101.3 | <0.0001 (****) |
| vehicle control (n=8) vs. 4-methyl-3-heptanol (n=11) | 88.72 | 64.44 to 113.0 | <0.0001 (****) |
| vehicle control (n=8) vs. blend (n=9) | 89.4 | 64.25 to 115.0 | <0.0001 (****) |

**Table S2. Statistical comparisons of behavioral effects, related to Fig. 1F.** Detailed statistical comparisons for the effects of alarm pheromones on the length of time that the original nest pile remained intact in the alarm behavior colony bioassay.

| Odorant | Category | Generated robust AL responses? | Vapor pressure (mmHg) at RT |
|---|---|---|---|
| ethanol | General odorant | Yes | 40 |
| isopropanol | General odorant | Yes | 33 |
| 3-hexanone | General odorant | Yes | 13.9 |
| 4-methyl-3-heptanone | Ant alarm pheromone | Yes | 5.03 (predicted) |
| propionic acid | General odorant | Yes | 2.9 |
| 6-methyl-5-hepten-2-one | Ant alarm pheromone | Yes | 1.78 (predicted) |
| ethylpyrazine | General odorant | Yes | 1.67 |
| 4-methyl-3-hexanol | Ant alarm pheromone | Yes | 1.55 (predicted) |
| 4-methyl-3-heptanol | Ant alarm pheromone | Yes | 0.43 (predicted) |
| butyric acid | General odorant | No | 0.43 |
| undecane | Ant alarm pheromone | No | 0.41 |
| linalool | General odorant | No | 0.16 |
| terpineol | General odorant | No | 0.04 |
| (+)-valencene | General odorant | No | 0.033 (predicted) |
| geranyl acetate | General odorant | No | 0.02 |
| dodecyl acetate | General odorant | No | 0.00047 |

**Table S3. Vapor pressures of odorant stimuli, related to Figs. 3-4.** Odors are listed according to vapor pressure in descending order. Vapor pressure values were obtained from the PubChem database (National Institute for Biotechnology Information: https://pubchem.ncbi.nlm.nih.gov). For records with missing values from PubChem, predicted values are given instead, generated from EPISuite [S20] and obtained from the ChemSpider database (Royal Society for Chemistry: https://www.chemspider.com).

| **Oligonucleotide name** | **Sequence 5'-3'** |
|---|---|
| Orco_F | tagttgtggtttgttgttcgcacaTATGTCACGTAATCAGCTTTTGACG |
| Orco_R | gcgcttgggtggcatgttgcaTCATATGTCTGCGAGCAAATGGAACG |
| ie1-A_F | ttatcgaattcctgcagcccgggggatccaACTAGTTGTTCGCCGAGCTCTTACG CGC |
| ie1-A_R | ctcggaggaggccatCCGCGGCGAACAGGTCACTTGGTTGTTCACGATC TTG |
| DsRed_F | acctgttcgccgcggATGGCCTCCTCCGAGAA |
| DsRed_R | ttattatatatatattttcttgttatagatGGCGCGCCCGAACACATATGCGAACAA CAAACCACAACTAGAATGCAGTG |
| QF2_F | aaccaagtgacctgttcgggccggACATATGCAACATGCCACCCAA |
| QF2_R | acccagtgacacgtgaccgCGAGCGCTGGATCTAAACGAGTTTTTAAGC |
| 15xQUAS_F | cggtcacgtgtcact |
| 15xQUAS_R | tgagaacccatcgaacaagcGTTTAAACAGATCTGTTAACGAATTGATC |
| GCaMP6s_F | gggccggcctgttcgAGCGCTTGTTCGATGGGTTCTCATCATCATC |
| GCaMP6s_R | atatattttcttgttatagatggCGCGCCGTAGCCCTAAGATACATTGATGAGT TTG |
| Ie1-B_F | ctgcattctagttgtggtttgttgttcgcaCATATGTGTTCGCCGAGCTCTTACGCG |
| Ie1-B_R | catcgaacaagcgctcgaacaggccggcccGAACAGGTCACTTGGTTGTTCAC |

**Table S4. Oligonucleotides used in plasmid construction, related to STAR Methods.** Gibson homology regions are shown as lowercase letters. The labels “F” and “R” denote forward and reverse orientation of oligonucleotides for a given target locus, respectively. All primers were designed for this study and purchased from Integrated DNA Technologies, Coralville, IA, USA.

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