Supplementary Material to "Sexual signaling strategy shows no influence on the morphometrics of firefly's primary olfactory organs"

2025-04-01

#### Contents

Figures: SM Figure 1 through Figure 2 Tables: SM Table 1 through SM Table 7

## Information about reproducibility of figures and statistics

Figures, tables, and statistical values are fully reproducible in the main manuscript as well as the supplementary material using RStudio version 2024.12.1+563 and R version 4.4.2 or higher from a R project in the Github repository: (redacted for review). All data, code, and images are available publicly in this repository. Instructions for directly reproducing this project are included in the repository.

# Full table of specimens

Individual samples of species were acquired from the entomology collection of the Los Angeles County Natural History Museum, representing 32 individual animals and 26 species, presented here in SM Table 1. Two specimens lacked abdominal segments and were unable to be sexed, and these are marked in Table 1 as "NA."

SM Table 1: Specimens used in analysis from the Los Angeles County Natural History Museum (LACM). Specimen ID is a specimen number unless BPC (Brian Brown personal collection) or NCC (individual specimens in the LACM collection not yet given a specimen number).

| Specimen ID       | Species                     | Signal   | Sex                | Body length (mm) |
|-------------------|-----------------------------|----------|--------------------|------------------|
| LACM Ent 574199   | Ellychnia californica       | chemical | NA                 | 10.3             |
| BPC 1             | Photinus macdermotti        | visual   | NA                 | NA               |
| LACM Ent 574198   | Ellychnia corrusca          | chemical | $_{\mathrm{male}}$ | 9.7              |
| LACM Ent 574194   | Ellychnia simplex           | chemical | $_{\mathrm{male}}$ | 6.7              |
| LACM Ent $574192$ | Lucidota sp                 | chemical | $_{\mathrm{male}}$ | 15.6             |
| LACM Ent 574191   | Lucidota punctata           | chemical | female             | 5.7              |
| LACM Ent $574190$ | Bicellonycha sp             | visual   | $_{\mathrm{male}}$ | 11.0             |
| LACM Ent 574189   | Bicellonycha wickershamorum | visual   | $_{\mathrm{male}}$ | 6.7              |
| LACM Ent 574188   | Phausis reticulata          | both     | $_{\mathrm{male}}$ | 7.7              |
| LACM Ent 574187   | Pyractomena angulata        | visual   | female             | 9.5              |
| LACM Ent 574186   | Pyractomena borealis        | visual   | $_{\mathrm{male}}$ | 11.2             |
| LACM Ent 574185   | Pyractomena lucifera        | visual   | female             | 10.4             |
| LACM Ent 574184   | Pyropyga nigricans          | chemical | $_{\mathrm{male}}$ | 6.2              |
| LACM Ent 574183   | Photinus marginellus        | visual   | NA                 | 4.6              |
| LACM Ent 574182   | Photinus pyralis            | visual   | female             | 17.3             |
| NCC Ent 1         | Photuris sp                 | visual   | $_{\mathrm{male}}$ | 15.9             |
| NCC Ent 2         | Bicellonycha wickershamorum | visual   | $_{\mathrm{male}}$ | 7.7              |
| NCC Ent 3         | Bicellonycha wickershamorum | visual   | female             | 9.1              |

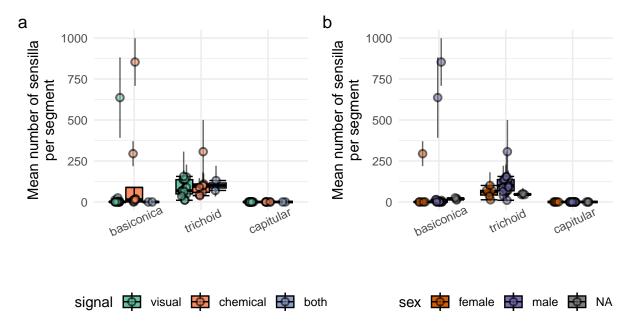
| Specimen ID | Species                | Signal   | Sex                | Body length (mm) |
|-------------|------------------------|----------|--------------------|------------------|
| NCC Ent 4   | Photinus scintillans   | visual   | male               | 7.6              |
| NCC Ent 5   | Photuris sp            | visual   | $_{\mathrm{male}}$ | 11.0             |
| NCC Ent 6   | Photuris flavicollis   | visual   | $_{\mathrm{male}}$ | 11.3             |
| NCC Ent 7   | Photinus pyralis       | visual   | $_{\mathrm{male}}$ | 15.7             |
| NCC Ent 8   | Photinus pyralis       | visual   | $_{\mathrm{male}}$ | 11.8             |
| NCC Ent 9   | Photinus indictus      | chemical | $_{\mathrm{male}}$ | 5.7              |
| NCC Ent 10  | Photinus consanguineus | visual   | $_{\mathrm{male}}$ | 9.4              |
| NCC Ent 11  | Microphotus angustus   | both     | $_{\mathrm{male}}$ | 10.4             |
| NCC Ent 12  | Cratomorphus sp        | visual   | female             | 25.5             |
| NCC Ent 13  | Aspisoma sp            | visual   | $_{\mathrm{male}}$ | 28.5             |
| NCC Ent 14  | Aspisoma sp            | visual   | female             | 13.3             |
| NCC Ent 15  | Ellychnia sp           | chemical | $_{\mathrm{male}}$ | 12.4             |
| NCC Ent 16  | Vesta sp               | visual   | $_{\mathrm{male}}$ | 7.2              |
| NCC Ent 17  | Vesta basalis          | visual   | male               | 11.7             |

### Antennal morphometrics are independent of body size

Data in the manuscript are not normalized by body length because no significant relationship with body length was found among the morphometric measurements using one-way ANOVAs. This includes body length with antenna length (F(1,19) = 3.92, p = 0.06), flagellar segment width (F(1,23) = 0.341, p = 0.6), olfactory sensilla width (F(1,23) = 0.406, p = 0.5), and olfactory sensilla length (F(1,23) = 0.21, p = 0.7).

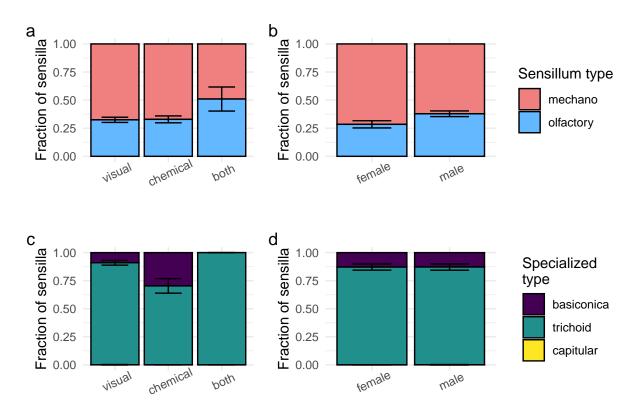
# Additional figures comparing values

Counts of olfactory sensilla per flagellar segment by type, signal, and sex are presented in SM Fig. 1.



SM Figure 1: Mean number of olfactory sensilla per flagellar segment by type, signal, and sex. a. Olfactory sensilla of each type (basiconica, trichodea, and capitular) for each type grouped by signaling strategy. b. Olfactory sensilla of each type (basiconica, trichodea, and capitular) for each type grouped by sex.

Fractions of mechanosensory and olfactor sensillum on the antennae are presented in SM Fig. 2.



SM Figure 2: Fraction of mechanosensory and olfactory sensillum coverage on the antennae. a,b: Fraction of mechanosensory or olfactory sensilla by sexual signaling strategy (a) and sex (b). c,d: Fraction of olfactory sensilla by type coverage on the antennae by sexual signaling strategy (c) and sex (d).

### Additional values from statistical comparisons

In the main manuscript's Tables 1 and 2, p-values were presented as a result of statistical comparisons. Here are additional values presented in SM Tables:

- SM Table 2: comparison values for all sensilla by signal, corresponding to comparisons in Table 1 and Fig. 2c,d.
- SM Table 3: comparison values for mechanosensory sensilla by signal, corresponding to comparisons in Table 1, Fig. 2a-d, and Fig. 5a,b.
- SM Table 4: comparison values for olfactory sensilla by signal, corresponding to comparisons in Table 1, Fig. 2a-d, Fig. 3a, and Fig. 5a,b.
- SM Table 5: comparison values for all sensilla by sex, corresponding to comparisons in Table 2 and Fig. 6c,d.
- SM Table 6: comparison values for mechanosensory sensilla by sex, corresponding to comparisons in Table 2 and Fig. 5a-d.
- SM Table 7: comparison values for olfactory sensilla by sex, corresponding to comparisons in Table 2 and Fig. 6a,b.

SM Table 2: Phylogenetically corrected comparisons of species means against signal type for all sensilla. Statistical scores (n, sample size; F-values; p-values) included for each comparison of sensillum width, sensillum length, sensilla density, mean distances between sensilla, mean count of sensilla per segment, and mean fraction of sensilla.

|                   | Mean value (visual) | Mean value (chemical) | Sample size | F value  | p value |
|-------------------|---------------------|-----------------------|-------------|----------|---------|
| Width             | n/a                 | n/a                   | n/a         | n/a      | n/a     |
| Length            | n/a                 | n/a                   | n/a         | n/a      | n/a     |
| Density           | $3480 \pm 1000$     | $3270 \pm 2000$       | 26          | 0.000671 | 0.76    |
| Mean distance     | $28.7 \pm 10$       | $27.6 \pm 9$          | 26          | 0.0849   | 0.82    |
| Count per segment | $337 \pm 300$       | $614 \pm 600$         | 26          | 1.2      | 0.24    |
| Fraction          | n/a                 | n/a                   | n/a         | n/a      | n/a     |

SM Table 3: Phylogenetically corrected comparisons of species means against signal type for mechanosensory sensilla. Statistical scores (n, sample size; F-values; p-values) included for each comparison of sensillum width, sensillum length, sensilla density, mean distances between sensilla, mean count of sensilla per segment, and mean fraction of sensilla.

|                   | Mean value (visual) | Mean value (chemical) | Sample size | F value | p value |
|-------------------|---------------------|-----------------------|-------------|---------|---------|
| Width             | $2.18 \pm 0.4$      | $2.51 \pm 0.7$        | 26          | 2.330   | 0.350   |
| Length            | $64.7 \pm 20$       | $71.2 \pm 20$         | 26          | 0.896   | 0.563   |
| Density           | $2330 \pm 1000$     | $2140 \pm 1000$       | 26          | 0.217   | 0.760   |
| Mean distance     | $34.2 \pm 10$       | $36.2 \pm 20$         | 26          | 0.153   | 0.820   |
| Count per segment | $205 \pm 90$        | $358 \pm 300$         | 26          | 3.550   | 0.240   |
| Fraction          | $0.683 \pm 0.1$     | $0.638 \pm 0.2$       | 26          | 0.346   | 0.730   |

SM Table 4: Phylogenetically corrected comparisons of species means against signal type for olfactory sensilla. Statistical scores (n, sample size; F-values; p-values) included for each comparsion of sensillum width, sensillum length, sensilla density, mean distances between sensilla, mean count of sensilla per segment, and mean fraction of sensilla.

| -                 | Mean value (visual) | Mean value (chemical) | Sample size | F value | p value |
|-------------------|---------------------|-----------------------|-------------|---------|---------|
| Width             | $1.87 \pm 0.6$      | $3.07 \pm 2$          | 26          | 5.610   | 0.112   |
| Length            | $13.3 \pm 3$        | $13.2 \pm 3$          | 26          | 0.003   | 0.980   |
| Density           | $1170\pm700$        | $1160 \pm 700$        | 26          | 0.001   | 0.990   |
| Mean distance     | $46.5 \pm 20$       | $44.2 \pm 10$         | 26          | 0.085   | 0.870   |
| Count per segment | $129 \pm 200$       | $248 \pm 400$         | 26          | 1.200   | 0.470   |
| Fraction          | $0.328 \pm 0.1$     | $0.368 \pm 0.2$       | 26          | 0.227   | 0.780   |

SM Table 5: Phylogenetically corrected comparisons of species means against sex type for all sensilla. Statistical scores (n, sample size; F-values; p-values) included for each comparison of sensillum width, sensillum length, sensilla density, mean distances between sensilla, mean count of sensilla per segment, and mean fraction of sensilla.

|                   | Mean value (female) | Mean value (male) | Sample size | F value | p value |
|-------------------|---------------------|-------------------|-------------|---------|---------|
| Width             | n/a                 | n/a               | n/a         | n/a     | n/a     |
| Length            | n/a                 | n/a               | n/a         | n/a     | n/a     |
| Density           | $3020 \pm 1000$     | $3450 \pm 1000$   | 23          | 2.77    | 0.88    |
| Mean distance     | $33.1 \pm 20$       | $27.6 \pm 9$      | 23          | 1.3     | 0.91    |
| Count per segment | $342\pm200$         | $479 \pm 500$     | 23          | 0.485   | 0.78    |
| Fraction          | n/a                 | n/a               | n/a         | n/a     | n/a     |

SM Table 6: Phylogenetically corrected comparisons of species means against sex type for mechanosensory sensilla. Statistical scores (n, sample size; F-values; p-values) included for each comparison of sensillum width, sensillum length, sensilla density, mean distances between sensilla, mean count of sensilla per segment, and mean fraction of sensilla.

|                   | Mean value (female) | Mean value (male) | Sample size | F value | p value |
|-------------------|---------------------|-------------------|-------------|---------|---------|
| Width             | $2.18 \pm 0.3$      | $2.28 \pm 0.6$    | 23          | 0.300   | 0.679   |
| Length            | $63.4 \pm 7$        | $68.1 \pm 20$     | 23          | 0.356   | 0.665   |
| Density           | $2260 \pm 1000$     | $2150 \pm 1000$   | 23          | 0.053   | 0.880   |
| Mean distance     | $36.7 \pm 10$       | $35.9 \pm 10$     | 23          | 0.019   | 0.910   |
| Count per segment | $225 \pm 100$       | $262\pm200$       | 23          | 0.136   | 0.780   |
| Fraction          | $0.732 \pm 0.1$     | $0.627\pm0.1$     | 23          | 2.620   | 0.280   |

SM Table 7: Phylogenetically corrected comparisons of species means against sex type for mechanosensory sensilla. Statistical scores (n, sample size; F-values; p-values) included for each comparsion of sensillum width, sensillum length, sensilla density, mean distances between sensilla, mean count of sensilla per segment, and mean fraction of sensilla.

|         | Mean value (female) | Mean value (male) | Sample size | F value | p value |
|---------|---------------------|-------------------|-------------|---------|---------|
| Width   | $2.86\pm2$          | $2.34 \pm 1$      | 23          | 1.060   | 0.469   |
| Length  | $13.6 \pm 1$        | $12.6 \pm 3$      | 23          | 0.560   | 0.604   |
| Density | $799 \pm 400$       | $1340 \pm 800$    | 23          | 2.770   | 0.220   |

|                   | Mean value (female) | Mean value (male) | Sample size | F value | p value |
|-------------------|---------------------|-------------------|-------------|---------|---------|
| Mean distance     | $54.1\pm20$         | $43.3 \pm 20$     | 23          | 1.300   | 0.420   |
| Count per segment | $115 \pm 200$       | $211 \pm 300$     | 23          | 0.485   | 0.620   |
| Fraction          | $0.291 \pm 0.1$     | $0.382 \pm 0.1$   | 23          | 2.110   | 0.300   |