**Reanalysis of an oft-cited paper on honeybee magnetoreception reveals random behavior**

**Baltzley and Nabity**

**Explanation of statistical tests with data output**

As described in the Materials and Methods section of our manuscript, the number of bees that reached criterion at any given magnetic field strength are not independent data points. The bees tested at 430 μT are the same bees that reached criterion at 1300 μT. To make the data points independent, we counted only the lowest level reached by each bee. In the table below, the first row represents the data reported by Kirschvink *et al.* (1997). 10 bees reached criterion at 1300 μT, and 9 of those 10 bees reached criterion at 430 μT—i.e., 1 bee was not able to reach criterion below 1300 μT.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Magnetic field strength (μT) | | | | | | | | |  |
|  | No learning | 1300 | 430 | 130 | 100 | 43 | 13 | 4.3 | 1.3 | 0.43 | Total |
| Original | 4 | 10 | 9 | 6 | 5 | 4 | 3 | 1 | 1 | 0 | 44 |
| Numbers used for analysis | 5 | 1 | 3 | 1 | 1 | 1 | 2 | 0 | 1 | 0 | 15 |

In their first experiment, Kirschvink *et al*. (1997) used 15 bees tested under 10 Hz ac magnetic fields. We calculated the expected results using the estimate that there was a 66.5% chance a bee would reach a criterion if it were choosing randomly. As described in the manuscript, the number of bees that reached a criterion at 1300 μT was ambiguous. Below are the results of our statistical analysis assuming 10 bees reached a criterion at 1300 μT:

**15 bees, 10 Hz ac field, 10 reached criterion at 1300 μT, data points are independent**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Magnetic field strength (μT) | | | | | | | | |
|  | No learning | 1300 | 430 | 130 | 100 | 43 | 13 | 4.3 | 1.3 | 0.43 |
| Observed | 5 | 1 | 3 | 1 | 1 | 1 | 2 | 0 | 1 | 0 |
| Expected proportions | 0.335 | 0.223 | 0.148 | 0.099 | 0.066 | 0.044 | 0.029 | 0.019 | 0.013 | 0.025 |

> xmulti(kirschvink\_15a,expected,detail = 3,histobins = T)

P value (LLR) = 0.4635

P value (Prob) = 0.1722

P value (Chisq) = 0.2031

Observed: 5 1 3 1 1 1 2 0 1 0

Expected ratio: 0.335 0.222775 0.1481454 0.09851667 0.06551359 0.04356654 0.02897175 0.01926621 0.01281203 0.02543284

Total number of tables: 1307504

Below are the results of our statistical analysis assuming 11 bees reached criterion at 1300 μT:

**15 bees, 10 Hz ac field, 10 reached criterion at 1300 μT, data points are independent**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Magnetic field strength (μT) | | | | | | | | |
|  | No learning | 1300 | 430 | 130 | 100 | 43 | 13 | 4.3 | 1.3 | 0.43 |
| Observed | 4 | 2 | 3 | 1 | 1 | 1 | 2 | 0 | 1 | 0 |
| Expected | 0.335 | 0.223 | 0.148 | 0.099 | 0.066 | 0.044 | 0.029 | 0.019 | 0.013 | 0.025 |

> xmulti(kirschvink\_15b,expected\_15,detail = 3,histobins = T)

P value (LLR) = 0.644

P value (Prob) = 0.2348

P value (Chisq) = 0.246

Observed: 4 2 3 1 1 1 2 0 1 0

Expected ratio: 0.335 0.222775 0.1481454 0.09851667 0.06551359 0.04356654 0.02897175 0.01926621 0.01281203 0.02543284

Total number of tables: 1307504

For comparison, below are the statistical results if we use all observations, even though they are not independent data points:

**15 bees, 10 Hz ac field, 10 reached criterion at 1300 μT, data points are NOT independent (pseudoreplication)**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Magnetic field strength (μT) | | | | | | | | |
|  | No learning | 1300 | 430 | 130 | 100 | 43 | 13 | 4.3 | 1.3 | 0.43 |
| Observed | 5 | 10 | 9 | 6 | 5 | 4 | 3 | 1 | 1 | 0 |
| Expected | 0.335 | 0.665 | 0.442 | 0.294 | 0.196 | 0.130 | 0.086 | 0.058 | 0.038 | 0.025 |

> xmulti(kirschvink\_15a\_ps,expected\_ps\_15,detail = 3,histobins = T,safety = 1e+10)

P value (LLR) = 0.95

P value (Prob) = 0.8063

P value (Chisq) = 0.9204

Observed: 5 10 9 6 5 4 3 1 1 0

Expected ratio: 0.335 0.665 0.442225 0.2940796 0.195563 0.1300494 0.08648283 0.05751108 0.03824487 0.02543284

Total number of tables: 4431613550

**15 bees, 10 Hz ac field, 11 reached criterion at 1300 μT, data points are NOT independent (pseudoreplication)**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Magnetic field strength (μT) | | | | | | | | |
|  | No learning | 1300 | 430 | 130 | 100 | 43 | 13 | 4.3 | 1.3 | 0.43 |
| Observed | 4 | 11 | 9 | 6 | 5 | 4 | 3 | 1 | 1 | 0 |
| Expected | 0.335 | 0.665 | 0.442 | 0.294 | 0.196 | 0.130 | 0.086 | 0.058 | 0.038 | 0.025 |

> xmulti(kirschvink\_15b\_ps,expected\_ps\_15,detail = 3,histobins = T,safety = 1e+10)

P value (LLR) = 0.9336

P value (Prob) = 0.7843

P value (Chisq) = 0.9027

Observed: 4 11 9 6 5 4 3 1 1 0

Expected ratio: 0.335 0.665 0.442225 0.2940796 0.195563 0.1300494 0.08648283 0.05751108 0.03824487 0.02543284

Total number of tables: 4431613550

In their second experiment, Kirschvink *et al*. (1997) used 11 bees tested under 60 Hz ac magnetic fields. We calculated the expected results using the estimate that there was a 66.5% chance a bee would reach criterion if it were choosing randomly. Below are the results of our statistical analyses:

**11 bees, 60 Hz ac field, data points are independent**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | Magnetic field strength (μT) | | | |
|  | No learning | 1300 | 430 | 130 | 100 |
| Observed | 4 | 3 | 1 | 3 | 0 |
| Expected | 0.335 | 0.223 | 0.148 | 0.099 | 0.20 |

xmulti(kirschvink\_11,expected\_11,detail = 3,histobins = T)

P value (LLR) = 0.1907

P value (Prob) = 0.1704

P value (Chisq) = 0.1845

Observed: 4 3 1 3 0

Expected ratio: 0.335 0.222775 0.1481454 0.09851667 0.195563

Total number of tables: 1365

**11 bees, 60 Hz ac field, data points are NOT independent (pseudoreplication)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | Magnetic field strength (μT) | | | |
|  | No learning | 1300 | 430 | 130 | 100 |
| Observed | 4 | 7 | 4 | 3 | 0 |
| Expected | 0.335 | 0.665 | .442 | .294 | .196 |

> xmulti(kirschvink\_11\_ps,expected\_ps\_11,detail = 3,histobins = T,safety = 1e+10)

P value (LLR) = 0.4705

P value (Prob) = 0.7322

P value (Chisq) = 0.7126

Observed: 4 7 4 3 0

Expected ratio: 0.335 0.665 0.442225 0.2940796 0.195563

Total number of tables: 7315

We also performed a simulation of the Kirschvink *et al.* (1997) experiment with 15 bees using a random number generator. When we analyzed the data using only independent observations, several of the categories in the simulated data had values of zero:

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Magnetic field strength (μT) | | | | | | | | |
|  | No learning | 1300 | 430 | 130 | 100 | 43 | 13 | 4.3 | 1.3 | 0.43 |
| Observed | 5 | 1 | 3 | 1 | 1 | 1 | 2 | 0 | 1 | 0 |
| Simulation | 4 | 3 | 2 | 1 | 0 | 0 | 2 | 2 | 0 | 1 |

Because the multinomial exact test cannot be performed with expected values of zero, we performed the data analysis using non-independent data points.

**15 bees, 10 Hz ac field, 10 reached criterion at 1300 μT, data points are NOT independent (pseudoreplication)**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Magnetic field strength (μT) | | | | | | | | |
|  | No learning | 1300 | 430 | 130 | 100 | 43 | 13 | 4.3 | 1.3 | 0.43 |
| Observed | 5 | 10 | 9 | 6 | 5 | 4 | 3 | 1 | 1 | 0 |
| Simulation | 4 | 11 | 8 | 6 | 5 | 5 | 5 | 3 | 1 | 1 |

> xmulti(kirschvink\_15a\_ps,R\_sim\_15bees,detail = 3,histobins = T,safety = 1e+10)

P value (LLR) = 0.8954

P value (Prob) = 0.9571

P value (Chisq) = 0.9386

Observed: 5 10 9 6 5 4 3 1 1 0

Expected ratio: 4 11 8 6 5 5 5 3 1 1

Total number of tables: 4431613550

**15 bees, 10 Hz ac field, 11 reached criterion at 1300 μT, data points are NOT independent (pseudoreplication)**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Magnetic field strength (μT) | | | | | | | | |
|  | No learning | 1300 | 430 | 130 | 100 | 43 | 13 | 4.3 | 1.3 | 0.43 |
| Observed | 4 | 11 | 9 | 6 | 5 | 4 | 3 | 1 | 1 | 0 |
| Simulation | 4 | 11 | 8 | 6 | 5 | 5 | 5 | 3 | 1 | 1 |

> xmulti(kirschvink\_15b\_ps,R\_sim\_15bees,detail = 3,histobins = T,safety = 1e+10)

P value (LLR) = 0.9162

P value (Prob) = 0.9765

P value (Chisq) = 0.9578

Observed: 4 11 9 6 5 4 3 1 1 0

Expected ratio: 4 11 8 6 5 5 5 3 1 1

Total number of tables: 4431613550

We also re-analyzed the results from Walker and Bitterman (1989). The results of the analysis are below.

**9 bees, dc field, data points are independent**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Magnetic field strength (μT) | | | | | | | | |  |  |  |
|  | No learning | 1200 | 120 | 12 | 5.6 | 2.6 | 1.2 | 0.56 | 0.26 | 0.12 | 0.056 | 0.026 | 0.012 |
| Original | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 0 |
| Expected | 0.67 | 0.22 | 0.07 | 0.02 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

> xmulti(walker,expected,detail = 3,histobins = T)

P value (LLR) = 5.472e-26

P value (Prob) = 1.065e-26

P value (Chisq) = 1.345e-05

Observed: 0 0 0 0 0 2 1 2 1 1 1 1 0

Expected ratio: 0.673 0.220071 0.07196322 0.02353197 0.007694955 0.00251625 0.0008228138 0.0002690601 8.798266e-05 2.877033e-05 9.407898e-06 3.076383e-06 1.494765e-06

Total number of tables: 293930