Data Sheet 1

*Experimental design:* Weiss devised two treatments for rearing caterpillars in individual containers where they could build shelters: a “clean” treatment where frass (caterpillar poop) was removed daily from the caterpillar’s container and a “frassy” treatment which allowed frass to accumulate in the caterpillar’s container throughout development. She measured the mass of 61 caterpillars in Year 1 (~30 in each treatment) and 50 caterpillars in Year 2 (25 in each treatment) throughout development (from five days to pupation) to create development curves assessing whether there was any fitness cost to living in “frassy” conditions.

*Figure 1.* Mean caterpillar mass ± 1 standard error of caterpillars raised under “clean” vs. “frassy” treatments. Weiss used a Repeated Measures Analysis of Variance to statistically analyze the mass of caterpillars from each treatment (Year 1: F=0.851, n=61, p > 0.05; Year 2: F=1.37, n=50, p > 0.05). *Source:* Weiss, M. R. (2003). Good housekeeping: Why do shelter-dwelling caterpillars fling their frass? *Ecology Letters* 6(4), 361–370. doi:10.1046/j.1461-0248.2003.00442.x. Used with permission of Wiley and Sons.
Data Sheet 2

Experimental Design: Weiss removed caterpillars from established leaf rolls at different frequencies to determine if there were any energetic costs to creating excess shelters that resulted in overall fitness costs. Weiss measured a number of variables that relate to lifetime fitness in the field: time to pupation, pupal weight, larval mass gain, and survival under these three removal frequencies. Caterpillars were removed from their shelters at four different frequencies: control (undisturbed), 50% (every 4 days), 100% (every 3 days), or 200% (every day) more often than the control treatment. This manipulation simulates a caterpillar abandoning their shelter because of space limitations.

Table 1: Number of shelters constructed and fitness measures in control and treatment (different frequencies of caterpillar removal from shelters). All measures besides survival to pupation are represented as mean ± standard error. Weiss used a two-tailed t-test to compare means between the control and each treatment: number of shelters constructed, days to pupation, larval weight gain, and pupal mass. For survival to pupation, Weiss used a chi-square test. Significant differences between control and treatment are represented by an asterisk.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of shelters constructed</th>
<th>Days to pupation</th>
<th>Larval weight gain, days 6–31 (g)</th>
<th>Pupal mass (g)</th>
<th>Survived to pupation</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.67 ± 0.34</td>
<td>33.07 ± 1.16</td>
<td>0.76 ± 0.09</td>
<td>0.72 ± 0.02</td>
<td>15 of 17</td>
</tr>
<tr>
<td>Increase</td>
<td>11.94 ± 0.32**</td>
<td>33.52 ± 1.09</td>
<td>0.78 ± 0.09</td>
<td>0.74 ± 0.02</td>
<td>17 of 19</td>
</tr>
<tr>
<td>100%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.84 ± 0.38</td>
<td>44.58 ± 0.95</td>
<td>0.78 ± 0.07</td>
<td>0.83 ± 0.03</td>
<td>19 of 33</td>
</tr>
<tr>
<td>Increase</td>
<td>15.76 ± 0.37**</td>
<td>44.24 ± 0.91</td>
<td>0.64 ± 0.06</td>
<td>0.78 ± 0.03</td>
<td>21 of 33</td>
</tr>
<tr>
<td>200%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.20 ± 0.73</td>
<td>43.67 ± 0.91</td>
<td>0.61 ± 0.07</td>
<td>0.83 ± 0.02</td>
<td>15 of 33</td>
</tr>
<tr>
<td>Increase</td>
<td>31.89 ± 0.69**</td>
<td>44.50 ± 0.86</td>
<td>0.56 ± 0.07</td>
<td>0.70 ± 0.02**</td>
<td>17 of 34</td>
</tr>
</tbody>
</table>

Asterisks indicate significant differences between control and increase treatments within a disturbance level. *P < 0.05; **P < 0.001.

Data Sheet 3

Experimental Design: Weiss conducted an experiment to test whether predators could be attracted by the smell of frass. She created artificial shelters similar to those of skipper caterpillars and placed either 6 frass pellets or 6 beads designed to look like frass in the shelter before placing a caterpillar in the shelter and presenting it to wasps, a natural predator of skipper caterpillars. Each wasp had to individually choose between a larva in a shelter with its frass and a larva in a shelter with pellets to visually imitate the frass (but not the smell). She recorded the number of times the wasp visited each shelter and time spent at each shelter for 10 different wasps.

![Graph showing comparison between average percent time spent by wasps per trial in bead shelter vs. frass shelter](image1)

Figure 2. Mean percentage of time spent ± 1 standard error on bead shelter and leaflet vs. frass shelter and leaflet (t-test=7.518, n=10, P < 0.001).

![Graph showing comparison between average percent visits per trial in bead shelter vs. frass shelter](image2)

Figure 3. Mean percentage of visits ± 1 standard error to bead shelter and leaflet vs. frass shelter and leaflet (t-test=6.417, n=10, P < 0.001).

Questions

1. Propose multiple hypotheses for why poop shooting behavior in caterpillars may be adaptive and design experiments to test these hypotheses. Be sure to identify the adaptation, fitness measure, and selective pressure in your experimental question.

2. You will be given the methods for three experiments Dr. Weiss conducted to determine why poop shooting may be adaptive. She proposed three main hypotheses: hygiene (frass in shelters changes survival), crowding (building new shelters to replace frass-filled ones uses energy), and natural enemies (frass attracts predators). For each experiment answer the following questions:
   a. Which hypothesis does this test?
   b. What is/are the measure/s of fitness in this experiment? Is this a direct measurement of fitness?
   c. How does this experiment address the hypothesis?
   d. Do the statistical results testing this hypothesis provide evidence that the behavior is adaptive? (Think about the statistical null hypothesis.)
   e. What does this experiment mean in a larger framework?

3. There were some statistically significant results in the crowding hypothesis. Given what you know, is this finding biologically relevant?

4. Are all traits a result of evolutionary adaptation?

5. Is caterpillar poop-shooting a perfect adaptation?