**Rearing protocol:**

1. Set up eggs from the colony, put on diet, and put at 25C until hatching. We want to do this in batches, so we don’t have too many bugs to take care of all at once.
2. Check eggs every day for new hatchlings. Gently transfer new hatchlings with a paintbrush to a large, shallow petri dish with diet. Label with the experiment name, the date of hatching, and the rearing temperature (25+/-10). Put in the 25+/-1o chamber.
3. Check hatchling dishes every day for newly molted 3rd instars. Also check for food freshness, and replace as needed.
4. Upon molt to the 3rd instar, caterpillars will enter the experiment, get an individual ID number, be parasitized, and be divided into a heatshock treatment group. (See Experimental protocol below)
5. Check all bins (in the rearing and both heat shock chambers) daily for new molts, food, and wasp emergence. New molts will be weighed, and the data recorded in the appropriate column (see Experimental Protocol below). Sometimes a caterpillars will not successfully shed its cuticle after a molt. If this happens, carefully remove the old cuticle with forceps and dissection scissors (ask Christina or Elizabeth if you need help). If any caterpillars are low on food or their food has gone bad (dried out, moldy, etc), replace with new diet. Also dump the old frass out of the petri dish. If wasps are emerging, following the Experimental protocol below.
6. Make any notes about general REARING in the gen.notes column (NOT the diss.notes column).

**Experimental Protocol:**

There is one rearing temperature regime (25+/-10), and two heat shock regimes (30+/-10 == heat shock of 40C; 30+/-12 == heat shock of 42). Caterpillars will stay in the heat shock treatments for either 1, 2, 3 or 4 days. So, there are 8 treatments of heat shock (40--1, 2, 3 or 4 days; 42--1, 2, 3 or 4 days), plus one control treatment that will stay at 25+/-10 (for a total of 9).

1. Once a caterpillar has molted to the 3rd instar, give it a unique ID number and put in a small petri dish. Write the ID, the date at 3rd, and the treatment group in the bottom of the petri dish--also record this in the data sheet. Weigh the caterpillar, and record the mass in the mass.3 column. Record the date in the date.3 column, and the time you weighed it in the time.3 column. Give the caterpillar a chunk of diet large enough to last at least 24 hours
2. Every day, divide the new 3rds as evenly as possible across all 9 treatments. Record the heat shock temperature in the hs.temp column (either 40, 42, or 0). Record the number of days in the heat shock treatment in the hs.num column (as 1, 2, 3, 4 or 0).
3. After weighing the caterpillar, take it to the wasp colony and parasitize it. If you haven’t parasitized 3rd instars before, have Elizabeth or Christina show you how. Try to only have the caterpillar oviposited in ONCE. Record the date of oviposition (date.ovp), the time of oviposition (time.ovp) and the number of ovipositions (num.ovp). Write the number of ovipositions in the bottom of the petri dish.
4. Separate caterpillars by treatment group, and put into plastic bins. Label the bins with the heat shock temperature and the number of heat shocks. Label the date that they should be put in the heat shock (same day as oviposition), and the date that they should be removed from heat shock. Put in the heat shock chambers BEFORE NOON!! Ideally it should be around 11am.
   * Caterpillars that are in the 1 day treatment group will need to be removed from the heat shock chamber the same day that they are put in (~4-5pm). All other caterpillars will be removed on the afternoon of the 2nd, 3rd, or 4th day after being put in the heat shock chamber.
   * Record the date and time that caterpillars were put into the heat shock treatment (date.hs.in; time.hs.in), as well as the date and time when they are removed. (date.hs.out; time.hs.out) ALWAYS record times as 24:00 (1pm==13:00, 2pm==14:00, etc)
5. After the completion of the heat shock experiment, return caterpillars to the 25+/-10 chamber. Continue to check for molting and food until wasp emergence OR 2 weeks from molt to the 5th instar.
6. At wasp emergence, remove caterpillar and gently place in a clean small petri dish. Label the new dish with ALL writing from the old dish. If any wasp larvae have fallen off the caterpillar, GENTLY move to the new petri dish with forceps. Record the date and time of emergence, the instar of the caterpillar and whether or not it bled (0 if you see no hemolymph, 1 if you do). Label the lid of the petri dish with “Count Cocoons \_\_\_\_”, where \_\_\_\_ is the date 2 days after wasp emergence (EX: if wasps emerge 1/22, cocoons should be counted 1/24). Do not give the caterpillar any food, as it will not eat.
7. 2 days after wasp emergence, use a clicker to count the number of wasp larvae that spun cocoons (num.coc), as well as the number that failed to (num.fail.spin). Put all cocoons in a clean condiment cup. Record the caterpillar ID, the treatment, the date of emergence, and the number of cocoons on the lid of the condiment cup. Return to the 25+/-10 chamber.
   * Label the lid of the petri dish of the caterpillar with ‘EHS’, tape closed, and put in the appropriate freezer and bin. These will be dissected later to determine their load
8. Check cups of cocoons every day for adult eclosion. Once you see eclosion, record it in the date.ecl column (put the time in the time.ecl column). Write “Freeze \_\_\_\_” on the lid, where \_\_\_\_ is the next day’s date. (EX: If wasps eclose on 1/22, they would be frozen on 1/23). Freeze any cups of wasps that need to be frozen. After at least 30 minutes to 1 hour, count the number of adult wasps and record in the num.ecl column.
9. For those caterpillars without wasp emergence in ~5-7 days after caterpillar molt to the 5th instar, we will keep under observation for 2 weeks after the molt to 5th. Continue to feed them, and check for molts to 6th (note the date and mass in the gen.notes column). After 2 weeks, if there is still no wasp emergence, weigh the caterpillar. Record the date in the date.end column, and the mass in the mass.end column.
   * If the caterpillar lived 2 weeks after the molt to 5th with no wasp emergence, put a 1 in the mongo.age column. If it had wasp emergence, put a 0.
   * If the caterpillar weighed >14g after 2 weeks, put a 1 in the mongo.mass column. If not, put a 0.
   * After weighing and recording the appropriate data, remove any food or frass in the petri dish. Label the lid with ‘EHS’, tape the lid, and put in the appropriate freezer and bin.