

Temperature adaptation experiment – Spring 2017 – “USA C”

Date

Sat 25 Feb:

Recharged SI USA populations: created 2 jars each for #1 (#1A & #1B) and #2 (#2A & #2B), each with 250 g mung beans and approx. 250-350 beetles.

Thurs 16 Mar:

By day 19: transfer fertilized beans from USA C#1B and #2B into 30-40 48-well plates of virgin chambers (VCs). This entire experiment will take place only at C conditions.

Fri 17 Mar – Tues 21 Mar:

How many ♂s and ♀s are hatching each day? If less than 50 of each, then use these beetles for the body size assay – for each treatment, need to weigh 48 newly hatched ♂s and ♀s (weigh sexes separately, in groups of 24, in pre-weighed 24-well VCs). Use equal numbers from each parent.

Tues 21 Mar:

On day 24, there should be a sufficient number of 1-day old beetles for the fitness assays. Randomly pair 50 ♂s and ♀s and place in 60-mm petri with 70 beans (“Day 0”). Make sure to use equal numbers of offspring per VC

Weds 22 Mar:

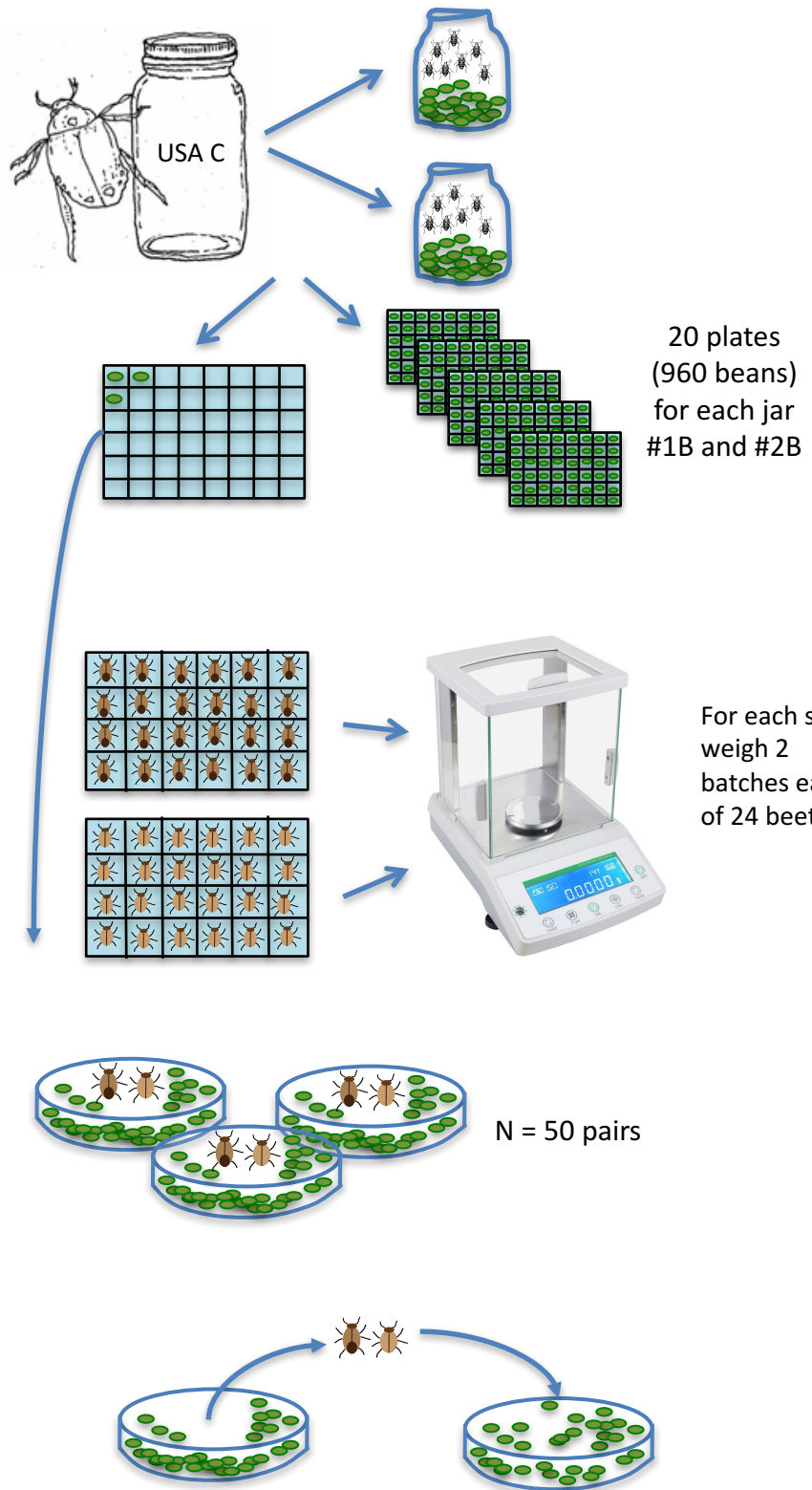
One day after pairing, move all ♂s and ♀s to new 60-mm petris with 60 beans (“Day 1”). Keep “Day 0” dishes.

Thurs 23 Mar:

2 days after pairing, move all ♂s and ♀s to new 60-mm petris with 50 beans (“Day 2”)

Fri 24 Mar:

3 days after pairing, move all ♂s and ♀s to new 60-mm petris with 40 beans (“Day 3+”). Leave there.



To quantify development (# days it takes for egg to develop into adult), must keep track of when eggs were laid. Thus, need to move pairs to new petris each day for 3 days. We will end up with 4 dishes per pair, eggs laid Day 0, Day 1, Day 2, and Days 3+.

Sun 9 Apr:

By day 19 of “Day 0” dishes, put all beans into VCs and monitor daily. Mark the date of hatch and sex of all offspring.

Mon 10 Apr:

By day 19 of “Day 1” dishes, put all beans into VCs and monitor daily. Mark the date of hatch and sex.

Tues 11 Apr:

By day 19 of “Day 2” dishes, put all beans into VCs and monitor daily. Mark the date of hatch and sex.

Weds 12 Apr:

By day 19 of “Day 3+” dishes, put all beans into VCs and monitor daily. Mark the date of hatch and sex.

Temperature adaptation experiment – Spring 2017 – “USA C to I”

Date

Tues 10 Jan:

Recharged SI USA populations: created 2 jars each for #1 (#1A & #1B) and #2 (#2A & #2B), each with 250 g mung beans and approx. 250-350 beetles.

Sun 29 Jan:

Day 19: transfer fertilized beans from USA C#1B and #2B into 20 48-well plates of virgin chambers (VCs) and leave in Control chamber.

Mon 30 Jan - Fri 3 Feb:

Monitor hatch. When have 60 ♂s and ♀s hatched (3 Feb), pair them randomly in 60 60-mm petris with 100 beans. Put all dishes into India chamber.

Mon 6 Feb:

Remove all pairs from petris after 72 hours. Leave petris in India.

Weds 22 Feb:

By day 19, choose 50 best petris and put 48 fertilized beans from each into individuals VCs.

Thurs 23 Feb – Mon 27 Feb:

Monitor hatch daily. When have 1 ♂ and 1 ♀ from each VC, randomly pair ♂s and ♀s and put into 50-mm petris with 80 beans each.

Thurs 2 Mar:

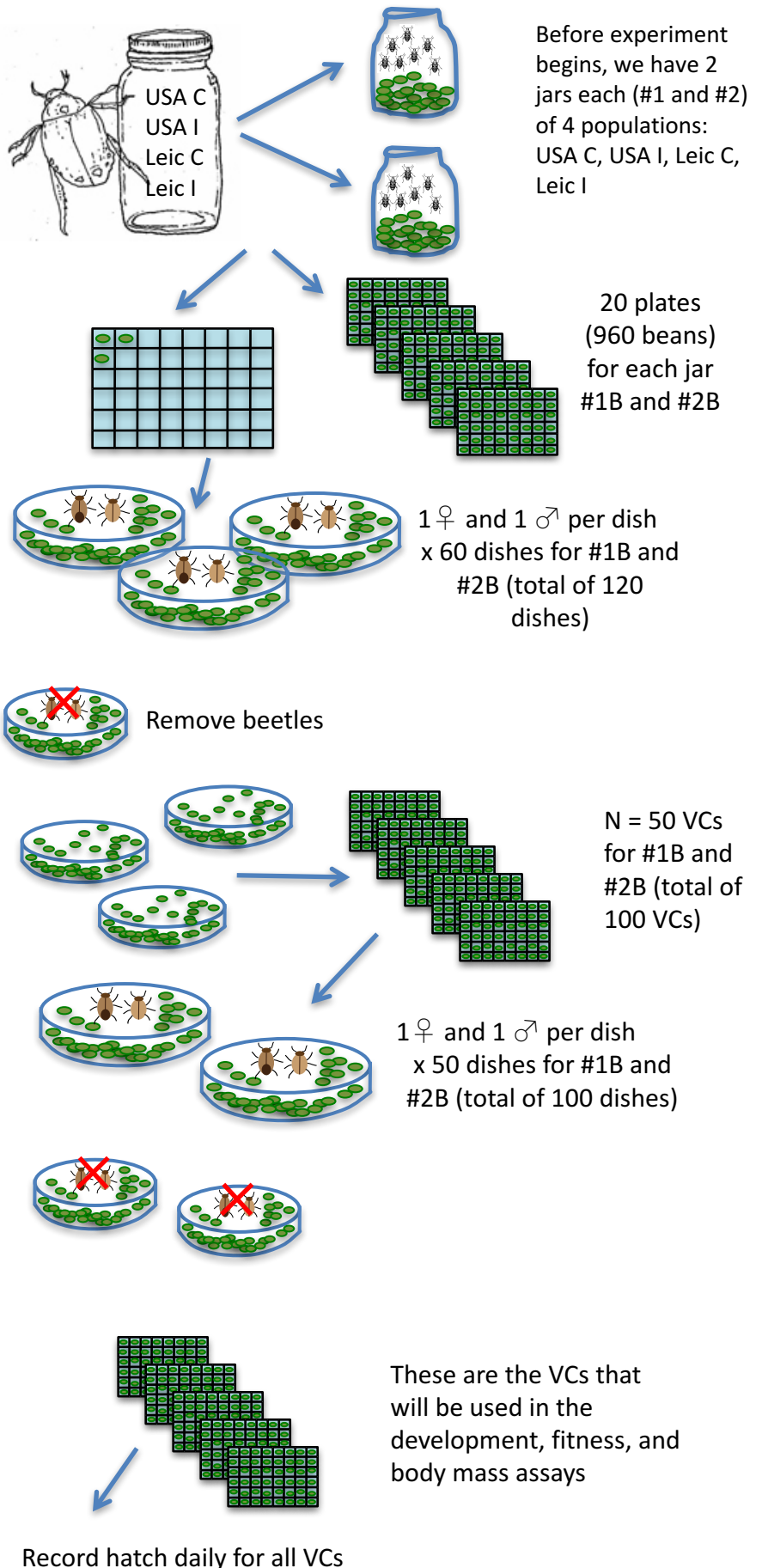
Remove adult beetles from dishes after 72 hours. Return petris to India.

Sat 18 Mar:

By day 19, transfer beans into one 48-well VC per pair (50 VCs)

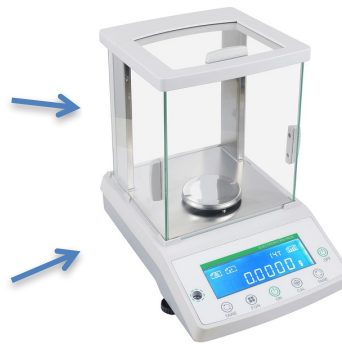
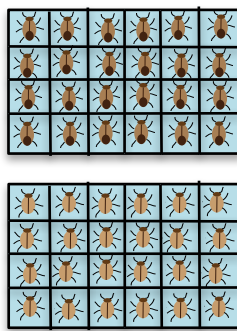
Sun 19 Mar – Thurs 23 Mar:

Monitor hatch daily, marking the date of hatch and sex on the VCs.



Tues 21 Mar or Thurs 23 Mar:

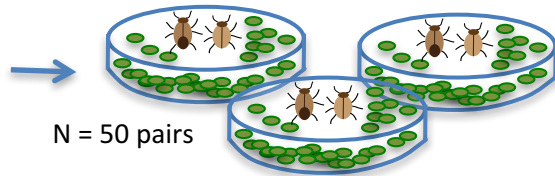
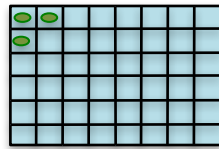
How many ♂s and ♀s are hatching each day? If less than 50 of each, then use these beetles for the body size assay – for each treatment, need to weigh 48 newly hatched ♂s and ♀s (weigh sexes separately, in groups of 24, in pre-weighed 24-well VCs). Use equal numbers from each parent.



For each sex, weigh 2 batches each of 24 beetles

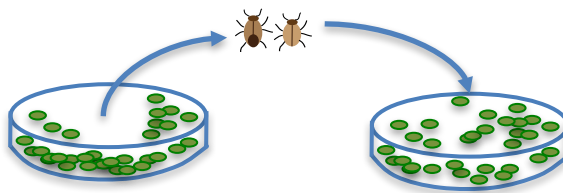
Thurs 23 Mar:

On day 24, there should be a sufficient number of 1-day old beetles for the fitness assays. Randomly pair 50 ♂s and ♀s and place in 60-mm petri with 70 beans (“Day 0”). Make sure to use equal numbers of offspring per VC



Thurs Fri 24 Mar:

One day after pairing, move all ♂s and ♀s to new 60-mm petris with 60 beans (“Day 1”). Keep “Day 0” dishes.



Sat 25 Mar:

2 days after pairing, move all ♂s and ♀s to new 60-mm petris with 50 beans (“Day 2”)

Sun 26 Mar:

3 days after pairing, move all ♂s and ♀s to new 60-mm petris with 40 beans (“Day 3+”). Leave there.

Tues 11 Apr:

By day 19 of “Day 0” dishes, put all beans into VCs and monitor daily. Mark the date of hatch and sex of all offspring.

Weds 12 Apr:

By day 19 of “Day 1” dishes, put all beans into VCs and monitor daily. Mark the date of hatch and sex.

Thurs 13 Apr:

By day 19 of “Day 2” dishes, put all beans into VCs and monitor daily. Mark the date of hatch and sex.

Fri 14 Apr:

By day 19 of “Day 3+” dishes, put all beans into VCs and monitor daily. Mark the date of hatch and sex.

To quantify development (# days it takes for egg to develop into adult), must keep track of when eggs were laid. Thus, need to move pairs to new petris each day for 3 days. We will end up with 4 dishes per pair, eggs laid Day 0, Day 1, Day 2, and Days 3+.

Temperature adaptation experiment – Spring 2017 – “USA I to C”

Date

Fri 10 Feb:

Recharged SI USA populations: 2 jars each for #1 (#1A & #1B) and #2 (#2A & #2B), each with 250 g mung beans and approx. 250-350 beetles.

Mon 6 Feb:

Day 20: transfer fertilized beans from USA I#1B and #2B into 20+ 48-well plates of virgin chambers (VCs) and leave in India chamber.

Tues 7 Feb – Fri 10 Feb:

Monitor hatch. When have 60 ♂ and ♀s hatched, pair them randomly in 60 60-mm petris with 100 beans. Put all dishes into Control chamber.

Fri 10 Feb - Mon 13 Feb:

Make note of dishes with dead beetles. Remove all pairs from petris after 72 hours. Leave petris in Control.

Weds 1 Mar:

By day 19, choose 50 best petris and put 48 fertilized beans from each into individuals VCs.

Thurs 2 Mar – Mon 6 Mar:

Monitor hatch daily. When have 1 ♂ and 1 ♀ from each VC, randomly pair ♂s and ♀s and put into 50-mm petris with 80 beans each.

Thurs 9 Mar:

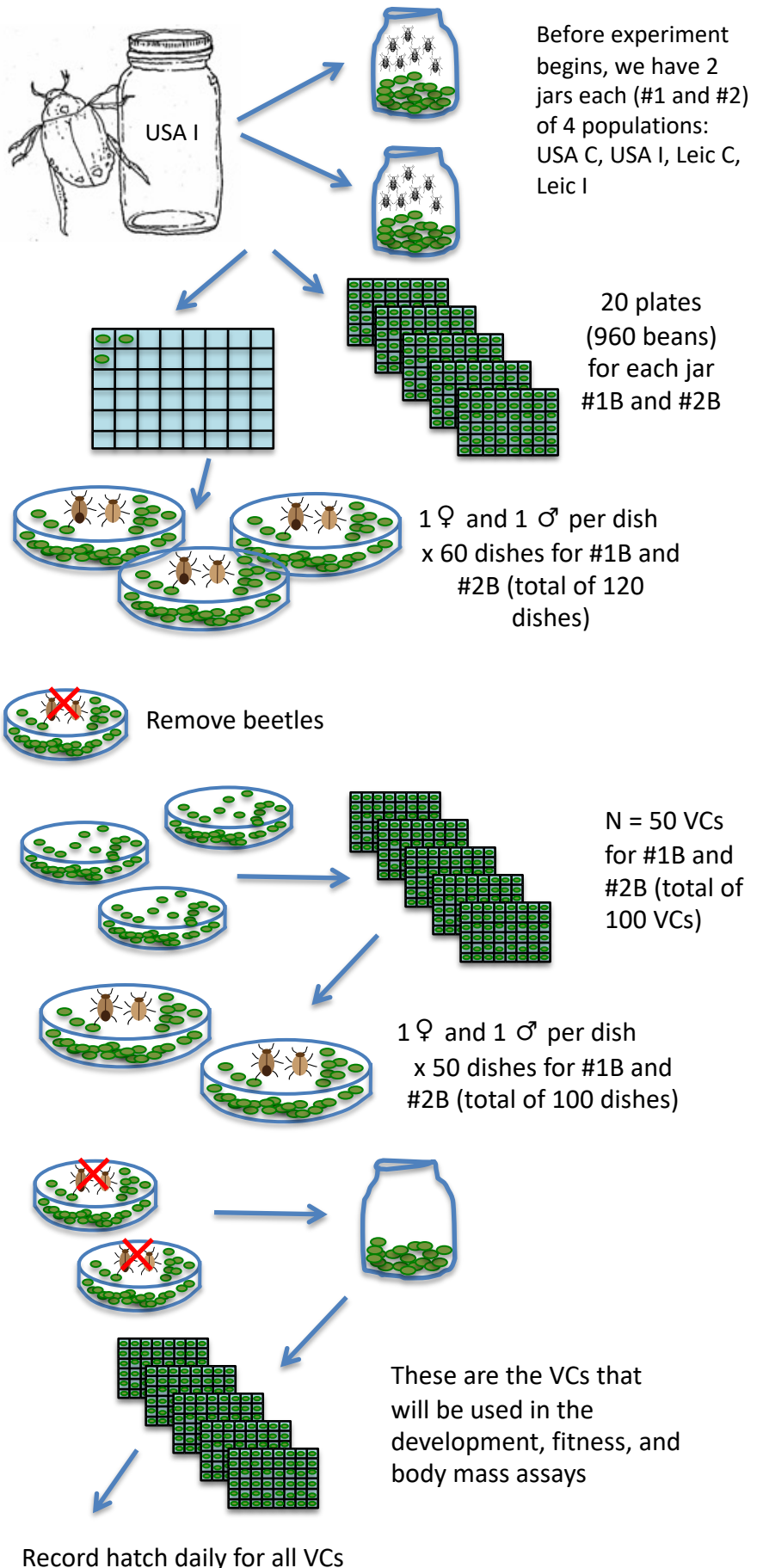
Remove beetles from dishes after 72 hours. Dump equal numbers of fertilized beans from each dish into a single jar and allow to develop.

Sat 11 Mar:

On day 19, move all of the beans into VCs (cannot know now how many VCs)

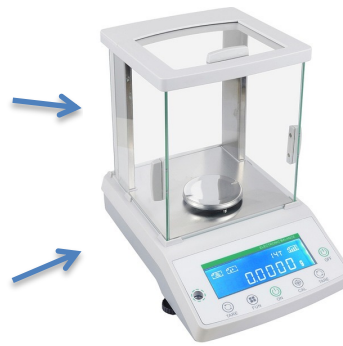
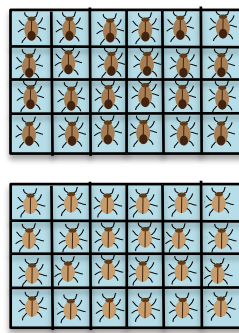
Sun 12 Mar – Weds 15 Mar:

Monitor hatch daily, marking the date of hatch and sex on the VCs.



Mon 13 Mar or Tues 14 Mar:

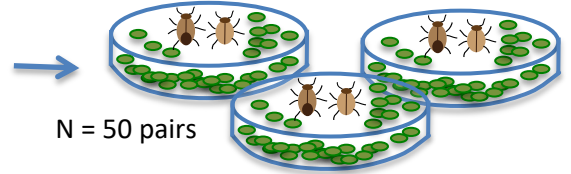
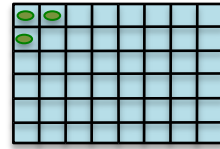
How many ♂s and ♀s are hatching each day? If less than 50 of each, then use these beetles for the body size assay – for each treatment, need to weigh 48 newly hatched ♂s and ♀s (weigh sexes separately, in groups of 24, in pre-weighed 24-well VCs).



For each sex, weigh 2 batches each of 24 beetles

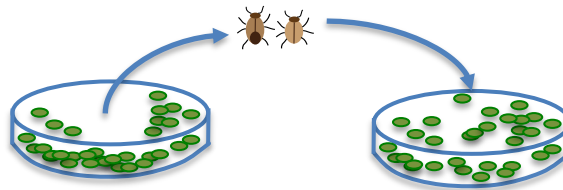
Weds 15 Mar:

On day 23, there should be a sufficient number of 1-day old beetles for the fitness assays. Randomly pair 50 ♂s and ♀s and place in 60-mm petri with 70 beans.



Thurs 16 Mar:

One day after pairing, move all ♂s and ♀s to new 60-mm petris with 60 beans.



Fri 17 Mar:

2 days after pairing, move all ♂s and ♀s to new 60-mm petris with 50 beans. Keep original dishes.

Sat 18 Mar:

3 days after pairing, move all ♂s and ♀s to new 60-mm petris with 40 beans. Leave there.

Thurs 20 Apr:

Freeze Day 0, Day 1, and Day 2 dishes (from 15-17 Mar). This corresponds to day 34-36.

Mon 24 Apr:

Freeze Day 1 dishes (from 16 Mar, day 37)

To quantify development (# days it takes for egg to develop into adult), must keep track of when eggs were laid. Thus, need to move pairs to new petris each day for 3 days. We will end up with 4 dishes per pair, eggs laid Day 0, Day 1, Day 2, and Days 3+.

8 treatment groups (2 replicates for each = 16 in total)

SI USA Control to India Acclimatized (USA C to I #1 and #2)

Callosobruchus maculatus beetles from the SI USA lab strain that have evolved at 29°C and then are kept for 2 generations under India conditions, without selection, before running body size, fitness, and development assays.

SI USA India to Control Acclimatized (USA I to C)

Beetles that have evolved at India conditions (temperatures fluctuating around mean of 33°C) and then are kept for 2 generations at 29°C, without selection, before running assays.

SI USA Control (USA C)

Beetles that have evolved at 29°C and are then assayed at 29°C.

SI USA India (USA I)

Beetles that have evolved at India conditions and are then assayed at India conditions.

SI Leicester Control to India Acclimatized (Leic C to I)

Callosobruchus maculatus beetles from the SI Leicester lab strain that have evolved at 29°C and then are kept for 2 generations under India conditions, without selection, before running body size, fitness, and development assays.

SI Leicester India to Control Acclimatized (Leic I to C)

Beetles that have evolved at India conditions (temperatures fluctuating around mean of 33°C) and then are kept for 2 generations at 29°C, without selection, before running assays.

SI Leicester Control (Leic C)

Beetles that have evolved at 29°C and are then assayed at 29°C.

SI Leicester India (Leic I)

Beetles that have evolved at India conditions and are then assayed at India conditions.

USA

Leicester