

Male Gain Curve Methods

Gain Curve

	Anthers/flower Plants	Anthers/Array
1	30	30
2	15	30
3	10	30
4	7	28
5	6	30
6	5	30

- Focal flower in centre with GUS marker
- 1, 3, 5 flowers/plant
- Array enclosed with a single, actively foraging bee at a time
- 4 hours of continuous foraging, with 2 15-minute observation periods per hour
- Anther collected from focal flower before and after foraging
- All stigmas collected next morning
- Stigmas stained for GUS marker and counted with light microscopy
- Fruits harvested once mature and seeds counted

Pollen Shadow

- Focal flower with GUS marker in centre of 24 plant array
- Focal flower with 2, 3, 4, 5 anthers compared with each of larger and smaller background
- Active foraging by pollinator until 24 visits to the focal flower
- All pollinator activity from the focal flower monitored until a full minute elapsed without foraging
- Stigmas of all flowers harvested next morning
- Stained for GUS marker and counted with light microscopy
- Seeds not collected

GUS Stain

Modified from Plant Physiol. 2002 130:1747-1753 with much help from Nam-il Park in Doug Muench's lab

Stock solution consisting of:

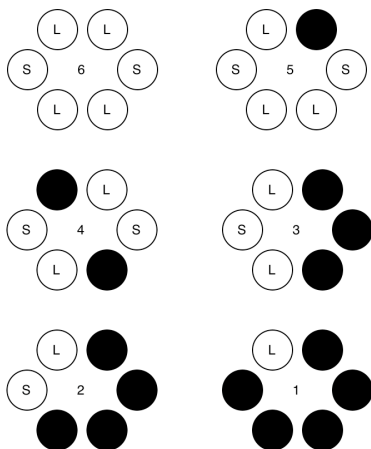
- 1 mL NaK
- 0.2 mL EDTA

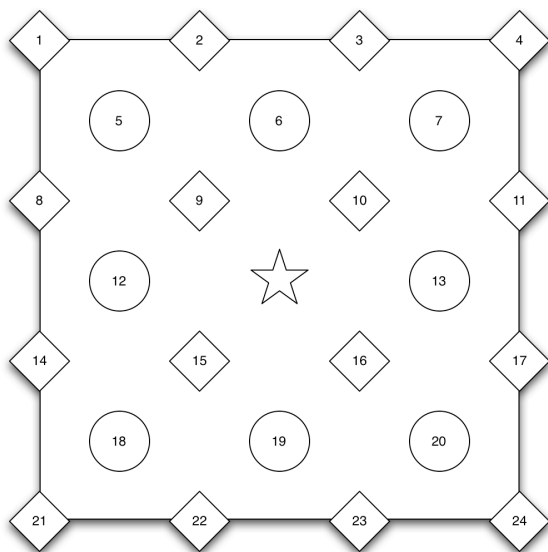
- 0.1 mL Triton
- 40 μL each of $\text{K}_3\text{Fe}(\text{CN})_6$ & $\text{K}_4\text{Fe}(\text{CN})_6$
- 8.7 mL dH_2O

which makes 10 mL. This is allocated as 0.5 mL volumes into 1 mL microcentrifuge tubes.

To activate the stock solutions:

1. add 0.001g of X-gluc to an empty centrifuge tube
2. set a 20 μL pipette to 12 μL and a 200 μL pipette to 74 μL
3. add 2x12 μL of dimethylforminade to the X-gluc and vortex
4. add 12 μL of the X-gluc dimethylforminade solution to each tube of stock solution and vortex
5. add 74 μL of the activated solution to each sample and ensure that the samples are submerged
6. incubate for an hour





Plant arrangement for the male gain curve experiment