

Jones, P. L. and Agrawal, A. A. 2019. Beyond preference and performance: host plant selection by monarch butterflies, *Danaus plexippus*. – Oikos doi: 10.1111/oik.06001

## Appendix 1

### Plant rearing

Experiments were conducted in the summers of 2015 and 2016 in Ithaca, NY. We reared plants under similar conditions for all of the experiments. *A. perennis* seeds were from a lineage of wild plants collected in Florida. *A. curassavica* seeds were obtained commercially from Everwilde Farms (WI, USA). *A. i. pulchra* seeds were from plants grown outdoors in Ithaca, NY (original seeds collected in New Jersey). *A. i. incarnata* seeds were from wild plants in Danby, NY.

### Monarch sources

Monarchs were purchased from two commercial breeders, one in Massachusetts that rears wild-caught butterflies on *Gomphocarpus physocarpa* or *G. fruticosa* and *A. syriaca* for <4 generations, and one in Florida that rears butterflies over many generations on *A. curassavica*, *Calatropis gigantea* and *C. procera*. In some experiments, monarch lines originally obtained from these sources were additionally reared in our laboratory (third source) for multiple generations on *A. syriaca* (oviposition preference choice test) or a combination of *A. i. incarnata* and *A. i. pulchra* (effect of previous oviposition experience experiment). All monarchs, whether arriving from commercial sources or reared in our lab were tested for the presence of spores of the protozoan parasite *Ophryocystis elektroscirrha* (Oe) by applying a 1 cm<sup>2</sup> piece of clear tape to each side of the abdomen and examining the piece of tape and attached scales for spores of Oe. Any butterflies with detectable Oe spores were immediately frozen and not used in experiments. Butterflies clear of Oe were placed in a 2 × 2 × 3m cage in the field for >5 days to have the opportunity to mate, provided with feeder dishes filled with Gatorade but no milkweed plants.

### Oviposition preference

In the summer of 2015 we examined the oviposition preferences of 16 monarch butterflies from three sources (our lab colony n = 3, and the commercial breeders in FL n = 6, and MA n = 7, USA). Monarchs in our lab colony were originally from the MA and FL sources and then allowed to interbreed in our lab for a few generations. To reduce any effect of plant flowers on oviposition preferences in this experiment, the day prior to testing we removed flowers from plants that were flowering and a leaf from non-flowering plants to control for clipping of flowers. Plants were all the

same age but differed in growth rates resulting in different plant heights at testing. *A. perennis* was the smallest averaging 40 cm tall, *A. curassavica* averaged 49 cm tall, *A. i. incarnata* 65 cm tall, and *A. i. pulchra* 55 cm tall. We tested each of the females in individual 1 m<sup>3</sup> field cages with a feeder dish with Gatorade, and four plants (one of each species). Females were allowed to lay eggs for one hour. Four females (2 from our lab colony, 1 from MA and 1 from FL) laid no eggs and were excluded. The remaining females (n = 12) were given a new set of four plants in a different configuration and laid for another hour. We analyzed number of eggs laid on each host plant by each of the 12 females for the combined 2 hours.

### Previous oviposition experience

We examined how experience ovipositing on a host plant affects later oviposition preferences in 2016. Freshly emerged adults obtained from the commercial breeders in FL and MA, and our own lab colony (reared on a combination of *A. i. incarnata* and *A. i. pulchra*), were placed in a mesh cage in the field with feeder dishes filled with Gatorade and non-milkweed flowering plants as nectar sources. No milkweed plants were available to the butterflies. After at least five days to have the opportunity to mate, females were randomly assigned to one of three treatments: experience with *A. i. pulchra* (n = 25: 11 from MA, 12 from our colony and 2 from FL), experience with *A. i. incarnata* (n = 21: 11 from MA, 8 from our colony, and 2 from FL), or no experience (n = 20; 8 from MA, 12 from our colony). During the tests females were also provided with feeder dishes filled with Gatorade.

### Competition effect with caterpillars and model adults

We examined the effect of the presence of caterpillars on female oviposition preferences using experiments in 1m<sup>3</sup> mesh cages in 2015. One female was placed in each cage and the experiment had three stages. Caterpillars used in the exposure phase were from our lab colony and were applied to plants 24-48 h before the experiment in order for them inflict damage on the plant leaves. We tested n = 12 females with the caterpillar on *A. i. pulchra* (6 from the FL source and 6 from the MA source) and n = 13 females with the caterpillar on *A. i. incarnata* (8 from the FL source and 5 from the MA source).