**Courtship under predation**

June 8, 2015

We will use flies from Ian’s 12 populations, evolved either under jumping spider predation (S), praying mantis predation (P), or no predation control (C) since spring 2011. We will mostly use 1-day old flies to reduce the magnitude of relaxation of anti-predatory behaviour in the currently no-predation setting. We will do all the early AM clearing under red LED (650 nm) and all fly collection by aspiration.

**2. Male courtship**

**Prediction**: A higher ratio of night-time to daytime courtship in the predation than in the control populations.

Conduct tests in 2 sessions, “night”, from 8 to 9 AM and “morning”, from 11 AM to noon. Transfer flies to test room in an opaque box or bag. Run the night session under red light and the day session under regular ceiling light.

Each vial will have 1 male and 2 females from the same population. Use 24 h old males and immature females.

**Day 1**. sex males and place individually in Ian food vials. Clear bottles at 4 PM (so max female age on day 2 at noon will be 20 h).

**Day 2**. Conduct tests. Each of 2 observers will have 3 successive sets of 4 vials.

**Fly needs**: 4 males and 8 females from each of the 12 lines.

**Collect**: 6 males (1 per vial) and 10 immature females (in 1 vial) from each of the 12 lines.

**Logistics**: 2 laptops with courtship program. Room B133B

**Vials**: 7\*12=84.

**Marking of vials**: 1-48 for males, 1-48 for females.

**Sample sizes:** we’ll have 2 full replicates per session per day. N=48 flies per day. 4 days= total of 96 for each session (dark and light); 8 males per population, 32 per trt, 196 total.

**Model**: Option 1: **dark / light activity as the dependent factor**, GLMM with day (random), population (random within trt); fixed: trt. A priori contrast: higher dark / light activity in 2 predation trt than control.

Option 2: **activity as the dependent factor**, GLMM with day (random), population (random within trt); fixed: trt, session, trt x session. 2 predictions: overall lower activity in predation than control; sig. trt x session interaction owing to higher dark activity in predation trts.