Developing Whole-Hive Pavlovian Conditioning to Promote Attraction of Apis mellifera to the Synthetic Scent of the Endangered Orchid Prosthechea cochleata for Conservation

**Background**

- Habitat fragmentation creates boundaries between populations causing:
  - Less visitation of plants by native pollinators and thus decreased genetic diversity and population size
  - Honeybees are efficient, but non-native and generalist pollinators:
    - Honeybees evaluate the quality of food sources as a hive, optimizing foraging by visiting sources with a high nectar and pollen yield
    - Did not co-evolve to forage from or pollinate native plants

Native pollinators are often the best suited for pollinating native plants but if their populations decline, it is important to investigate methods to encourage honeybees to aid in pollinating endangered plant species like *Prosthechea cochleata*

**Methods**

- **Setup:** EAG records electrical activity in antennae to determine strength of signals sent to the brain after contact with stimuli
  - Antenna creating single path for neuronal currents to flow through in EAG setup
  - Micro EAG measures changes in electrical responses of the antenna upon introduction of VOs

- **Stimuli:**
  - Introduce only filter paper (negative control), pseudocumene, nonanal, mesitylene, limonene, decanal, benzaldehyde, and α-pinene

- **Analysis of Data:**
  - Peaks indicate deviation from the resting state
  - Photoresistor helps align data

- **PER exhibition**
  - Wait synthetic scent of VOC over bee for 15 seconds and observe PER exhibition

**Stimulus**

- Wait synthetic scent/VOC or hexane (control group) over bee for 15 seconds

**Reward**

- Reward is a 50% (by weight) sucrose and water solution
- Introduce sucrose reward let sit for 10 minutes
- Repeat previous steps 5 times

**Repetition, Concentration, and Persistence:**

- Repeat Pavlovian conditioning in 6 groups conditioned 1-6 times to observe the impacts of repetition
- Feed bees with a mixture of synthetic scent and sucrose reward at various concentrations
  - Observe PER after 5 rounds of conditioning
  - Observe sustained PER after multiple unrewarding experiences and a singular round of conditioning

**Results**

- No measurement tool available in EAG software, so relative units (RU) used to quantify results with average control response=1 RU
- Revised EAG setup resulted in more reliable data with an R&R value of 16.78%
- Tukey-Kramer tests found 5 VOs yielded higher results than filter paper (p<0.05)
  - α-pinene and benzaldehyde results were not significantly different from filter paper (p>0.10)

- Logistic regression confirmed experimental group for 5 rounds of conditioning to be significantly higher than the experimental groups for 1,2,3, and 6 rounds of conditioning (p<0.05), but not 4 rounds

- Logistic regression confirmed 0.06g/10mL yields significantly higher PER exhibition than all other groups tested (p<0.05)

- Percent of bees that exhibited PER in response to repeated unrewarding experiences with synthetic scent after one feeding with 0.006g, 0.006, 0.006, 0.075, 0.15, 0.3, 0.6g hexane (left) or the synthetic scent (right) per 10mL of sucrose and water solution
  - Logistic regression confirmed 0.06g/10 mL elicits highest sustained PER exhibition of tested groups throughout each unrewarding exposure (p<0.05)

- Logistic regression confirmed 0.06g/10 mL elicits highest sustained PER exhibition of tested groups when averaged across unrewarding exposure (p<0.05), but is not statistically higher than 72 rounds at later unrewarding experiences

All images, figures, charts, and graphs were created by the researcher unless otherwise cited.