

Class mean *77%*

90-100	40
80-89	220
70-79	315
60-69	90
0-59	27

# Nest Recognition in Harvester Ants



# Objectives

1. Test the hypothesis that harvester ants can recognize their own nest soil, using quantitative and unbiased procedures to reject a null hypothesis.
2. Learn some basic statistical procedures for evaluating quantitative data and use them to test a hypothesis.
3. Observe the behavior of members of an animal species that forms very complex social organizations.

## Ants are social insects with specific roles to play...

- Haven't changed in last 35,000,000 years
- Drones \*, males are short lived, may fertilize 1 queen (provide set of genes)
  - \* Old English, "dron" parasites living on labor of others.
- Queen lays eggs (fertilized and unfertilized)
- Workers switch professions several times, soldier, forager, larva care

# Harvester ant nest

(gravel mounds in center of large clearings)

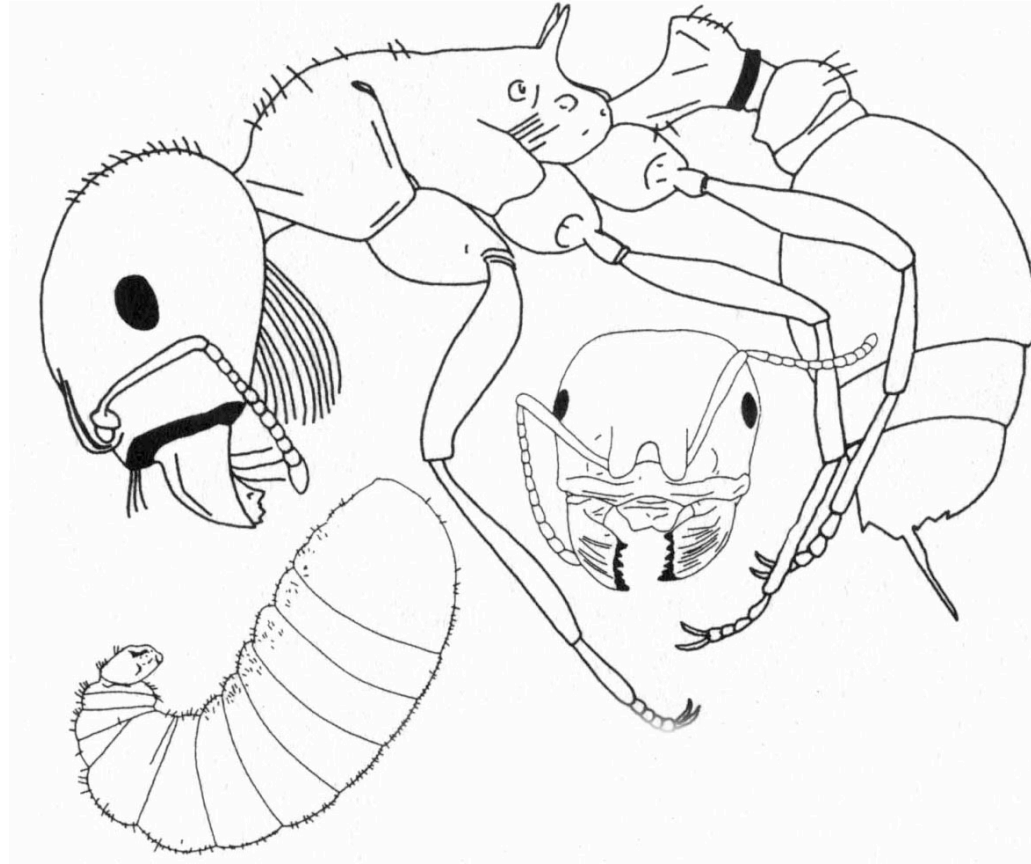




## Harvester Ants (*Pogonomyrmex barbatus*)



*Pogonomyrmex* {bearded ant}  
*barbatus* {hairs/setae on ventral surface of petiole}



Taber (1998). *The world of the Harvester Ant*. p.104



Worker carries a seed home



You discover wingless ants in your box of cookies. What is the sex of those ants?

- A. Males or drones
- B. Female
- C. A mixture of drones and workers

# Stages in life of ant nest

- Founding stage
- Exponential growth stage
- Maturity state
- Death of queen: end of nest

# Stages of Development

- Stored sperm used to fertilize some eggs.
- Eggs laid
  - 1N (unfertilized) eggs become winged males.
  - 2N (fertilized) eggs become females

# What determines fate of ant females?

- Females have similar or same genes.
- Environmental factors during larval development: temperature, food
- Physical condition of queen  
(secretions from healthy queen inhibit development of new queens)

# Metamorphosis among order Hymenoptera

- Egg hatches into grublike larva (feeding machine)
  - Female larva capable of mating become queen ants
  - Female larva not capable of mating become workers
- After a period of growth, larva changes into a pupa.
- Pupa transforms into an adult.

# Establishing a new colony

- Drone and virgin queen ants leave the nest ("marriage flight")
- Virgin winged queen ant mates with a single male
- Sperm cells transferred to seminal receptacle of queen.
- Male discarded (sperm can be stored for up to 15 years)
- Female finds a suitable environment to start new nest.
- Wings drop off or are chewed off.

## (honeymoon over)

- Queen's bulky wing muscles degenerate.
- Queen lays eggs.
- Newly hatched larva fed with salivary secretions.
- Small workers develop and begin foraging.
- Queen continues to lay eggs for up to 10-15 years.



# Life as a Queen

- Out of 1000 newly established nests, only 20-50 survive the first year.
- First year, queen uses stored sperm to produce several hundred workers.
- After five year, queen needs to produce 10,000 workers per year.
- Colony may last 15 years.
- Colonies wither away after queen dies

## Why should worker ants forego reproducing and work for the nest?

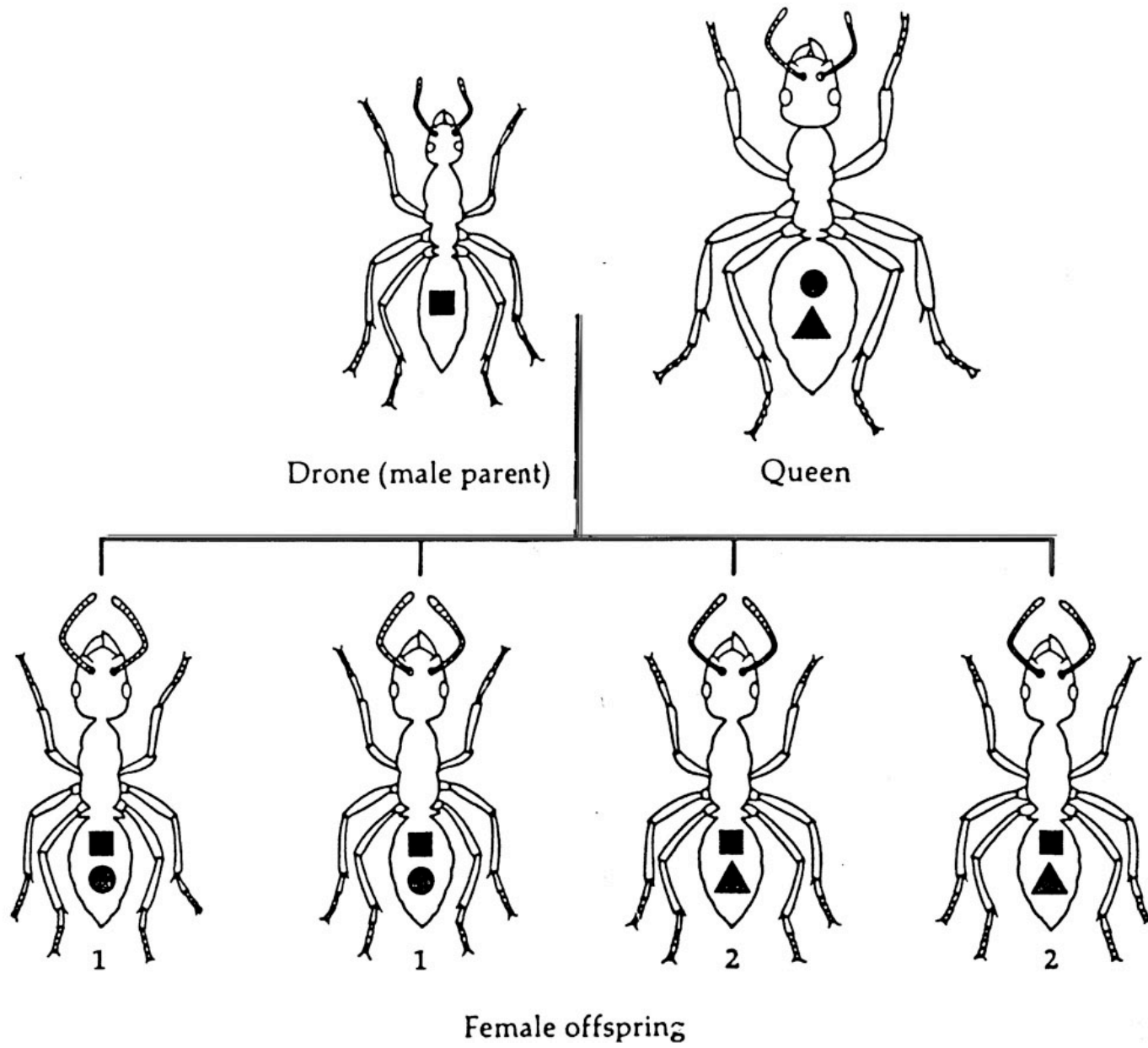
- Coefficient of relatedness
- Individual altruism can result in more genes identical to its own being passed on to the next generation.
- Sisters are more related to one another than to the queen.

# Genetics of Nest (haplodiploidy)

- Males
  - haploid, single allele for each gene
  - Pass same set to each offspring
- Queen
  - diploid, two alleles for each gene
  - Pass half to each of her offspring
- Sisters (workers & new queens)
  - 100% related or 50% related (average 75%)

# Summary of genetic relationships

- Workers (and future queens) are all sisters
- Sisters share 100% of same genes from dad
- Sisters share 50% of genes from queen
- Sisters on average share 75% of genes
- Sisters are more related to each other than to parents.



# Genetics of Ant nest summary

	SEX	Number of individuals	Chromosome number
Queen	Female	1	2N
Worker	Female	Numerous	2N
Drone	Male	Few	1N

# Pheromones

(ectohormone, sociohormone)

- Ants depend upon smell
- Detect smell with antennae
- Substances secreted externally in small quantities to elicit specific responses from members of the same species.

# Glands produce pheromones

- Affect other individuals of same species
- Sex attractants
- Recognition of same colony (social insects)
- Mark trail from nest to food



Pheromones mark trail from nest to food

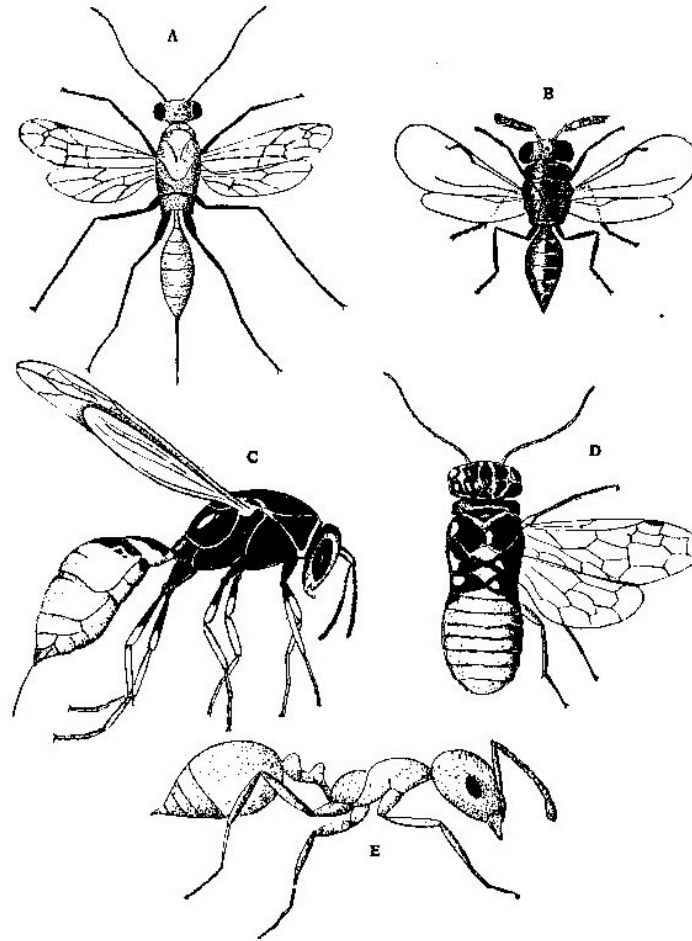


A = Pheromones      B= Hormones      C=Both

- Produced by glands?
- Small quantity secreted?
- Secreted internally?
- Secreted externally?
- Affects single organism?
- Affects many organisms?

# Order: Hymenoptera

Ovipositor can serve for sawing, piercing, or stinging



# Lab Safety

- Handle ants with care. They can sting!
- Toxicity measured as  $LD_{50}$
- The amount of toxic agent that is sufficient to kill 50% of a population of an animal within a certain time.
- Venom 100 times less toxic to arthropods than to mammals

# LD<sub>50</sub> Values in mice of toxins found in Hymenoptera (venom:neurotoxin, formic acid, proteins, peptides)

W. L. Meyer, W.L. (1996). Most toxic insect venom, *University of Florida Book of Insect Records*, University of Florida,

Common Name	LD <sub>50</sub> (mg/Kg)
Honey bees	2.5
Velvet ants	71
Paper wasps	2.4
Yellow jackets	3.5
Harvester ants ( <i>Pogonomyrmex</i> spp.)	0.66

# Lab Procedure

Investigate soil preference of ants

- Establish research question concerning ants' soil preference.
- Decide on a refutable Null hypothesis.
- Gather "blind" or unbiased observational data on ant behavior when test subjects are provided 2 soil samples.
- Statistically test the Null Hypothesis.
- Arrive at a conclusion based on the statistical test.
- **Goal is to reject the null hypothesis!**

# Null Hypothesis $H_0$

- $H_0$  states: The ants demonstrate no preference for either soil sample.
- Statistical procedure allows us only to reject the null hypothesis in favor of the alternative hypothesis,  $H_A$ .
- Rejection of the null hypothesis does not necessarily make an alternate hypothesis true.

# Wilcoxon Rank Sum test

- Obtain unbiased observations
  - Behavior of ants with 2 soil samples
    - Home soil vs. foreign ant soil
    - Home soil vs. other soil
  - Don't know which soil is home (unbiased)
- After data obtained, identity of home soil revealed.
- Wilcoxon Rank Sum test comparing % time ant spent on home soil compared to % of time expected to spend on home soil if there is no preference (50%).



## Some Assumptions for Statistical Analysis

- Scores must be interval or ratio in nature.
- Scores must be measured on random samples from the respective populations.
- The populations from which the samples were drawn must be normally distributed.
- The populations from which the samples were drawn must have approximately the same variability (homogeneity of variance).

# Test for Significance?

- If the results are not significant, you cannot reject the null hypothesis. The ants displayed no preference for either soil sample.
- If your data are significant, you reject the null hypothesis that the ants displayed no preference for either soil.

# Errors in making decisions

- Type I error is committed if the null hypothesis is rejected when it actually is true.
- The probability of a Type I error is under our direct control, since we are responsible for setting the significance level.

# Errors in Making Decisions

- A Type II error is committed if the null hypothesis is accepted when actually it is false.
- If we decrease the probability of a Type I error we increase the probability of a type II error.

## Decision on the basis of sampling

	REJECT $H_0$	ACCEPT $H_0$
$H_0$ is TRUE in population	Type I error	correct
$H_0$ is FALSE in population	correct	Type II error

# Significance Levels

- "The probability that a result is due to sampling errors, and, if this probability is small enough, we reject the notion that sampling error is the cause."

# 0.05 Significance Level

- Probability that our results happened by chance is 0.05 (5%) or less.
- Results are significant at the 0.05 level.

## 0.10 Significance Level

- Probability that our results happened by chance is 0.10 (10%) or less.
- Results are significant at the 0.10 level.



## Wilcoxon Signed Test for Two Matched Samples

- The test statistic is  $T$
- $T$  = the total number of observations in a finite population
- Comparison between a calculated  $T$  value of the sum of the ranks of the smaller value and the 0.05 tabular  $T$  value.

# In the end...

- If  $T_c$  is  $>$  than  $T_+$ , the data are not significant and  $H_0$  is not rejected.
- If  $T_c$  is  $<$   $T_+$ , the data are significant and the  $H_0$  is rejected.

## Wilcoxon Signed Test for $n=5$ and $n=20$

- Observed time - Expected time (if  $H_0$ )
- Rank (Obs. - Exp.) by absolute value
- Calculate the sum of the negative values.
- Calculate the sum of the positive value.
- The lower of the two values becomes  $T_c$
- Compare  $T_c$  to  $T_+$  and come to a conclusion about  $H_0$

(Observed - Expected) by absolute value

Observed Time	Expected Time	Observed-Expected
70	50	+20
45	50	-05
65	50	+15
60	50	+10
48	50	-02

(Observed - Expected) by absolute value

Observed- Expected	Rank by absolute value	Calculated T values
+20		
-05		
+15		
+10		
-02		

Calculate the sum of the negative values.  
Calculate the sum of the positive values.

Observed- Expected	Rank by absolute value	Calculated T values
+20	5	$\Sigma^+ = 5+4+3=12$
-05	2	$\Sigma^- = 2+1=3$
+15	4	
+10	3	
-02	1	

## Compare Calculated T value with T value in table

- If  $T_c > T_t$  then data are not significant
  - If  $T_c < T_t$  then data are significant
  - $T_c = 3$  &  $T_t = 0.6$  for  $n=5$
  - $T_c > T_t$  :Data are not significant
- 
- A. Reject Null Hypothesis?
  - B. Can't reject Null Hypothesis?
  - C. Not enough data to make a decision.

# Ant Books

