International Centre of Insect Physiology and Ecology (*icipe*)

African Insect Science for Food and Health

Dr. Segenet Kelemu, **Director General**

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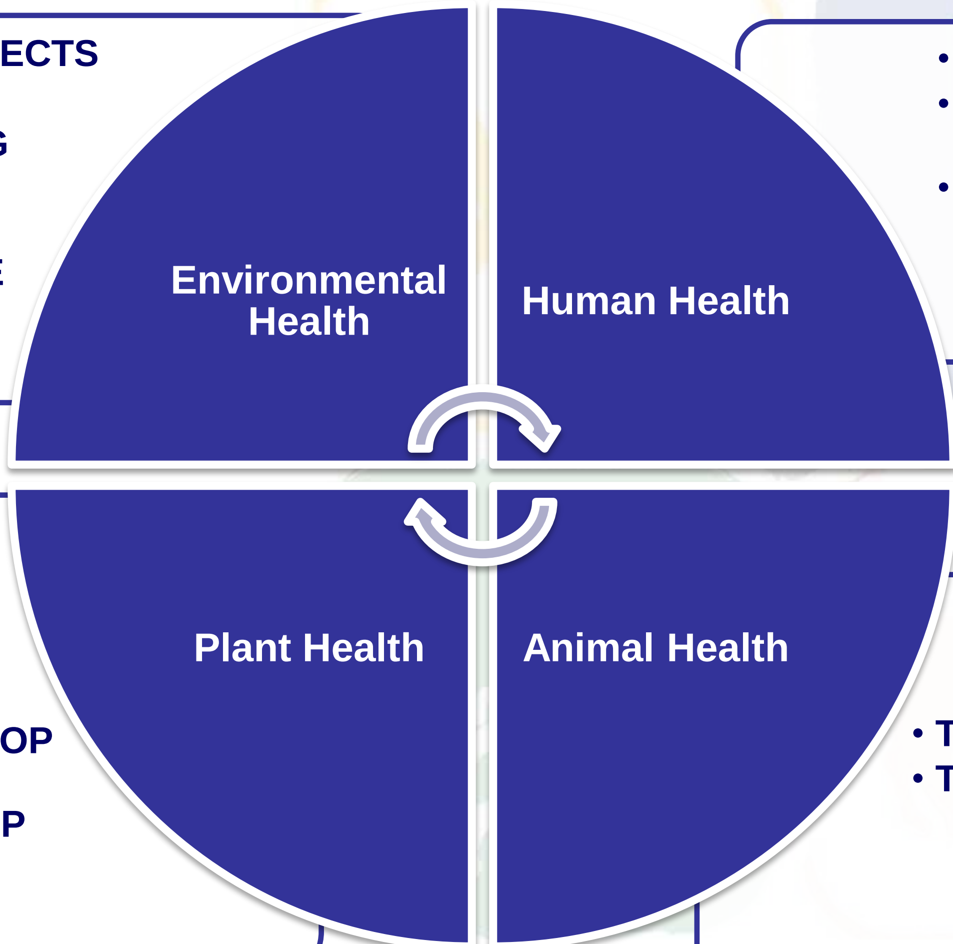
Major Programme Areas

- COMMERCIAL INSECTS
- BEE HEALTH
- BIOPROSPECTING
- BIODIVERSITY CONSERVATION
- CLIMATE CHANGE

- MALARIA
- SLEEPING SICKNESS
- ARBOVIRAL INFECTIONS



- HORTICULTURAL CROP PESTS
- STAPLE FOOD CROP PESTS
- PLANTATION CROP PESTS

- TSETSE
- TICKS

CONTINENTAL TRAINING OF TRAINERS (TOTs) ON BEE DISEASES AND PESTS, PREVENTION AND CONTROL

March 31 – April 4 2014

Honeybee biology and queen breeding

Suresh Raina, *icipe*

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THE TAXONOMY AND IDENTIFICATION OF BEES

Bees all belong to:

Kingdom:	Animalia
Phylum:	Arthropoda
Class:	Insecta
Order:	Hymenoptera
Superfamily:	Apoidea

- The Apoidea comprises two groups, the Anthophila (bees) and the Spheciformes (sphecid wasps).
- The Anthophila has six families in the Afrotropical Region: Colletidae, Andrenidae, Halictidae, Melittidae, Megachilidae and **Apidae**.

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Subfamilies Apinae, Nomadinae and Xylocopinae

Apidae

The Apidae comprising the common **honey bees**, **stingless bees**, **carpenter bees, orchid bees, cuckoo bees, bumblebees,** and various other tribes and groups. Many are valuable pollinators in natural habitats and for agricultural crops.

Apidae has three Subfamilies

1. Apinae: honey bees, bumblebees, stingless bees, orchid bees, digger bees, and 14 other tribes, the majority of which are solitary and whose nests are simple burrows in the soil. However, honey bees, stingless bees, and bumblebees are eusocial or colonial..

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Subfamily Nomadinae

2. Nomadinae

The subfamily Nomadinae, or cuckoo bees, has 31 genera in 10 tribes which are all cleptoparasites bees for their habit of invading nests of solitary bees and laying their own eggs

Thyreus splendidulus Democratic Republic of Congo.
Image: Nicolas Vereecken (Free University of Brussels)

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Subfamily Xylocopinae

3. Xylocopinae

The subfamily Xylocopinae, which includes carpenter bees, are mostly solitary, though they tend to be gregarious. Some tribe lineages, such as the Allodapini, contain eusocial species. Most members of this subfamily make nests in plant stems or wood.

carpenter bee (Kakamega Forest Kenya)

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Apis mellifera subspecies originating in Africa

A general trait of the African subspecies is absconding behaviour, during the time of the year when food-stores are low.

1. *Apis mellifera scutellata*, classified by Lepeletier, 1836 - (African honey bee) East, Central and West Africa, now hybrids also in South America, Central America and the southern USA.
2. *Apis mellifera capensis*, classified by Eschscholtz, 1822 - the Cape bee from South Africa (workers can lay eggs)
3. *Apis mellifera monticola*, classified by Smith, 1961 - High altitude mountains at elevation between 1,500 and 3,100 metres of East Africa Mt. Elgon, Mt. Kilimanjaro, Mt.Kenya, Mt.Meru
4. *Apis mellifera sahariensis*, classified by Baldensperger, 1932 - from the Moroccan desert oases of Northwest Africa.
5. *Apis mellifera intermissa*, classified by von Buttel-Reepen, 1906; Maa, 1953 - Northern part of Africa in the general area of Morocco, Libya and Tunisia.

Apis mellifera Subspecies originating in Africa

6. Apis mellifera major, classified by Ruttner, 1978 - from the Rif mountains of Northwest Morocco -.
7. Apis mellifera adansonii, classified by Latreille, 1804 - originates Nigeria, Burkina Faso
8. Apis mellifera unicolor, classified by Latreille, 1804 - Madagascar
9. Apis mellifera lamarckii, classified by Cockerell, 1906 - (Lamarck's honey bee) of the Nile valley of Egypt and Sudan
10. Apis mellifera litorea, classified by Smith, 1961 - Low elevations of east Africa
11. Apis mellifera nubica, (Nubian honey bee) of Sudan
12. Apis mellifera jemenitica, classified by Ruttner, 1976 - Somalia, Uganda, Sudan, Yemen

Subspecies originating in Middle East and Asia

1. *Apis mellifera macedonica*, classified by Ruttner, 1988 - Republic of Macedonia and Northern Greece
2. *Apis mellifera meda*, classified by Skorikov, 1829 - Iraq
3. *Apis mellifera adamii*, classified by Ruttner, 1977 - Crete
4. *Apis mellifera armeniaca*, Mid-East, Caucasus, Armenia
5. *Apis mellifera anatolica*, classified by Maa, 1953 - This race is typified by colonies in the central region of Anatolia in Turkey and Iraq (Range extends as far east as Armenia). .
6. *Apis mellifera syriaca*, classified by Skorikov, 1829 - (Syrian honeybee) Near East and Israel
7. *Apis mellifera pomonella*, classified by Sheppard & Meixner, 2003 - Endemic honey bees of the Tien Shan Mountains in Central Asia. This sub-species of Apis mellifera has a range that is the farthest East.

Subspecies originating in Europe

1. *Apis mellifera ligustica*, classified by Spinola, 1806 - the Italian bee. North America, South America and southern Europe.
2. *Apis mellifera carnica*, classified by Pollmann, 1879 - Carniola region of Slovenia, the Eastern Alps, and northern Balkans
3. *Apis mellifera caucasica*, classified by Pollmann, 1889 - Caucasus Mountains
4. *Apis mellifera remipes*, classified by Gerstäcker, 1862 - Caucasus, Iran, Caspian Sea.
5. *Apis mellifera mellifera*, classified by Linnaeus, 1758 - the dark bee of northern Europe also called the German honey bee

Subspecies originating in Europe

6. The hybrid populations of *A. m. mellifera* x *A. m. ligustica*, found in North America and Western Europe, have the reputation of stinging people

7. *Apis mellifera iberiensis* (*aka Apis mellifera iberica*), classified by Engel, 1999 - the bee from the Iberian peninsula (Spain and Portugal)

8. Apis mellifera cecropia, classified by Kiesenwetter, 1860 - Southern Greece

9. Apis mellifera cypria, classified by Pollmann, 1879 - The island of Cyprus –

10. Apis mellifera ruttneri, classified by Sheppard, Arias, Grech & Meixner in 1997- is a sub-species originating in the Maltese islands.

11. Apis mellifera sicula, classified by Montagano, 1911 - from the Trapani province and the island of Ustica of western Italia

Apis mellifera species: Sex/Caste Differentiation

Fertilized egg

↓

Fed royal jelly

Unfertilized egg

↓

Fed heavy brood food & then honey/pollen

Fertilized egg

↓

Fed light brood food & then honey/pollen

The Worker Bee

- Female but not fertile
  - Normally does not lay eggs
  - If she does, they will be drones
- About 20,000 to 60,000 in a colony
- Has several functions throughout her life

- Lives about 4 – 6 weeks
- Stinger has barbs and stays in your skin – used to defend the hive and herself

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Hive Bee "Work"

- Includes…
  - cleaning
  - nursing
  - nectar ripening & honey storage
  - pack pollen
  - secrete wax
  - guarding

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Jobs - Never unemployment or a layoff

- **Workers do the work in the bee society**. Employment is based on the age of the bee and the needs of the colony. During their life they pass through many job promotions:

- **Nurse Bee**
  - ✓ 1 – 12 days
  - ✓ Clean own cell and others
  - ✓ Feeding brood (larvae)

- **House Bee**
  - ✓ 10 – 20 days old
  - ✓ Comb building
  - ✓ House keeping
  - ✓ Undertaker
  - ✓ Ripening honey
  - ✓ Climate control
  - ✓ Secreting/molding wax into cells
  - ✓ Accept and store pollen and nectar from foragers

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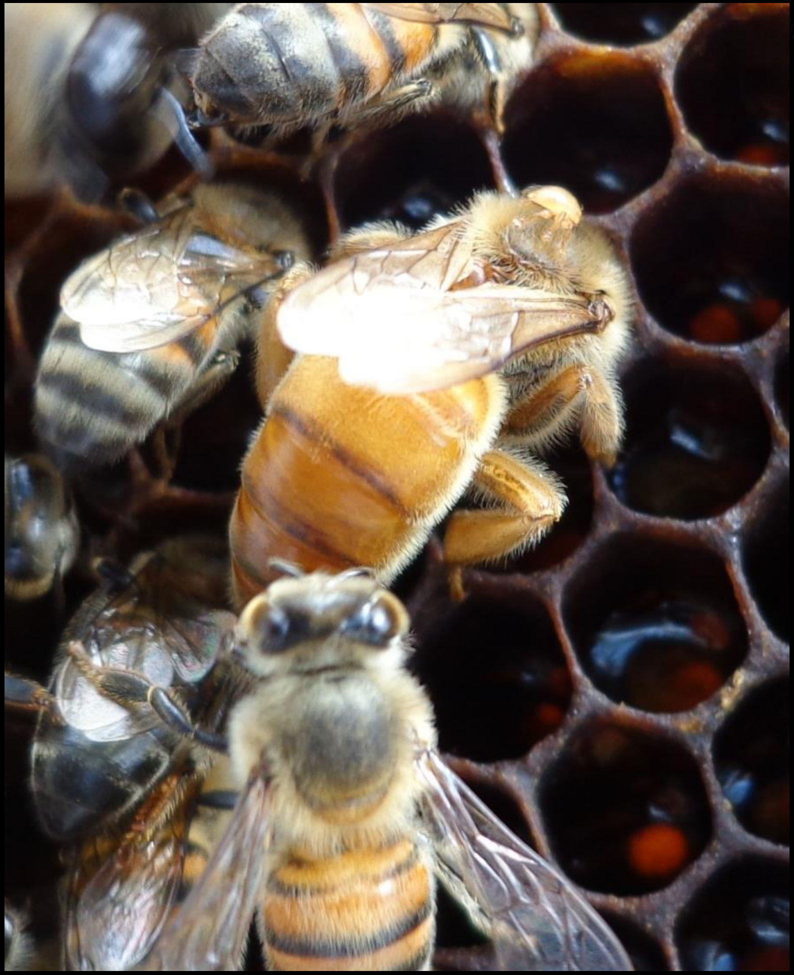
Jobs - Never unemployment or a layoff

- **House Security**
  - ✓ Guard hive and its entrance (some say only about 5% of bees perform this job)
  - ✓ Orientation flights to learn surroundings

- **Field Agent**
  - ✓ After about three weeks the girls are ready to spend the rest of their lives as **foragers** gathering pollen, nectar, tree resin (that they turn into propolis) and water for the hive. During this time they work themselves to death – literally
  - ✓ Worker bees in Africa live about six weeks

The Queen

- Queen can lay 1500 eggs a day at height of season live 2 to 5 years



- Produces air-borne pheromones ("queen substance") that keep the colony functioning orderly, loyal and protective to that queen
- Stinger does not have barbs – only uses it to kill rival queens

The Drone

- Male, develops from unfertilized egg
- Larger than workers, has big eyes and no stinger

- Sexually mature at 2 weeks
- Mate with virgin queens once, in drone congregation areas at about 300 feet above ground, then dies
- Survivors are forced out of hive and die

Life stages of honey bees

The Life Cycle of Honey Bees

Table 1: Developmental stages of the three castes of bees.

DEVELOPMENTAL STAGE	DURATION OF STAGES		
	QUEEN	WORKER	DRONE
	Days		
Egg	3	3	3
Larval stage	5 ½	6	6 ½
Pupal stage	7 ½	12	14 ½
Total developmental time	16	21	24

Drone & Worker Cells

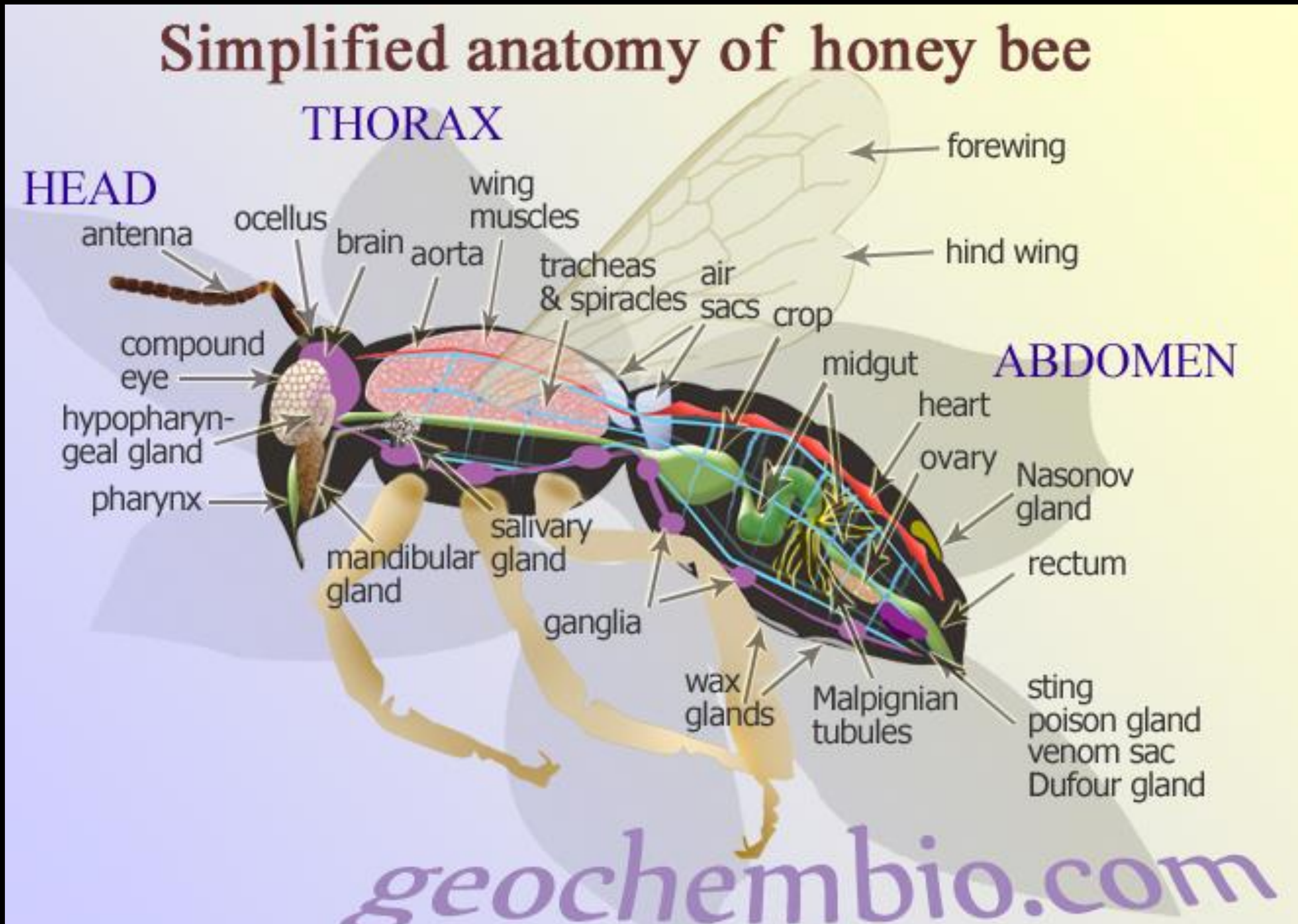
Worker – cap flush with cells

Drone – larger & raised cap, usually found at the outer edges of frames.

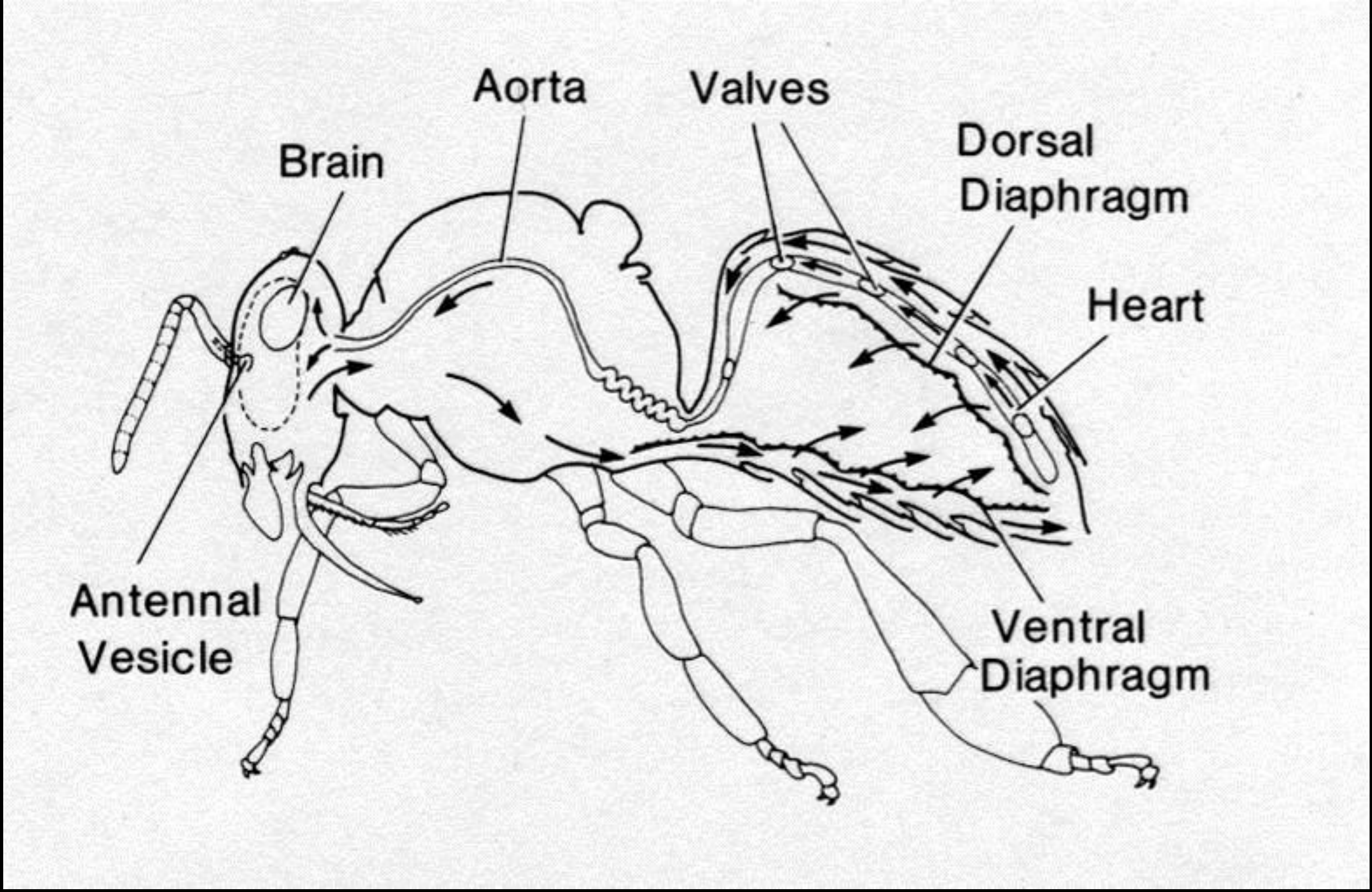
Queen Cells

Worker cells are horizontal while queen cells are vertical. As the queen larva grows, the cell enlarges and becomes peanut-shaped when capped for the pupal stage of development.

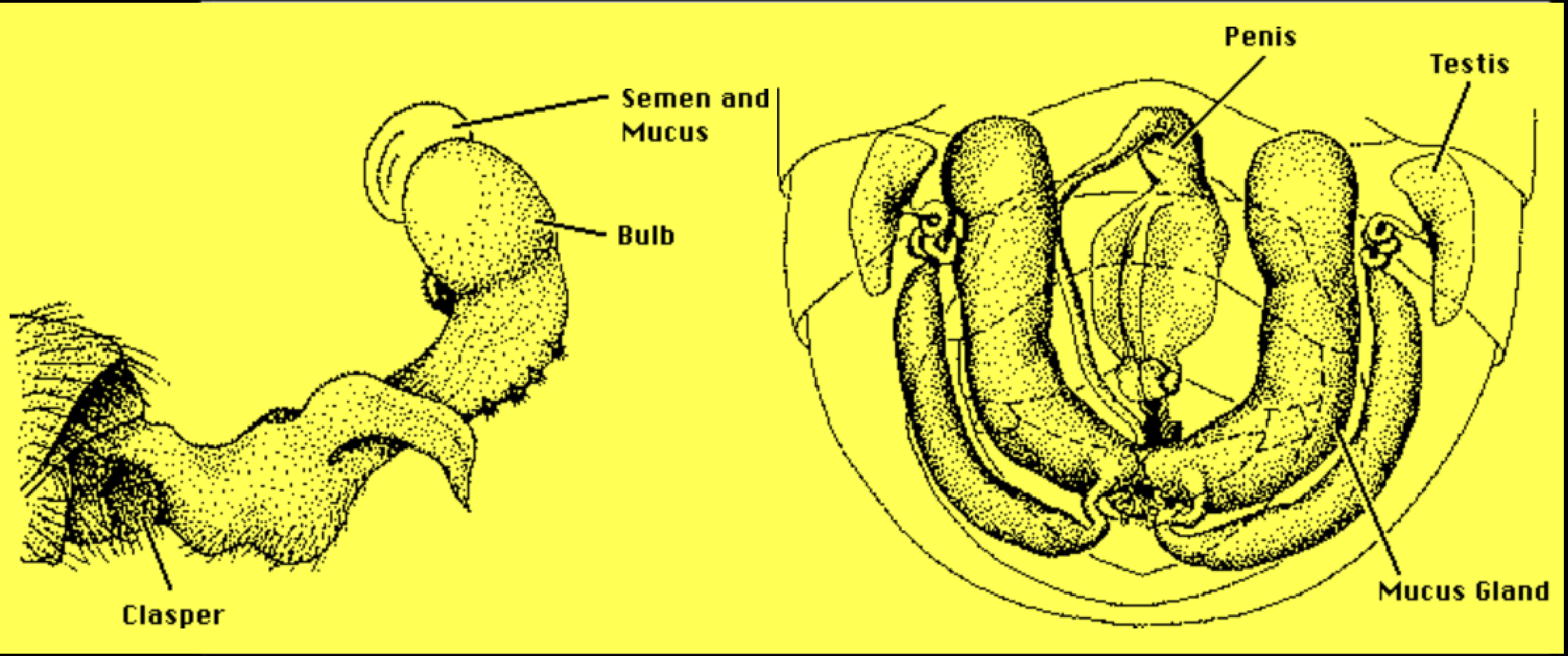
Internal Anatomy



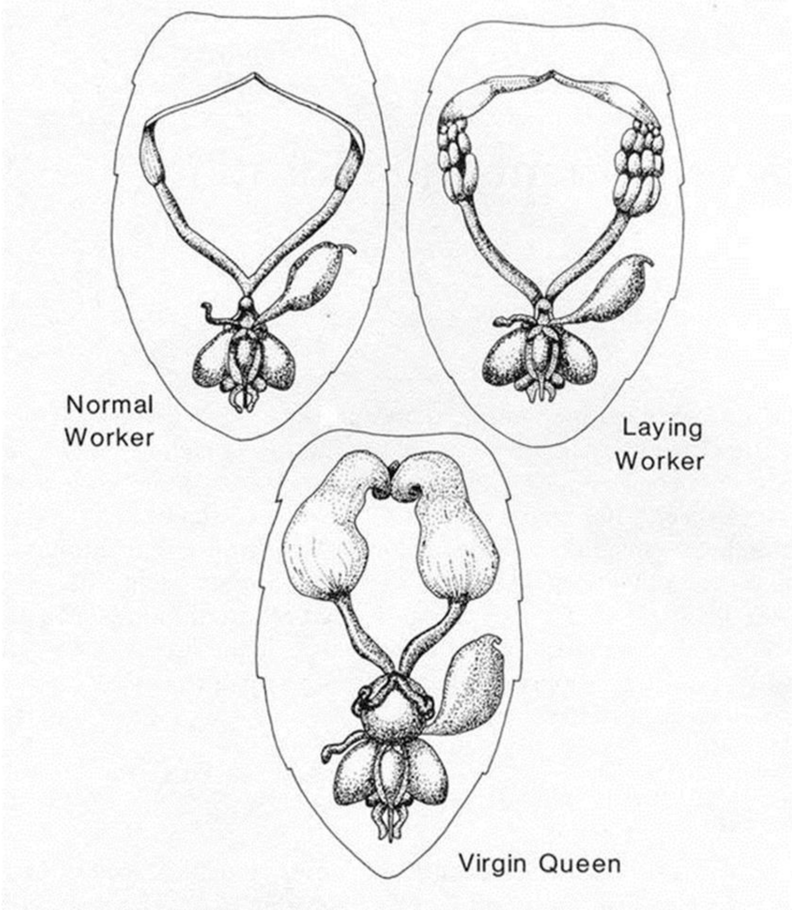
Internal Anatomy

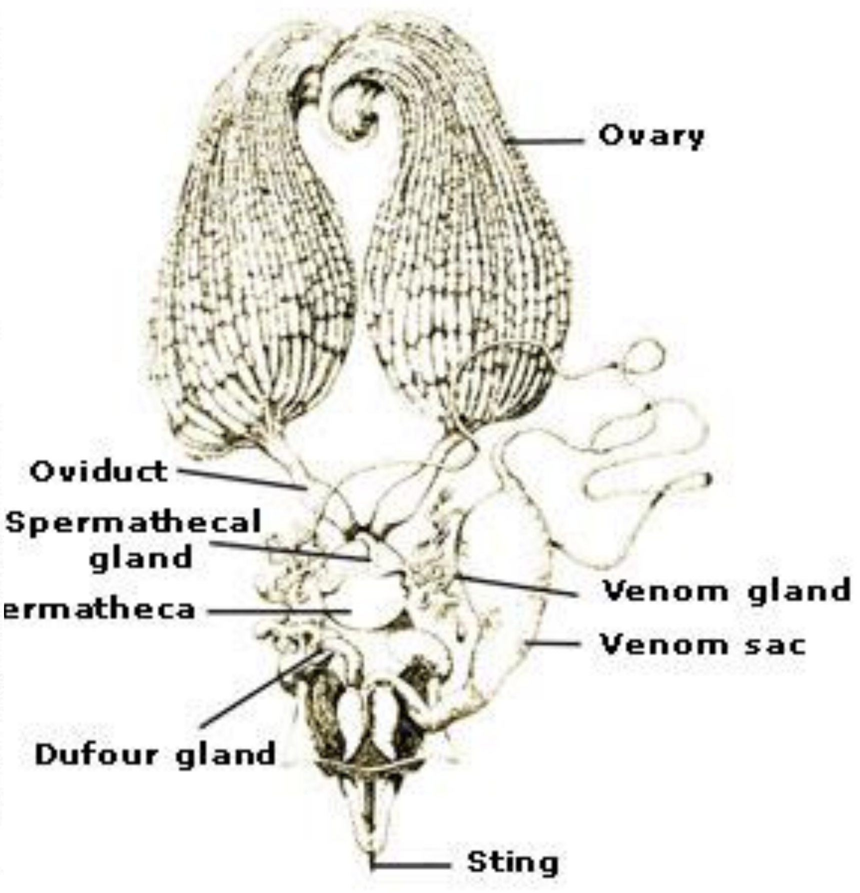


Male reproductive organ of honey bee

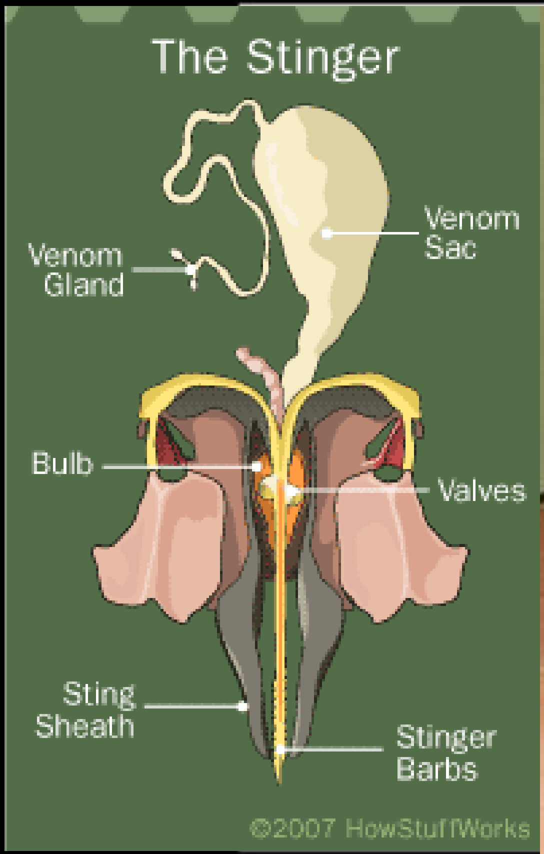


Female reproductive organs

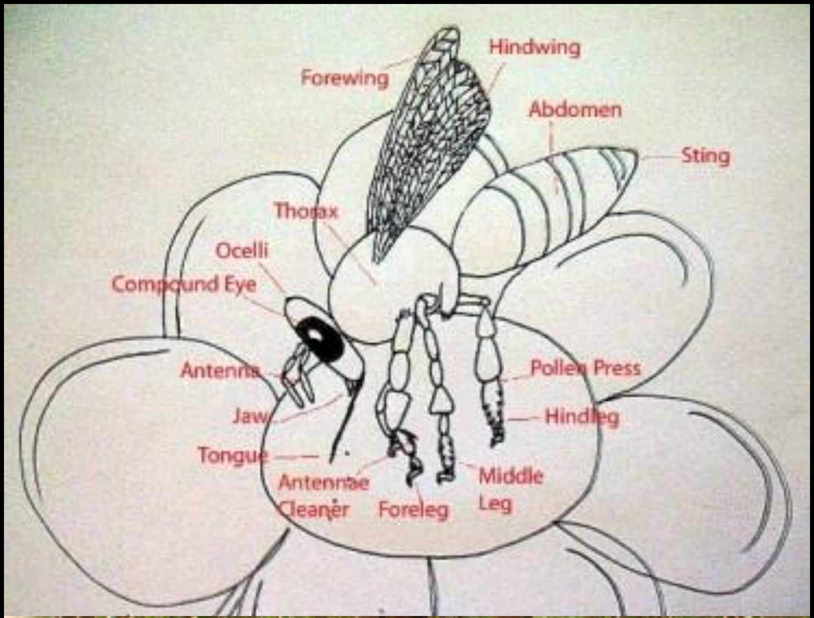




Honey bee Stinger



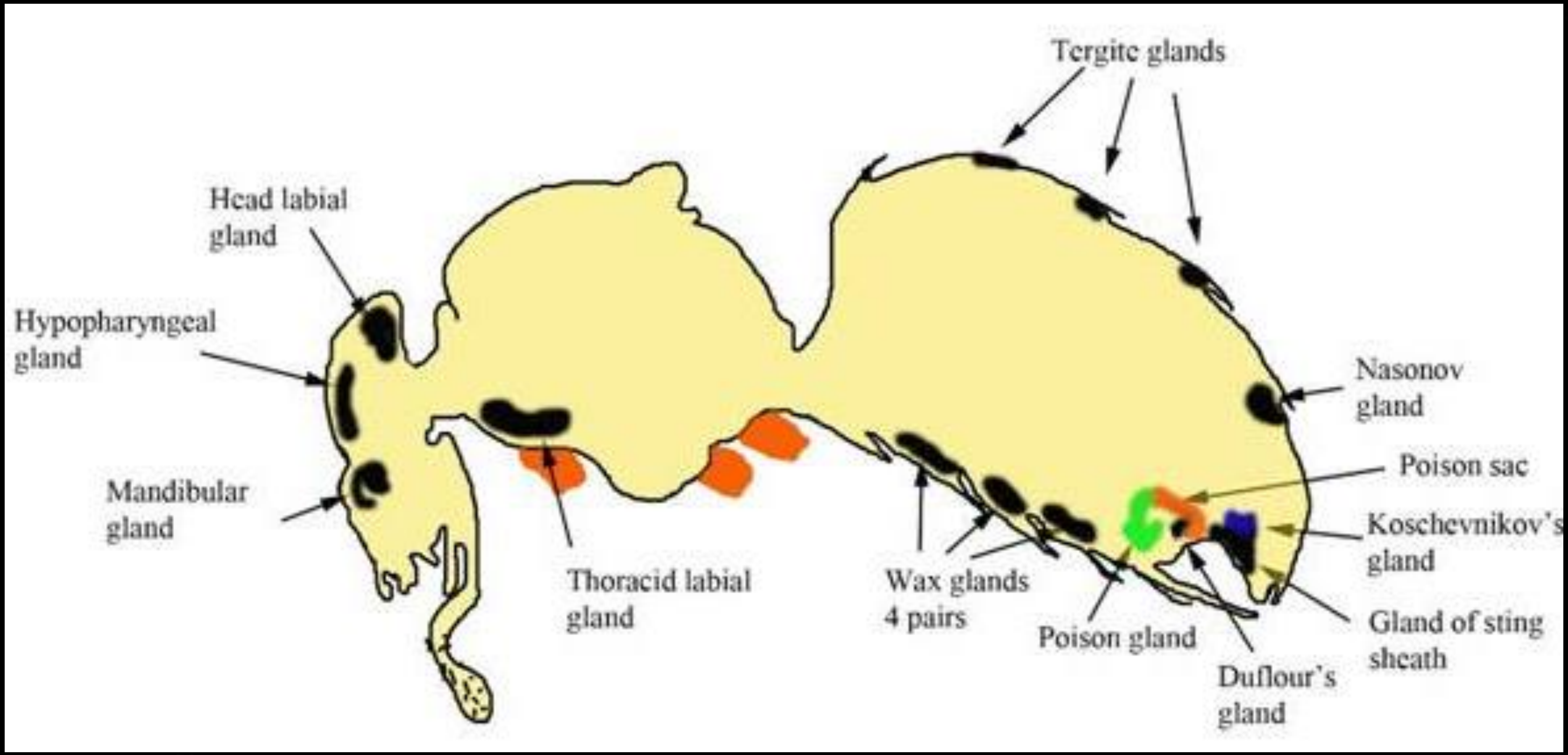
How do bees receive chemical messages?



- The antennae and mouthparts are equipped with sensory receptors which interpret the odors and bring about an excitement within a hive.
- Bees produce several pheromones or chemical signals through several glands in the body to communicate in the colony.
- Only queen has the capability of producing a "queen substance", that evokes a sense of well-being and calm within a colony.

Pheromones glands

The honey bee queen produces pheromones that function in both releaser and primer roles such as attracting a retinue of workers around her, attracting drones on mating flights, preventing workers from reproducing at the individual (worker egg-laying) and colony (swarming) level, and regulating several other aspects of colony functioning.



Pheromones glands (workers and Queen)
The bee contains several glands.

- **Nasanov gland**

Produce chemicals (terpenoids including geraniol, nerolic acid, citral and geranic acid) which the bee uses to assist identification of the entrance of the hive.

- **Koschevnikov gland**

Releases alarm pheromone – attracts other bees to attack and sting the same part of the body of the offending animal.. The pheromone contains more than 40 different compounds, including pentylacetate, butylacetate, 1-hexanol, n-butanol, 1-octanol, hexylacetate, octylacetate and 2-nonanol

In the **queen** this gland products are responsible for the formation of the clusters of court bees that surround the queen.

Pheromones glands (workers and Queen) The bee contains several glands.

- **Dufour's gland**

The products of this gland line the entrance to the hive and may assist recognition of family or nest ownership.
The gland secretes its alkaline products into the vaginal cavity of the queen , and is deposited on the eggs as they are laid.
Indeed, Dufour's secretions allow worker bees to distinguish between eggs laid by the queen, which are attractive, and those laid by workers.

- **Forager pheromone**

Ethyl oleate is released by older forager bees to slow the maturing of nurse bees. This primer pheromone acts as a distributed regulator to keep the ratio of nurse bees to forager bees in the balance that is most beneficial to the hive.

Pheromones glands (workers and Queen)

- **Mandibular glands**
  - In **young workers**(5-15days old) this gland produces the lipid-rich white substance mixed with the hyopharyngeal gland secretions resulting in royal jelly.
  - In **older worker** this produces part of the alarm pheromone.
  - In the **queen,** this gland has a number of important functions – produces queen substance (queen mandibular substance) and is associated with:
    - Suppression of construction of emergency queen cells
    - Inhibits ovary development in the workers
    - Attracting drones during the mating flight in drone congregation areas (DCA's)

Pheromones glands (workers and Queen)

Arnhart or footprint glands on each foot :

- This produces pheromone which is left by bees when they walk and is useful in enhancing Nasonov pheromones in searching for nectar.
- **In the queen**, it is an oily secretion of the queen's tarsal glands that is deposited on the comb as she walks across it. This inhibits queen cell construction (thereby inhibiting swarming), and its production diminishes as the queen ages.

Egg marking pheromone

- This pheromone, helps nurse bees distinguish between eggs laid by the queen bee and eggs laid by a laying worker.

Pheromones glands (workers and Queen)

- **Hypopharyngeal glands**
  - Produce protein-rich sections (Royal jelly) when the worker is a nurse bee.
  - When the worker becomes a forage bee it produces invertase which helps break down sucrose into fructose and glucose.
- **wax gland**
  - produce wax in 4th and 7th segments of abdomen
- **Sting pheromone gland**

  makes the alarm 'isopental'. produced in the stinger

Pheromones glands (workers and Queen)

- **Queen retinue pheromone**

The queen produces a synergistic, multiglandular pheromone blend of at least nine compounds for retinue attraction, the most complex pheromone blend known for inducing a single behavior in any organism

Pheromones glands (workers and Queen)

Queen mandibular pheromone (QMP), emitted by the queen, is one of the most important sets of pheromones in the bee hive. The following compounds have been shown to be important in retinue attraction of workers to their queen and other effects

- **(*E*)-9-Oxodec-2-enoic acid** (9-ODA) – inhibits queen rearing as well as ovarian development in worker bees; strong sexual attractant for drones when on a nuptial flight; critical to worker recognition of the presence of a queen in the hive
- **(*R*,*E*)-(-)-9-Hydroxy-2-enoic acid** (9-HDA) promotes stability of a swarm, or a "calming" influence
- (*S*,*E*)-(+)-9-HDA
- **Methylparaben** (HOB)
- **4-Hydroxy-3-methoxy phenylethanol** (HVA)

Slessor *et al.,* 1991. patented Synthetic queen mandibular pheromone (QMP), a mixture of five components: 9-ODA, (-)-9-HDA, (+)-9-HDA, HOB and HVA in a ratio of 118:50:22:10:1.

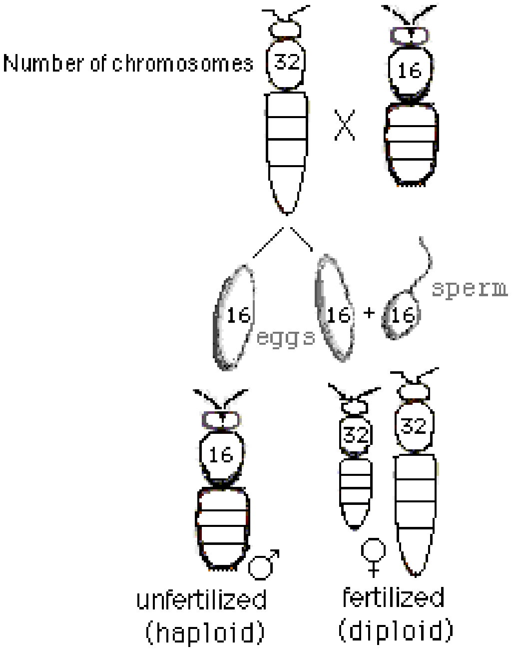
Honey Bee Queen: Why I am Unique ?

"I laid 3,000 eggs today, and you expect me to dress for dinner?"

- You've probably all heard that Humans share 99% of their genes with chimpanzees.
- But did you know that we also share 25% of our genes with bananas.
- Still, every species is obviously unique and honeybees have their share of odd genetic quirk (reproductive behavior). It's the Unique quirk (**Haplodiploidy)** that make honeybees what they are.

Haplodiploidy: A sex-determination system in bees

- All eggs and sperm carry 16 chromosomes each.
- Drones result from unfertilized eggs (parthenogenesis).They have no father.
- Each egg contains a unique combination of 50% of the queens genes.
- All 10 million sperm produced by a drone are identical clones.
- Since each queen mates with 10-15 drones, colonies are comprised of subfamilies, each having the same mother but different fathers.



- Workers of the same subfamily are related by 75% of their genes.
- This "extra" close relatedness may explain the cooperative, and altruistic/unselfish behaviors found in colonies.
- It also explains why workers forego their own reproduction in favor of helping their queen mother raise more sisters.
- Their sisters are more closely related to them than their own offspring would be. (75% vs 50%)

Haplodiploidy

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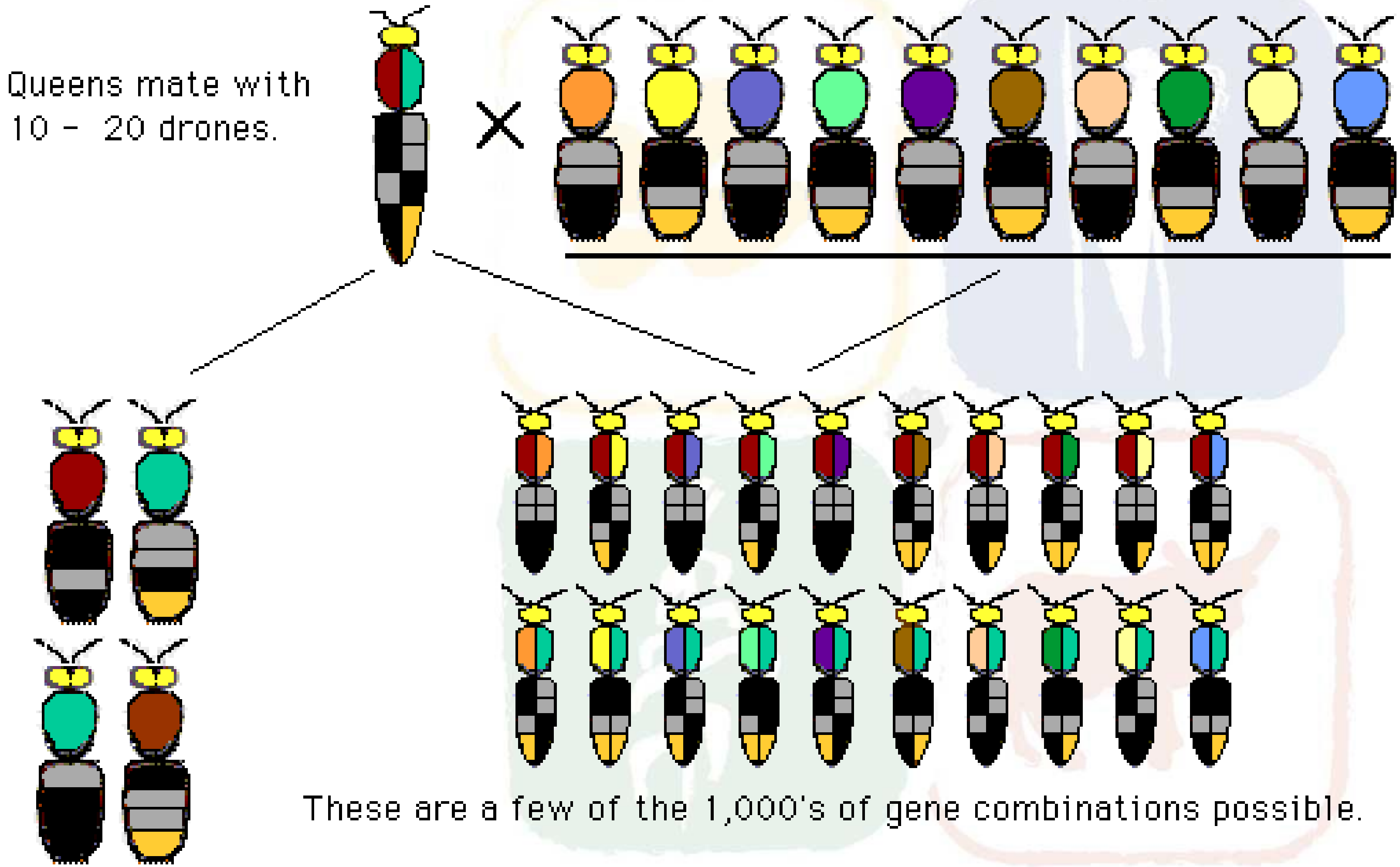
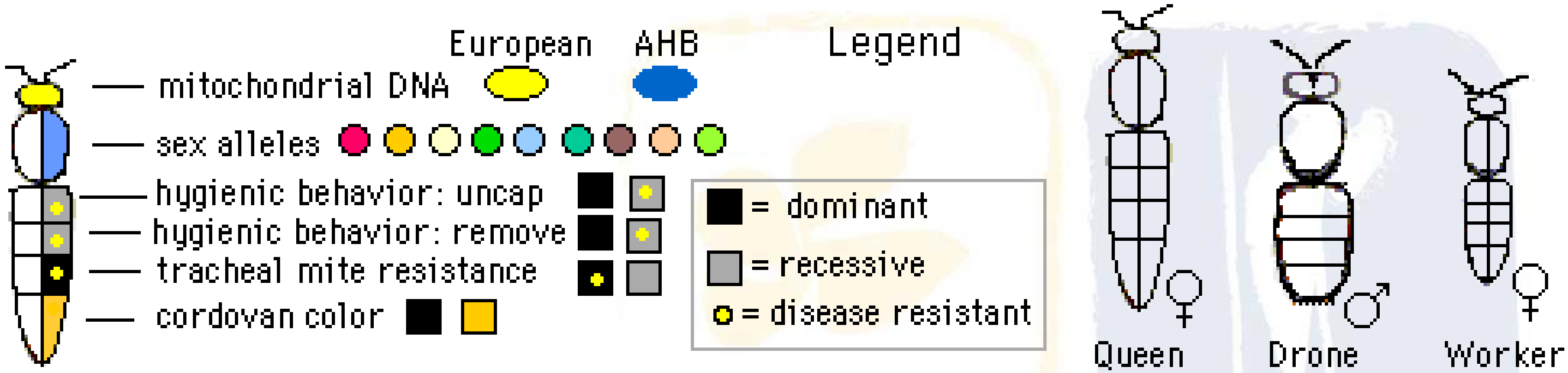
Mating Behaviour

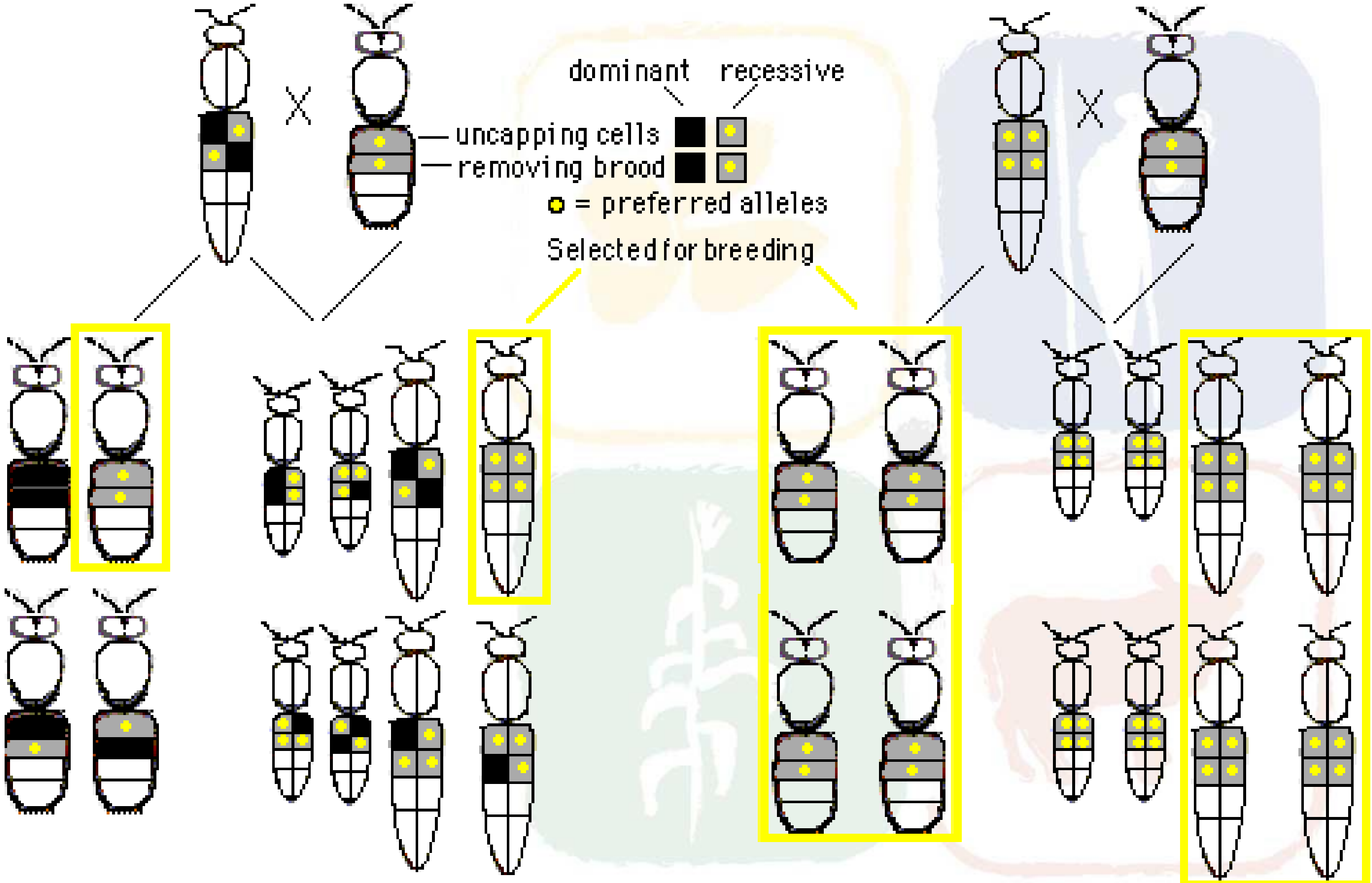


Genetic Mechanisms



Mating behavior - multiple mates make for a complex family.
Cordovan color- a useful genetic marker, controlled by a recessive gene.
Hygienic behavior: two recessive genes for uncapping and removing diseased brood.
Tracheal mite resistance - thought to be controlled by dominant gene(s).
Mitochondrial DNA - used to trace the maternal lineage of bees.

Hygienic behavior

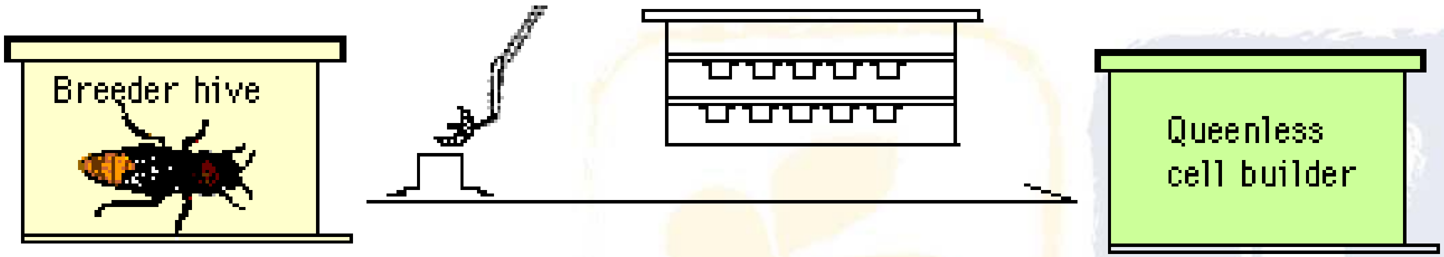


Breeding of Queen bee

Requirements for successful queen rearing

- A good breeder queen to graft larva from.
- Grafting requires good light, good eyesight or appropriate magnification.
- Grafting larva of the proper age (1-24 hrs old).
- Queen rearing equipment (grafting tool, cell cups, cell bars and frame) can be made or purchased.
- Natural mating requires 20-25% degree temps. and mature drones (15 days old)

A Simple Queen Rearing Technique



1 Breeder lays eggs	2	3 Eggs hatch	4 Larva grafted Into cell builder	5	6	7
8 Cells are sealed	9	10	11	12	13	14 Out builder In mating nuc
15	16 Cells hatch	17	18	19	20	21
22 Queen mates	23	24	25	26	27 Queen begins laying	28
29	30	31				

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Grafting Honey bee queens

- How to graft queens: Grafting is simply the process of transferring larva from the worker cell of the breeder's hive to an artificial queen cell.

Grafting

The Breeder hive

- Graft from your best colony,
- Use the youngest (smallest) larva.
- By placing an empty brood comb in the brood nest 4 days before you graft, the larva will be the right age.

Confining grafted queen cells

Setting up the Cell Building Colony

- Any strong hive can serve as a cell builder.
- Remove the queen from the hive one day before you graft cells.
- Place grafted cells in center of the brood nest.
- Place about 30 cells per colony.
- Large cells will be produced by well nourished colonies.
- Feeding is not necessary if a light honeyflow is on and pollen is abundant.

Queen Cells – Karura Forest

Matured Queen Cell- *Apis mellifera scutellata*

Developing queen cells

Queen cell introduction into the hive

Setting up Mating Nucs

- Cells are placed in queenless colonies the day before they hatch.
- The mating process is usually only 75% successful.
- Small mating colonies minimize the losses due to unsuccessful queens.
- Mini-nucs are convenient for raising large numbers of queens.
- Cells can be placed in any queenless colony.
- Most queenless colonies will accept cells without queen cell protectors.
- Queen quality is best when they are left to lay eggs in the nuc for about a month.

Mating conditions

- Queens are ready to mate 5-7 days after hatching.
- Temperature must be at least 20-25 degrees C with no strong winds.
- Virgin queens mate with 10 to 20 drones on one or more flights.
- Drones and queens may fly a mile or more to drone congregation areas.
- Queens will begin laying eggs 2 to 4 days after mating.

http://www.harunyahya.com/en/Books/4193/the-miracle-of-the-honeybee/chapter/5024

Artificial Insemination: Semen Collection

1. Selection of a breeder colony with the best traits such as Hygienic behaviour, Varroa grooming behaviour
2. Collection of embryo to be transported to the breeding laboratory to inseminate virgin queens in captivity.
3. Characterization of local and regional population to conserve these subspecies
4. Cryopreserve aliquots of the semen for use in the near or distant future.

Drone Semen Collection

Artificial insemination

Artificial Insemination

Introducing inseminated queen in the hive

What is a bee stock?

- The term “stock” is defined as a loose combination of traits that characterize a particular group of bees.
- Such groups can be divided by species, race, region, population, or breeding line in a commercial operation.
- Honey bees vary among themselves in traits such as temperament, disease resistance, and productivity.
- The environment has a large effect on differences among bee colonies), but the genetic makeup of a colony can also impact the characteristics that define a particular group.
- Through breeding programme, *icipe* shall utilize different bee strains to suit their particular purpose, whether it be a pollination, a honey crop, or Royal jelly, propolis etc.

Long term Solution to Maintain Bee Health in Africa

- Conserve African bee genetic diversity through breeding and select best traits for pollination services and hive products improvements.

- Provide training in Queen rearing techniques to advance the development, establishment and maintenance of productive commercial African breeding stock with economically valued traits

- Africa does not have any stock of bees for commercial production, however, few subspecies of *A. mellifera* have demonstrated high honey productivity in several regions of Sub Saharan Africa.

- ICIPE'S strategy is to initiate the Stock improvement program in Kenya using three subspecies of *Apis mellifera* : *Apis m. scutellata, A.m. monticola* and *A. m. litorea* (Raina and Kimbu, 2004)

African Honeybee Germplasm Repository: Cryopreservation,

Germplasm is a collection of genetic resources (Sperms/eggs) for an organism/bees

Repository commonly refers to a storage location, often for safety or preservation. ("Man made Spermatheca'')

Cryopreservation or **cryoconservation** is a process where cells, whole tissues, or any other substances susceptible to damage caused by chemical reactivity or time are preserved by cooling to sub-zero temperatures.

- Generally the ultra cold of liquid nitrogen at −196 ° C (77 K; −321 ° F) is required for successful preservation of the more complex biological structures to virtually stop all biological activity.

Germplasm repository

Aliquots of honey bee semen ready for cryopreservation. Note the red and yellow internal rods used to mark samples during cryo-storage. The semen is the tan liquid near the bottom of each tube

Cryopreservation in action. Semen samples are in the small black unit in the liquid nitrogen filled "cryobath" on the left. The computer is used to control the freezing rate.

Gene Bank: Cryopreservation

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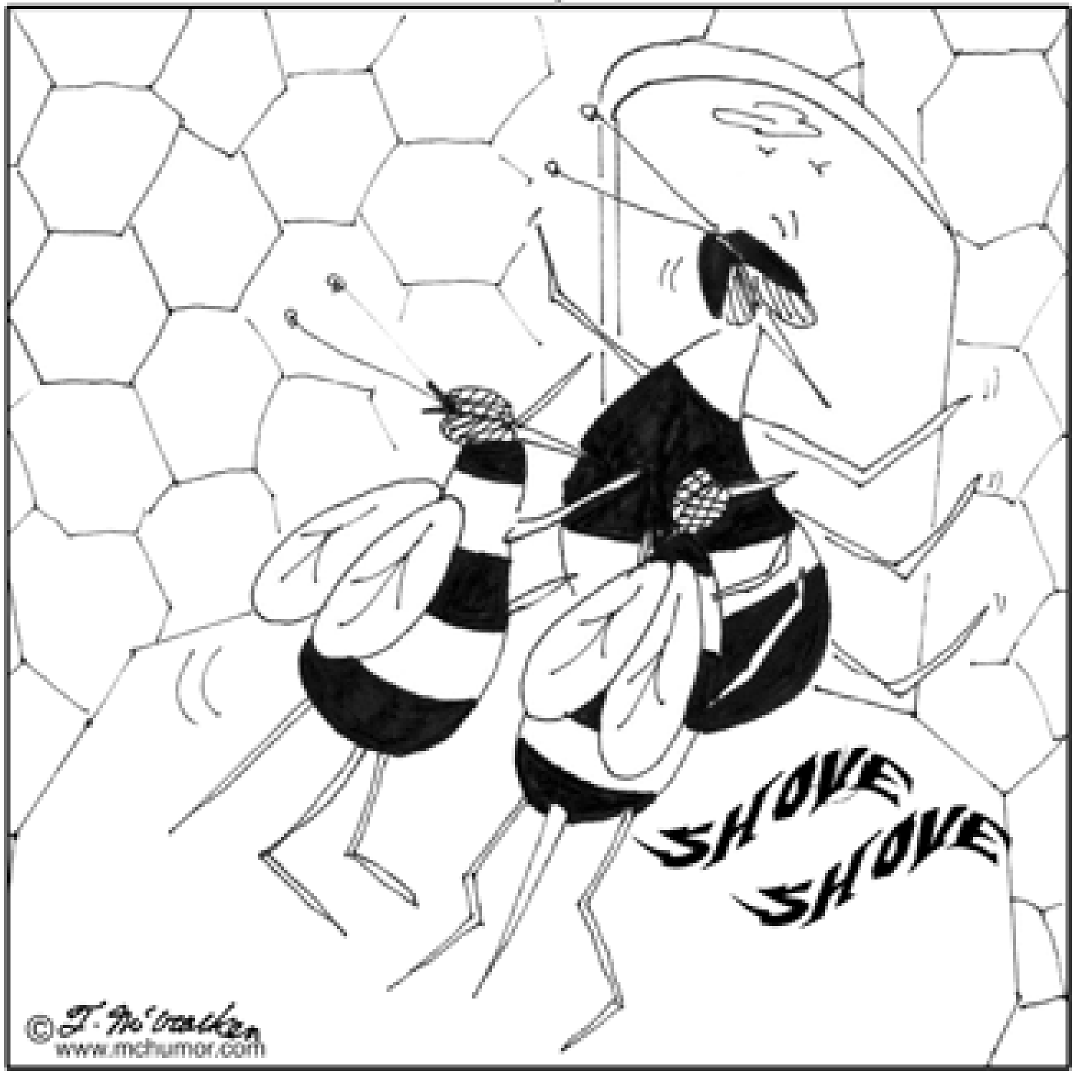
MCHUMOR.com by T. McCracken



"You're at that awkward age where the others have figured out that you're a drone and you don't do anything."

Queen Reigns

Thank You

AFRICAN UNION
INTERAFRICAN BUREAU FOR ANIMAL RESOURCES

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

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