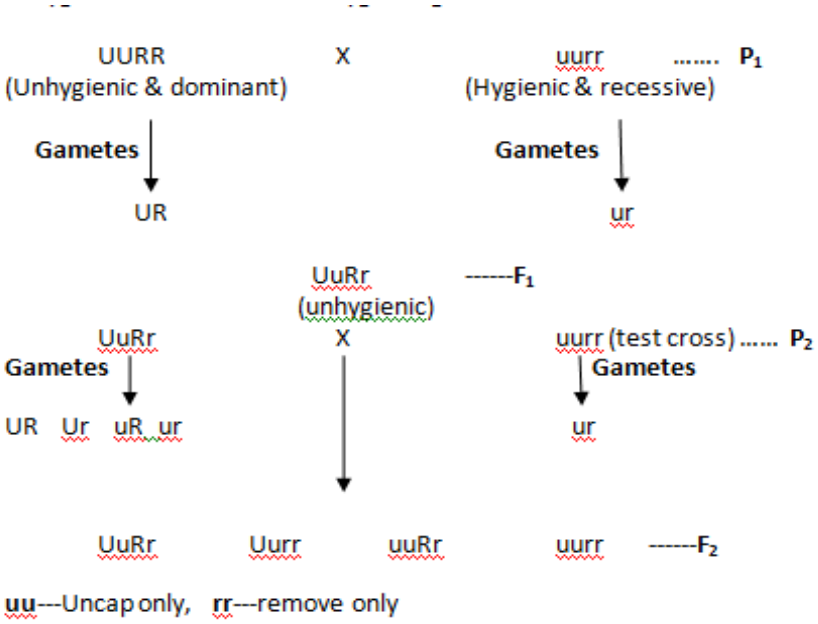
M.SC. II SEMESTER
ZOPBLT3: ANIMAL BEHAVIOUR
(Lab. Exercises)

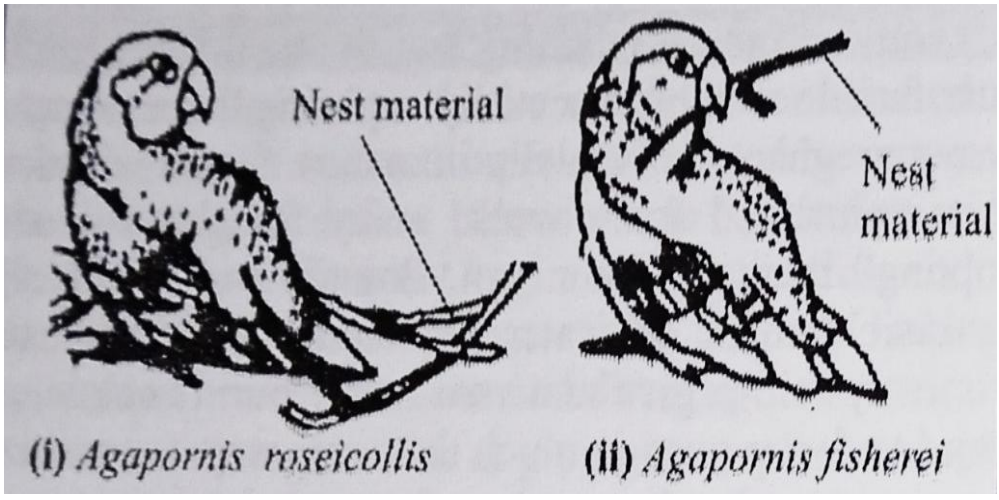
Ex.1. Write the comments on the spots

Genetic basis of behavior

- **1. Nest cleaning behavior of honey bee:**
- It was observed by Rothenbuhler in 1964 by taking of example of brood foul disease.
- -It is a single gene controlling behavior. eg. Hygienic bee.
- -Hygienic bee cleans the nest but unhygienic bee neither uncap the cells nor remove infected/dead larvae.
- -When UURR of honey bee was crossed with uurr. F_1 bee were unhygienic (UuRr). This indicates that unhygienic is dominant over hygienic genes.
- -When hybrid of F1 generation (UuRr) was crossed with hygienic strain (uurr), then F_2 generation gave four types of bees:
- 1. uurr- Hygienic
- 2. uuRr- Uncapped the comb chamber, containing infected larvae but did not removed the disease larvae
- 3. Uurr- Did not uncap the comb chamber but remove the infected larvae, if the cap of the chambers were uncapped by any bee
- 4. UuRr- Would neither uncap comb chambers nor would remove infected larvae.
- -Above results show that different genes control the uncapping of the comb chamber and removal of diseased larvae.
- -No physical or physiological differences have been found among totally hygienic, partially hygienic or totally unhygienic worker bee.



- **2. Nest material carrying behavior of love birds (Willium Dilger, 1962):**
- One species of love bird uses its bill to transport the nesting materials while other species tucks these under its rump/base of feathers.
- Hybrids appeared confused while carrying nest materials.
- -They attempted to tuck the nest materials under their feathers, then tried to carry it in their beaks.



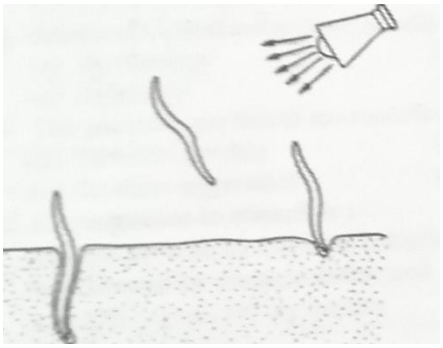
Patterns of behaviour

1. Kinesis

- **a. Orthokinesis:**

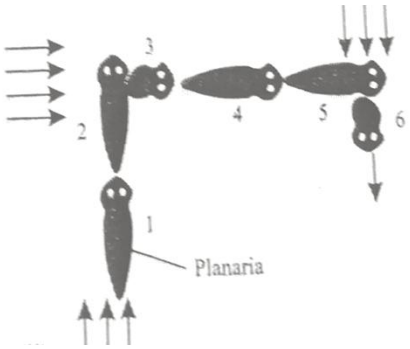
- The animal orient straight and velocity of its movement is directly proportional to the strength of the stimulus.

- eg. Dendrocoelum larva (Fresh water planarian).



b. Klinokinesis:

- Velocity of turning of animal in response to the stimulus is directly proportional to the strength of stimulus.
- -In dim light, it turns occasionally but in bright light, the rate of turning increases accordingly.
- eg. *Planaria*.

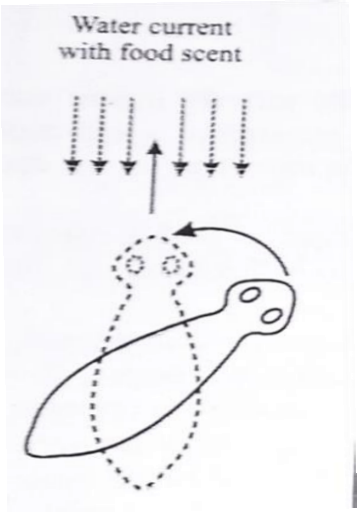


2. Taxes

a. Tropotaxis:

-When a stimulus is received simultaneously by two or more receptors of an animal, it can then compare and steers a course towards or away from the stimulus.

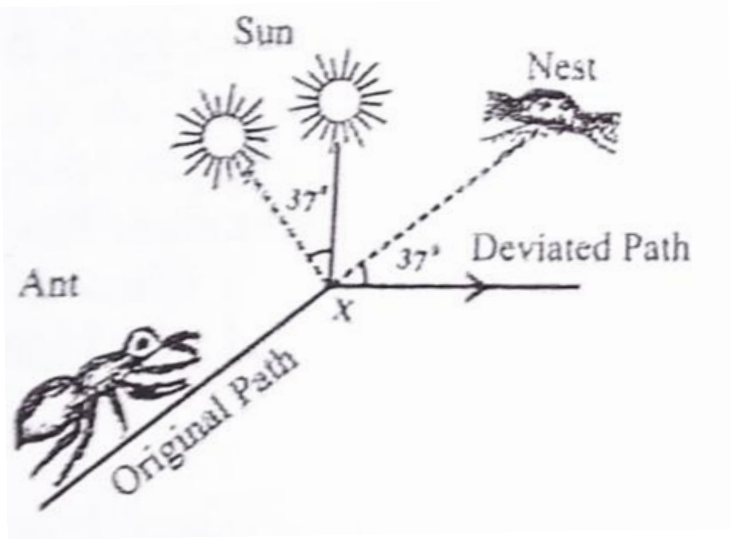
eg. *Planaria* shows positive tropotaxis response to the water current carrying scent of food (chemoreceptors) instead of plane water current (rheoceptors).



b. Menotaxis:

-The orientation of an animal at a constant angle to the direction of source of stimulation is called menotaxis.

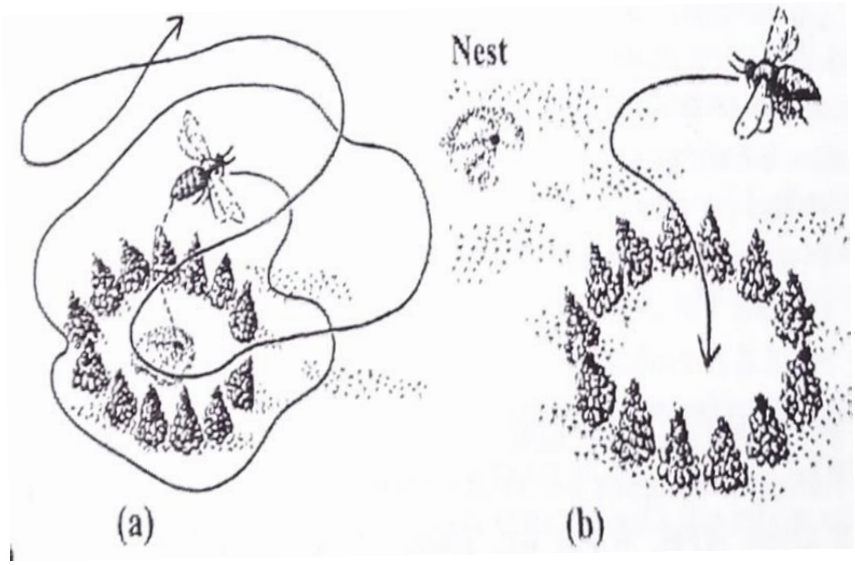
-eg. Homing ants maintain orientation towards nest by progressing at right angle to the sun. -Ant captured and held at a point, and during this time, now sun moves at 37^{o}, ants path continue at right angle to the sun and then ants deviate from their path to the nest by 37^{o}.



c. Mnemotaxis:

-It depends on complex stimulus situation and memory of the animal. eg. Tinbergen (1928) observed that digger or hunter wasp (*Pseneo punctatus*) remembers and uses landmarks while homing.

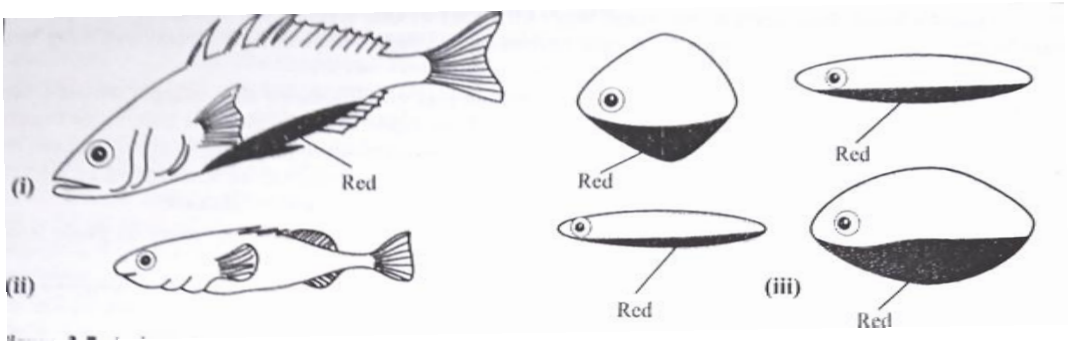
-When the ring of pine cones is moved from position 'a' to 'b', the wasp behaved as if her nest was still located at the center because she learned it position in relation to the cones. -The wasp responds to the arrangement of cones. She is unable to locate the nest left nearby.



3. Sign stimulus

a. Stickleback fish:

- This concept was given by Niko Tinbergen (1952).
- -Releasers are those parts of surrounding that trigger instinctive acts or characteristics such as colour or shape, that evoke FAP or the simple and specific stimuli that cause release of FAP are termed as sign stimulus.
- -eg.1. Sticklebacks are small fresh water fishes.
- -In the breeding season, the male develops a red colouration (sign stimulus) on the underside of their bodies and become highly territorial.
- -It selects a site to form a tubular nest and defend it actively by fighting and chasing an intruder male.
- -Niko Tinbergen (1952) used some models to study the specificity of sign stimulus.
- -He took a realistic model of stickleback without red spot but two other models he used were of odd shapes but with red spots.
- -Models with sign stimuli released attack behavior in other male sticklebacks.
- -Therefore, the red belly is the specific sign stimulus responsible for releasing territorial defense in male sticklebacks.



4. Imprinting

a. Baby duck and dog:

- This theory was developed by Konrad Lorenz (1903-1989).
- -It is the unique process of learning to recognize and become attached in memory only to an object in the early period of life.
- -This does not subsequently modifies in their life time.
- -Imprinting is concerned with behavior elicited by particular visual, auditory, olfactory or tactile stimuli.
- -Imprinting establishes the bond between parents and offspring.
  eg. Baby duck has recognized a dog as its mother.

5. Motivation

a. Hungry dog:

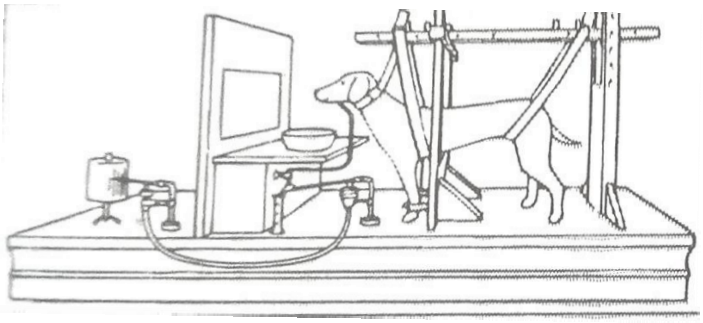
- -A hypothetical state of the individual that arouses a goal directed activity is motivation or
- Mood, drive, urge or readiness (strong desire) of an animal to perform a certain behavior is motivation.
- -Each instinctive behavior has a certain motivational level at all times. This value is declined when the act is performed, then rises again.

- -At different times, the animals respond in different ways to the same stimulus. eg. Hungry dog.
- (a) Appetitive or searching behavior- Food searching (motivational level is highest)
- (b) Consummatory behavior- Finds the goal/food and eats (motivational level is minimum)
- (c) Refractory behavior- Quiescent period; dog will not eat for some time (after this motivational level rises gradually and so on).
- -Hence all instinctive behaviour (genes determined) are regulated by motivation.
- -Large motivational systems are- feeding, reproduction, fight and flee.
- -In a motivational system, there are many sub-units of behavior. e**g**. Reproduction- sexual behavior, nest buildings, care of young, etc.(each has own drive mechanism).

6. Conditioned reflex

a. Salivation in dogs:

- It is a type of learning and an association is formed between some normal body function and a new stimulus.
- -It is based on the work of Ivan Pavlov.
- -Animals have inbuilt reflexes but he found that new reflexes could be conditioned (learned).
- Pavlov rang a bell just before he fed a dog; the dog formed an association between the sound of bell and the food, and started salivation.
- -Eventually even when the bell was rang in absence of food, the dog salivated.
- -Food is unconditioned stimulus while bell is conditioned stimulus, salivation with food and ring is unconditioned response and salivation without food but with ring is conditioned response.
- -If the bell no longer signaled food, the dog eventually stopped responding to it (extinction).



Animal signals and communication

1. Dishonest communication

a. Female *Photuris* and Male *Photinus*:

- It is the manipulated communication which is beneficial to only sender.
- Female *Photuris* (firefly) mimic the flesh pattern of female *Photinus*. When *Photinus* male approaches the female *Photuris* for mating, she kills him and eats him quickly.

2. Stridulation

a. Rubbing of Wings and hind legs:

- Various insects produce sound by rubbing two body parts, mainly for mating.
- They also have receivers of sound (Trichoid Sensilla, ear on the leg or abdomen, Johnston organ at the base of antennae, tympanic organs on the sides of abdomen or on the tibial segments of forelegs of grasshoppers and antennae).

Mimicry

1. Leaf insect/moth

-Leaf insect (*Phyllium*) that uses their physical appearance to blend in with their surroundings.
-It shows extreme degree of camouflage.
-Its body, wings and even legs are flattened and green coloured like plants.
-They resemble and match so much with the foliage on which they feed that they become invisible.

2. Stick insect:

-Stick insect (*Carausius*) camouflaged as a eucalyptus twig.
-Its selender body resembles a twig or stick.
-It shows changes in colour (grey, green, yellow, etc.) like environment.

3. Non-poisonous snake and coral snake:

-When a harmless snake develops the red, yellow, and black pattern of a poisonous coral snake**.**

4. Viceroy and Monarch butter flies:

- The viceroy butterfly (*Limenitis archippus*) is a palatable member of a Müllerian complex with the monarch butterfly (*Danaus plexippus*) which is unpalatable, sharing coloration patterns and display behaviour of monarch.
- -The viceroy has subspecies with somewhat different coloration, each closely matching the local Monarch species.

Ex.2. Study the functioning of brain in rats by T-maze.

Aim: To study the functioning of brain in rats by T-maze.

Principle: T- maze spontaneous alteration is a behavioural test for measuring exploratory behaviour in animals, especially rodents, mice and rats. Brain areas involved this test includes hippocampus, septum, basal forebrain and prefrontal cortex.

The maze is a T-shaped apparatus featuring a stem (start arm) and two lateral goal arms (left and right arms). The procedure is based on the natural tendency of rodents to prefer exploring

a novel arm over a familiar one,which includes them to alternate the choice of the goal arm across repeated trails.

Requirements: Albino rats, T-maze, alcohol, cotton, etc.

Procedure:

1. Left and right arm gates are opened, animal is placed in start arm and recoding begins.

2. Start gate is opened quietly when animal is facing away from goal arms and the timing begins.

3. When all four paws enter into the one arm, other arm gate is closed and arm entry is recorded as R or L.

4. Arm gate is closed upon return from arm, and start gate is closed upon re-entry into starting arm. If animal is motionless in goal arm for longer than 90 sec, rolled paper towel is used to guide the mouse towards the start arm.

5. This procedure is repeated for total 5-6 times and time recorded.

6. Arena is cleaned with alcohol between the trails and then with another rat/mice this procedure is repeated.

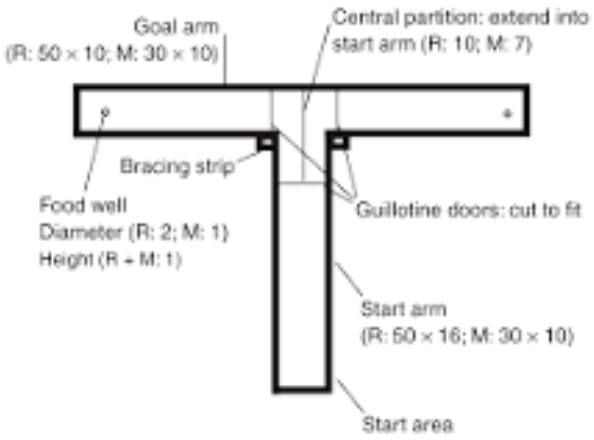
Data analysis and tabulation:

Rat- A					Rat-B				
No.	Left arm	Right arm	Latency (Sec)	Reference	No.	Left arm	Right arm	Latency (Sec)	Reference
0		✓	04		0	✓		03	
1		✓	16	-1	1		✓	06	+1
2	✓		02	+1	2	✓		11	+1
3		✓	03	+1	3		✓	17	+1
4	✓		23	+1	4	✓		27	+1
5		✓	20	+1	5	✓		19	-1
6		✓	03	-1	6	✓		11	-1

Results:

-Average of latency of mouse A- 70/7 = 10 sec

- Average of latency of mouse B- 89/7 = 12.7 sec (Avoid the given data)



T-maze

Ex.3. Study the functioning of brain in rats by elevated plus maze.

Aim: To study the functioning of brain in rats by elevated plus maze.

Introduction: The elevated plus maze (EPM) test is widely used behavioural assay for rodents and it has been validated to assess the anti-anxiety effects of pharmacological agents and steroid hormones and to define brain regions and mechanisms underlying anxiety related behaviour. The elevated plus maze apparatus consists of a T- shaped maze elevated above the floor with two oppositely positioned closed arms, two oppositely positioned open arm over closed arm is calculated to measure anxiety- like behaviour.

Materials required:

-Any stain of rat
-Elevated plus maze apparatus
-Alcohol

Procedure:

1. Clean the whole apparatus with alcohol before starting the experiment.
2. Take rodent out of its cage and place at the junction of the open and closed arm, facing the open arm opposite to where experimenter is.
3. Set 03 minutes timer and note the observed movements of the rat.
4. Calculate the time, how much it spend its time on closed and open arms.
5. An open arm entry is counted when all four paws of the rodent are on the open arm.
6. At the end of 05 min of the test, remove the rodent from the maze and place it into cage carefully.
7. Clean the maze with alcohol before testing with another rodent.

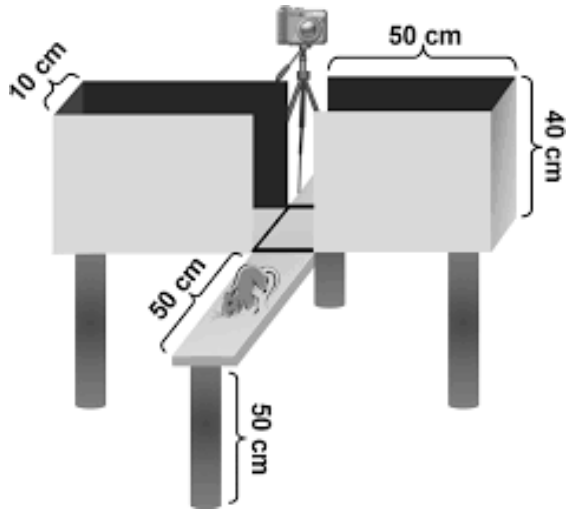
Data Analysis and Tabulation:

Time spend (Sec) on each arm	
Close arm	**Open arm**
19.6	11.18
19.5	25.7
17.63	34.13
32.42	4.33
24.97	13.15
1.17	19.23

Results:

-Mean of time spend on closed arm- 30.88 sec (Avoid the given data)

- Mean of time spend on open arm- 16.67 sec



Elevated Plus Maze

Ex.4. Study the functioning of brain in rat by rotarod.

Aim: To study the functioning of brain in rat by rotarod.

Principle: The rotarod test is conducted to evaluate motor co-ordination and balance. It provides a quick and simple estimation of neuromuscular co-ordination. Mice have to keep their balance in rotating rod. The time (latency) it takes the rat to fall off the rod rotating at different speeds around continuous acceleration (eg. From 4 to 40 rpm) is recorded as well as the season for trail end (eg. Falling a jumping) is recorded.

Requirements:

1. Any stain of rats, those require training and should be acclimated to testing environment and experimenter before testing.

2. Appropriate rotarod system for rat, including rotating rod with separate lanes and fall sensors.

3. Virkons used between trials to estimate visual and olfactory residue in arena.

4. Alcohol, cotton, stopwatch, rate cage, etc.

Procedure:

Before testing:

1. Acclimatization:

-Rats in home cage are placed in testing room for at least one hour before testing to minimize the effects of stress on behaviour during testing.

2. Training:

-Rats should be able to walk forward on rotating rod.

-Animals from cage are placed in separate lanes on rod rotating at 5 rpm such that animals may walk forward to keep balance.

-After three minutes on rod animals are returned to home cage and apparatus is cleaned with alcohol between trails.

-Procedure is separated for total of three trails/two trails separated by 10 minutes intertrailinterval.

-Trails may be repeated once if animal fall off rod before 60 sec cut off but no more than five trails should be run per animal.

-Subject/rat must be able to stay on rod rotating at 5 rpm for 60 sec before proceeding to testing.

Testing procedure:

1. Apparatus is set to accelerate from 4 to 40 rpm in 180 sec and animals from some cage are placed in separate lanes on rod initially rotating at 5rpm.

2. Trails begin when acceleration is started and ends when animals fall off rod. If animals cling to rod and complete fall passive rotation time is stopped for animal.

3. Apparatus is cleaned with alcohol between trails.

4. Procedure is repeated for total of two trails separated by 10 minutes interval. At the end of trails animal should be weighed.

Data analysis:

The following parameter are collected for analysis:

-Latency to fall

-Speed at fall

Observation table:

Speed of rotarod (rpm)	**Latency/time to fall (Sec)**	
	Rat (A)	**Rat (B)**
15 rpm		
Mean value		

Result:

Latency to fall at 15 rpm : Rat (A)- Rat (B-)

Ex.5. Nest making behavior of birds.

Aim: Study of the nesting behaviour in some common birds.

Principle/Introduction: Bird nests are designed to contain and protect parents, eggs and nestlings and to facilitate optimal safety and climatic conditions from egg-laying to fledging offspring. Birds use a variety of materials to construct their nests and they can literally use a wide range of materials like twigs, leaves, cotton fibres, hairs and even mud to build their nest. The use of twigs and grasses are a common practice.

Type of nests: There are several types of nests build by raptors, wetland birds, ground-nesting birds and colony-nesting birds to contribute specifics related to those taxa.

1. Indian Houses Crow (*Corvus Splendens*):

House crow is a common bird. Body length is 40 cm. The forehead, crown, throat and upper breast are a richly glossed black, whilst the neck and breast are a lighter grey brown in color. The wings, tail and legs are black. It feeds largely or refuses around human habitations, small reptiles, insects, small invertebrates, eggs, nestlings, grain, fruits, etc.

Construction of Nest:

Nests are placed 18-60 feet above ground, they are usually 12 inches wide and they are built using twigs, sticks and are lined with bark strip grass noses leaves, feathers and human hair. They built the nest in same trees and local environment seem to be necessary for its successful breeding, although they occasionally nest on telephone towers, etc. They lay 3-6 eggs in a typical stick nest.

2. Indian Weaver Bird (*Polceus Philippinus*):

Baya is a weaver bird found across south and Southeast Asia. Flocks of these birds are found in grasslands, cultivated areas, and shrub. They are widespread and common within their range but prone to local, seasonal movements mainly in response to resin and foods availability.

They are 15cm, sparrow sized and in their non breeding plumage both males and females resemble female house sparrow. They have stout conical bill and a short square tails. Non breeding males and females look alike, dark brown streaked fulvous buff above, plain whitish fulvous below eyebrow long and buff colored, bill is horn colored and no mask. Breeding males have bright yellow crown, dark brown mask, blackish brown bill upper parts

are dark brown streaked with yellow, with a yellow breast and cream buff below.

Construction of Nest:

During breeding season males begin building nests. Nests are build mainly in colonies. Pendulous nests are retort shaped, with a central nesting chamber and a long vertical tube that leads to a side entrance to the chamber. Nests woven with long strips of paddy leaves, rough grasses and long strips to from palm fronds. Each strip can be below 20-60 cm. in length. Females lay about 2 to 4 white eggs.


3. Red Vented Bulbul (*Pycnonotus cafer*):

Red vented bulbul is a passerine birds. It is resident breeder in tropical southern Asia from India, Srilanka, Burma and China. It is identified by its short crest giving the head a squarish appearance. Body is dark brown with a scaly pattern white head is darker or black. The rump is white the vent is red. The black tail is tipped in white. They are about 20cm. in length. It feeds mainly on fruits, petals of flowers, nector, insects and occasionally geckos.

Construction of Nest:

It builds its nest in a bush at a height of around 2-3 meter. They occasionally built inside houses or in a hole in a mud bank. In one instance a nest was found on a floating mat of water hyacinth leaves, nest in tree cavities have also been noted. They breed from June to September. Eggs are pale pinkish with spots of darker red more dense at broad end. They use small twigs and metal wires for nesting. The eggs hatch after about 14 days.


4. Rock Pigeon (*Columba livia*):

Rock pigeon or rock dove is a member of the bird family columbidae. Habitats include various open and semi open environments including agricultural and urban areas. Originally found wild in Europe, North Africa and Western Asia, feral rock pigeons have become established in cities around the world.

These are pale grey with black bars on each wing. It is 32-37 cm. long has dark bluish grey head, neck and chest with glossy yellowish, greenish and reddish purple iridescence along its neck and wing feathers. The iris is orange bare skin around the eye is bluish Greybull is grey black with a conspicuous off white cere and feet are purplish red. They fed on food grains.

Construction of Nest:

They breed at any time of year, but peak times are spring and summer. Nesting sites are situated along coastal cliff faces as well as artificial cliff faces created by apartment buildings with accessible ledges or roof spaces. The type of nest constructed is a family platform of straw, sticks, twigs and grasses, put on ledge under cover. Two white eggs are laid with incubation that is shared by both parents lasting from 17 to19 days and the fledging period is 30 days.

Finding nest: You may become aware of a nest when you see a bird with nesting material in its beak, or one carrying food in its beak (birds rarely fly around with food in their beaks except when they are feeding nestlings. An obvious exception is raptors, or birds that are engaged in courtship feeding, or carrying food items that are too large to swallow whole). If you are going to search for the nest, it is highly recommended to first try and learn the nesting habits of the species, to get a sense of where you should look for the nest. Always be very careful when walking through habitats like dense undergrowth or grassland so that you do not disturb, step upon, or dislodge nests of other birds.

Nest measurements:

-dimensions of the nest cavity and entrance

-Size

Observation of nests:

Sr. No.	Name of bird	Nest type	Construction materials	Occupied/abandoned	Location of nest	Visiting date and time
1.						
2.						
3.						
4.						
5.						
6.						
7.						

Precautions:

-Minimizing disturbance caused by the observer at nests is paramount.

-The observer should always put the safety and welfare of the bird(s) before the success of their research.

-Utmost care should be taken to make sure that research methods do not jeopardize the nest(s) or the birds in any way.

-Do not touch the eggs or nestlings. Otherwise birds may leave them.

Ex.6. Geotaxis test (RING test) in Drosophilla.

AIM: Determination of *Drosophila* climbing ability by RING (Rapid Iterative Negative Geotaxis) assay

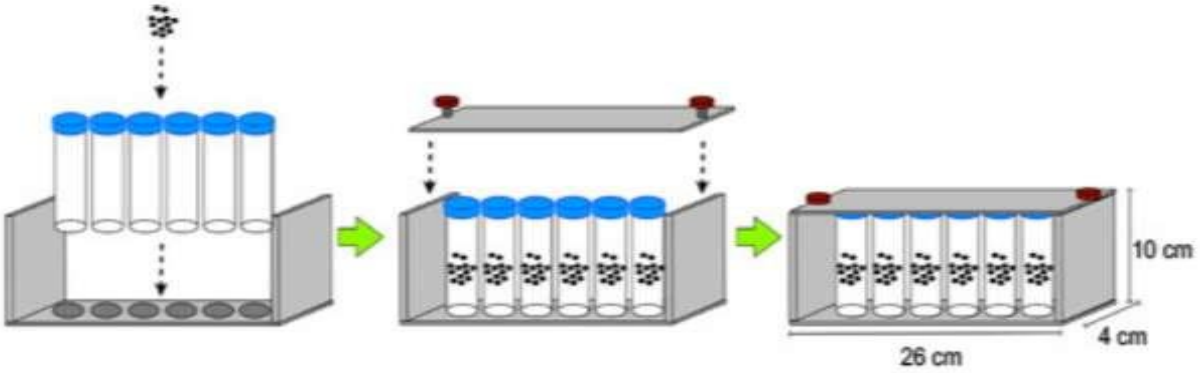
Requirements: Adult *Drosophila,* polystyrene vials, Brush, Camera, Test tube stand, timer.

Introduction: This is a simple and robust behavioral assays for determining adult climbing ability (RING assay), of *Drosophila.* In the RING assay, several genotypes or drug treatments can be tested simultaneously using large number of animals.

Protocol (Gargano et al.)

1. Collect newly emerged adult male/Female flies under cold anesthetization and place into astandard vial containing food.
2. Maintain flies at room temperature (25 - 30°C) for 1 hour to allow for recovery from cold shock.
3. Transfer about 25 flies in polystyrene vials and assemble vials with flies into the RINGapparatus.

4. Allow flies to acclimate to the environment, undisturbed, for 15-20 minutes.
5. During this time place digital camera ~1 m in front of the apparatus (mid-height of the vials), and set a timer to 3.0 seconds.
6. Sharply tap the apparatus down on the surface of the bench three times, ensuring that the tap ishard enough to knock down all the flies to the bottom of the vials.
7. Simultaneously with completion of the third tap, start the 3 second countdown timer.
8. At three seconds take a picture.
9. Reset the timer for 1 minute and start. During this time reset the camera and focus on the apparatus, and set another channel of the timer for three seconds.
10. After 1 minute, repeat steps 7 to 10
11. After a total of 5-6 trials, upload images onto a computer and use your favourite image viewerto open, and score the average height climbed for each vial.
12. Perform statistical analysis on your different groups comparing the mean height climbed.Observation:



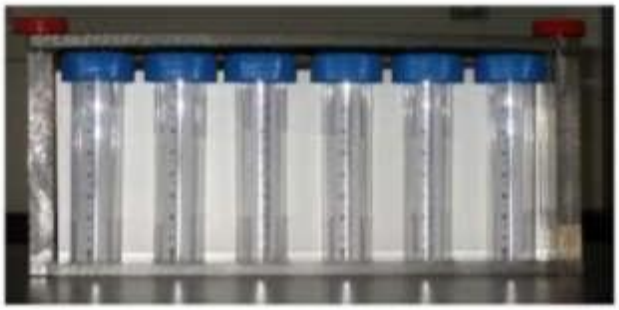
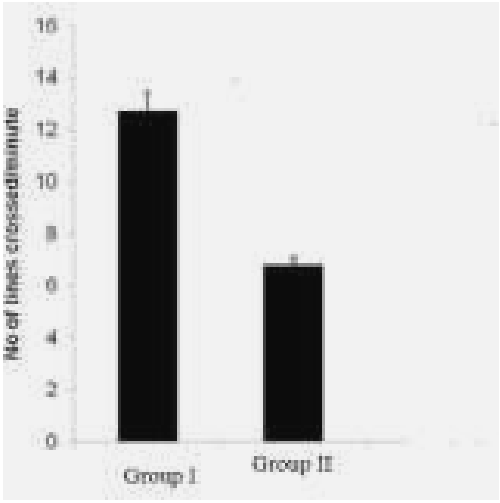


Figure 1. Setup for the RING Assay. The digital camera is placed ~1 m in front of the apparatus containing flies in the polystyrene vials; focus and zoom the camera onto the apparatus; and set a timer to 3.0 seconds.

Results:



Precaution:

-Carefully take hold of the RING apparatus with your left hand so as not to disturb the flies, and hold the timer with your right hand.

-Young wild-type adult flies should have an average climbing height of ~4-5 cm in a 3-second time period (the time can be adjusted from 3 seconds to accommodate different strains or activity levels to define a given average height for a given strain/treatment).

-Flies that remain at the bottom are assigned a value of 0. -It is not advised to use more than 25 flies per vial because it then becomes difficult to determine the location of each individual fly to measure height.

-No desensitization has been observed at up to the 6 consecutive trials spaced 1 minute apart that we have employed.

-It is critical NOT to reuse the polystyrene testing vials in this assay after the initial sets of data are gathered because new fliesplaced into used vials will not climb to the same extent as in fresh vials.

Ex.7. Study of interspecific association between cattle and egrets.

Aim: Study of inter-specific association between cattle and cattle egrets.

Principle: The interaction that exist between cattle egret and cattle is known as commensalism. In this type of interaction, one species is benefited whereas the other is neither benefited nor harmed. The cattle, as they move, stir up and flush out insects from the vegetation that they graze, that otherwise might be difficult for the egrets to find and catch. The cattle is neither benefited nor harmed but the egret is benefited.

Study location: The present study location is GGU campus. In the campus, cattle egrets and cows are normally seen which live in association, particularly for feeding.

Pilot study: The observations should be recorded in 15 minutes time intervals. 10 minutes for behavioural observations, followed by 05 minutes dedicated to keeping in pace the cattle.

Behavioural observations:

1. Flying: Use of wings to move through the air, with the wings either flapping or used to assist gliding.

2. Walking: Standing in an upright position with movement at regular pace by lifting and setting each foot down in turn, both feet are not off the ground at once.

3. Vigilance: Remaining in a single area with head pointed upwards either motionless or with an occasional scanning motion of the head:

4. Leaning stationary: The head is leaning forward and down almost parallel with the body with the legs stationary.

5. Leaning whilst walking: The head is leaning forward and down almost parallel with the body whilst in a walking motion.

6. Pecking: Strike forward with the beak to bite.

7. Running: Standing in an upright position with movement at regular pace by lifting and setting each foot down in turn, both feet are not off the ground at once. At a faster pace than walking.

Observation table: Observation started at the time of: …….

Sr. No.	Activities to be observed	Observation of activities (Yes/No) and Time
1.	Flying	
2.	Walking	
3.	Vigilance	
4.	Leaning stationary	
5.	Leaning whilst walking	
6.	Pecking	
7.	Running	

Leaning whilst walking

Pecking

Leaning stationary

Vigilance

Flying

Running