ECOLOGY AND PHYSIOLOGY

1. Chemical ecology

   a. Plants, nutrition and insect reproduction

2. The physiology of migration in insects
3. The physiology of diapause in insects (several examples)

   a. Monarch butterfly-a classic model in integrative studies on insect physiology and chemical ecology

      (1) Migration and diapause

   b. Large milkweed bug-case study into the physiology of migration

4. Photoperiodism
5. Communication systems

TABLE 1.1 Ecological Hierarchy and the Structural and Functional Properties Characterizing Each Level

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Population	Density Dispersion Age structure Genetic structure	Natality Mortality Dispersal Gene flow Temporal and spatial changes
Individual	Morphology Physiology Behavior	Resource acquisition Resource allocation Learning

1. Chemical ecology-Plant insect interactions

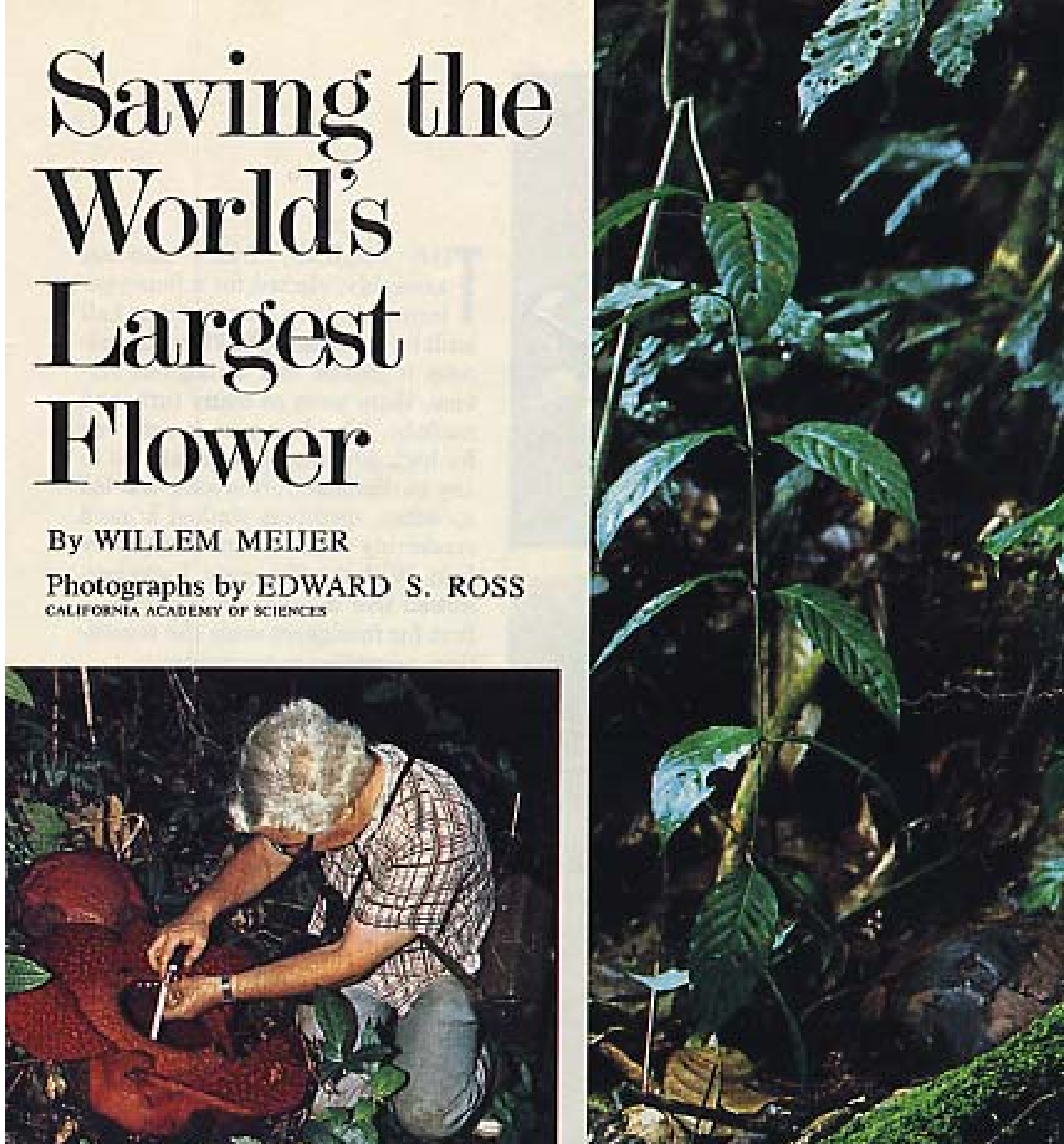
a. Plants and fungi – nutrition, deception and insect reproduction

Stapelia, the starfish flower, smells rotten but blowflies love it. It even has the color of ‘meat’ and has tiny hairs (see below) that resemble hairs on a carcass. Blowflies are attracted to it and lay their eggs on it. The role of the larva in pollination has not been explored. No benefit to the fly.

K. Spender (ed.). The chemical mediation of coevolution. Pergamon Press, NY. Article by M. Stowe on **Chemical mimicry**

Rafflesia arnoldii

1. **No roots**
2. **No photosynthetic tissue**
3. **Is a parasite on roots of wild vines of the grape family**
4. **Produces a stinky odor**
5. **Pollinated by flies**

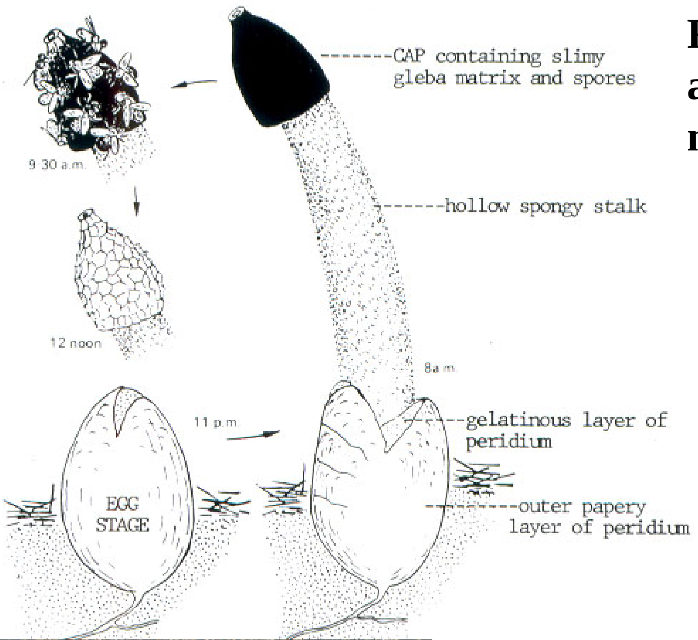


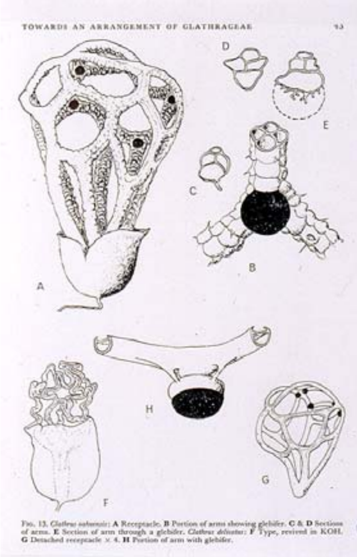
Ed Ross is a famous photographer and is also a noted entomologist

Stinkhorn fungi-Phallales

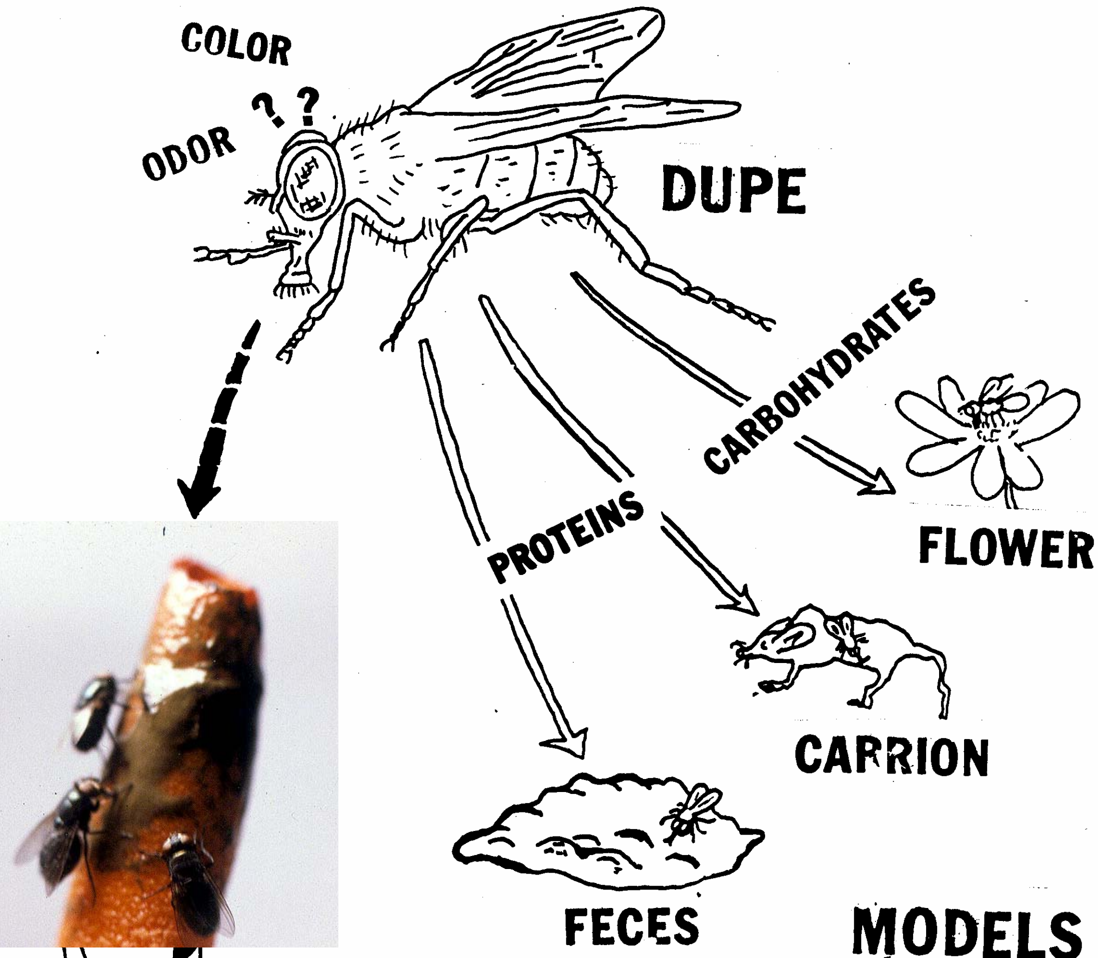
Flies are attracted to the stink that mimics a dead animal and feed on the gleba or sticky matrix containing the fungal spores.

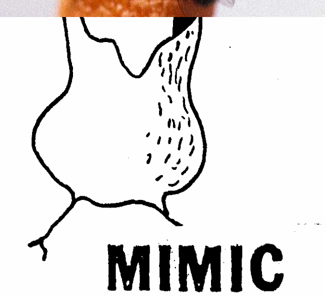
Taken from: C.T. Ingold. 1971. Fungal Spores-Their Liberation and Dispersal.





CHEMICAL MIMICRY





WHAT IS THE BENEFIT TO THE FUNGUS?

Since blowflies feed on the stinkhorn's gleba, one can develop the following hypotheses:

1. Does the gleba provide the female fly with the necessary nutrients to produce egg?

2. Does the gleba taste like carbohydrates and thus act as a phagostimulant?

3. Does the gleba produce an odor that mimics feces or carrion?

EFFECT OF DIFFERENT DIETS ON VITELLOGENESIS OR OVARY DEVELOPMENT IN *PHORMIA REGINA*

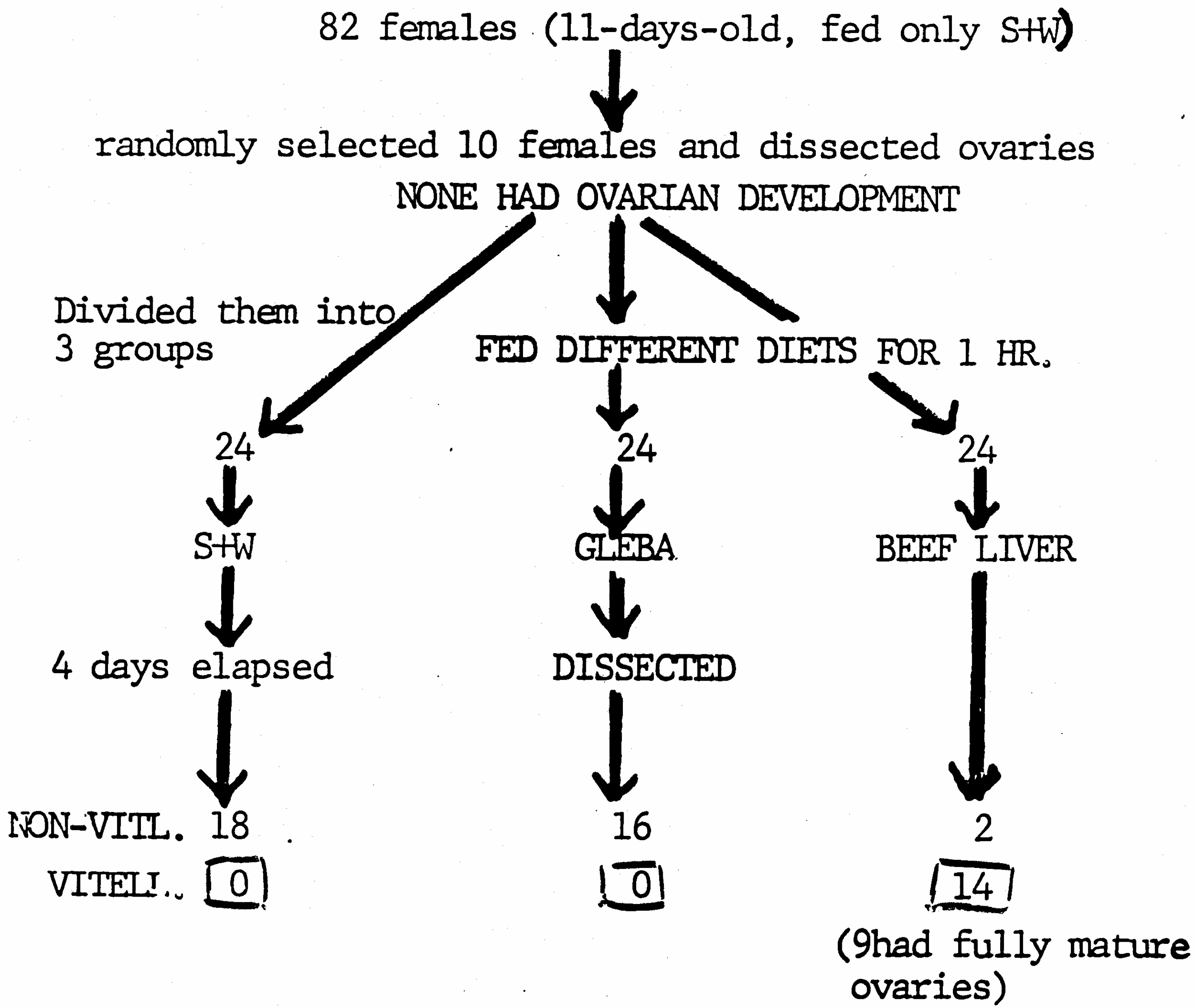


Table 1. Effect of different diets on the average total protein concentration in female hemolymph and ovary of *P. regina*

Days[a]	Diet					
	Sugar		*M. caninus* gleba		Liver	
	Hemolymph μg/μl	Ovary μg	Hemolymph μg/μl	Ovary μg	Hemolymph μg/μl	Ovary μg
1	1.68	5.0	7.25	21.04	8.13	28.75
2	2.89	6.0	17.76	46.84	27.00	302.50
3	1.50	4.5	24.57	287.50	50.00	422.50

[a] Days following feeding on the diet.

Table 2. Effect of different diets on average vitellogenin concentration in the hemolymph and vitellin concentration in the ovary of *P. regina*

Days[a]	Diet					
	Sugar		*M. caninus* gleba		Liver	
	Hemolymph μg/μl	Ovary μg	Hemolymph μg/μl	Ovary μg	Hemolymph μg/μl	Ovary μg
1	ND	ND	1.30	ND	2.30	ND
2	ND	ND	2.08	ND	5.30	51.5
3	ND	ND	3.10	4.53	1.00	115.3

[a] Days following feeding on the diet; ND, none detected.

Stoffolano, Yin and Zou. 1989. Reproductive consequences for female black blowfly (Diptera: Calliphoridae) fed on the stinkhorn fungus, *Mutinus caninus*. Ann. Ent. Soc. Amer. 82: 192-195.

Stoffolano, Zou and Yin. 1990. The stinkhorn fungus, *Mutinus caninus*, as a potential food for egg development in the blowfly, *Phormia regina*. Entomol. Exp. Appl. 55: 267-273.

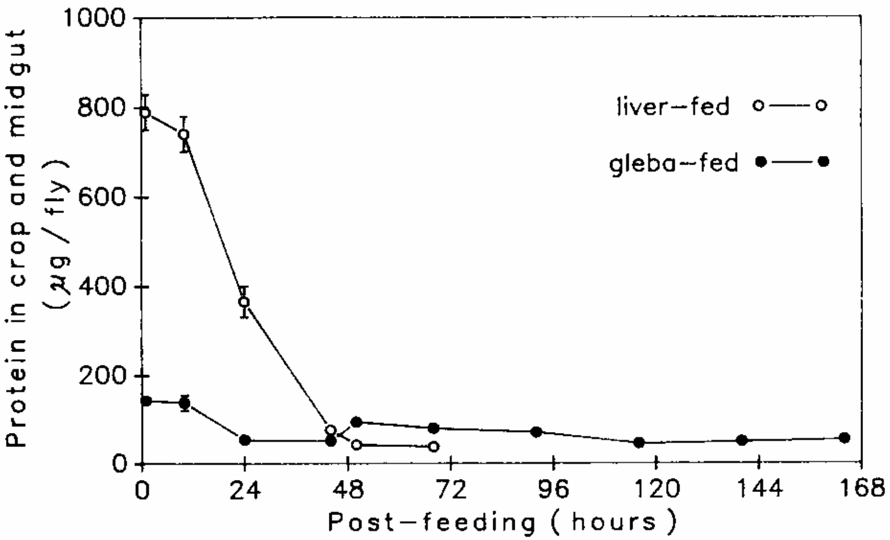


Fig. 1. The total amount of protein in the midgut and crop of *Phormia regina* females at various times (hours) following a 1 h exposure to the diets and feeding to repletion. All females initially fed diet for only 1 h at 72 h post-emergence. Gleba-fed flies were given continuous access to gleba at 44 h after the first gleba diet exposure.

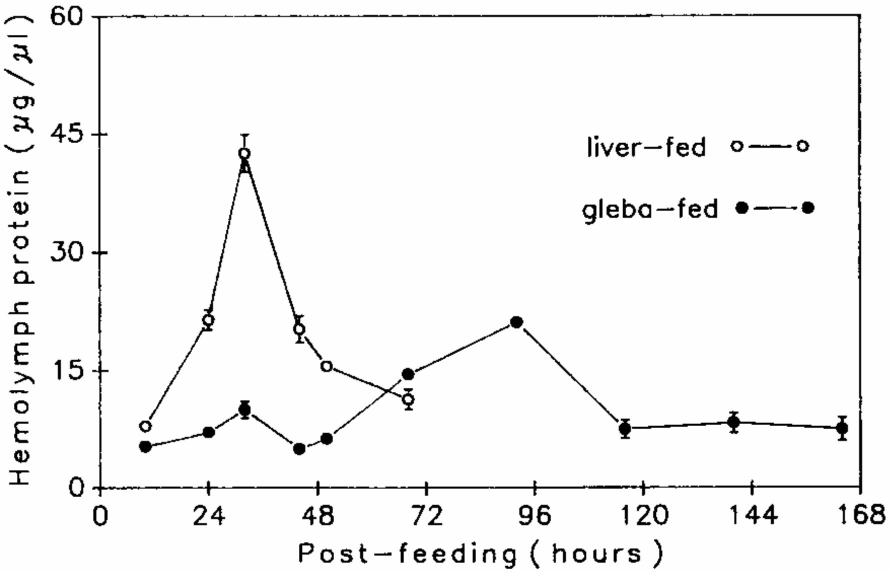


Fig. 2. The total amount of protein in the hemolymph of *Phormia regina* females at various times (hours) following a 1 h exposure to the diets and feeding to repletion. All females initially fed diet for only 1 h at 72 h post-emergence. Gleba-fed flies were given continuous access to gleba at 44 h after the first gleba diet exposure.

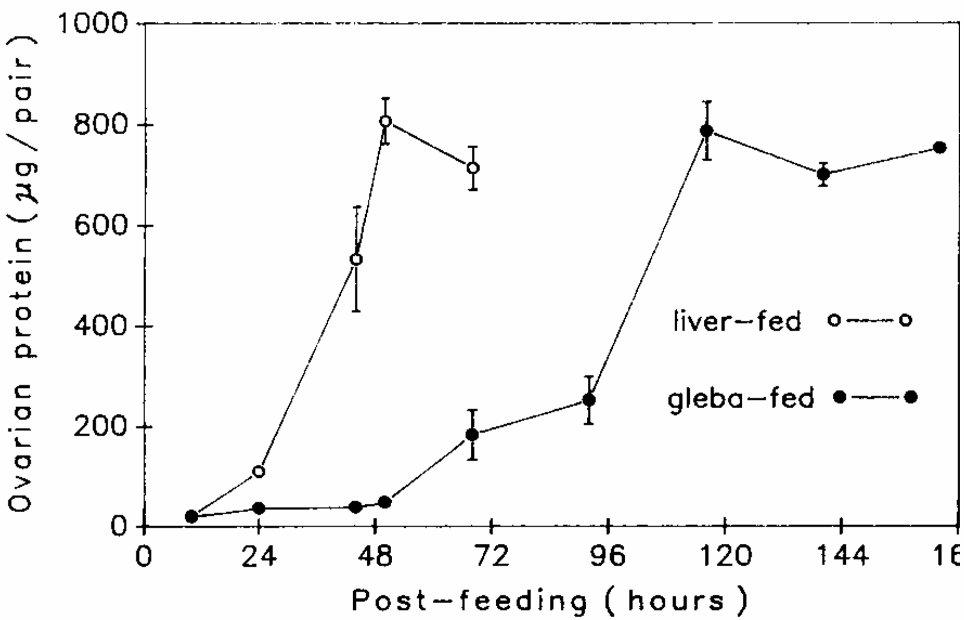


Fig. 3. The total amount of protein in the ovaries of *Phorm regina* females at various times (hours) following a 1 h exp sure to the diets and feeding to repletion. All females initial fed diet for only 1 h at 72 h post-emergence. Gleba-fed fli were given continuous access to gleba at 44 h after the fir gleba meal.

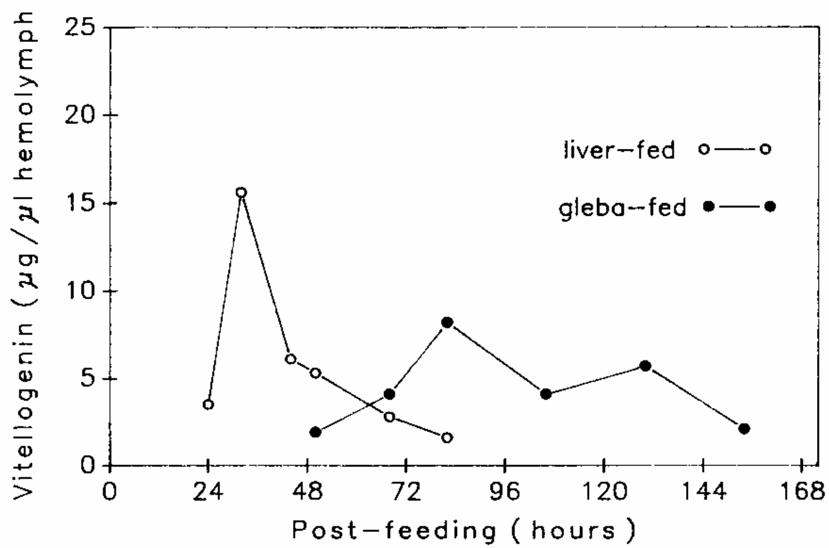


Fig. 4. The amount of vitellogenin in the hemolymph of *Phormia regina* females at various intervals (hours) following a 1 h exposure to diet and feeding to repletion. All females initially fed diet for only 1 h at 72 h post-emergence. Gleba-fed flies were given continuous access to gleba at 44 h after the first gleba diet exposure. A pooled sample of 5 ul of hemolymph taken from 5 flies was used for each Vg determination; replicated 2 times.

TABLE 4. The total amount (ug/mg) of protein in beef liver and gleba

Diet	wet wgt.	dry wgt.
liver	94.1 (± 4.7)	274.9 (± 13.8)
gleba	6.6 (± 0.3)	22.3 (± 1.1)

replicated 3 times

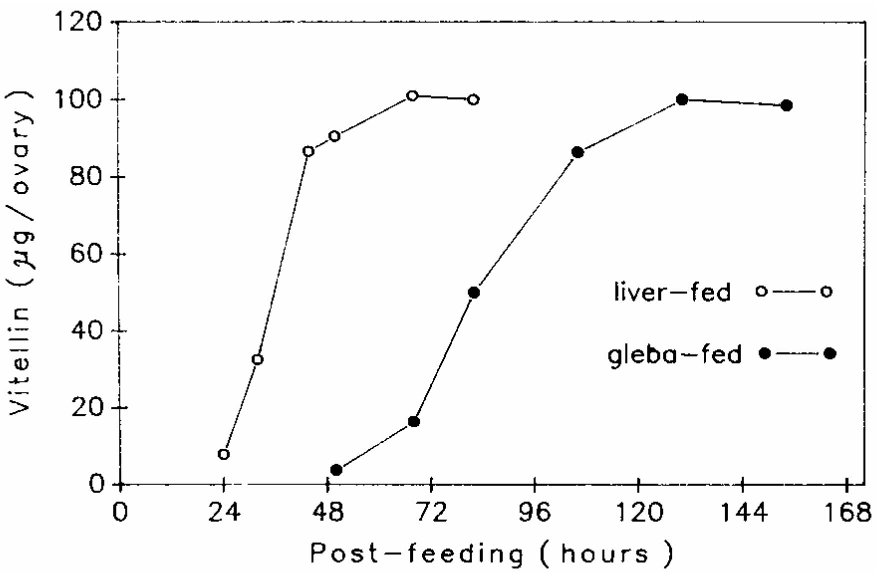
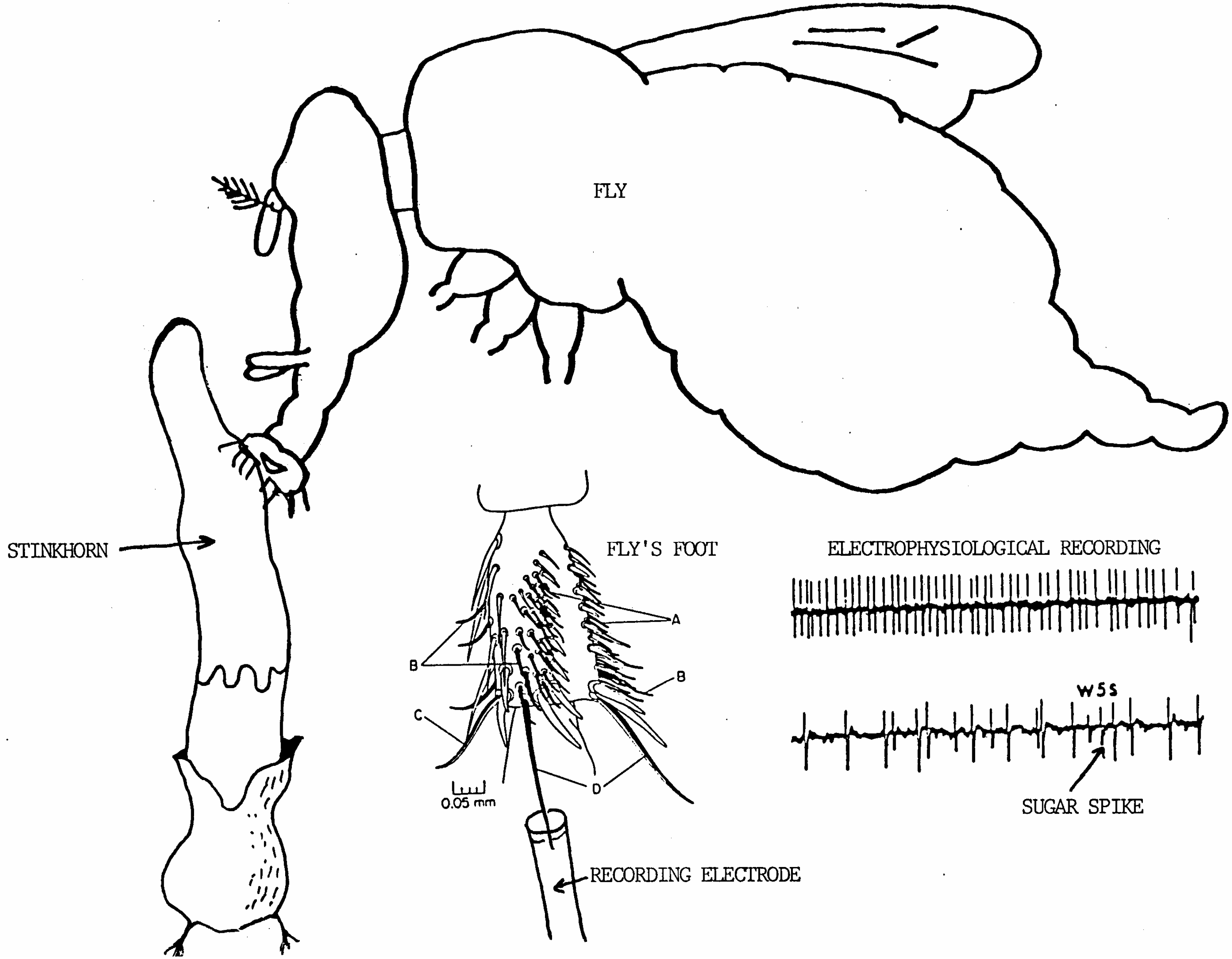


Fig. 5. The amount of vitellin in the ovary of *Phormia regina* at various times following a 1 h exposure to the diet and feeding to repletion. All females initially fed the diet for only 1 h at 72 h post-emergence. Gleba-fed flies were given continuous access to gleba at 44 h after the first gleba diet exposure. Each test replicated 2 times.

Table 1. The total amount of protein and vitellin in eggs[1] of *Phormia regina* fed two different diets

Diet	Protein (μg/egg)[2]	Vitellin (μg/egg)[3]
Liver-fed	2.56 (±0.1)	1.36
Gleba-fed[4]	2.72 (±0.3)	1.08

[1] Without egg shell; [2] replicated 3 times, 3 eggs/replicate; [3] replicated 2 times, 3 eggs/replicate; [4] eggs produced by *Phormia* only after a continuous supply of gleba was provided 44 h after the initial gleba meal.



Electrophysiological recordings from the tarsal or labellar hairs of the gleba shows that the action potentials are those that would induce a sugar, positive phagostimulatory response by the fly

HYPOTHESES

- Does the gleba provide the female fly with the necessary nutrients to produce egg?
- Does the gleba taste like carbohydrates and thus act as a phagostimulant?
- Does the gleba produce an odor that mimics feces or carrion?

CONCLUSIONS

1. With only one meal, the gleba does not provide the female with enough protein to develop her ovaries. This is mainly due to the low protein content of the gleba but, on continuous feeding that benefits the fungi, it can.
2. Based on electrophysiology, the gleba tastes like sugar to the fly.
3. Based on the literature, it appears the gleba mimics a fecal odor and not carrion.

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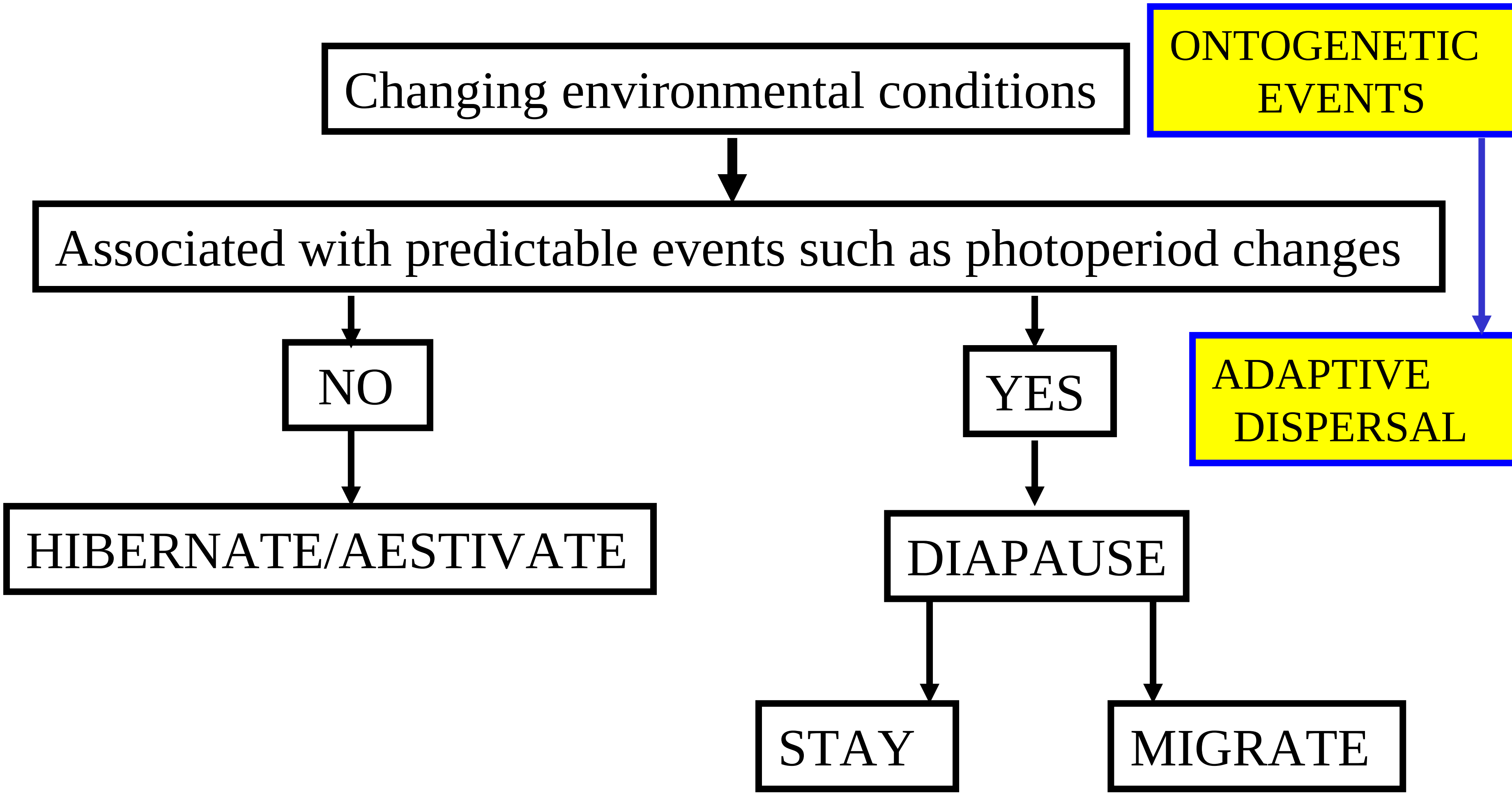
"The population responses to changing environmental conditions reflect the net physiological and behavioral responses of individuals that determine their survival and reproduction." pg. 7 from Insect Ecology- an ecosystem approach by T. D. Schowalter. 1996. Acad. Press, N.Y.



SOME INDIVIDUALS DIVIDE INSECT MOVEMENTS INTO:

1. **Random dispersal**-found in many aphids and Lepidoptera, the caterpillars that use silk lines to balloon. Insects showing this type of disperal often are at the mercy of the wind.

2. **Directed dispersal**-insects that are strong fliers and fly to a particular stimulus. These insects have a better chance of finding food.

3. **Migration**-Active mass movement of individuals and usually a two way movement.

IMPORTANT ENVIRONMENTAL FACTORS

1. Food
2. Temperature
3. Humidity

These factors can fluctuate in dramatic ways

4. **Photoperiod**-the most predictable factor used by animals for long-term survival.

INSECT MIGRATION

1. Feed-back loop involved in the tarsal/flight response
2. Migration versus adaptive dispersal
3. Environmental cues leading to migration
4. Physiology of insect migrants
   a. Monarch butterfly, *Danaus plexippus*
   b. Large milkweed bug, *Oncopeltus fasciatus*
   c. Ladybugs
   d. Locust

REFERENCES:

Dingle, H. (ed.). Evolution of insect migration and diapause. Springer-Verlag, N.Y.

Drake, V.A. and A.G. Gatehouse (eds.). 1995. Insect migration: tracking resources through space and time. Cambridge Univ. Press, Cambridge, England

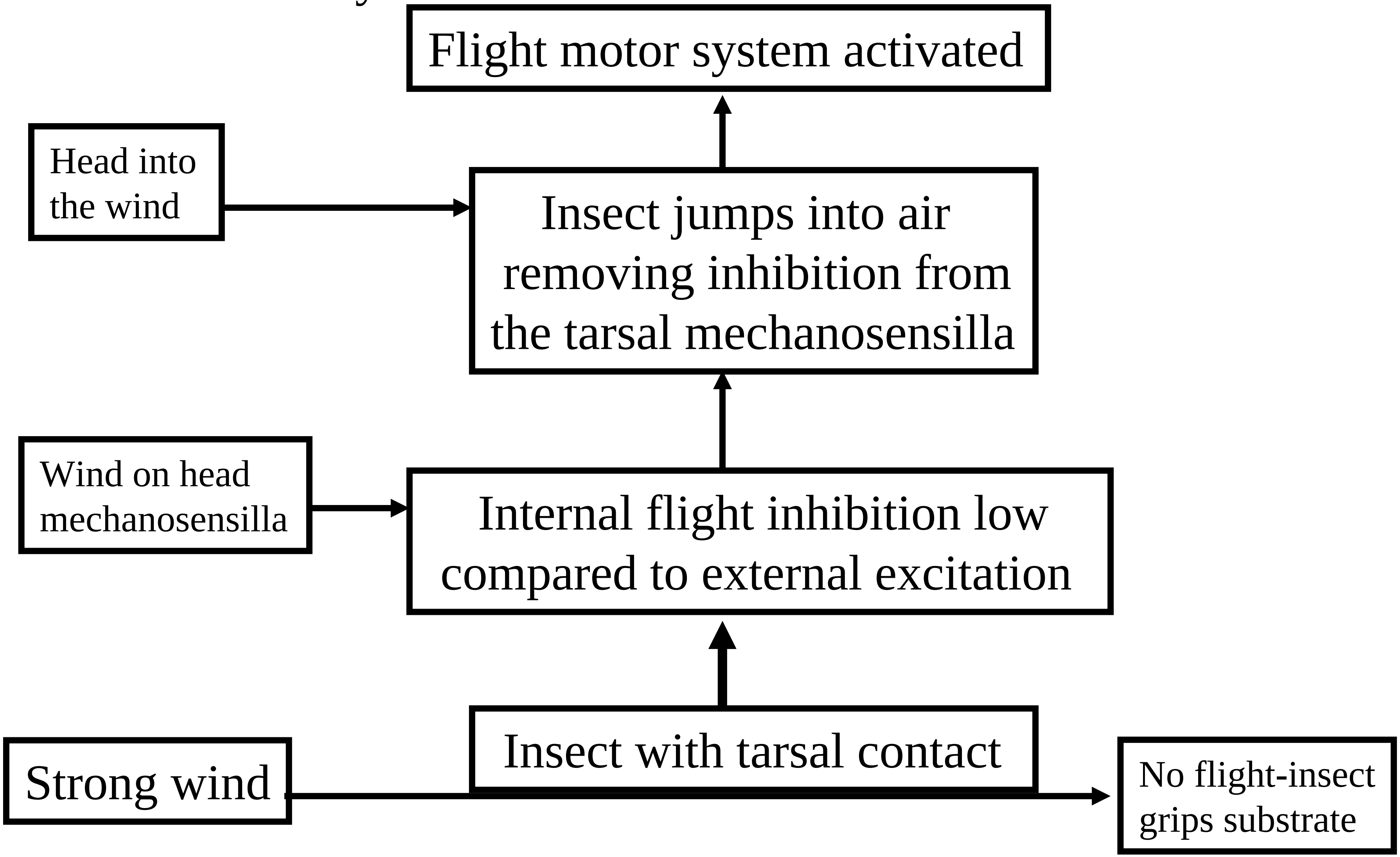
Goldsworthy, G.J. and C. H. Wheeler. 2000. Insect Flight. CRC Press, Boca Raton, FL.

Johnson, C.G. 1969. Migration and dispersal of insects by flight. Methuen & Co., Ltd., London.

Feed-back loop involved in the tarsal/flight take-off response

Two important aspects of flight are

1. Tarsal/flight take off response system
2. Wind hair system on head



MIGRATION/ADAPTIVE DISPERSAL

Migration-Usually involves a two way movement of an organism.

Adaptive dispersal-Is an ontogenetic event, usually occuring when the adults emerge and usually associated with taking the members of the population to a new food source.

Dispersal-The one-way movement of an organism

Environmental cues leading to migration

FOOD QUALITY AND SHORTAGE
TEMPERATURE
DAYLENGTH
DENSITY OF POPULATION

OOGENESIS-FLIGHT SYNDROME

ENERGY

The energy path used by the insect is influenced by food, crowding, temperature, and in some cases daylength (photophase)

REPRODUCTIVE EVENTS
MATING & EGG DEVELOPMENT

MIGRATORY EVENTS
FLIGHT MUSCLES, FLIGHT LOAD & FUEL FOR FLIGHT

Food for energy in the natural situation is an expensive item and insects have evolved various strategies to best utilize and, at the same time, conserve energy. Migration is an expensive event but insects have solved this in an energy efficient way.

Physiology of insect migrants

Monarch butterfly-case studies into ecological chemistry & migration

Where do the monarchs overwinter?

F.A. Urquhart. 1960. The monarch butterfly.Toronto Univ. Press. 361 pp

First to find site

Sitting on log when Brower arrived

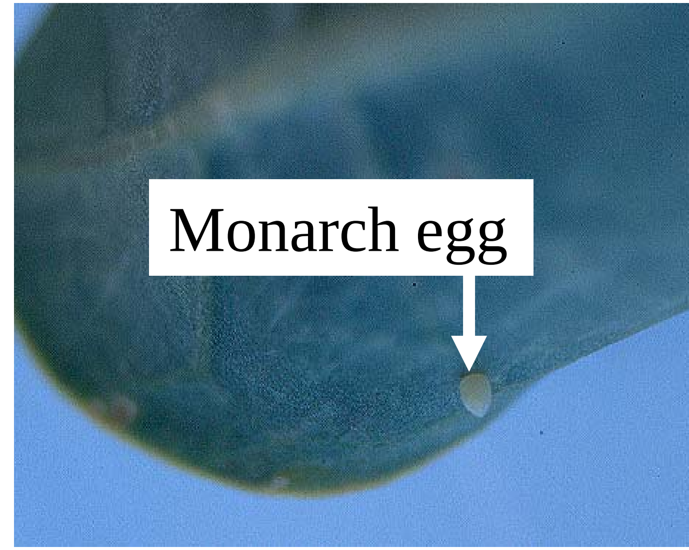
Mexican refugia

1960's at Amherst College

Bill Calvert-Postdoc at Umass

The monarch in the Pacific Islands

1. Sequestration of cardiac glycoside
2. How does the monarch handle this?
3. Ecological chemistry of plants and the benefit to the plant
4. Migration in North America



Survival of 1st instar by vein cutting

ENDOCRINOLOGY OF ADULT MONARCH

Juvenile hormone is a major hormone for this adult insect

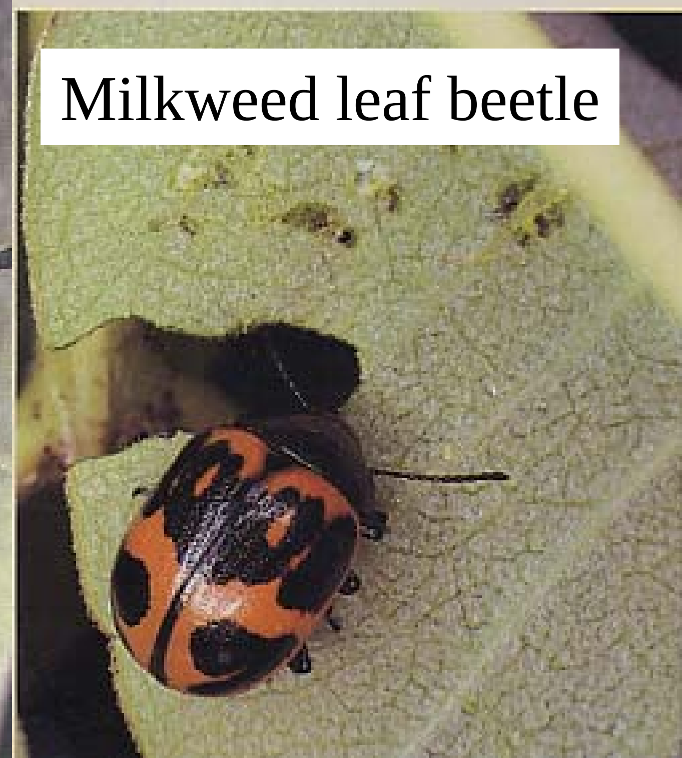
JH is essential in:

1. High titers essential for reproductive events in both sexes
   a. Growth of ovaries, female ARG and tract, including the bursa
   b. Accessory reproductive glands and male reproductive tract
2. High titers accelerate senescence in adults
3. Low titers promote reproductive diapause and migration
4. Adipokinetic hormone acts to elevate circulation of diglycerides, that are essential for flight or migration

By cutting the veins and letting the ‘milk’ or sap containing the cardiac glycosides, the insect avoids being trapped or affected by this substance.

Dussourd, D.E. and T. Eisner. Vein-cutting Behavior: insect counterpoly to latex defense of of plants. Science. 237: 898-901

Arrows point to white latex



Asclepias physocarpa (=*Gomphocarpus physocarpus*) is found in Hawaii.

Note the monarch photos below. Is there something unsual about them?

How could this white mutant be maintained in Hawaii?

AT THE OVERWINTERING SITE & AGGREGATION

1. The multicolored Asian lady beetle, *Harmonia axyridis*

2. The monarch butterfly

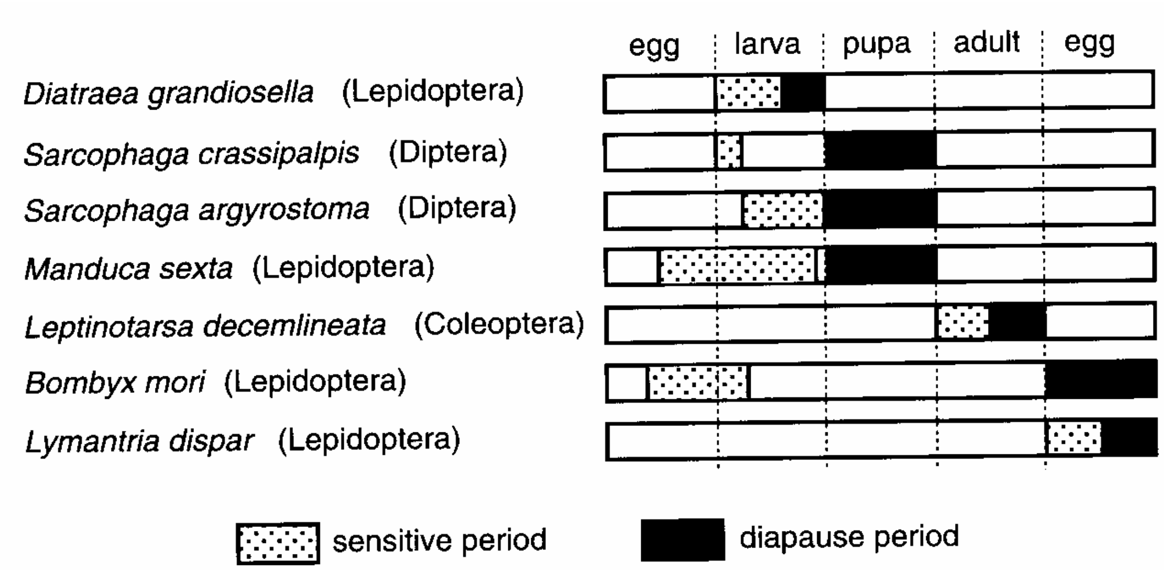
At the site:

1. Low JH levels
2. Group protection by sequestered chemicals and aposematism
3. Flight muscle mitochondria or sarcosomes usually degenerate

Smoke elicited this massive panic response of millions of aggregated monarchs in the refugia in the Sierra Chincua in central Mexico

DIAPAUSE-a physiological delay in development caused by the lack of a specific hormone in response to regularly recurring periods of adverse environmental conditions (winter and dry seasons).

Diapause can occur in any stage of insect development but in a species it is only found in one stage of development.



In diapausing embryos or larvae/nymphs, what major hormone could cause such a physiological delay and what major event does it delay?

In diapausing adults, what major hormone could cause such a physiological delay and what major event(s) does it delay?

Diapause can be

1. **Obligate**-Every individual in every generation enters diapause. These insects usually have 1 generation/yr, thus a univoltine life cycle. Genetically controlled.

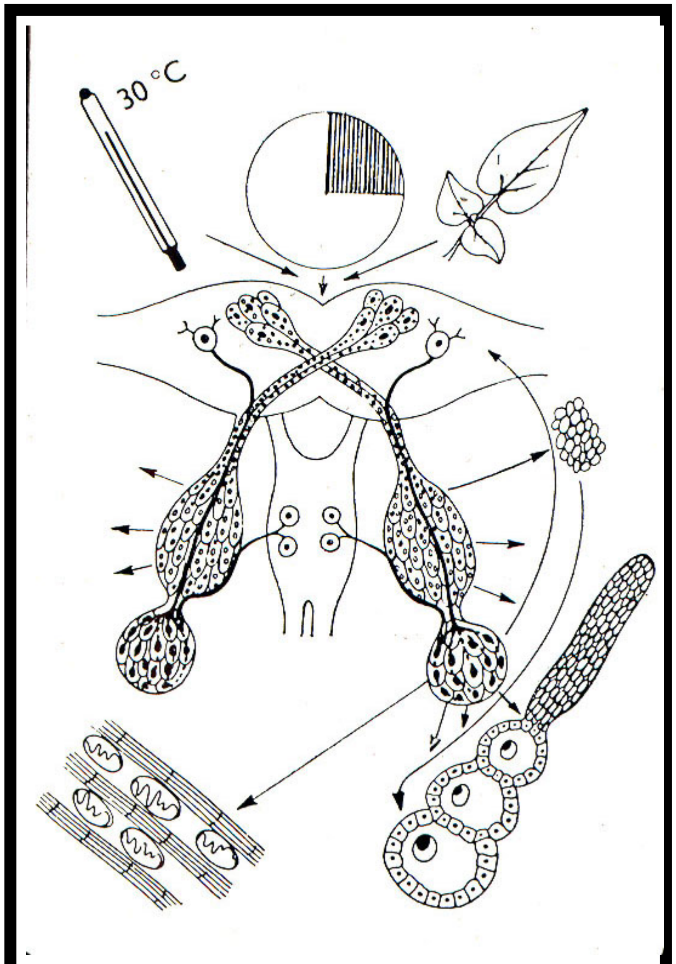
2. **Facultative**-Some generations may be completely free of diapause while some or all of the individuals in a generation may enter diapause. These insects usually have several generations/yr and are known as multivoltine. Environmentally controlled.

Diapause is a neurohomonally mediated, dynamic state of low metabolic activity. Associated with this are reduced morphogenesis, increased resistance to environmental extremes, and altered or reduced behavioral activity. Diapause occurs during a genetically determined stage of metamorphosis, and its full expressions develops in a species-specific manner, usually in response to a number of environmental stimuli that precede unfavorable conditions. Once diapause has begun, metabolic activity is suppressed even if conditions favorable for development prevail. Definition of Tauber

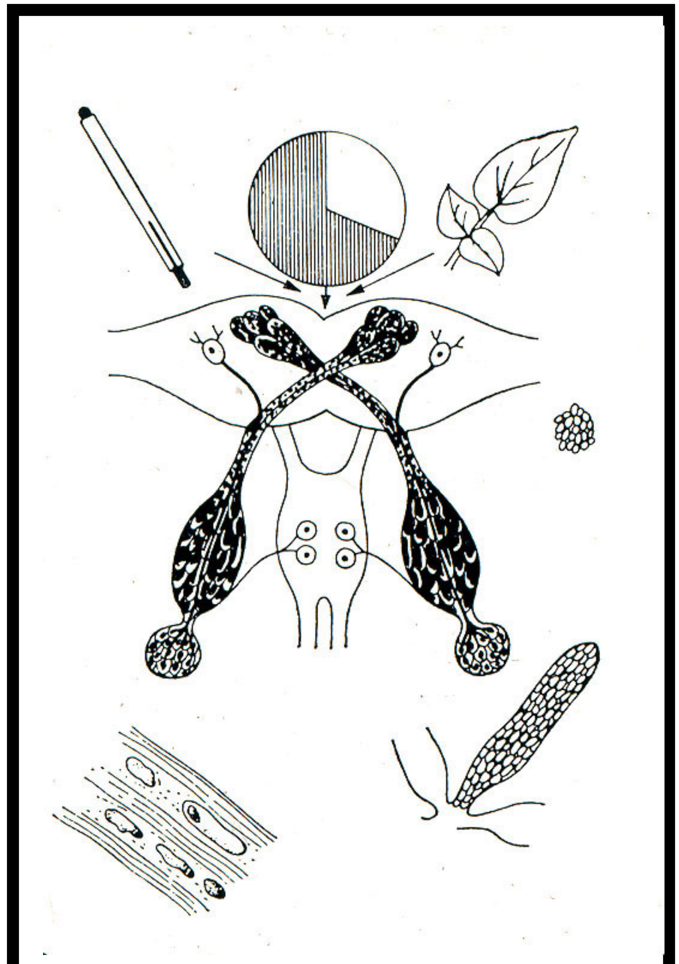
Colorado Potato Beetle-has an adult, facultative diapause

Extensive research by Jan de Wilde

Non-diapause conditions

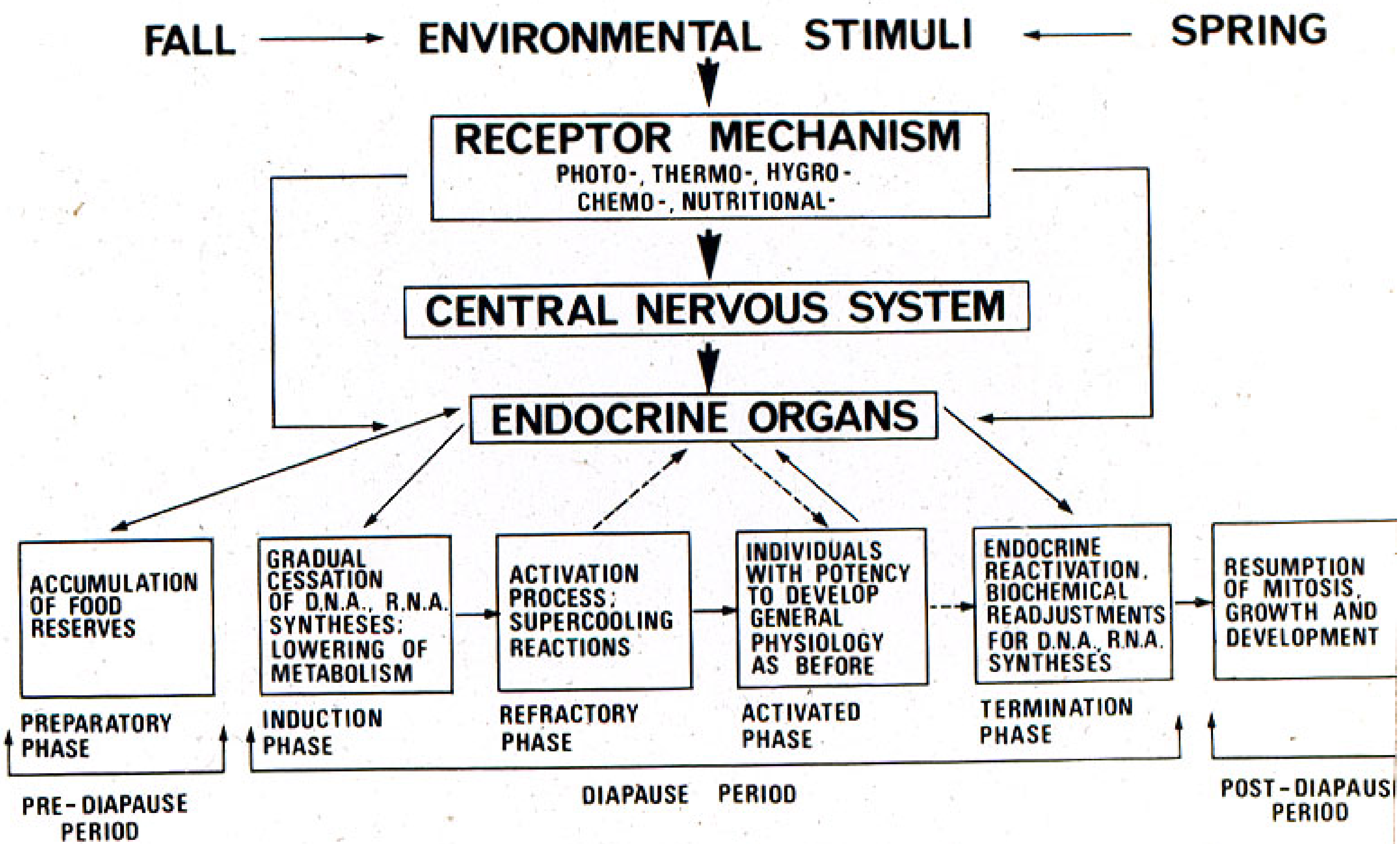


Diapause conditions



The boll weevil, *Anthonomus grandis*- a facultative, adult diapause

One of the most unusual attractions in Alabama is the Boll Weevil Monument, which its hometown of Enterprise often touts as the world's only monument honoring a pest. In the early 1960’s Perry Adkisson did extensive research on diapause in this species. Individuals at the time thought that they could control the insect by disrupting its diapause, as they wanted to do in other pests insects, thus causing the insect to die. They even believed they could do this by flooding fields with lights to lengthen the day length, thus breaking diapause or preventing it.



FALL
ENVIRONMENTAL STIMULI
SPRING
RECEPTOR MECHANISM
PHOTO-, THERMO-, HYGRO-
CHEMO-, NUTRITIONAL-
CENTRAL NERVOUS SYSTEM
ENDOCRINE ORGANS
ACCUMULATION OF FOOD RESERVES
GRADUAL CESSATION OF D.N.A., R.N.A. SYNTHESES; LOWERING OF METABOLISM
ACTIVATION PROCESS; SUPERCOOLING REACTIONS
INDIVIDUALS WITH POTENCY TO DEVELOP GENERAL PHYSIOLOGY AS BEFORE
ENDOCRINE REACTIVATION, BIOCHEMICAL READJUSTMENTS FOR D.N.A., R.N.A. SYNTHESES
RESUMPTION OF MITOSIS, GROWTH AND DEVELOPMENT
PREPARATORY PHASE
INDUCTION PHASE
REFRACTORY PHASE
ACTIVATED PHASE
TERMINATION PHASE
PRE-DIAPAUSE PERIOD
DIAPAUSE PERIOD
POST-DIAPAUS
PERIOD

Morphologic and metabolic differences between non-diapausing and diapausing insects

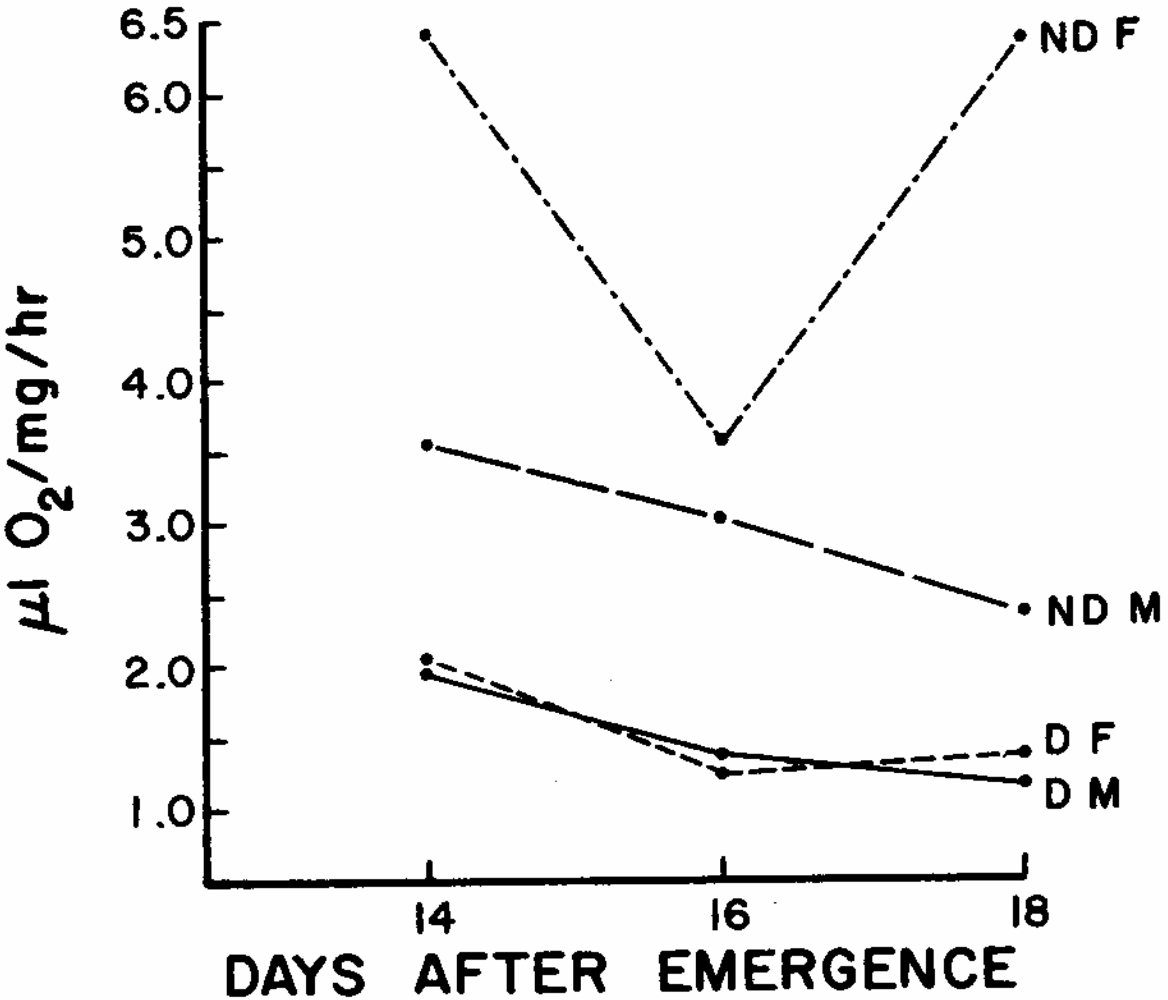
Non-diapausing	Diapausing
1. Fat bodies small	1. Fat body hypertrophy
2. Ovaries develop	2. No ovary dev.
3. Low lipids in blood	3. High lipid conc.
4. Oenocytes large	4. Oenocytes small
5. High oxygen consump.	5. Low oxygen consump
6. Accessory glands dev.	6. Accessory gl. small
7. Salivary glands dev.	7. Salivary gl. small
8. Sarcosomes large and numerous	8. Sarcosomes small and few
9. Juvenile hormone	9. No juvenile hormone

Behavioral differences between non-diapausing and diapausing insects

Non-diapausing	Diapausing
1. Normal feeding	1. Stop feeding
2. Photopositive	2. Photonegative
3. No hiding or digging	3. Hide and dig
4. No congregations	4. Congregate
5. Normal mating	5. Refuse to mate
6. Normal locomotor rhythm	6. Little activity
7. Attracted to baits	7. Not attracted to baits

Diapause is a physiological condition caused by lack of a specific hormone that leads to various morphological, metabolical and behavioral changes in the insect.

Respiration in diapausing (D) *Phormia regina* is considerably lower than that of nondiapausing (ND) adults



Calabrese, E. and J. Stoffolano. 1974. The influence of diapause on respiration in adult male and female black blowflies, P. regina. Ann. Ent. Soc. Amer. 67: 715-717.

EFFECT OF DIAPAUSE ON MTAT IN *P. REGINA*

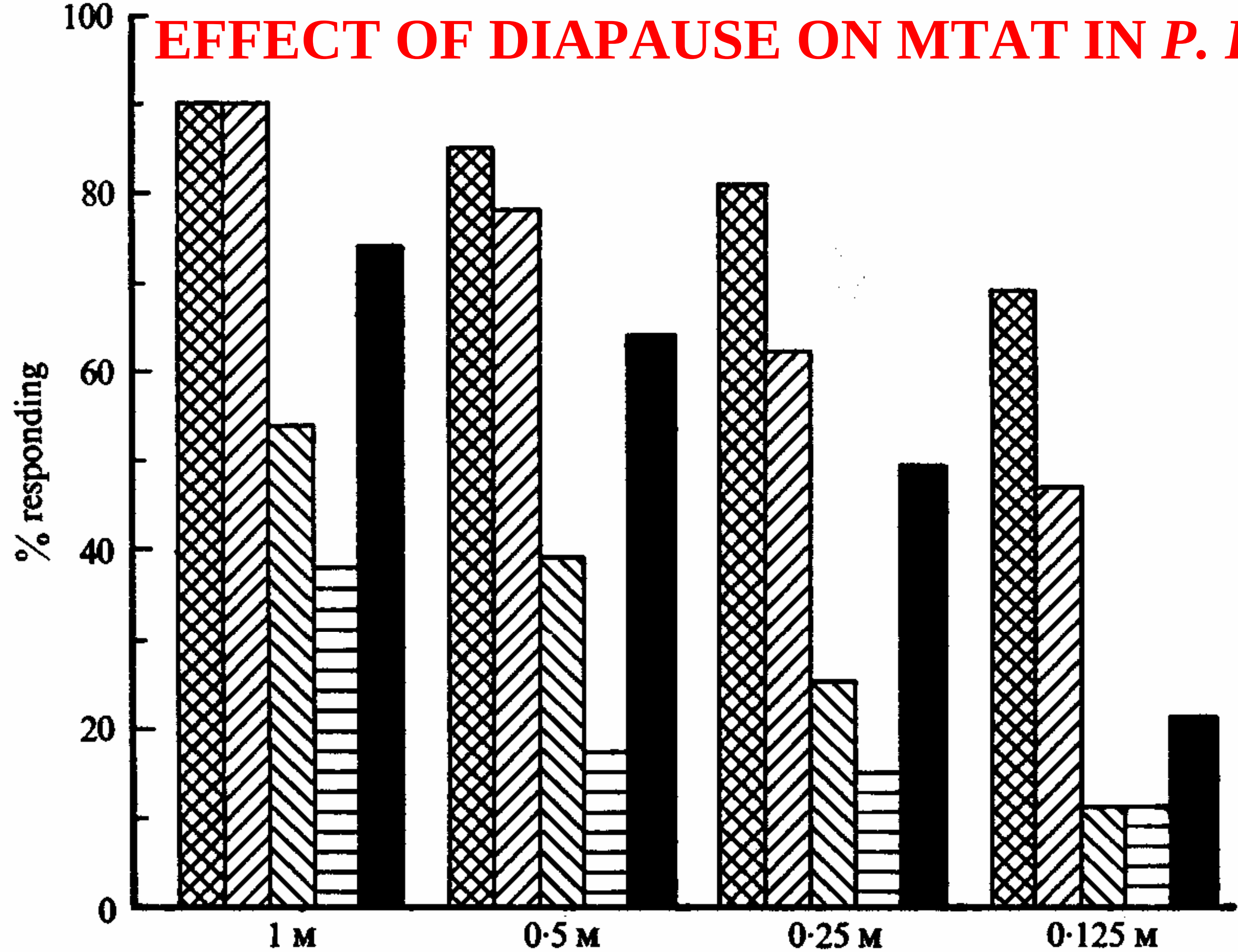


Fig. 1. The percentage of *P. regina* adults responding to various concentrations of sucrose at different ages following eclosion and at different physiological conditions. ▩, $3\frac{1}{2}$ days old and non-diapausing; ▧, 39 days old and non-diapausing; ▤, 39 days old and diapausing; ▧, 10 days old and non-diapausing; ■, 41 days at diapausing inducing conditions and 14 days at non-diapausing inducing conditions. The smallest number of flies tested for each group was 162 for the 10-days-old, non-diapausing group, and the maximum number was 355 for the 39-days-old, diapausing group.

Stoffolano, J.G., Jr. 1975. Central control of feeding in the dipaausing adult blowfly *Phormia regina*. J. exp. Biol. 63: 265-271.

Table 1. *Mean* (± S.D.) *intake and duration of drink of diapausing and non-diapausing* P. regina *fed a* 0·125 M *sucrose solution*

Treatment	Mean intake (μl)	Mean duration (sec)	No. tested
N-D*	24·7 (±4·2)	140·6 (±30·5)	29
D*	6·0 (±4·8)	37·5 (±29·8)	33
D, 41 days; N-D, 8 days	22·0 (±4·6)	168·1 (±64·5)	20

N-D = non-diapausing, D = diapausing.
* Forty days old.

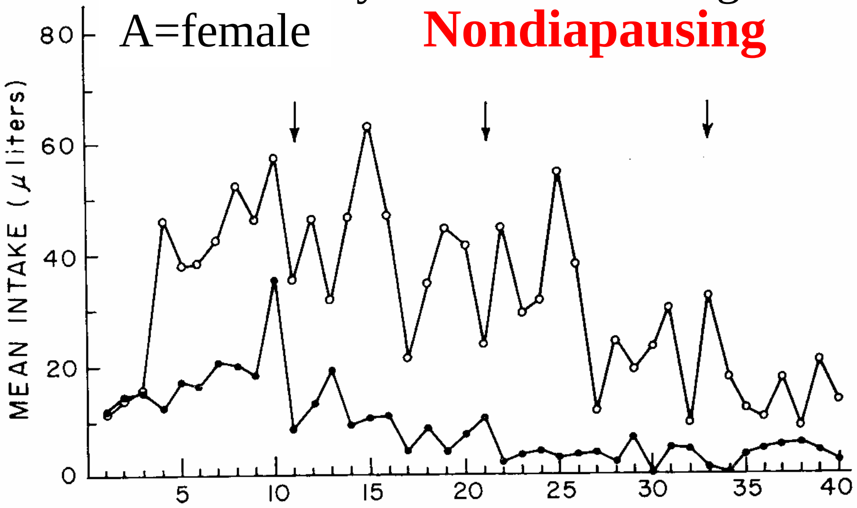
Table 2. *Effect of diapause on the mean impulse frequency of labellar sugar chemoreceptor sensilla of* P. regina *using* 0·5 M *sucrose as the testing solution*

Age (days)	Frequency (imp/sec)	No. of flies tested	No. of sensilla recorded from
N-D, 60	34·0	6	70
D, 60	27·3	11	70

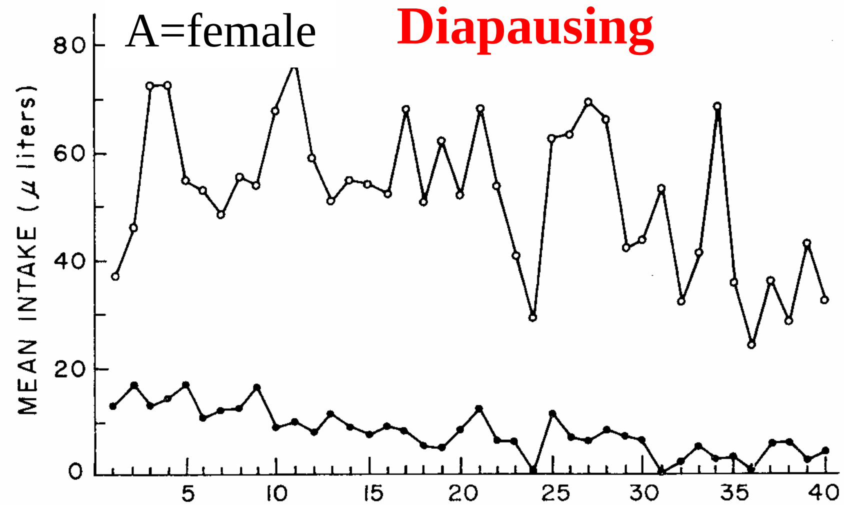
N-D = non-diapausing, D = diapausing.

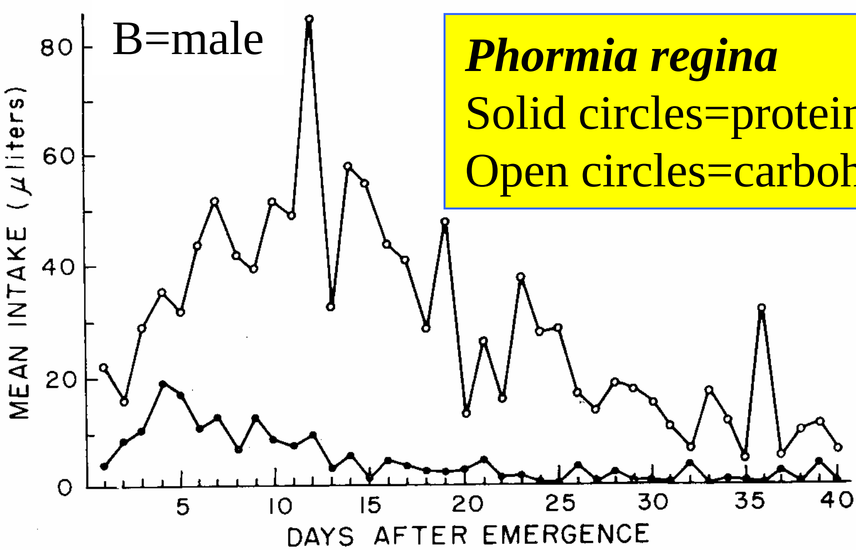
DO INSECTS, LIKE MAMMALS, EXPERIENCE GREATER INTAKE PRIOR TO ENTERING THE WINTER OR DIAPAUSE STATE?

Total intake for both sexes
Protein---------17.99 ul
Cabohydrate-- 0.38 ul/mg

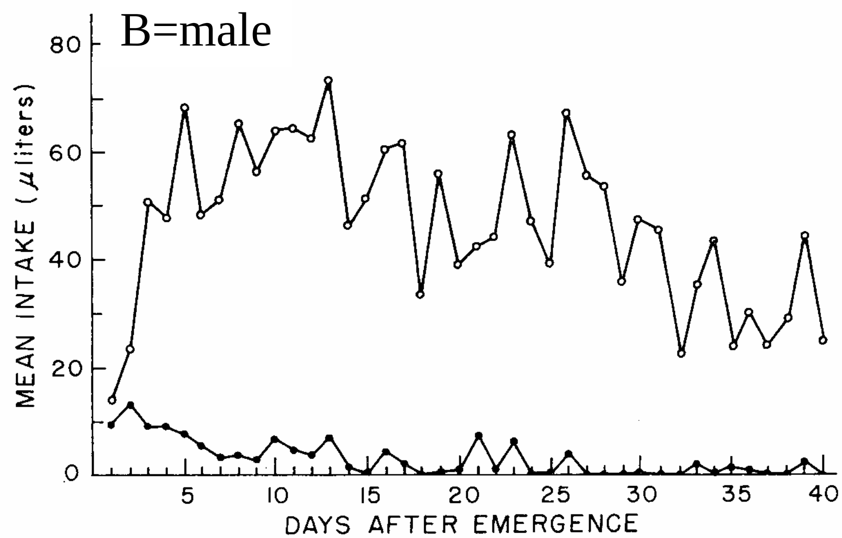


Total intake for both sexes
Protein---------27.18 ul
Cabohydrate-- 0.65 ul/mg

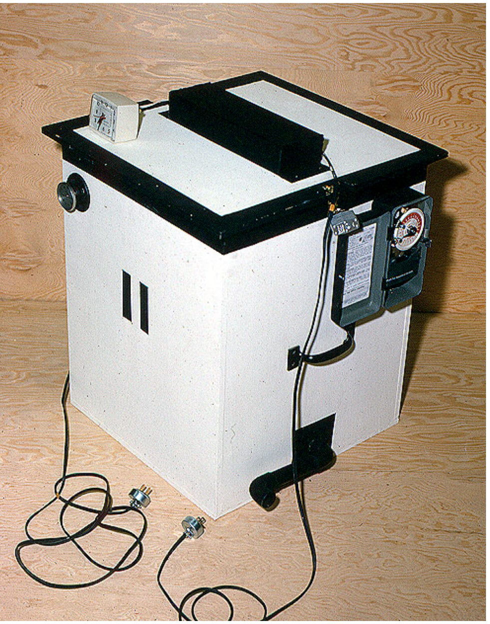


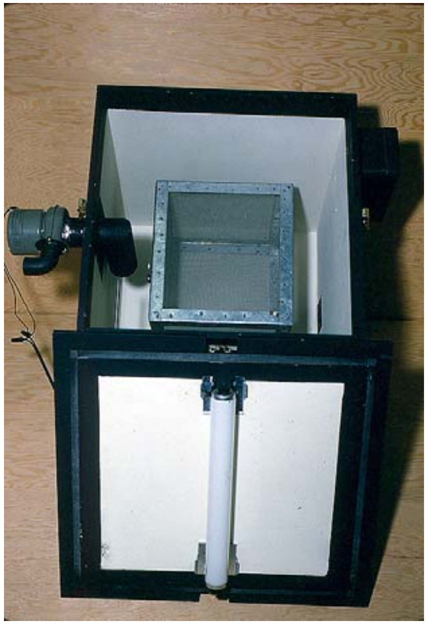


Phormia regina
Solid circles=protein
Open circles=carbohydrate



Facultative, adult diapause in face fly, *Musca autumnalis*





Stoffolano, J.G., Jr. and J.G. Matthysse. 1967. Influence of photoperiod and temperature on diapause in the face fly, *Musca autumnalis* (Diptera: Muscidae). Ann. Entomol. Soc. Amer. 60:1242-1246.

Above is a photoperiod chamber

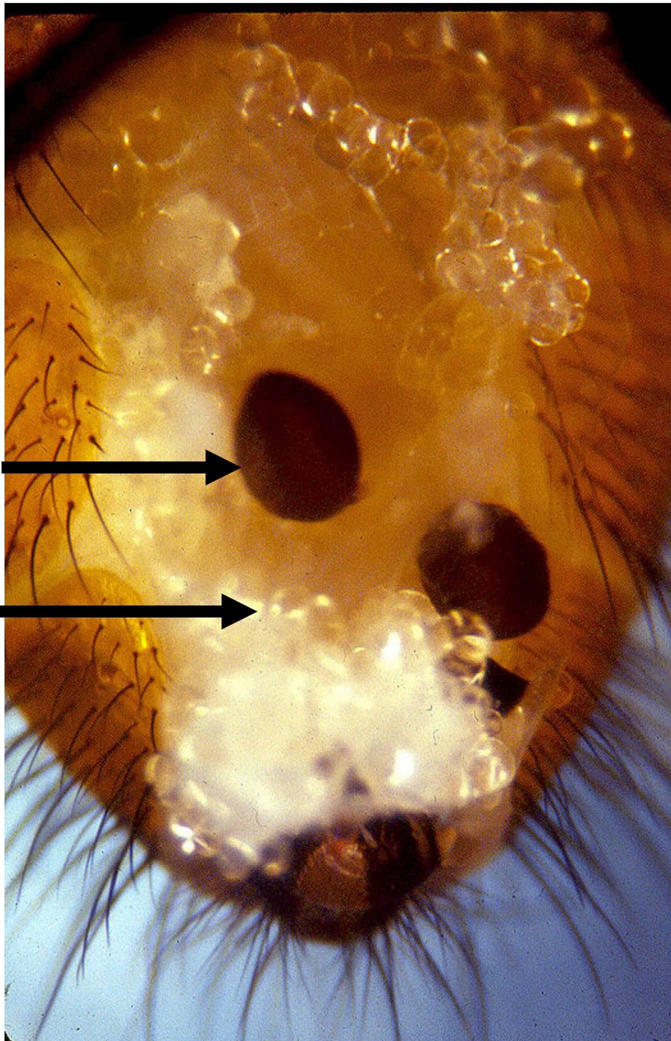
Undeveloped ovaries

Spermathecae

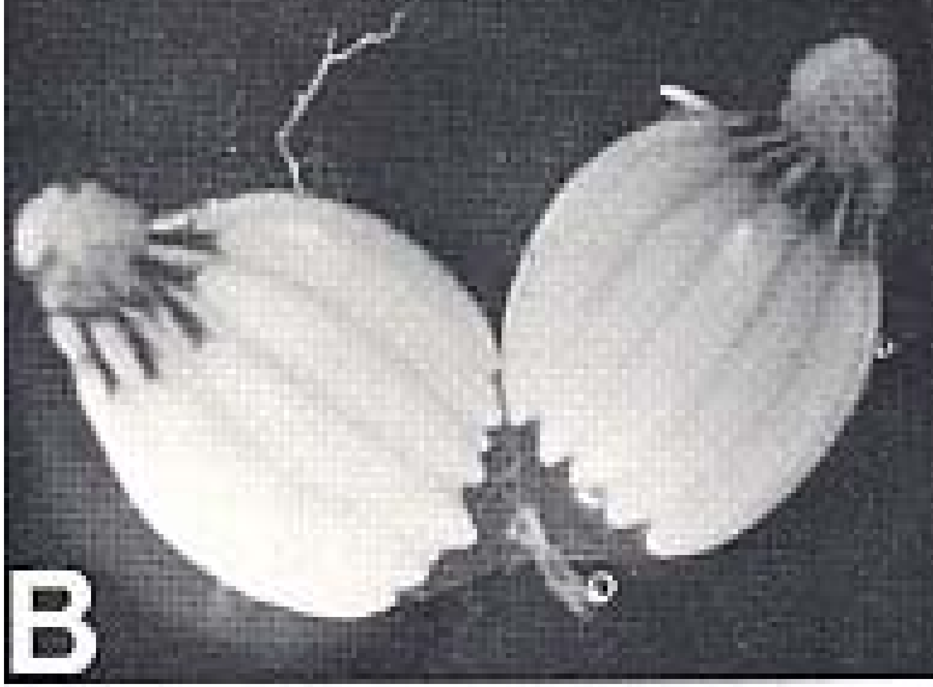
MALE ON LEFT; FEMALE ON RIGHGT

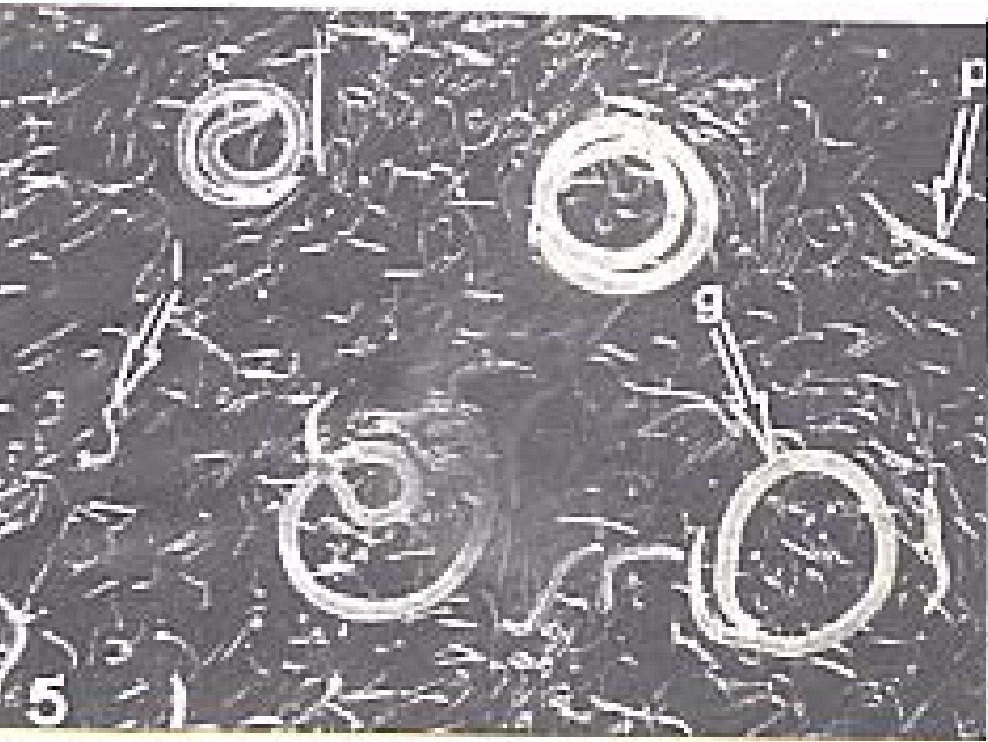
Testes

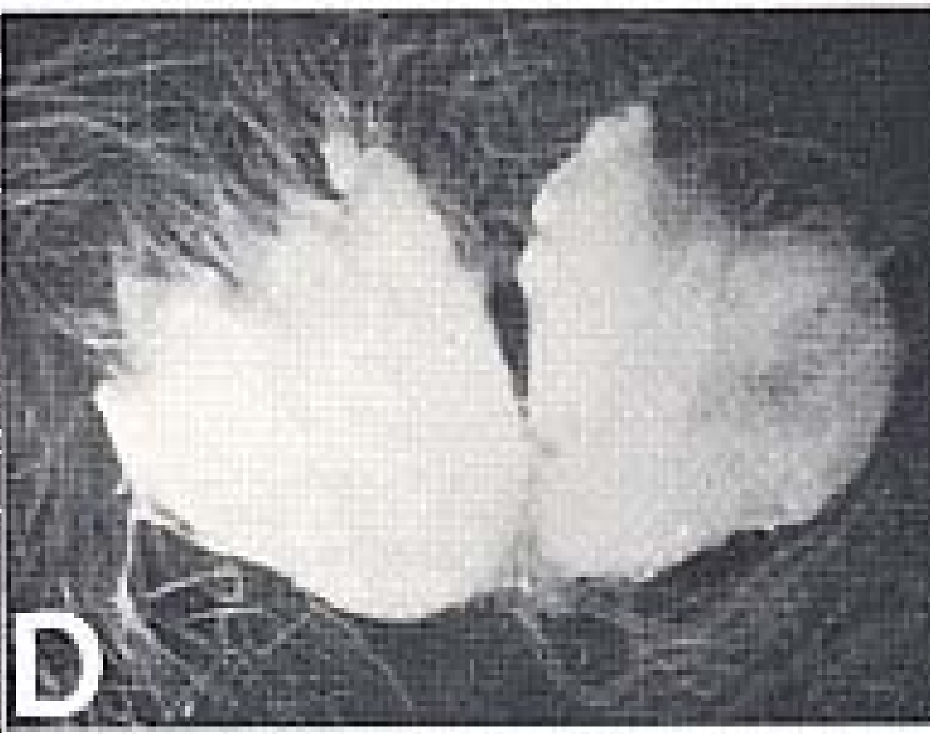
Hypertrophied fat body

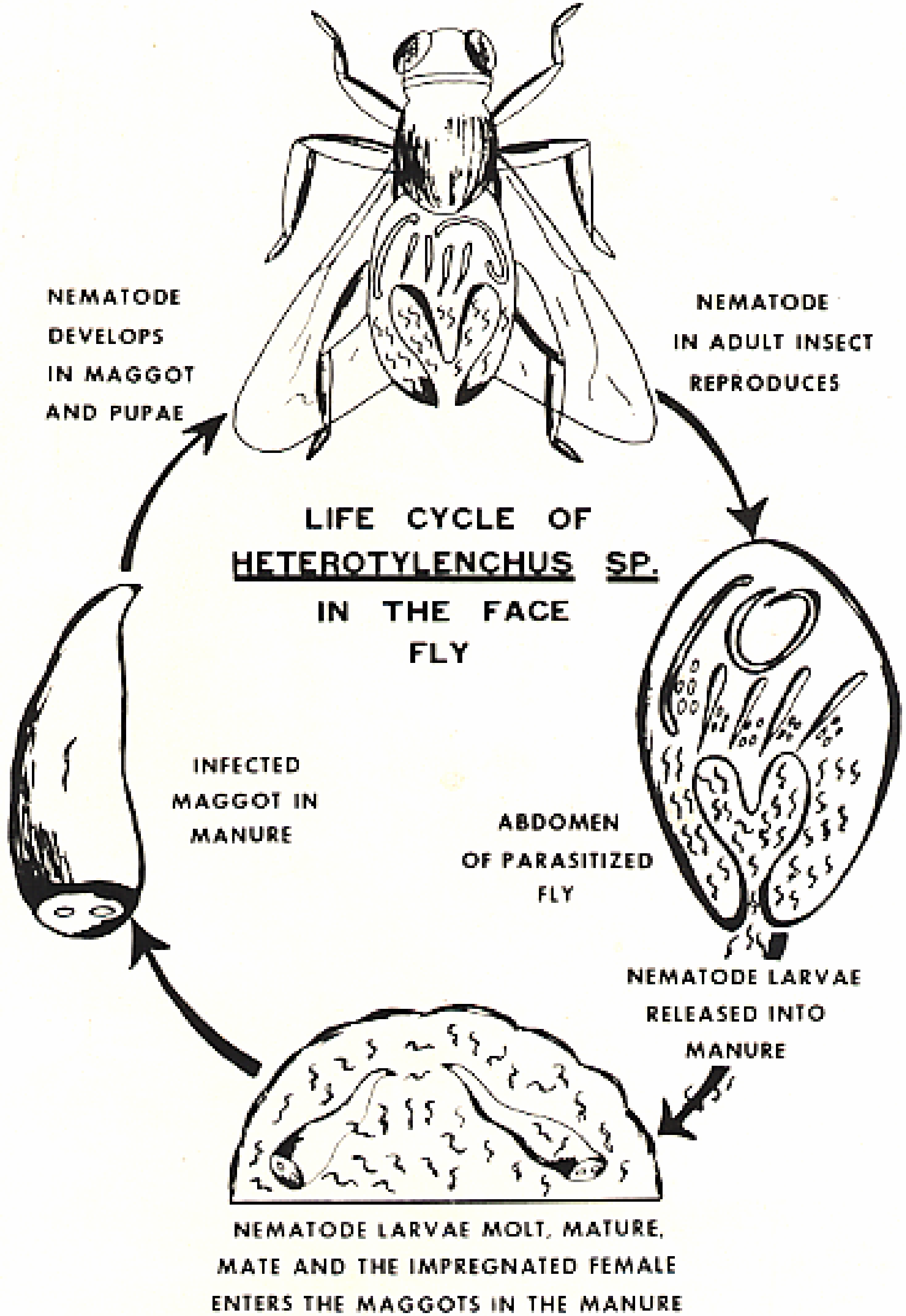


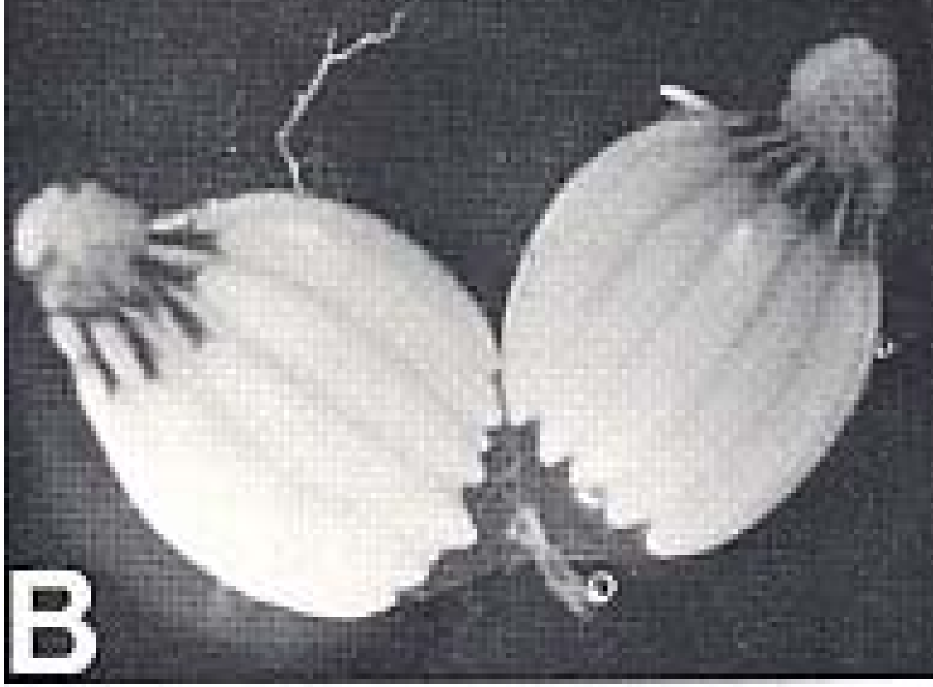
The nematode, *Paraiotonchium muscadomesticae,* has so far been found only in Serra Talhada, a small town in northeastern Brazil. University of Florida graduate student Reginald R. Coler is credited with the discovery.

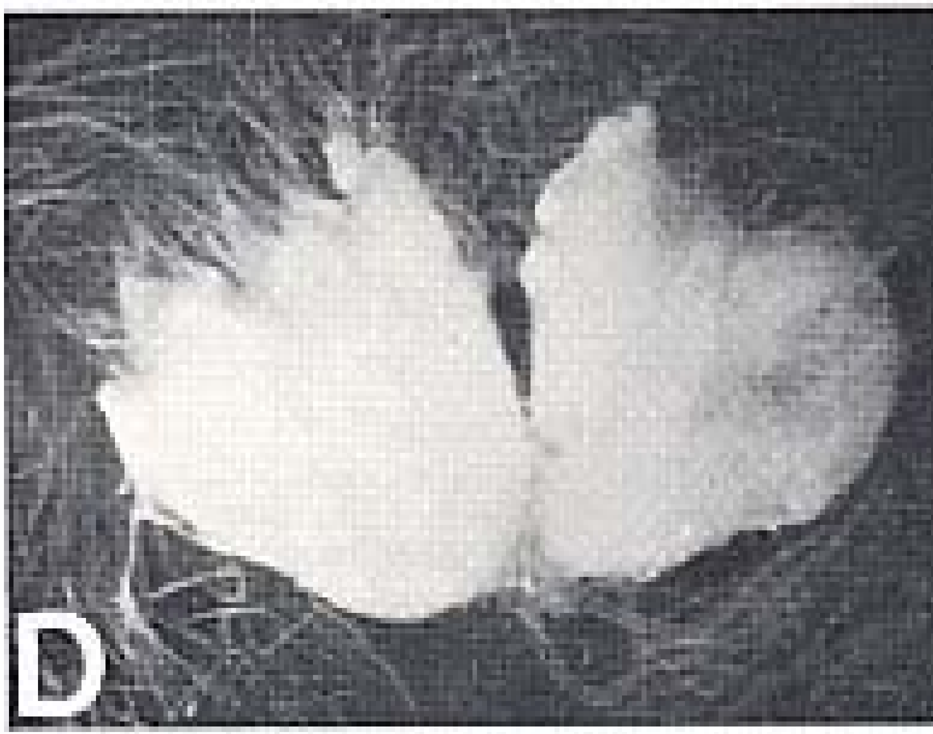







NEMATODE
DEVELOPS
IN MAGGOT
AND PUPAE
NEMATODE
IN ADULT INSECT
REPRODUCES
LIFE CYCLE OF
HETEROTYLENCHUS SP.
IN THE FACE
FLY
INFECTED
MAGGOT IN
MANURE
ABDOMEN
OF PARASITIZED
FLY
NEMATODE LARVAE
RELEASED INTO
MANURE
NEMATODE LARVAE MOLT, MATURE,
MATE AND THE IMPREGNATED FEMALE
ENTERS THE MAGGOTS IN THE MANURE


B

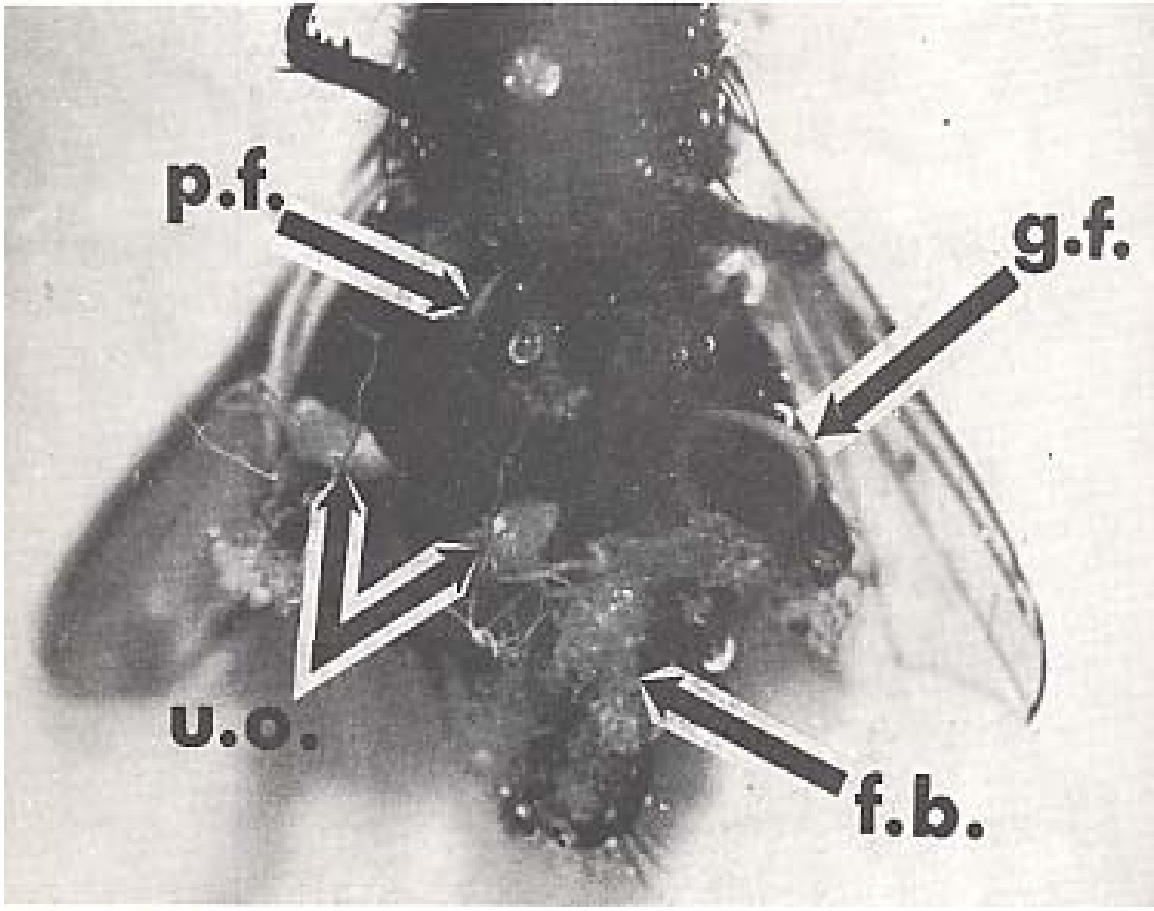

D

EFFECT OF DIAPAUSE ON INTERNAL PARASITES

Stoffolano, J.G., Jr. 1967. The synchronization of the life cycle of diapausing face flies, *Musca autumnalis*, and of the nematode *Heterotylenchus autumnalis*. J. Invert. Pathol. 9:395-397.

Face fly has a facultative adult diapause. It is also parasitized by the nematode in the fall and must remain in the diapausing flies throughout the winter. What kind of adjustments do you think the nematode must incur to survive?

In the diapausing fly various chemicals such as sterols are low. Nematodes have been shown to need sterols for reproduction. In this parasite, only gamogenetic (g.f.) and parthenogenetic (p.f.) are present. Reproduction in the p.f females is stopped, thus no infective stages produced. This is the stage that penetrates and fills up the ovaries, which in a diapausing female do not develop. Notice the fat body and undeveloped ovaries (u.o.).



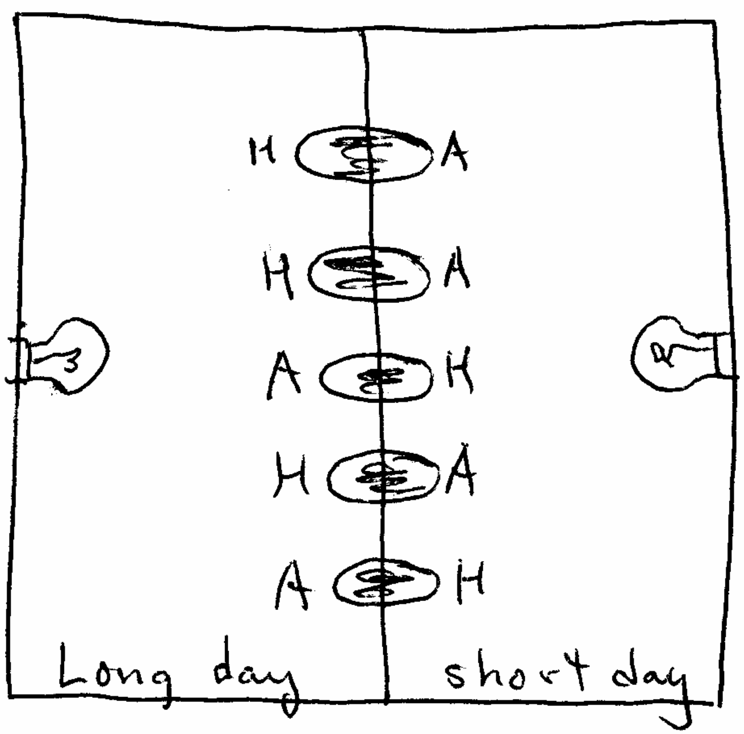
PARASITIC DIAPAUSE

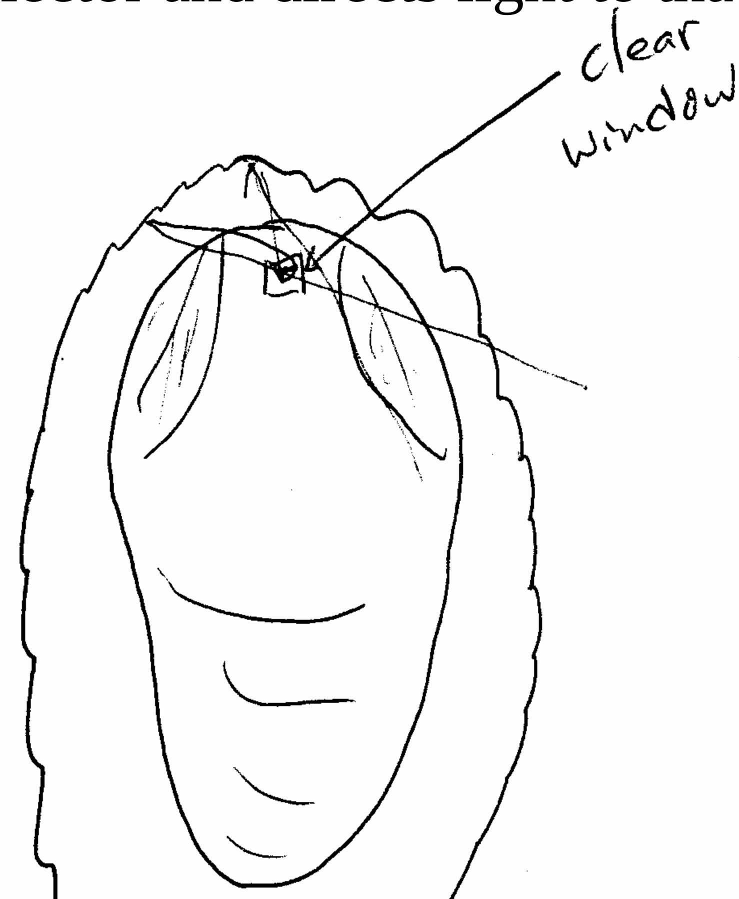
Nasonia vitripennis enters diapause as a mature larva. These larvae are committed to either diapause or not diapause depending on the temperatures and photoperiods experienced by their female parent.

Young females produce non-diapausing offspring while older females produce diapausing offspring. One can prevent this in older females by exposing them to long days and warm temperatures.

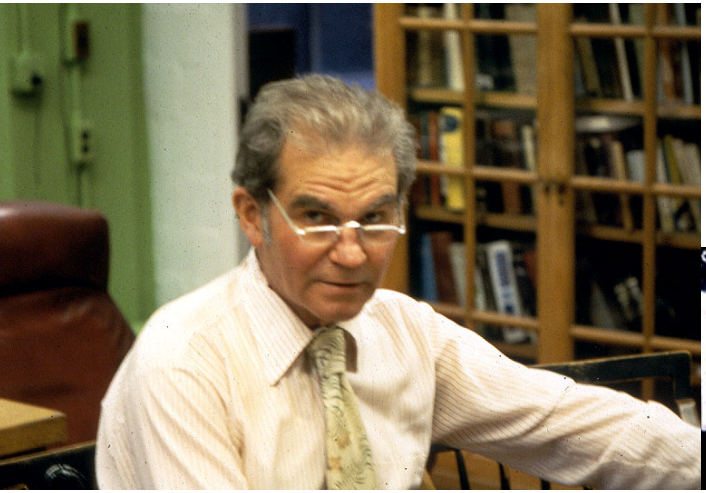
Nasonia vitripennis ovipositing into a fly pupa

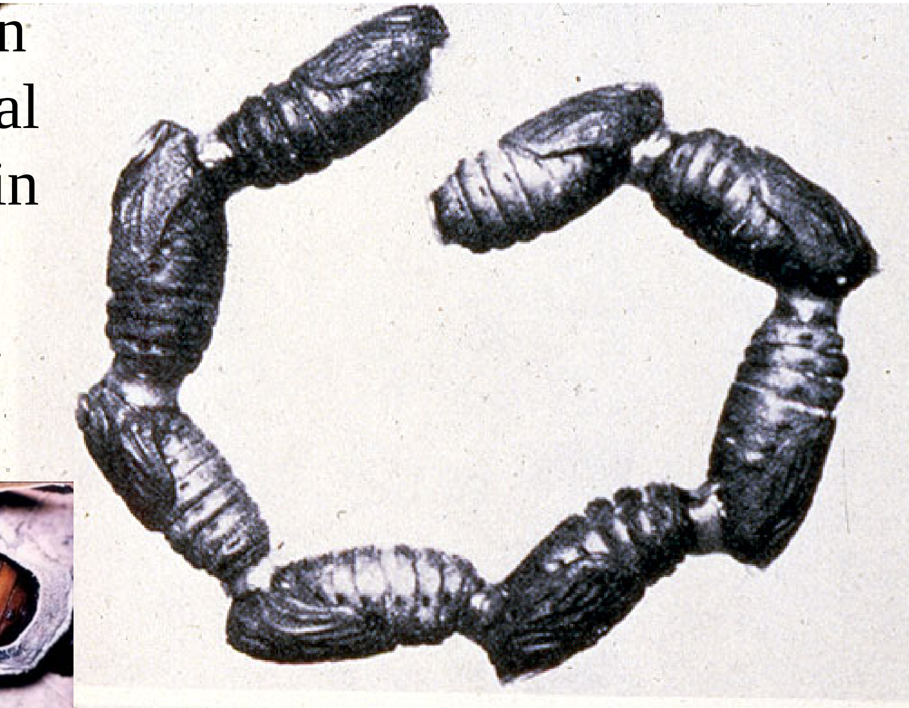
In an early experiment, Carroll Williams took pupae and put them in a divided box separated by a black sheet. He then cut a hole in the sheet and put in a cecropia pupa. Some had the head (H) going one way and the abdomen (A) the other way. Only those exposed to long days and with their heads in that section broke diapause. Also he found a transparent window just above the brain that was important for light to enter. The cocoon acts like a parabolic reflector and directs light to that area.

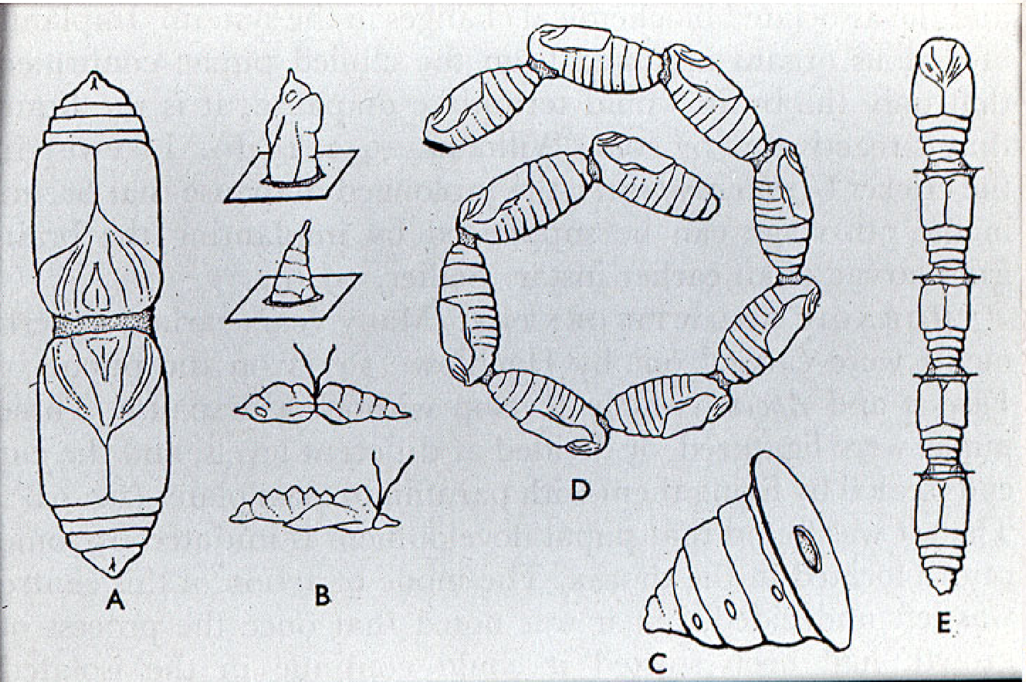


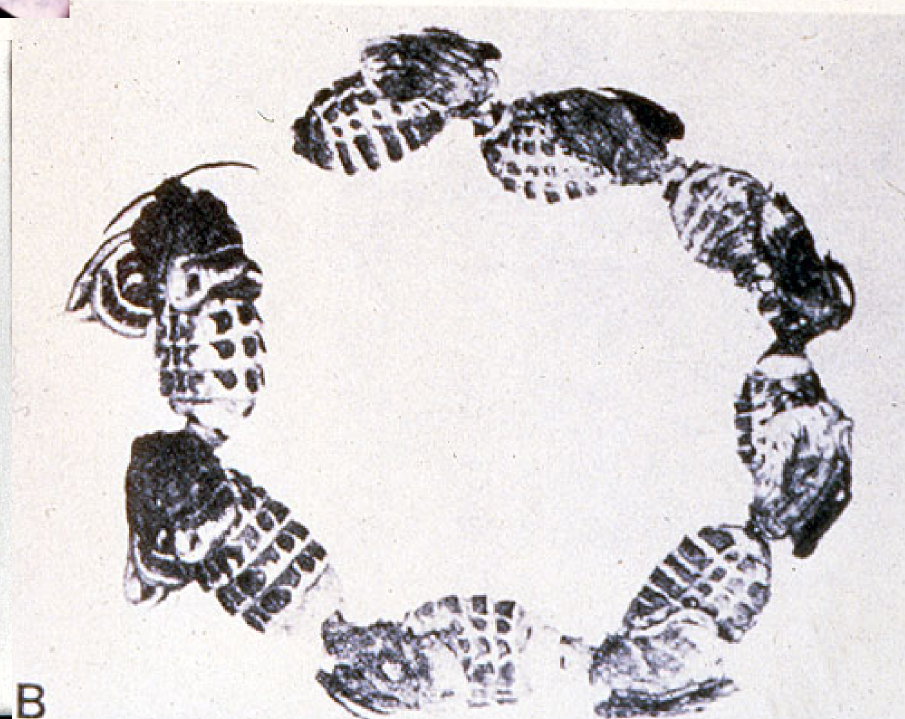


Carroll Williams and pupal diapause in *Hyalophora cecropia*. Obligatory pupal diapause can be broken by chilled brain and long daylengths.









Embryonic diapause in *Bombyx mori*

Short photopase

↓

Larva

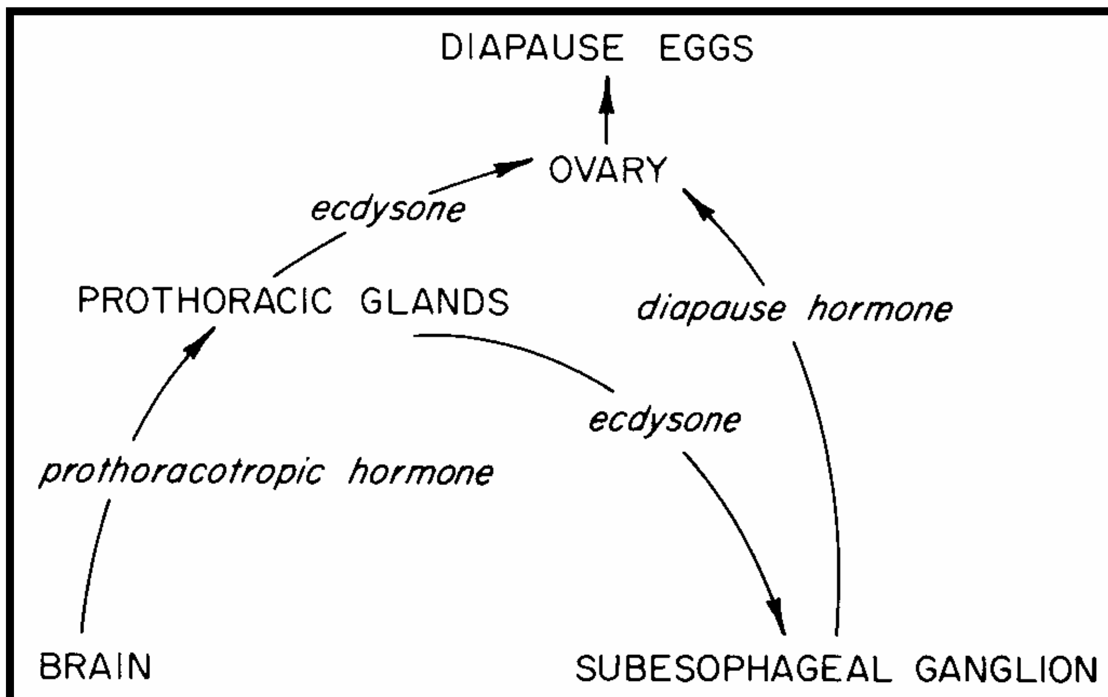
↓

Adult

↓

Produces a diapausing hormone from the subesophageal ganglion that is put into her eggs, thus causing those embryos to cease development and remain in diapause

Diapausing hormone is a 24 amino acid peptide that is produced in the subesophageal ganglion. Male brains are rich in this peptide but its function is unknown.



Physiology of insect migrants

Large milkweed bug-case study into the physiology of migration

In this bug, as in other migrants, diapause and migration are linked. Having an adult diapause, low JH results in cessation of reproductive events and stimulates diapause and migration.

Dingle, H. (ed.). 1978. Evolution of insect migration and diapause. Springer-Verlag, N.Y.

Rankin, M.A. Hormonal control of insect migratory behavior. In above book of Dingle.

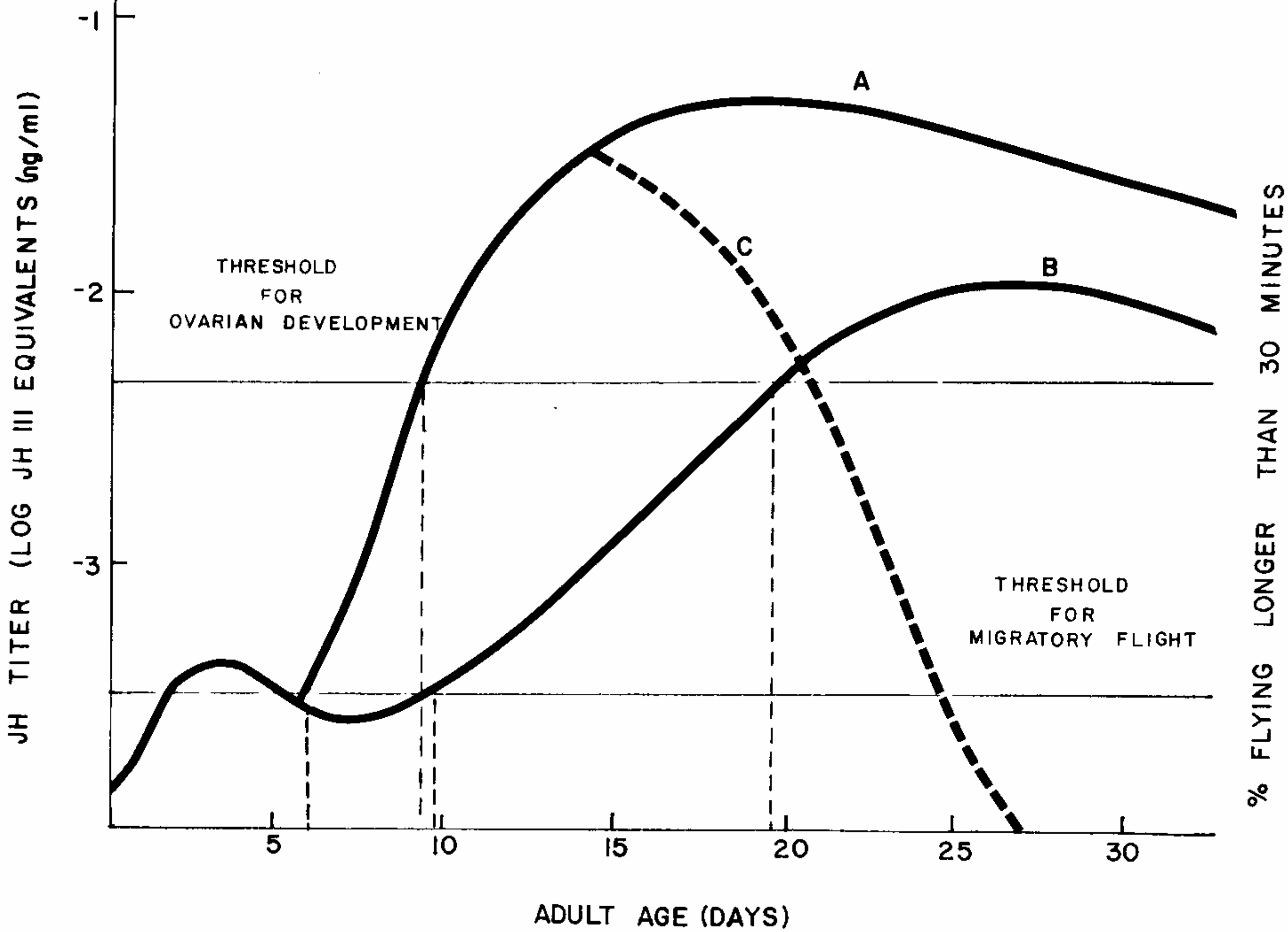


Fig. 14. A model indicating the proposed response of the corpus allatum to the environmental stimuli of photoperiod, temperature, and food deprivation; the relationship of JH titers to ovarian development and migratory behavior. High temperatures and long photoperiods (A) stimulate high JH titers and immediate reproduction, lower temperatures and short photoperiods (B) result in migratory flight in response to intermediate titers, presumably below the threshold for ovarian development, while poor quality food or starvation (C) results in lower JH titers and flight activity followed by a decrease in JH titer and cessation of flight if lack of food persists (from Rankin and Riddiford, 1977b).

ECOLOGICAL CHEMISTRY

Garcia effect and avoidance based on association of bad experience with color in this case

BIRD

↑

MONARCH

↑

Cardiac glycosides

↑

MILKWEED

Larvae of
Danaus plexippus
Danaus gilipus
Danaus chrysippus
all store cardiac glycosides from milkweeds

Many chemicals are synthesized by animals *de novo* while many phytophagous insect species sequester unique chemicals from the plants and incorporate them for their own use.

Hypothetical case-Which came first, sequestration of the cardiac glycoside or the predisposition by some caterpillars to eat their own egg shell that might have contained these chemicals, thus preferring to feed on plants containing them.

To date, no one has tested the phagostimulatory effect of the milkweed plant on the monarch caterpillar and no one has tested the phagodeterrent effect on species of caterpillars that don’t feed on the plant.

AUTOTOXICITY

Why aren't the monarch caterpillars affected by the cardiac glycosides?

Let's briefly strategize about why this is so?

These chemicals are toxic to most animals because they inhibit the enzyme Na^+/K^+-ATPase but in animals that sequester these toxins, this enzyme is 100 times less sensitive to inhibition than in susceptible insects.

In *Danaus plexippus*, insensitivity to these chemicals is due to a change in a single amino acid on a binding site of the enzyme

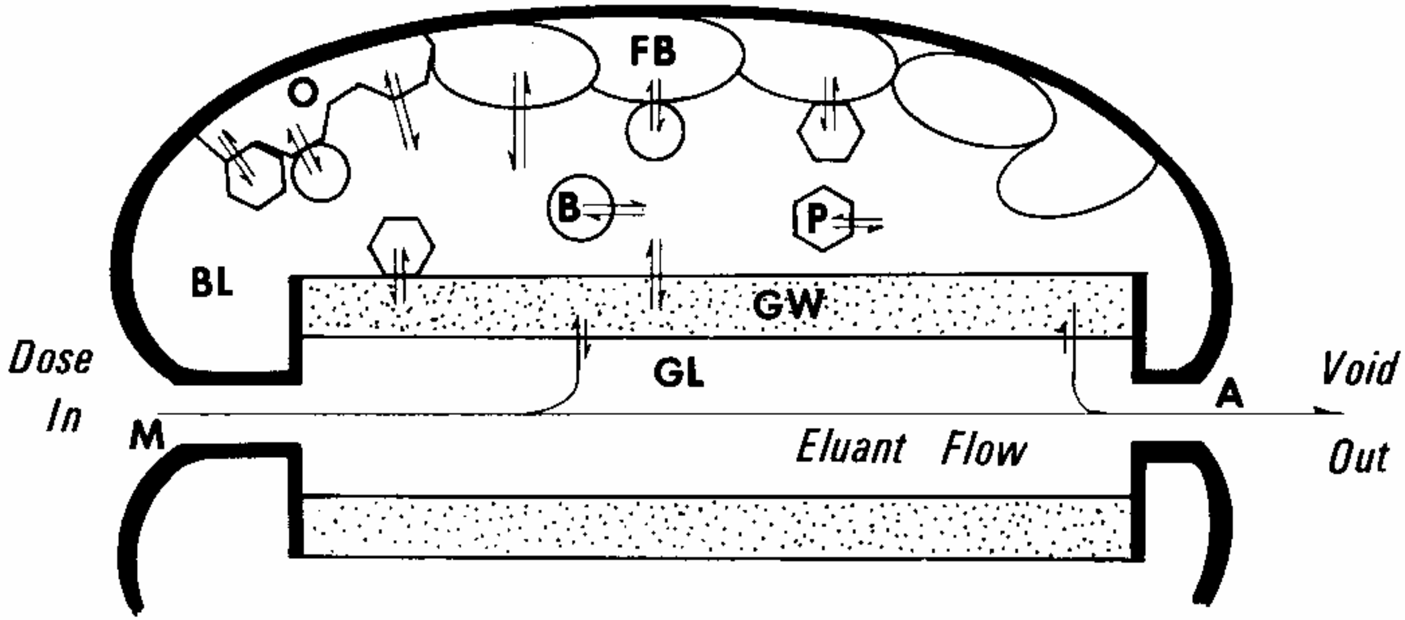


Figure 2 Diagram of a chromatographic insect. *A* = anus; *B* = blood cells: *BL* = blood: *FB* = fat body; *GL* = gut lumen; *GW* = gut wall; *M* = mouth; *O* = organs; *P* = carrier-proteins in blood. *Arrows* between compartments imply a ratio of two rate constants, the influx, and efflux of a given molecule, i.e. $\underset{K_o}{\overset{K_i}{\rightleftharpoons}}$.

Remember the statement from the digestive system concerning feeding on plants when it was stated that "feeding on plants is like feeding from a poisoned platter"

Above taken from Duffey, S.S. 1980. Sequestration of plant natural products by insects. Ann. Rev. Entomol. 25: 447-477.

Table 4 Some phytochemicals sequestered by insects for self-defense

Chemical(s)	Organism(s)	Source: Comments	Depot	References
Anthraquinones	*Dactylopius* sp., scale	carminic acid from plant or symbiotic microbes?	fat	p. 205 (65)
Amaryllidaceous alkaloids	*Xanthopastis* sp.,	from amaryllidaceous host	droplets	(149)
Aristolochic acids	some papilionids	from *Aristolochia* sp.	?[b]	(149, 155)
Bufadienolides	*Photinus* sp., lampyrid	some unknown precursor from host converted to a lucibufagin	?	(56)
Cannabinoids	an arctiid and pyrgomorphid	from *Cannabis* sativa	?	(153)
Cardenolides	lygaeids, pyrgomorphids	from apocynaceous hosts	glands, sinuses	(162, 182)
	danaids, arctiids, ctenuchids, nymphalids, coccinellids, etc.	from apocynaceous hosts	wing, abdomen, elsewhere?	(142, 149, 151, 156)
	some chrysomelids	noncardenolide precursor derived from host	glands	(127)
Carotenes[a]	insects generally	derived from host; some metabolism; protective coloration?	cuticle, fat body	(64, 114–116, 146, 150, 154)
	Romalea sp., acridid	an allenic sesquiterpene derived from carotene breakdown?	gland	(106)
Cocaine	*Eloria* sp. lymantrid	from *Erythroxylon* sp.	?	pers. commun. M. S. Blum
Cycasin	*Seirarctia* sp., arctiid	from *Zamia* sp.	body fluids	(173)
Flavonoids[a]	*Cionus* sp., curculionid	anthocyanins; from host?	fat cells	p. 210 (65)
	Asilus sp., asilid	anthocyanins; from host?	?	p. 210 (65)
	Leptocoris sp., corizid	flavones; from *Acer* sp.?	cuticle	p. 210 (65)
	Bombyx sp.,	flavones; from *Morus* sp.?	?	p. 212 (65)
	some satyrids	flavones and isoflavones from *Dactylus* sp.	wing	(112, 174)
	Dytiscus sp., dytiscid	aurone, marginalin, from host?	gland	(160)
Insecticide residues	*Romalea* sp., acridid	2,5-dichlorophenol from plant	gland	(54)
	Iridomyrmex sp., dolichoderine	dibutylphthalates and chloroanisoles	gland	(30)
Mustard oils	*Pieris* sp., pierid	allylisothiocyanate from sinigrin in *Brassica* sp.	?	(5)

Table 4 (*Continued*)

Chemical(s)	Organism(s)	Source: Comments	Depot	References
Polyacetylenes	*Chauliognathus* sp. cantharid	dihydromatricaria acid from a composite?	gland	(107)
Pyrrolizidines	variety of danaids, arctiids, ctenuchids, and ithiomids	derived from flowers of composites, borages, and some legumes	gland	(15, 53, 131, 149a)
Steroids	some dytiscid water beetles	steroid precursors from host converted to pregnones and pregnadienes	gland	(160)
Terpenoids	*Neodiprion* sp., diprionid	pinenes, abietic acid, etc from *Pinus* sp.	gut diverticulum	(55)

[a] = chemicals used for protective coloration.
[b]? = exact site of deposition unknown.

The ability of the insect to sequester various substances into various depots of their body makes the chemical ineffective as a toxicant to the insect sequestering them.

Table 5 Generalized summary of factors that contribute to sequestration[a]

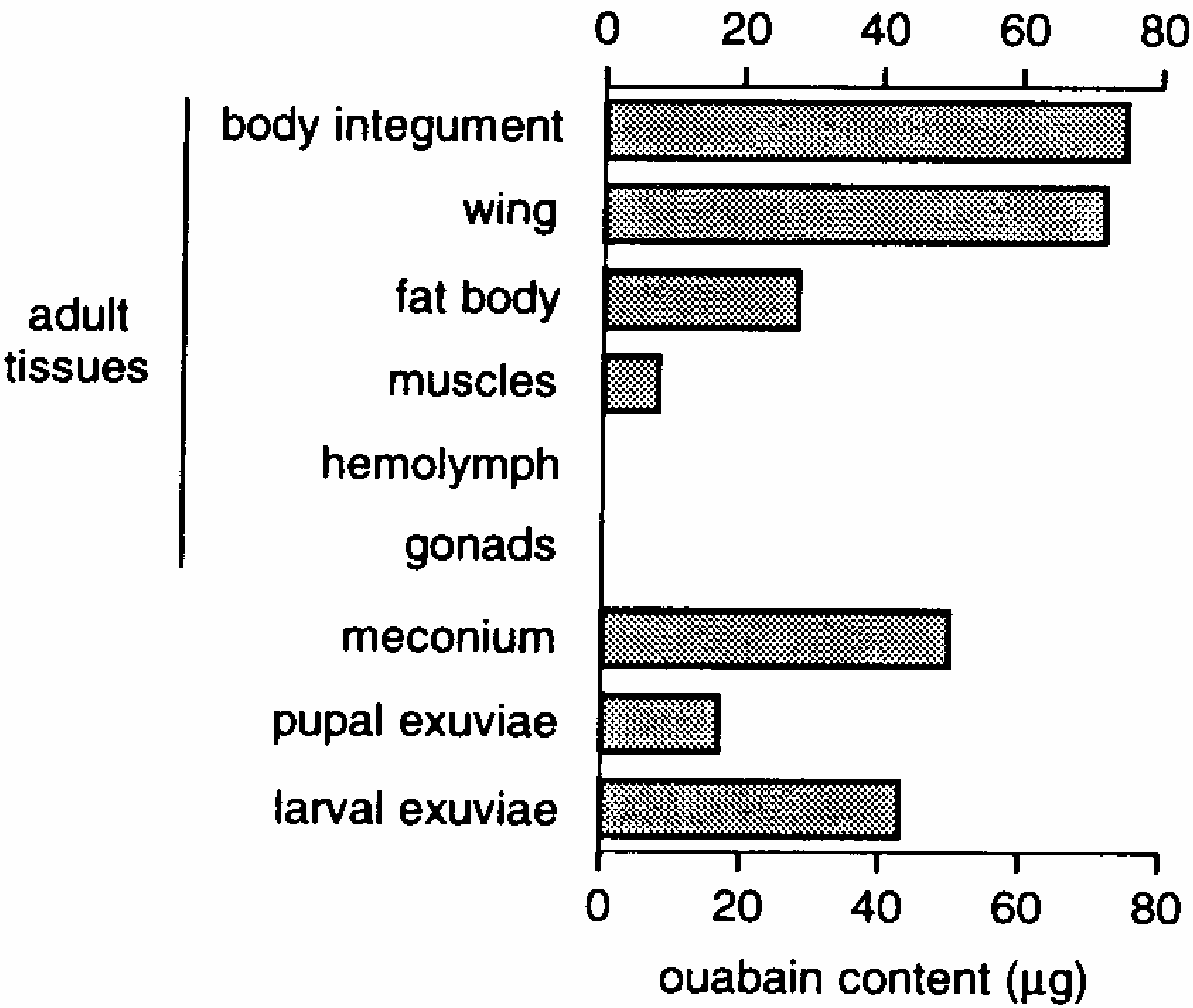
Physical (identity not altered)	
Diffusional	passive, facilitative, through pH or concentration gradients
Solvational	solution; lipo- and hydrophily, charge-transfer
Adsorptive	binding to proteins, carbohydrates and other polymers; facilitative diffusion
Phasic	bulk phase formation; micelles, emulsions, colloids, membranes
Entrapping	physical trapping as salt, insoluble or nondiffusive chemical in depot
Chemical (identity altered)	
Derivatizing	via alkylation, acylation etc
Decompositional	labile functions disintegrating spontaneously or via physical catalysis from light, heat, or chemicals
Enzymatic (identity usually altered)	
Active	active transport of chemical or conjugate of it
Incorporative	incorporation into polymers (protein, carbohydrates, melanin), lipids, and other conjugates
Depositional	deposition e.g. into cuticle as melanine, chitin, sclerotin; active deposition in cells or organelles

[a]Factors are not absolute in their categorization.

Where do insects store sequestered chemicals?

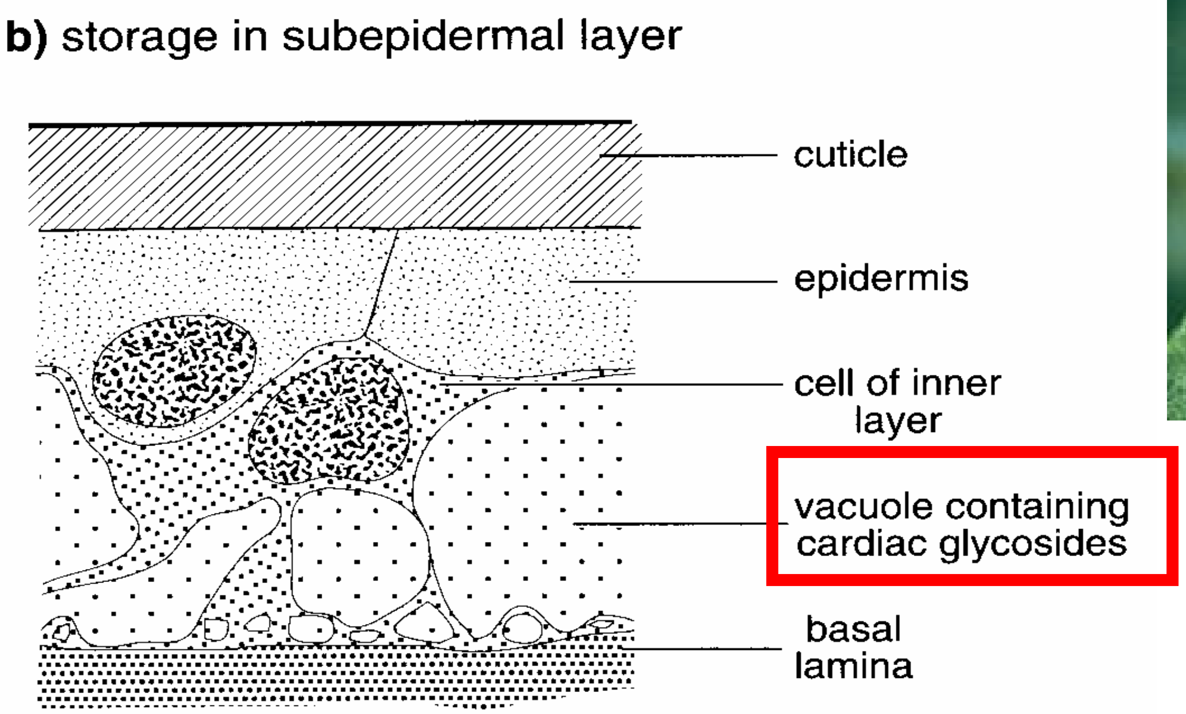
Storage of ouabain, a cardiac glycoside in adults

1. **Various adult tissues in monarch**



Cont. **Where do insects store sequestered chemicals?**

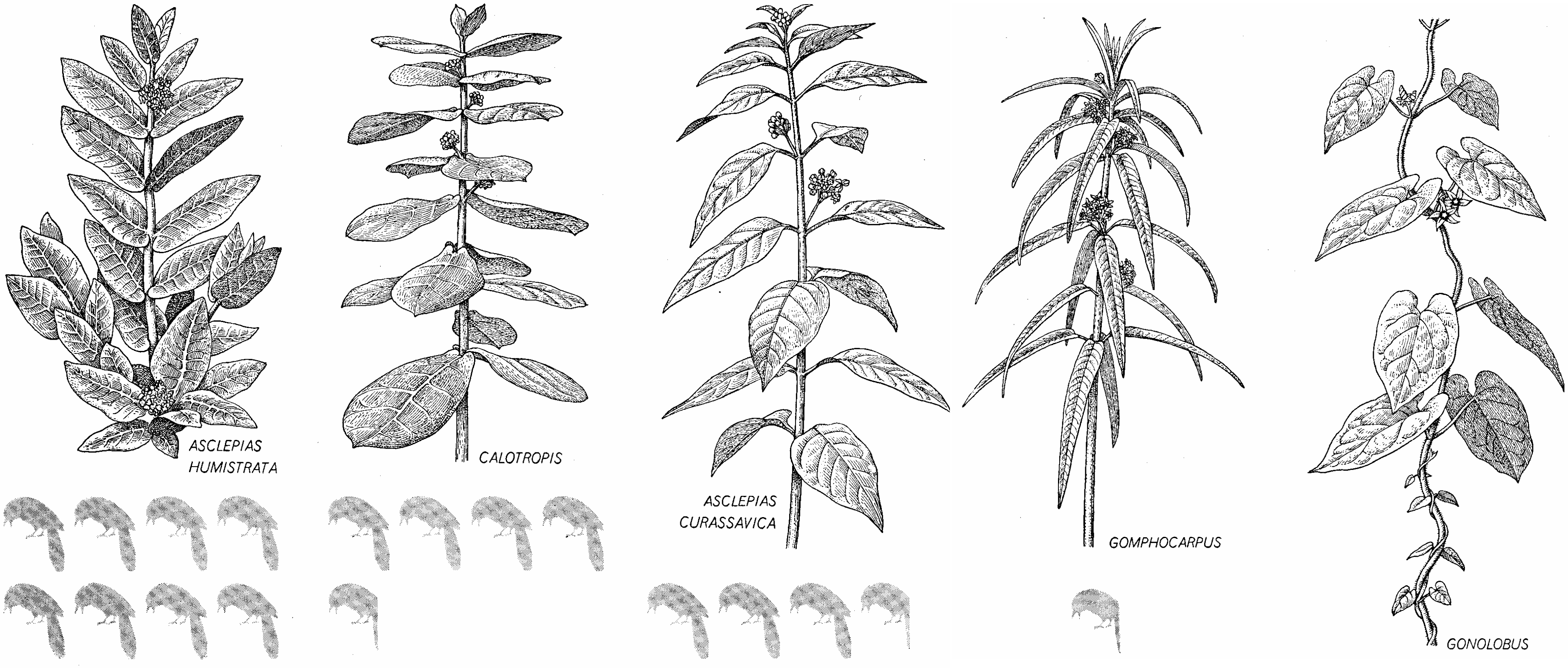
2. Integument for
a. milkweed bug



3. Hemolymph-most frequent amongst Coleoptera (Meloidae store cantharidin (reflex bleeding & rove beetle store pederin)

4. Eggs of some insects but derived from female and sometimes from the male

Cardiac glycosides (calactin, calotropin and calotoxin) or cardenolides act on a nerve center of the brain that causes vomiting. A great way to get rid of a toxic compound.



Brower ground up wings and made pills he forced fed to the blue jays. On the left is *A. humstrata* that one pill will make 8 blue jays sick while all the way to the right is a plant that lacks the cardiac glycoside.

Different milkweed species have different levels of cardiac glycosides, thus this chemical spectrum is transferred over to the monarchs that end up with different concentrations that they have sequestered.

Level of energy efficiency in this experience for the bird increases as you go down the system

Jay eats monarch and throws up

Jay learns to associate bitter taste of cardiac glycosides with throwing up so makes choice based on taste. Beak marks on monarch wings then lets go.

Jay learns to associate throwing up with the monarch's color

TABLE 1.1 Ecological Hierarchy and the Structural and Functional Properties Characterizing Each Level

Ecological level	Structure	Function
Global	Biome distribution Atmospheric condition Climate Sea level	Gas, water, nutrient exchange between terrestrial and marine systems Total NPP
Biome	Landscape pattern Temperature, moisture profile Disturbance regime	Energy and matter fluxes Integrated NPP of ecosystems Migration
Landscape	Disturbance pattern Community distribution Metapopulation structure	Energy and matter fluxes Integrated NPP of ecosystems Colonization and extinction
Ecosystem	Vertical and horizontal structure Disturbance type and frequency Biomass Functional organization	Energy and matter fluxes Succession NPP, herbivory, decomposition, pedogenesis
Community	Diversity Trophic organization	Species interactions Temporal and spatial changes
Population	Density Dispersion Age structure Genetic structure	Natality Mortality Dispersal Gene flow Temporal and spatial changes
Individual	Morphology Physiology Behavior	Resource acquisition Resource allocation Learning

COMMUNICATION & SPECIES INTERACTIONS

Espelie, K.E. and H.R. Hermann. 1988. Congruent cuticular hydrocarbons: biochemical convergence of a social wasp, an ant, and a host plant. Biochem. System. And Ecol. 16: 505-508.

Chemical structuring of the interaction between 3 different species: ACACIA, ANT AND WASP

1. Howard, R.W. and G.J. Blomquist. 1982. Chemical ecology and biochemistry of insect hydrocarbons. Annu. Rev. Ent. 27:149-172.

2. Howard, R.W. and G.J. Blomquist. 2005. Ecological, behavioral, and biochemical aspects of insect hydrocarbons. Ann. Rev. Ent. 50: 371-393.

CHEMICAL MIMICRY-by mimicking the cuticular hydrocarbons of its host, several insects gain access to their hosts.

a. Staphylinid beetle (termitophile) accepted by termite hosts

b. Myrmecophilous crickets accepted by ponerine ants

POLYPHENISM-The presence of several different phenotypes or forms of a given species, each of which develops facultatively in response to the internal and external environmental cues

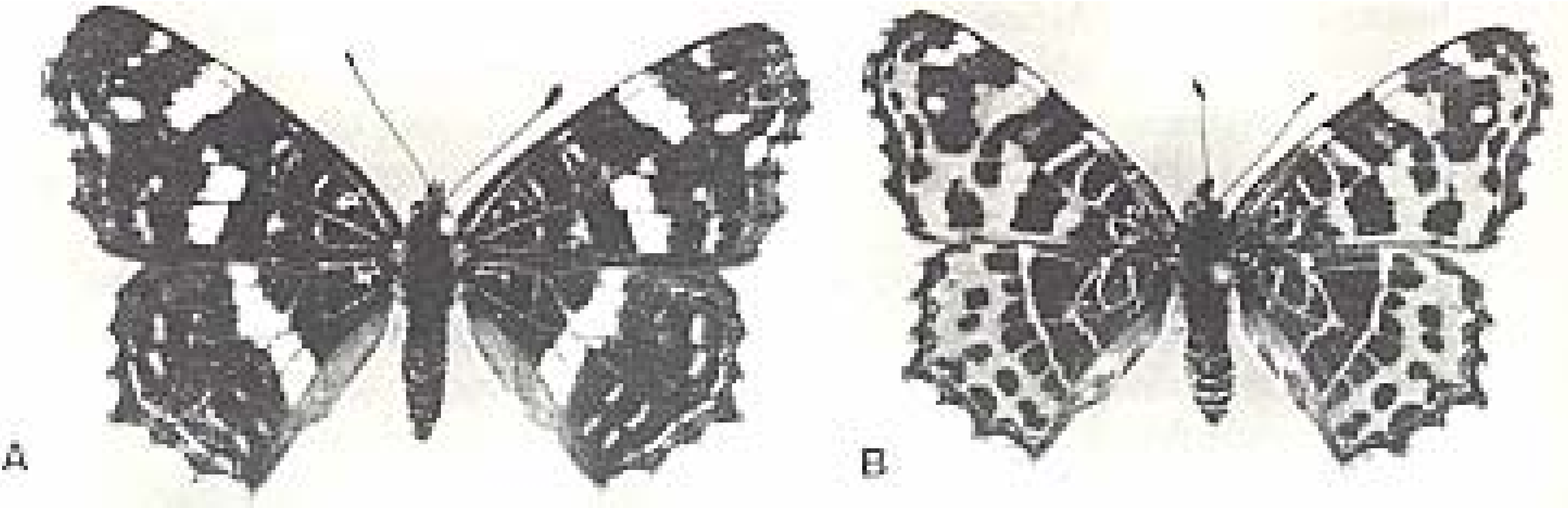
"It appears at this time that all polyphenisms in insects come about by hormonally controlled switches in developmental pathways." Nijhout

POLYMORPHISM-Due to the genetic differences among individuals

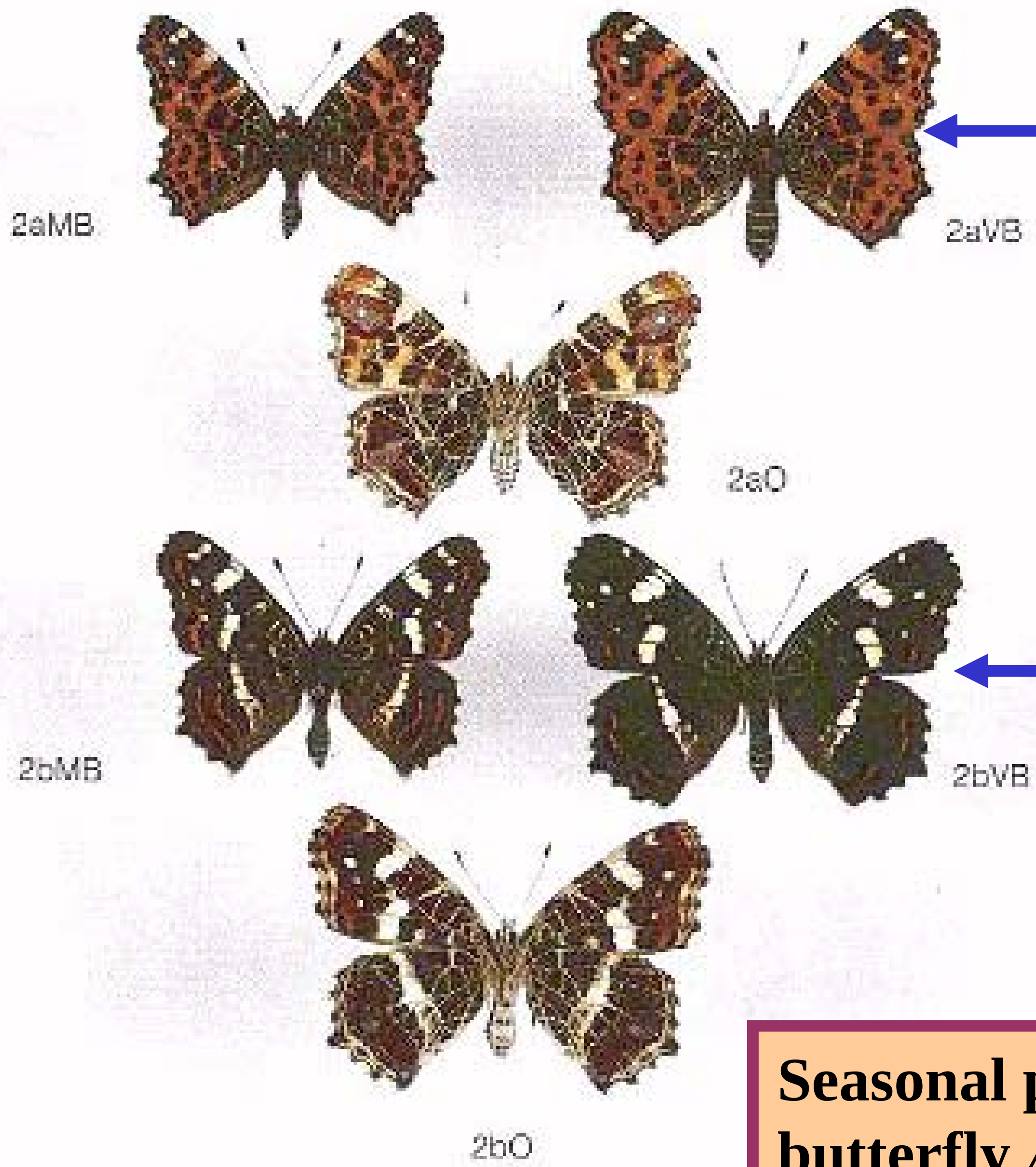
POLYPHENISM-Due to developmental difference in individuals that are genetically identical

1. Sequential polyphenisms
   a. Metamorphosis
2. Alternative polyphenisms
   a. Seasonal polyphenism
   b. Caste polyphenism (social insects)
   c. Phase polyphenism (locusts, aphids and moths)

SEASONAL POLYPHENISM



Butterfly *Araschnia levana*. (A) is the black and white summer form that emerges from nondiapausing pupae. (B) The orange and brown spring form that emerges from diapausing pupae

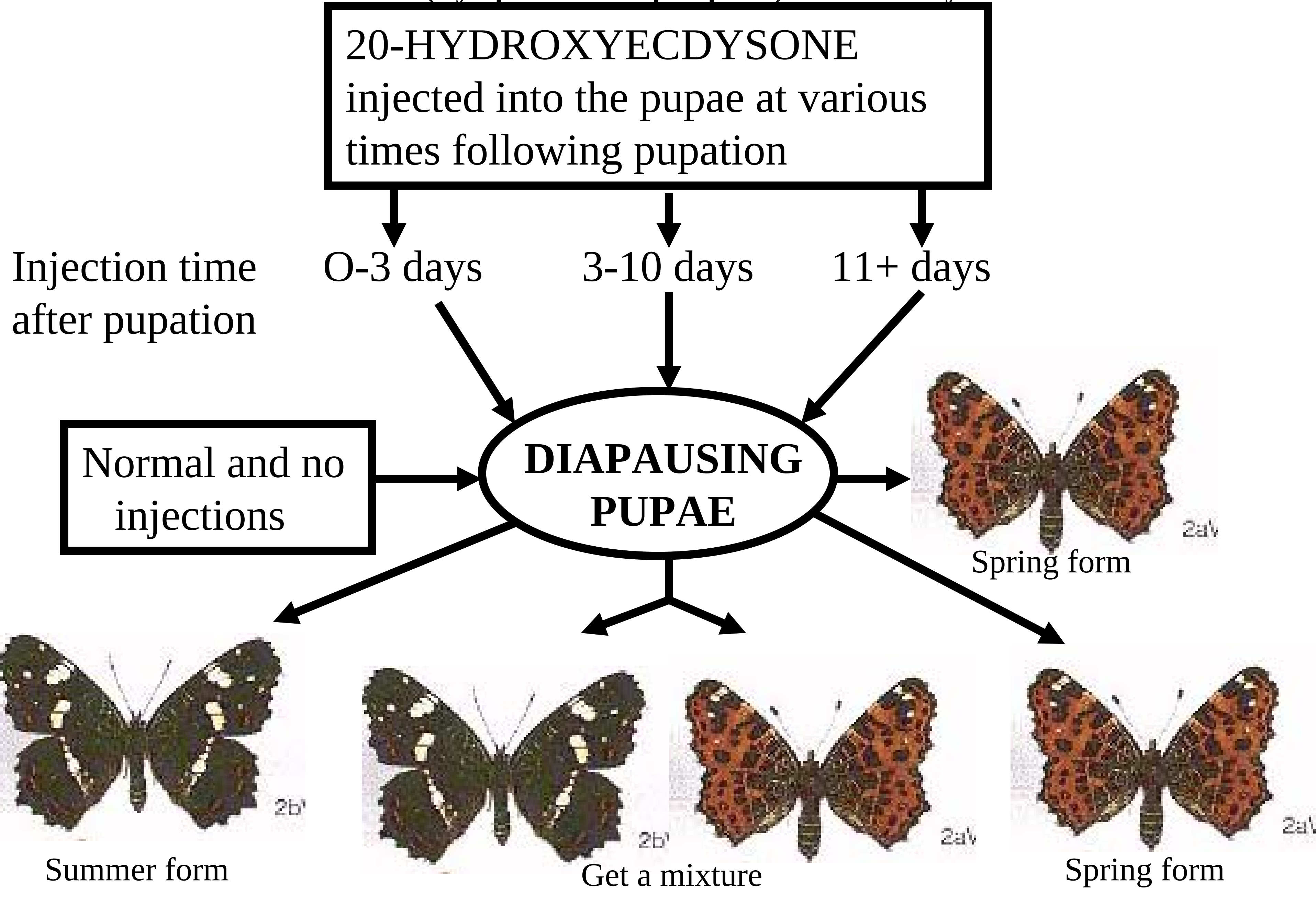


Orange and brown spring form. Emerges from diapausing pupae

Black and white summer form. Emerges from nondiapausing pupae

Seasonal polyphensim in the butterfly ***Araschnia levana***

Koch, P.B. and D. Buckmann. 1987. Hormonal control of seasonal morphs by the timing of ecdysteroid release in *Araschnia levana* (Nymphalidae: Lepidoptera). J. Insect Physiol. 33: 823-829.



1962, 1977- "…a gregarizing pheromone which is believed to play a role in promoting the phase change from *solitaria* to *gregaria*."

Crowded mothers lay eggs that produce nymphs that differ from those of solitary mothers

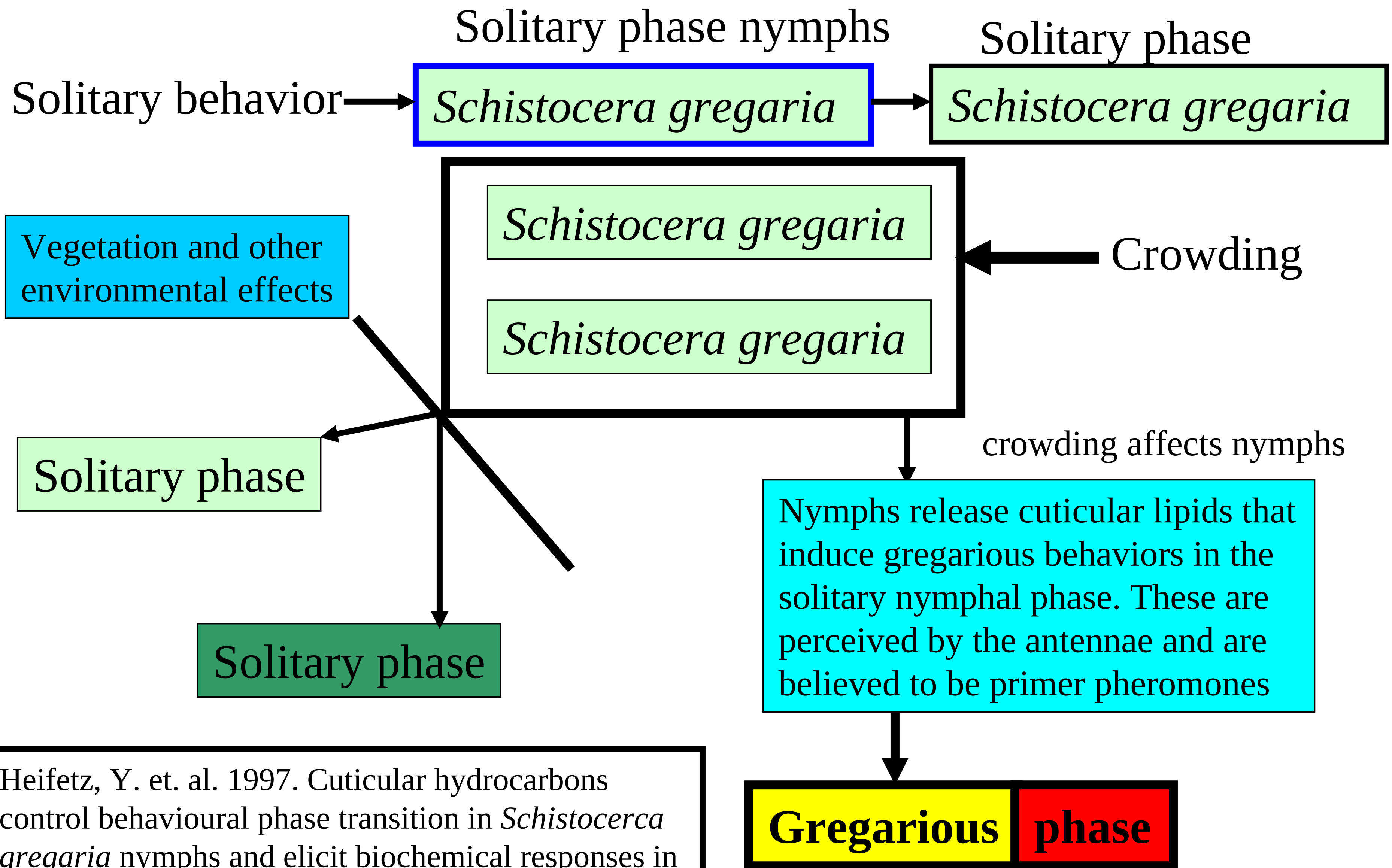
↓

Environmental factors

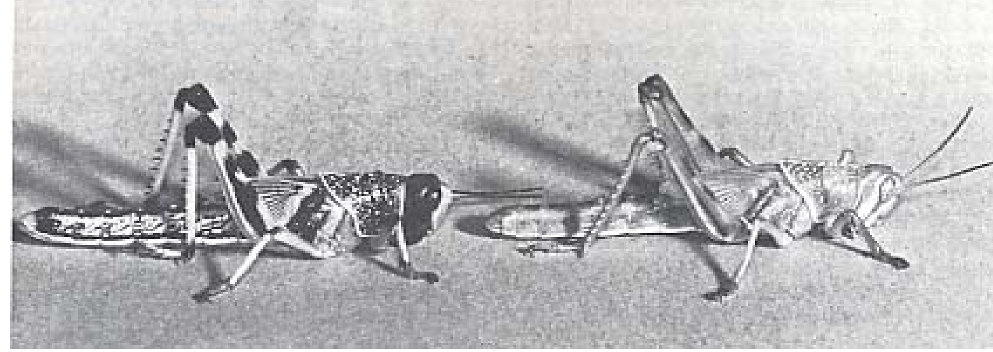
↓

Crowding effects are believed to be cumulative throughout the nymphal instars

Phase polyphenism (locusts)



Heifetz, Y. et. al. 1997. Cuticular hydrocarbons control behavioural phase transition in *Schistocerca gregaria* nymphs and elicit biochemical responses in antennae. Insect Biochem. Mol. Biol. 27: 563-568.

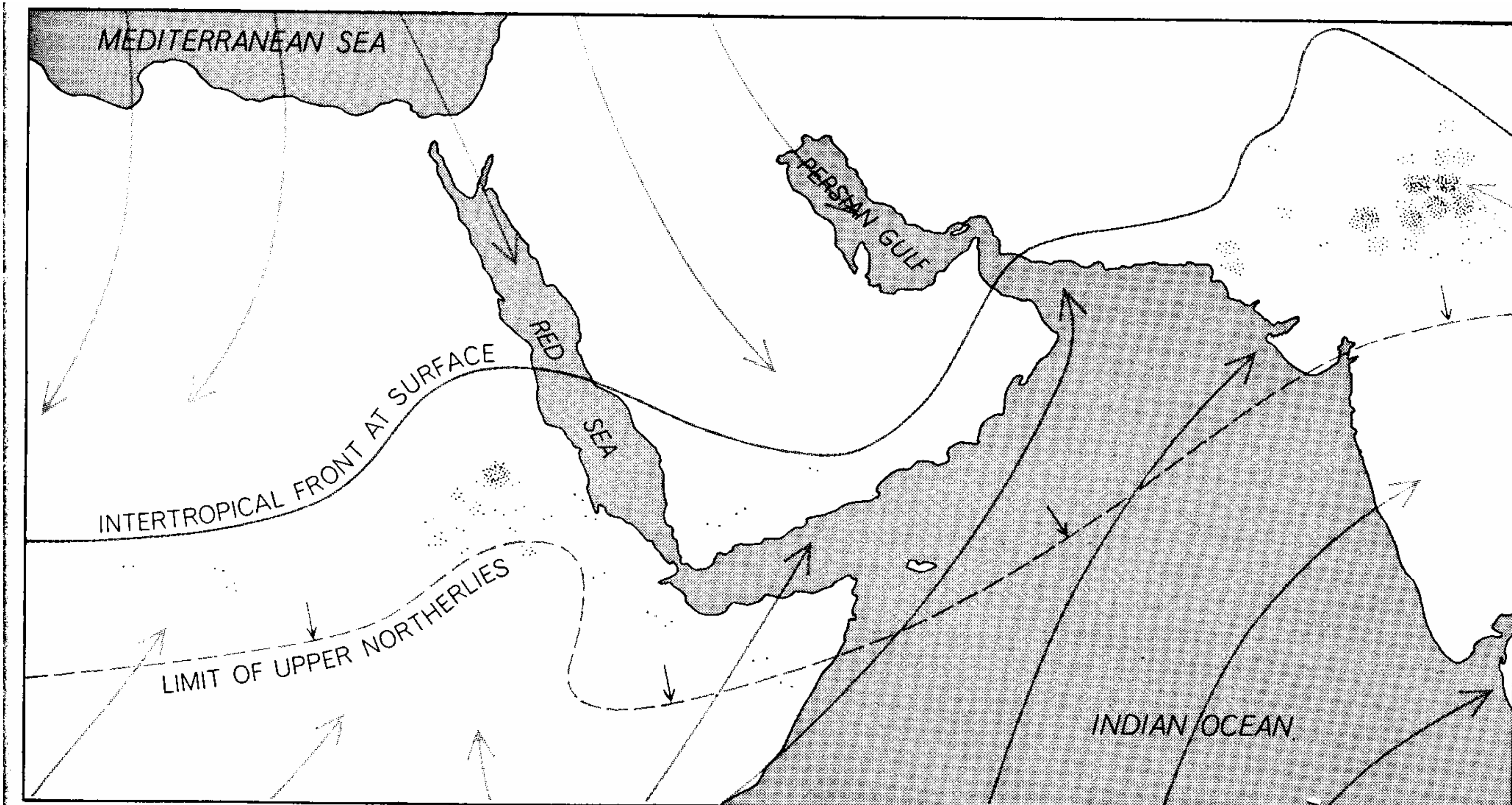


The extremely robust desert locust Schistocerca-Gregaria.

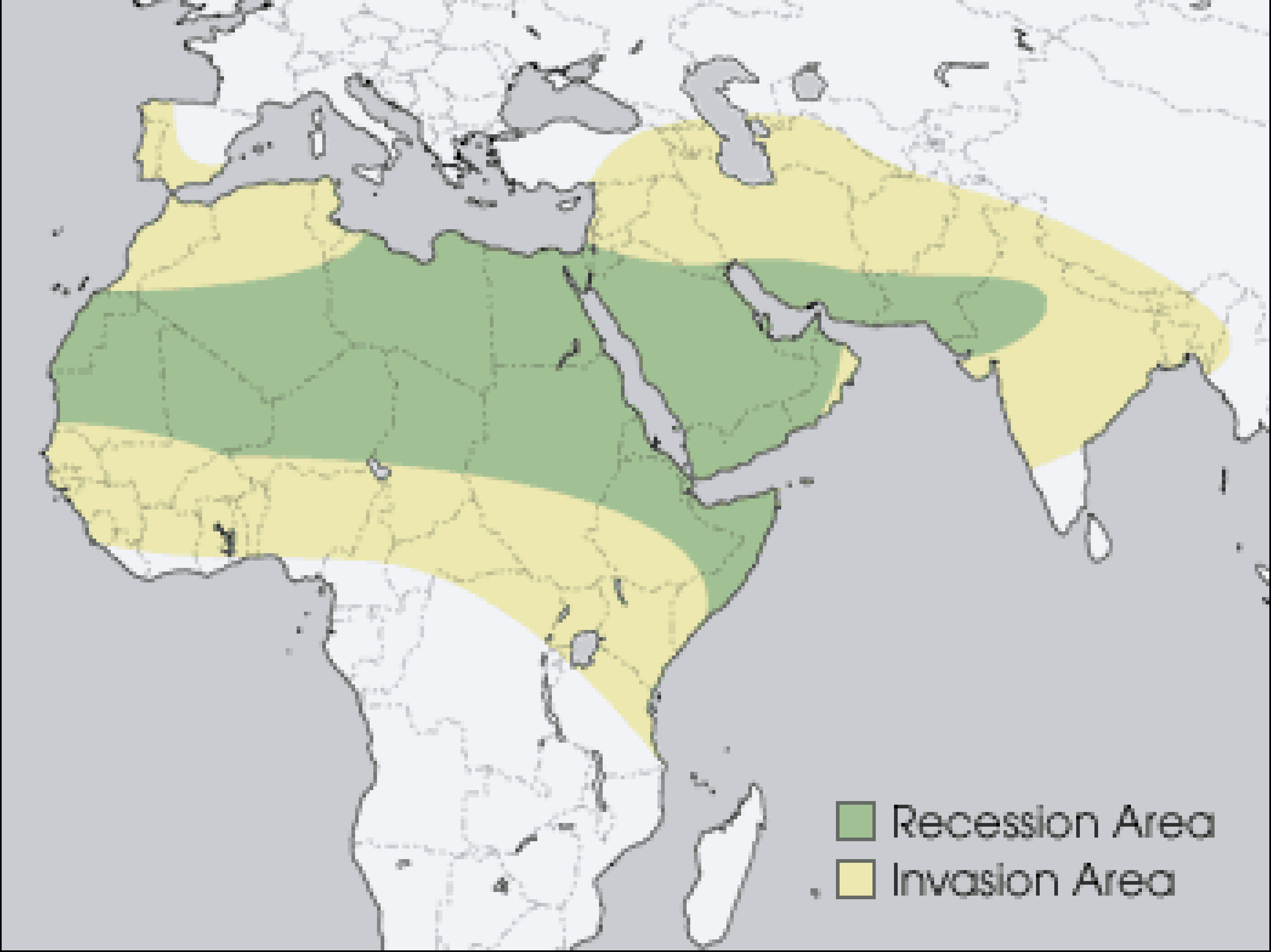
"And Moses stretched his rod over the land of Egypt and the Lord brought an east wind upon the land all that day and all that night and when it was morning the east wind brought the locust." Exodus 10:13

	GREGARIOUS	SOLITARY
Physiology	JH III low	JH III high
Behavior	Active to fly	Sedentary
Color	Black, yellow & organge	Matches the color of vegetation
Ecology	Migrate	Remain and eat
Morphology	Longer wings Pronotum depressed	Shorter wings pronotum keel shaped
Ovaries	Small	Large
Prothoracic glands	Retained for several wks after becoming adults	Breakdown immediately when adult

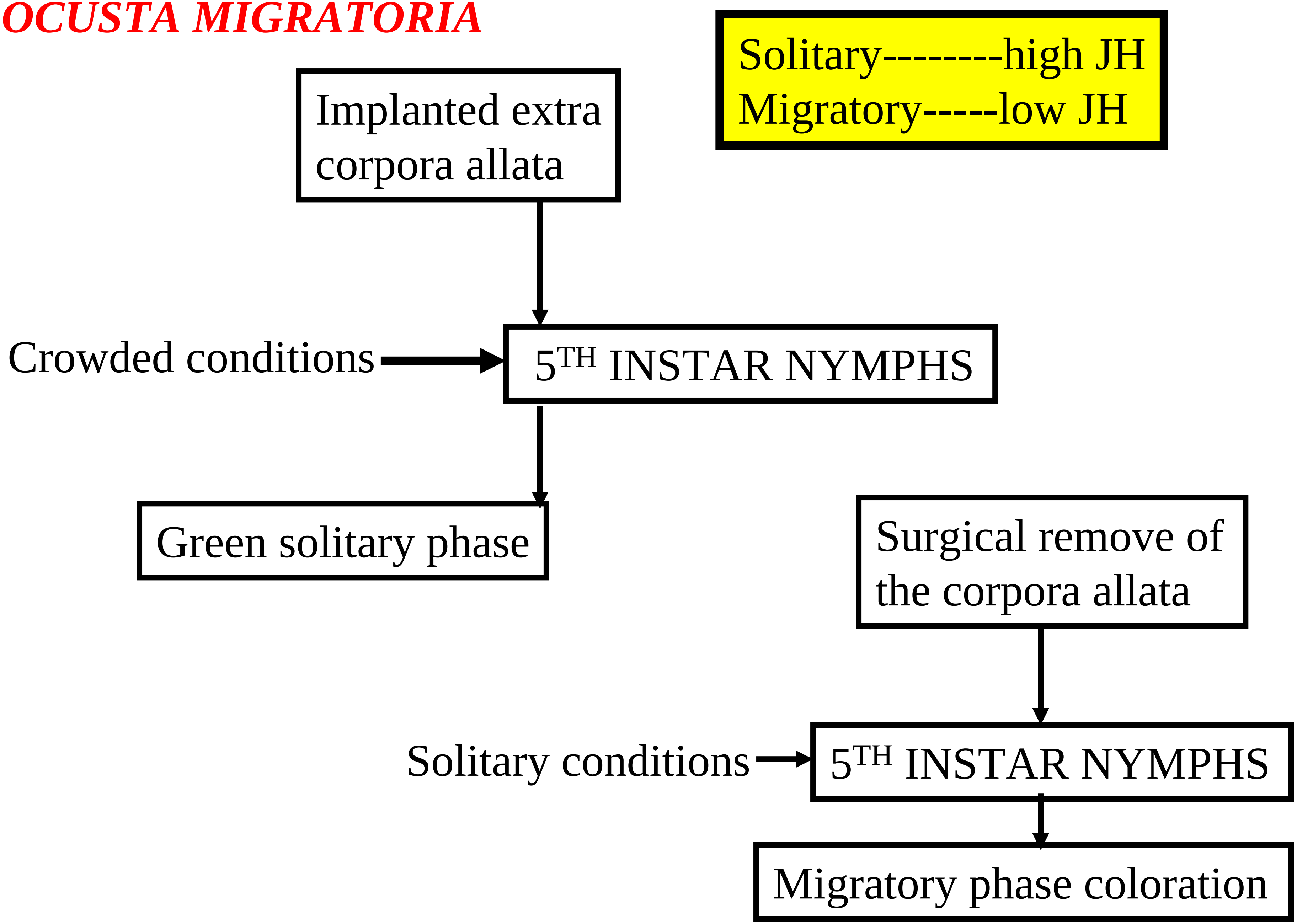
Migratory locusts migrate in the winds that will take them to areas where winds from the north meet those from the south and produce rain, which is essential for food for the future nymphs.



INTERTROPICAL CONVERGENCE ZONE, where winds from north meet those from south (*colored arrows*), producing rain, provides moisture that enables locusts to breed. The rain also brings growth of vegetation, on which locust larvae can feed. Thus, travel with the wind serves ecological needs. Each colored dot denotes a report of a locust swarm between July 12 and July 31, 1950.


Recession Area
Invasion Area

ENDOCRINE CONTROL OF PHASE DETERMINATION IN *LOCUSTA MIGRATORIA*



Role of JH on phase in *Locusta migratoria*

SOLITARY PHASE	GREGARIOUS PHASE
1. JH titer is higher than in gregaria	1. JH titer is lower
2. CA volume is larger	2. CA volume is smaller
3. Higher rate of JH III synthesis	3. Lower rate of JH III synthesis

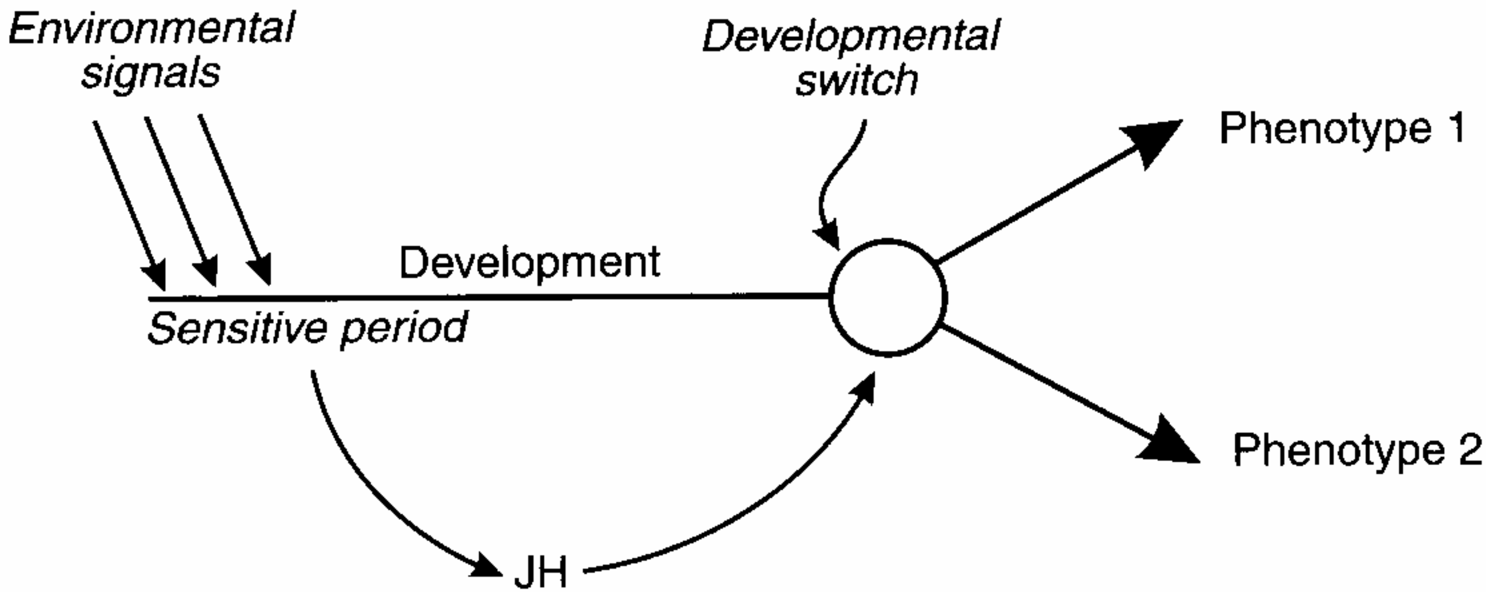


FIGURE 5.6 The control of polyphenisms by juvenile hormone. Environmental signals during a sensitive period of development trigger the release of JH later in development. The JH can switch between alternative phenotypes. Adapted from Nijhout (1999). Reprinted with permission.

TABLE 1.1 Ecological Hierarchy and the Structural and Functional Properties Characterizing Each Level

PHOTOPERIODISM

Ecological level	Structure	Function
Global	Biome distribution Atmospheric condition Climate Sea level	Gas, water, nutrient exchange between terrestrial and marine systems Total NPP
Biome	Landscape pattern Temperature, moisture profile Disturbance regime	Energy and matter fluxes Integrated NPP of ecosystems Migration
Landscape	Disturbance pattern Community distribution Metapopulation structure	Energy and matter fluxes Integrated NPP of ecosystems Colonization and extinction
Ecosystem	Vertical and horizontal structure Disturbance type and frequency Biomass Functional organization	Energy and matter fluxes Succession NPP, herbivory, decomposition, pedogenesis
Community	Diversity Trophic organization	Species interactions Temporal and spatial changes
Population	Density Dispersion Age structure Genetic structure	Natality Mortality Dispersal Gene flow Temporal and spatial changes
Individual	Morphology Physiology Behavior	Resource acquisition Resource allocation Learning

PHOTOPERIODISM-One of the more important topics in biology since life on earth has been continually exposed to an alternating cycle (i.e., photoperiod) of light (i.e., photophase) and dark (i.e., scotophase). This important and reliable cycle is the calendar by which many biological events are controlled. Thus, everyone knows that some insects are diurnal, some nocturnal and come crepuscular. Think about yourself. Are you a morning, midday, or night person?

This universal event has its effect at all ecological levels

Photoperiod-a cycle consisting of a period of light and a period of dark.

Photophase-the day light period of the photoperiod cycle. Also known as day length.

Scotophase-the night or dark period of the photoperiod cycle

INHERENT UNIVERSAL RHYTHMS

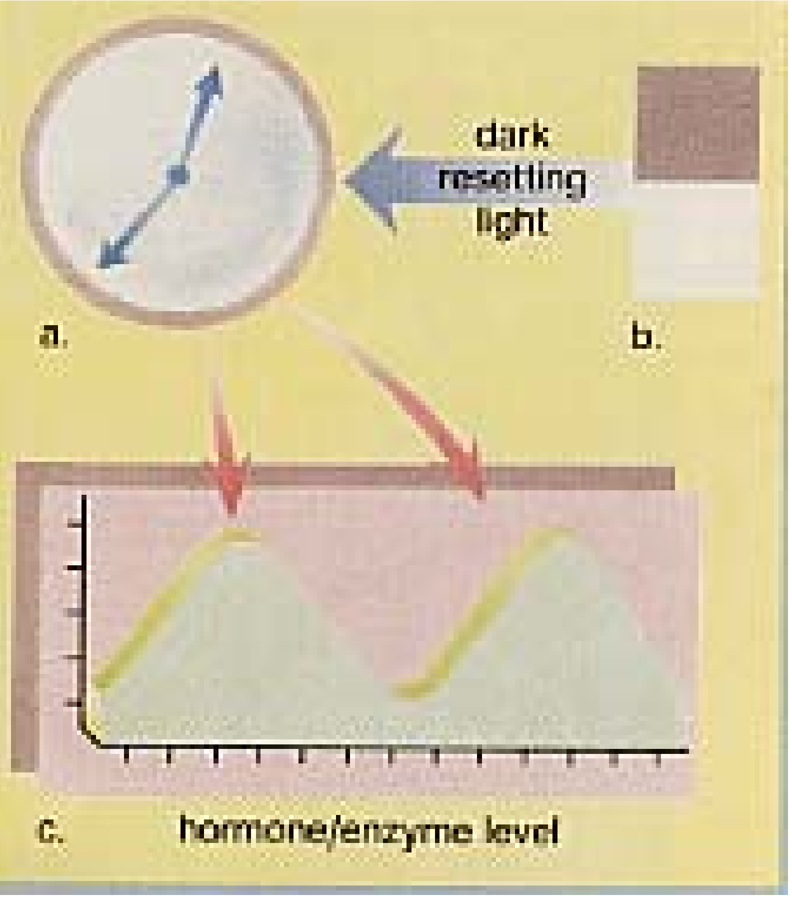
1. **SOLAR DAY**-Earth rotates around the sun every 24 hrs.

2. **LUNAR DAY**-Using the moon as the point of references, the earth seems to rotate once in about 24 hrs. and 50 min.

3. **SIDEREAL DAY**-Using stars as the point of reference, the day is called a sidereal day and is about 4 mins. shorter than the solar day

4. **SYNODICAL MONTH**-Averages about 29.53 days. Both sun and moon contribute to this. Once every synodical month the sun and moon rise and set at nearly the same times.

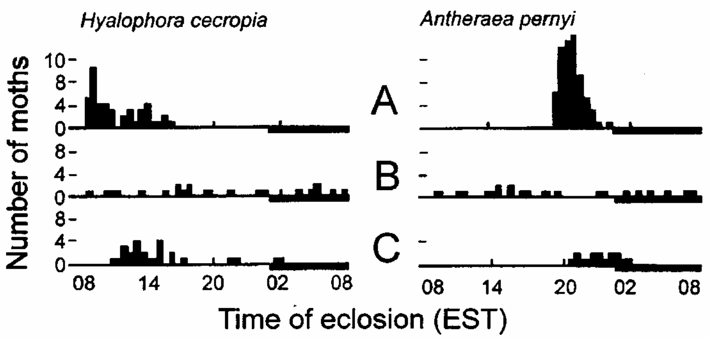
BIOLOGICAL CLOCKS HAVE 3 COMPONENTS



1. INTERNAL TIMEKEEPER
2. WAY OF DETECTING LIGHT/DARK
3. WAY OF COMMUNICATING THE TIME AND DETECTION DEVICE WITH WHATEVER BRINGS ABOUT THE EVENT

TRUMAN'S ECLOSION BEHAVIOR SYSTEM

1. INTERNAL TIMEKEEPER-the **brain**

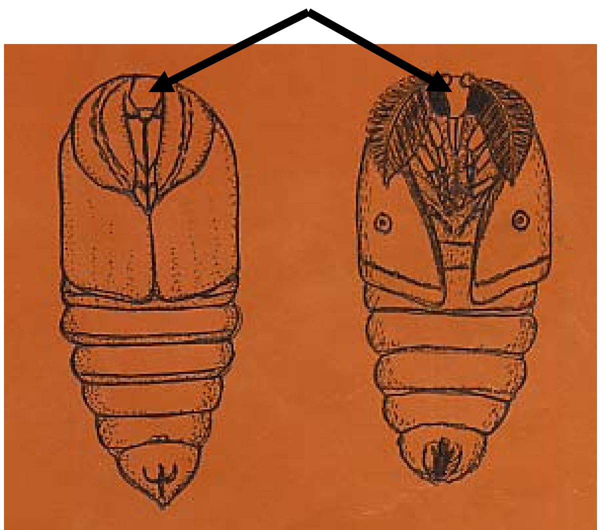


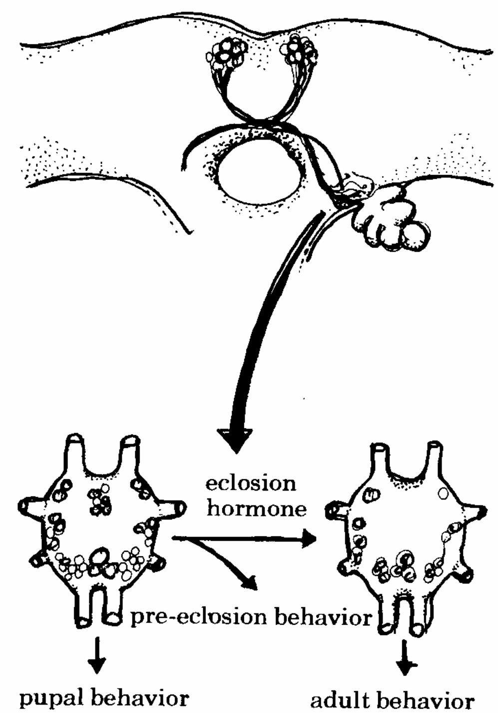
3. WAY OF COMMUNICATING THE TIME AND DETECTION DEVICE WITH WHATEVER BRINGS ABOUT THE EVENT

Ecdysis triggering and eclosion hormones

2. WAY OF DETECTING LIGHT/DARK

Clear window over the brain





THE PER GENE OF *DROSOPHILA*-circadiam rhythms in behavior

In *Drosophila melanogaster* a gene at the "per" locus controls rhythmic locomotor activity. There are several allelic forms shown in the table below.

The "Per" locus also controls interpulse rhythm in male courtship song, which is species specific. *D. simulans* and *melanogaster* differ in the intervals between song pulses.

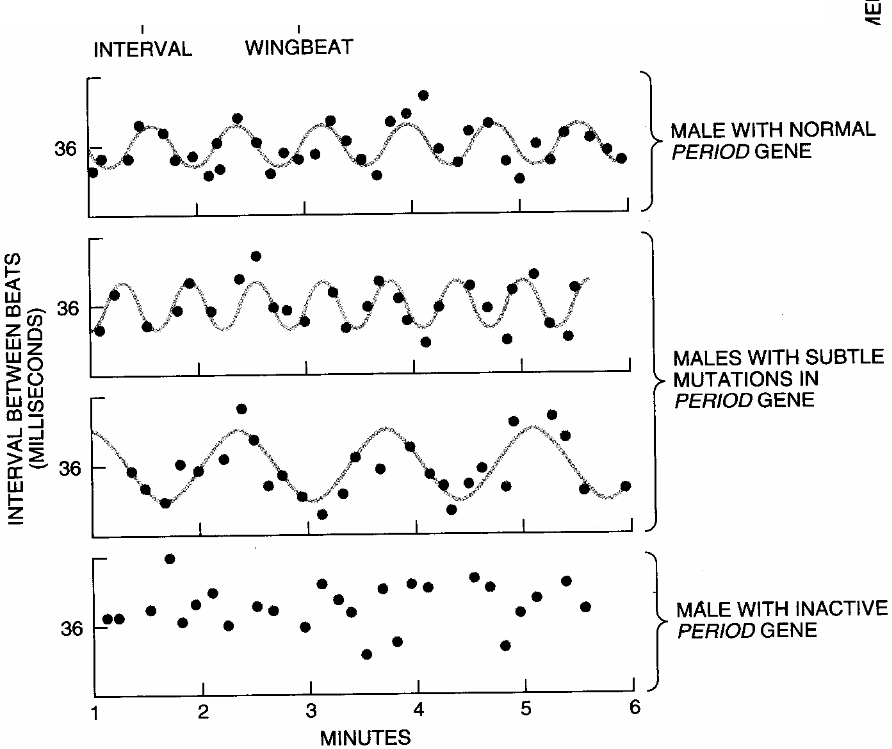
Table 8.1 Average Period Length for the Interpulse Song Interval for *Drosophila melanogaster per* mutants and *D. simulans*[a]

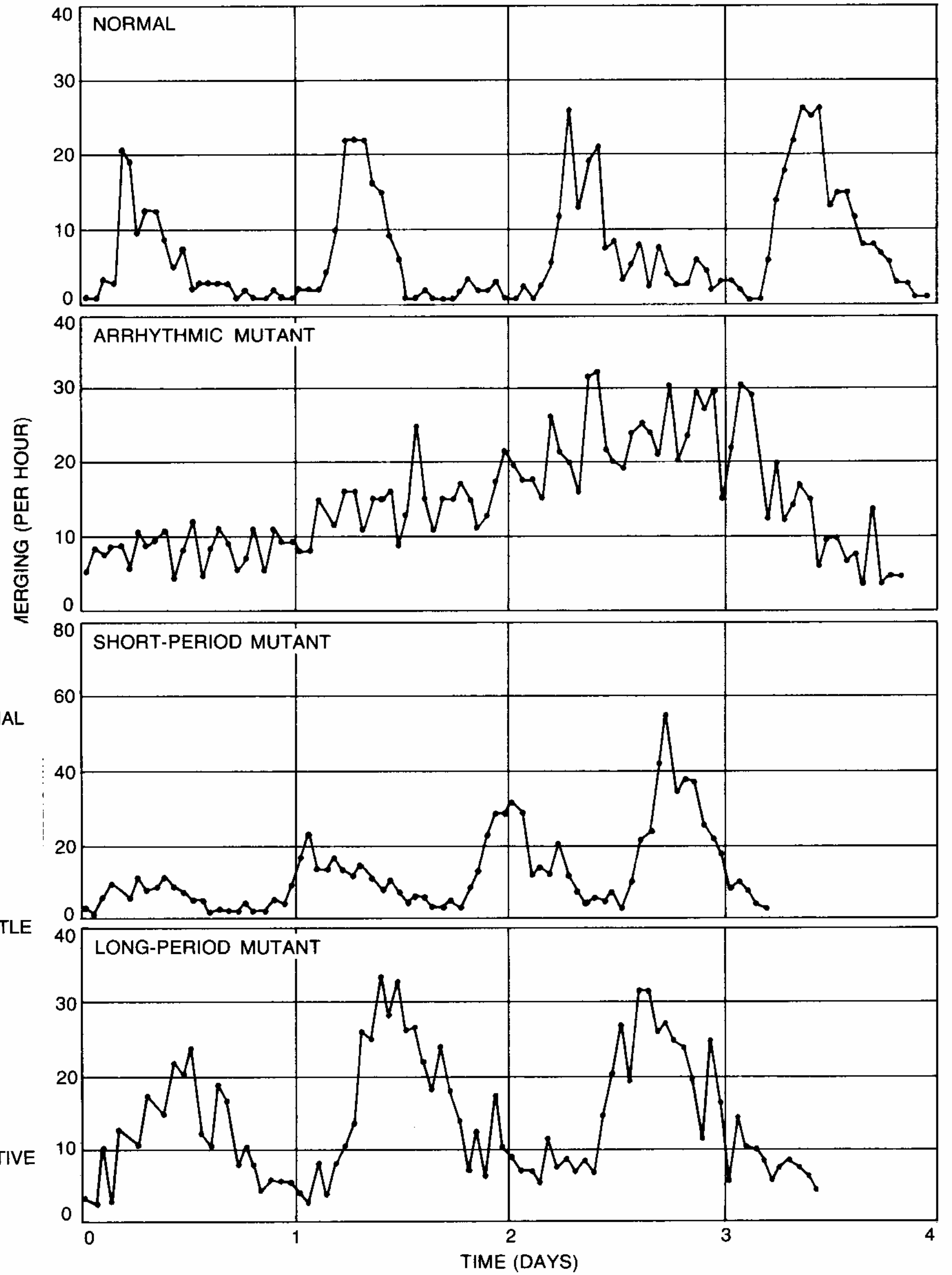
Species	Secs (Range)
D. melanogaster	
per$^+$ or normal, wild-type	50–65
per^s or shortened	35–45
per^L or lengthened	75–95
per^o or abolished	Arrhythmic
D. simulans	
per$^+$ or normal, wild-type	30–40

[a]Data from Wheeler et al. (1991).

D. melanogaster per^o = arrhythmic courtship song Cloned a copy of a DNA segment from the per$^+$ locus of *D. simulans*, using P-element construct and introduced gene into *D. melanogaster* per^o and the gene introduction rescued the arrhythmicity in the behavior. However, it now had the interval song of *D. simulans*.


3 "SINGING"


INTERVAL
WINGBEAT
INTERVAL BETWEEN BEATS (MILLISECONDS)
36
MALE WITH NORMAL *PERIOD* GENE
MALES WITH SUBTLE MUTATIONS IN *PERIOD* GENE
MALE WITH INACTIVE *PERIOD* GENE
1
2
3
4
5
6
MINUTES


NORMAL
ARRHYTHMIC MUTANT
SHORT-PERIOD MUTANT
LONG-PERIOD MUTANT
ERGING (PER HOUR)
0
10
20
30
40
60
80
0
1
2
3
4
TIME (DAYS)

IF ONE WAS TO PREPARE A SWITCH TO TURN GENES ON AND OFF, WHAT INSECT CHEMICAL MIGHT BE A GOOD CANDIDATE FOR TURNING ON AND OFF GENES IN MAMMALS?

There is great need to develop switches that can turn on and turn off specific genes. Chemicals such as the antibiotic tetracycline have been used in both mice and cell cultures.

The insect hormone, ecdysone, however, may provide the most effective gene switch yet. This hormone has no effect on mammalian cells. Couldn't be done until the ecdysone receptor was found.

Made mammalian cells and strains of mice with genes that turn on when ecdysone reaches them.

Insect hormone inspires switch for genes. Science. D. No, T.-P. Yao and R. M. Evans. Proc. Natl. Acad. Sci. April 16, 1996.

HOW DOES ONE LOCATE THE SITE OF ACTION OF A PARTICULAR GENE?

1. Must be able to turn gene on at will. How does one do that?

   Heat shock genes

2. Must be able to visualize the gene products. How does one do that?

   Luciferase gene from the firefly

Fuse the luciferase gene to a known PROMOTER

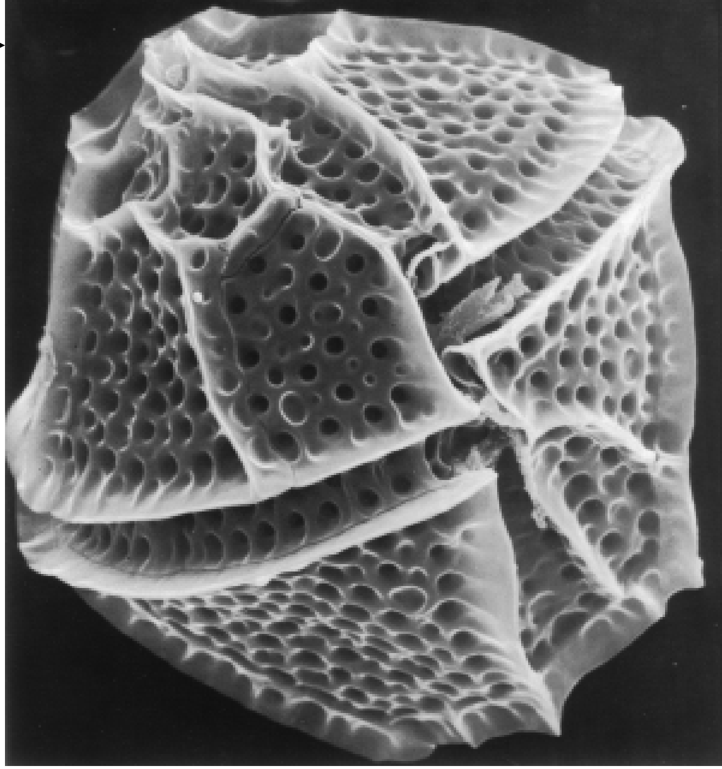
PROMOTER=is a regulatory element that in this case had belonged to a gene that also showed circadian rhythm

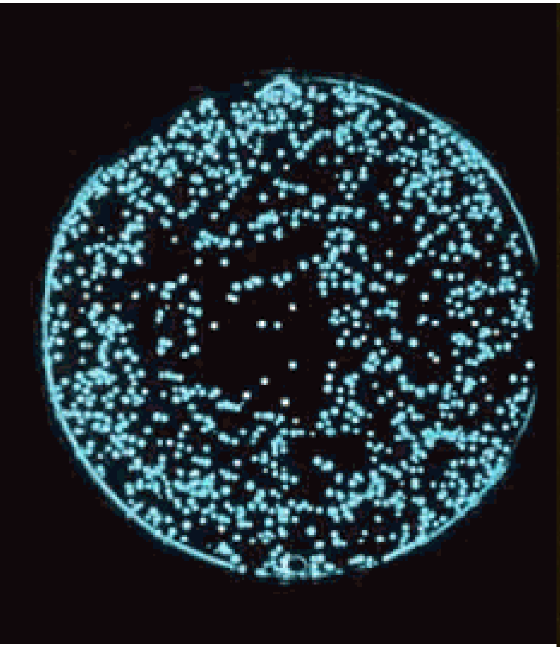
Develop the DNA segment below

<u>Circadian rhythm promoter ▌luciferase gene</u>

Now insert this DNA segment into the genome of the organism

SEM of *G. polyedra*

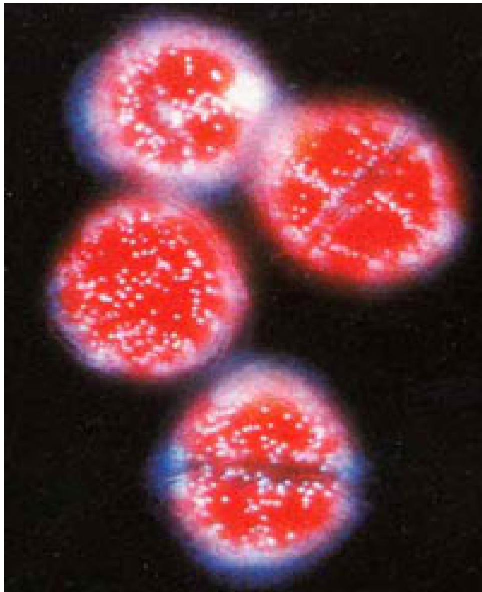




bioluminescent bacteria

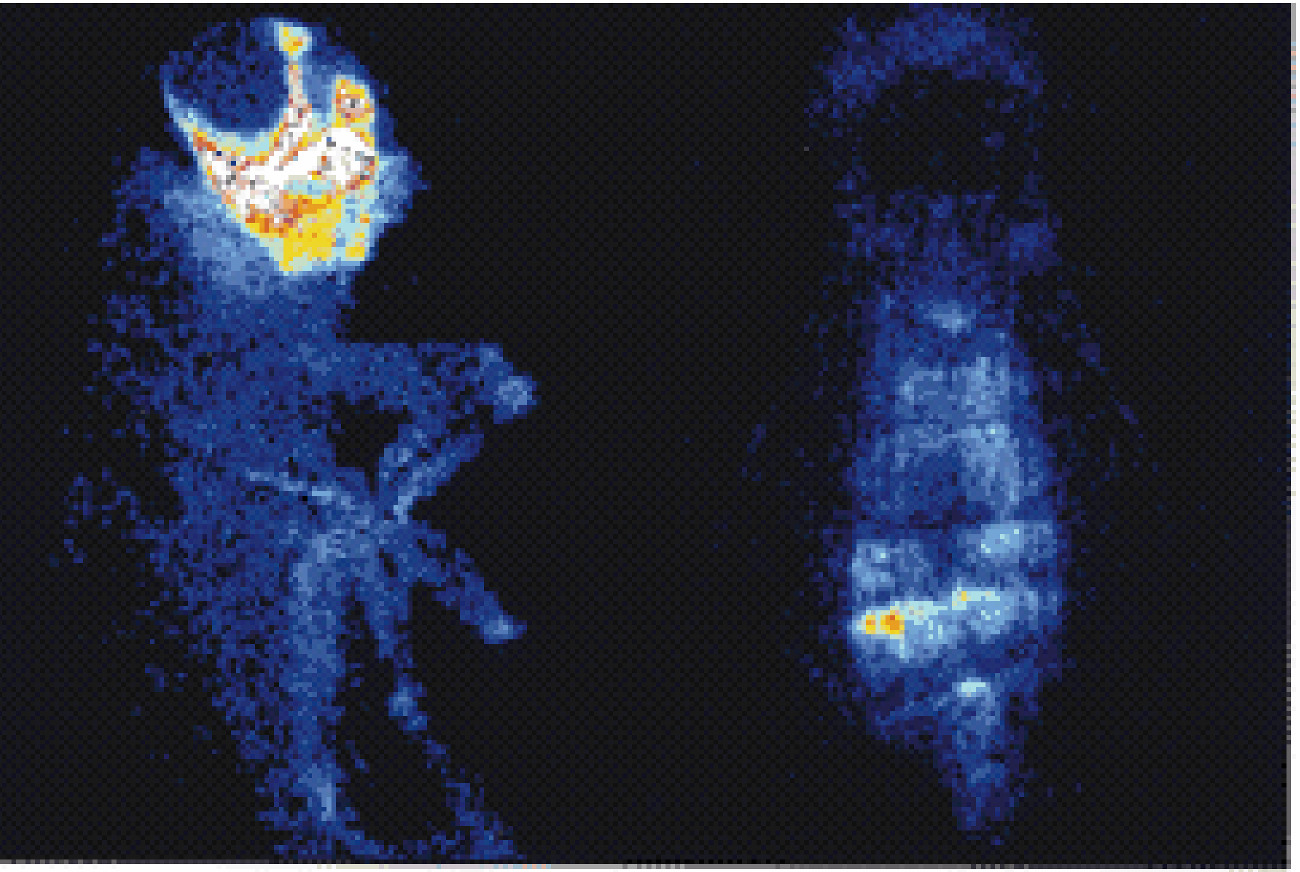
Gonyaulax polyedra), day

Gonyaulax polyedra), day



Photos to the right of screen show: Bioluminescent dinoflagellates showing the fluorescence of scintillons, the organelles where light emission occurs, and the difference between cells and organelles from day to night. Red fluorescence from chlorophyll. The circadian rhythm of light production.

USING A BIOLUMINESCENT MARKER SYSTEM TO LOCATE GENE EXPRESSION



Circadian rhythms are controlled by pacemaker cells that are located in the brain and optic lobes

These cells are probably neurons or glial cells or both

The genes for PER are expressed here where they produce PER protein in a cyclical manner

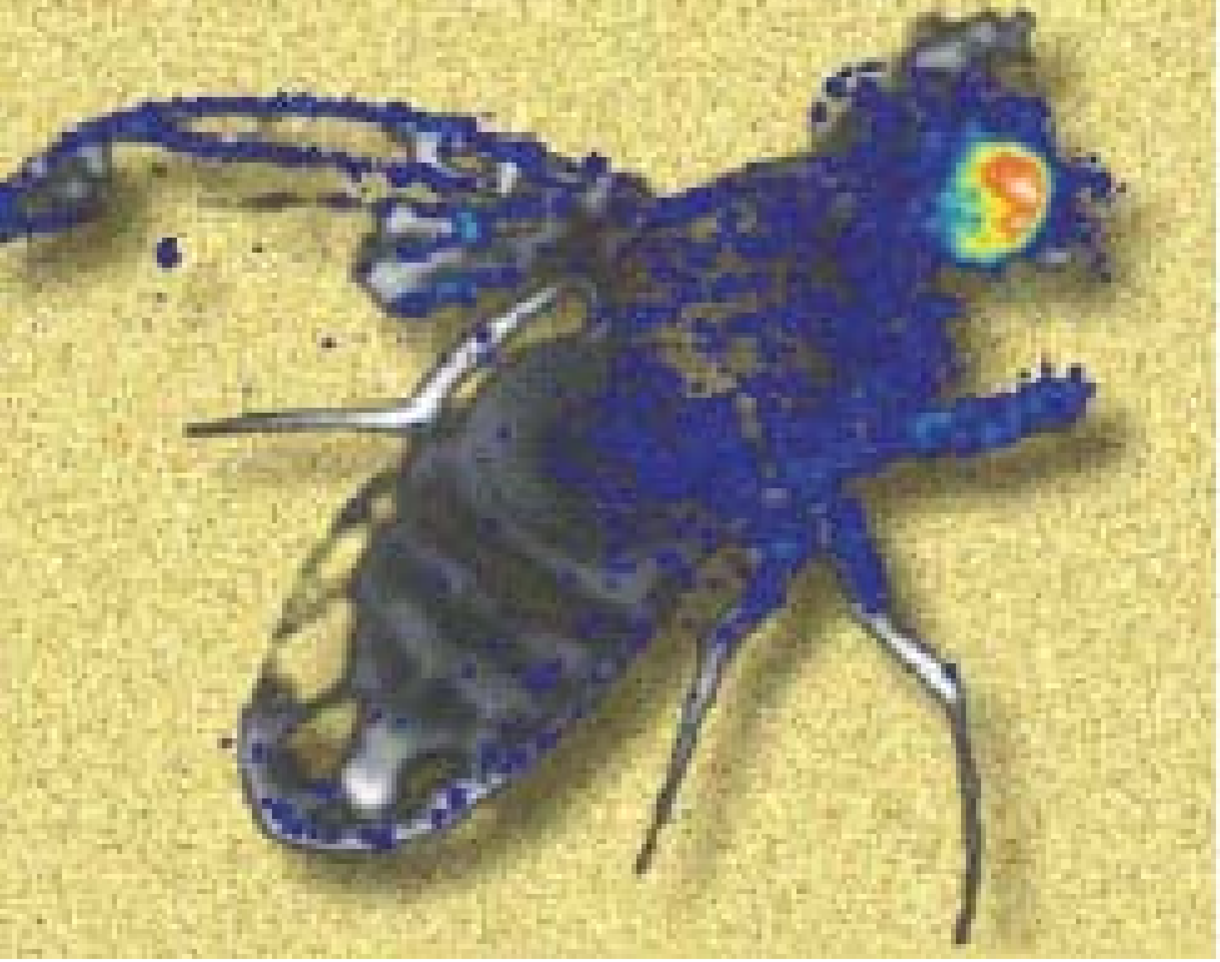
Figure 5. Hsp70::*luc* transcription in living *Drosophila*. Adult *Drosophila* bearing an hsp70::*luc* fusion were heat shocked in a dry vial at 40°C for 40 minutes, followed by a 30-minute recovery period at room temperature. The animals were then anesthetized with ether, sprayed with 5mM luciferin and imaged for 10 minutes using a 10X objective on a Nikon upright microscope attached to the Hamamatsu VIM camera.

USING THE BIOLUMINESCENT MARKER TO ASSAY POPULATIONS OF FLIES and INCREASING THE NUMBER OF INDIVIDUALS MONITORED

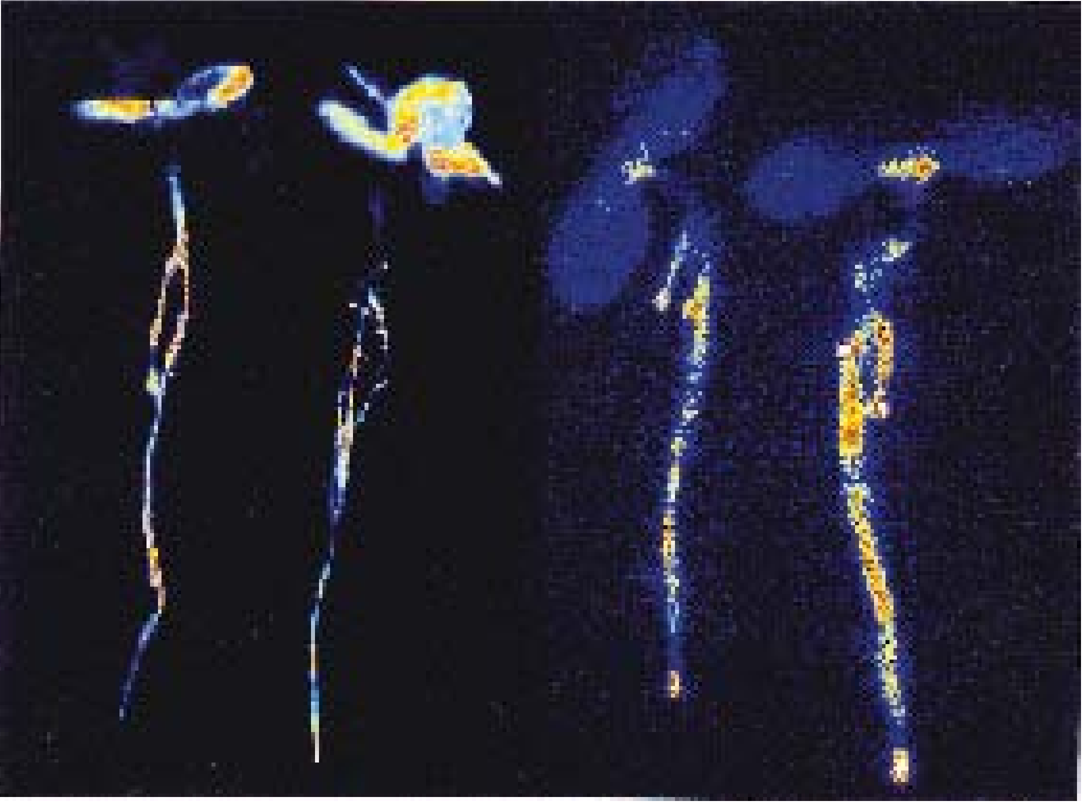
Assaying large populations of Drosophila for the circadian gene PER Requires lots of time. They must grind up the flies and then check for the PER gene protein.

Hall and his group tried a simple experiment that has saved them a lot of time. They put luciferin in the diet of the genetically altered flies and they lit up. By then putting a light detection device into the cages of these flies they could follow their cyclical activity for days.

They then documented the circadian rhythms of 1,500 individual flies for days. An experiment they would never have been able to do without these techniques.



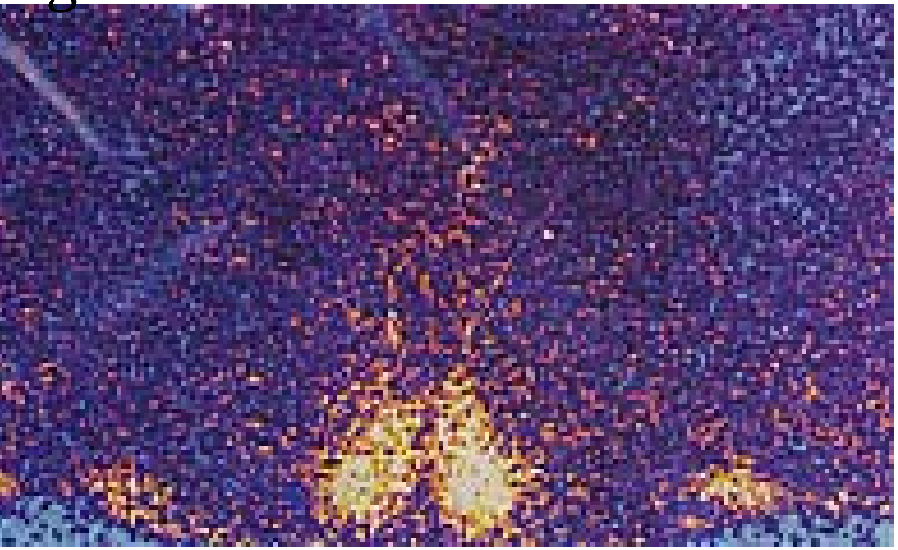
Genes that code for the clock protein, PER, glow in the head and other body parts of a fruit fly. Researchers made the clocks glow by engineering transgenic strains of flies in which the same genes that illuminate a jellyfish and a firefly’s abdomen are attached to PER. The gene for luciferase, the enzyme that glows intermittently in fireflies, was expressed along with PER to reveal when the clock protein was being produced. Flies were also molecularly altered to brightly mark the clock sites with Green Fluorescent Protein, which glows constantly in jellyfish.



Cab2 one of a family of nuclear genes that encodes the chlorophyll a/b-binding proteins of the photosynthetic light-harvesting complexes.

Figure 4. Firefly luciferase reports spatial regulation of transcription. The left hand panel shows two seedlings bearing a -198/-33 *cab*2::35S::*luc* fusion which were imaged using a Nikon 105mm f2.8 lens attached to a Photometrics liquid nitrogen-cooled CCD camera. The image was processed using PMIS software and Adobe Photoshop. The right panel shows two seedlings containing a -322/-74 *cab*2::35S::*luc* fusion. These images were acquired using a Nikon 105mm f/2.8 lens attached to the Hamamatsu VIM camera for 5 minutes.

They have found the *clock* gene in mammals.



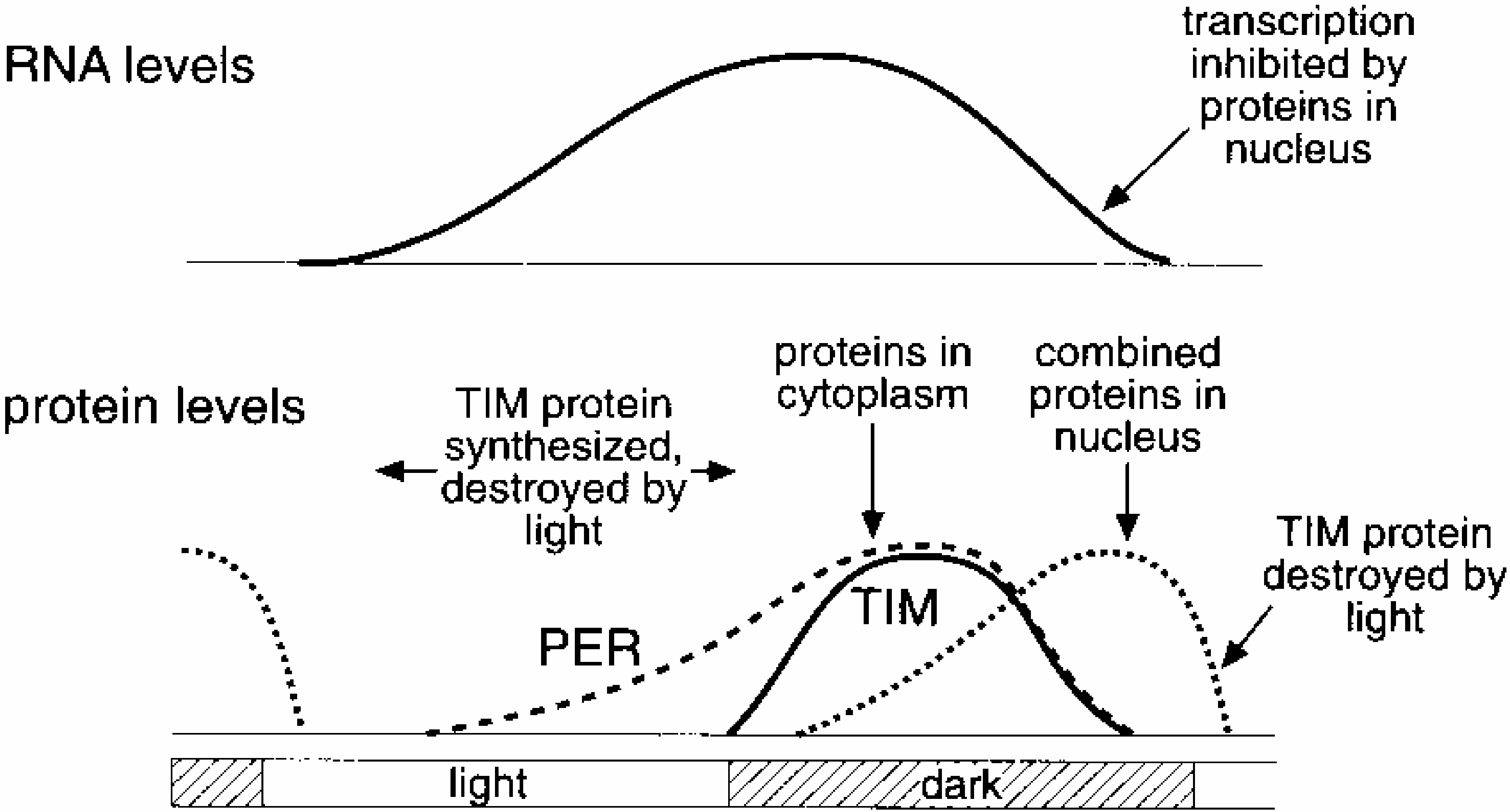
The suprachiasmatic nuclei, brain regions that govern most circadian rhythms in mammals, exhibit high activity (yellow and black arrow) of a gene called clock.

1. INTERNAL TIMEKEEPER
   Pacemaker-brain suprachiasmatic nucleus (SCN)
2. DETECTING LIGHT/DARK
   Eyes
3. WAY OF COMMUNICATING
   Hormones, possibly melatonin

Clock gene is active in retina and brain. Also found in brain, heart, liver and kidneys

Hamster retinas contain independent biological clocks

a) normal cycle



ADAPTIVE BEHAVIORS ASSOCIATED WITH ENDOGENOUS RHYTHMS

1. Locomotor rhythms
2. Feeding rhythms
3. Oviposition rhythms
4. Mating rhythms
5. Adult emergence rhythms-Truman’s eclosion behavior
6. Metabolic rhythms

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Population	Density Dispersion Age structure Genetic structure	Natality Mortality Dispersal Gene flow Temporal and spatial changes
Individual	Morphology Physiology Behavior	Resource acquisition Resource allocation Learning **Communication**

PHYSIOLOGY OF INSECT COMMUNICATION

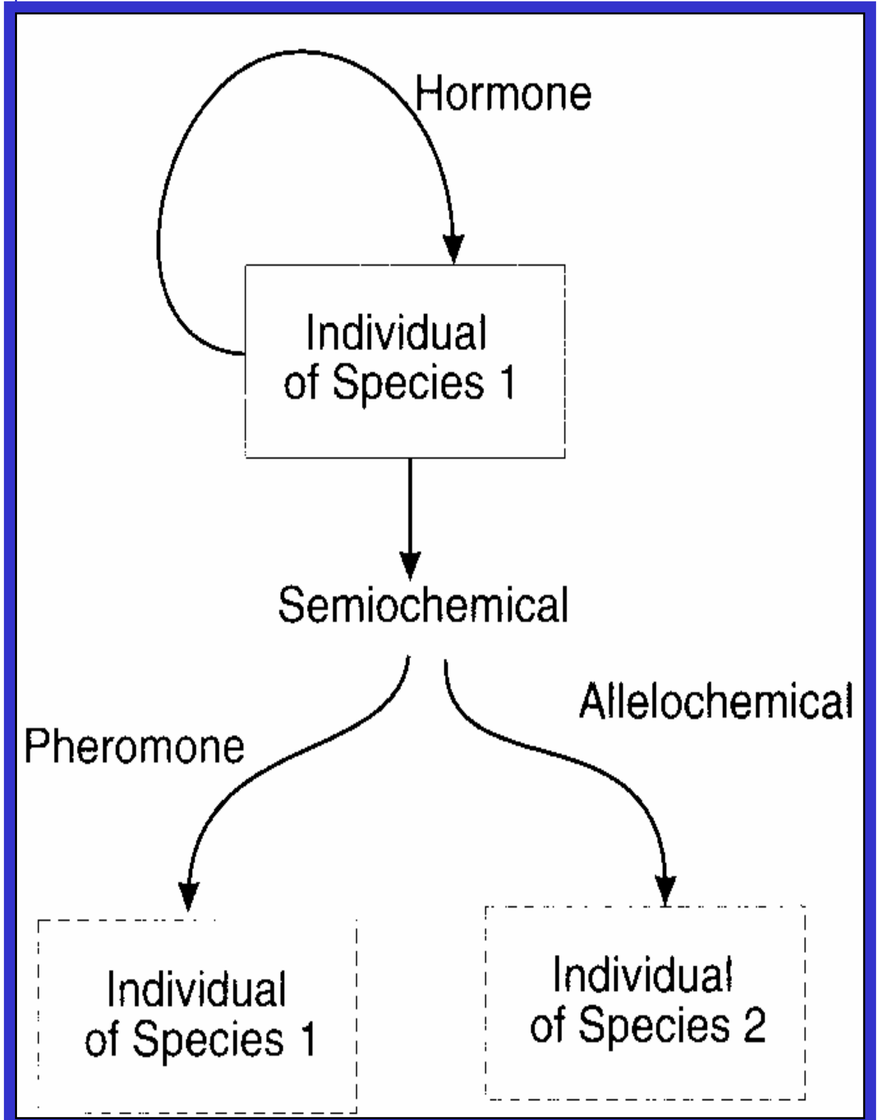
1. Visual communication
   a. Mating
2. Acoustical communication
   a. Mating and territoriality
3. Tactile communication
   a. Mating-sex and species recognition using cuticular hydrocarbons

4. Chemical communication-Use of chemicals for
   a. Finding mates
   b. Information about sources of food
   c. Identifying nest mates
   d. Defense against predators

CHEMICAL COMMUNICATION

Chemicals mediating physiological and behavioral responses can be categorized into:

1. **Hormones**-Chemicals produced that are generally transported in the blood and have their action somewhere else in the body of the organism producing the chemical
2. **Semiochemicals-**A chemical that initiates an interaction between two or more organisms, regardless of whether they are of the same species or different species
   - a. **PHEROMONES-**Mediate intraspecific interactions
     - (1) **Releaser pheromones**-stimulates an immediate and irreversible response
     - (2) **Primer pheromones**-mediates a physiological response that reprograms the individual response and is usally not immediate
   - b. **ALLELOCHEMICALS-**Mediate interspecific interactions



CHARACTERISTICS OF PHEROMONES:

1. Produced in exocrine glands, which are modified epidermal cells

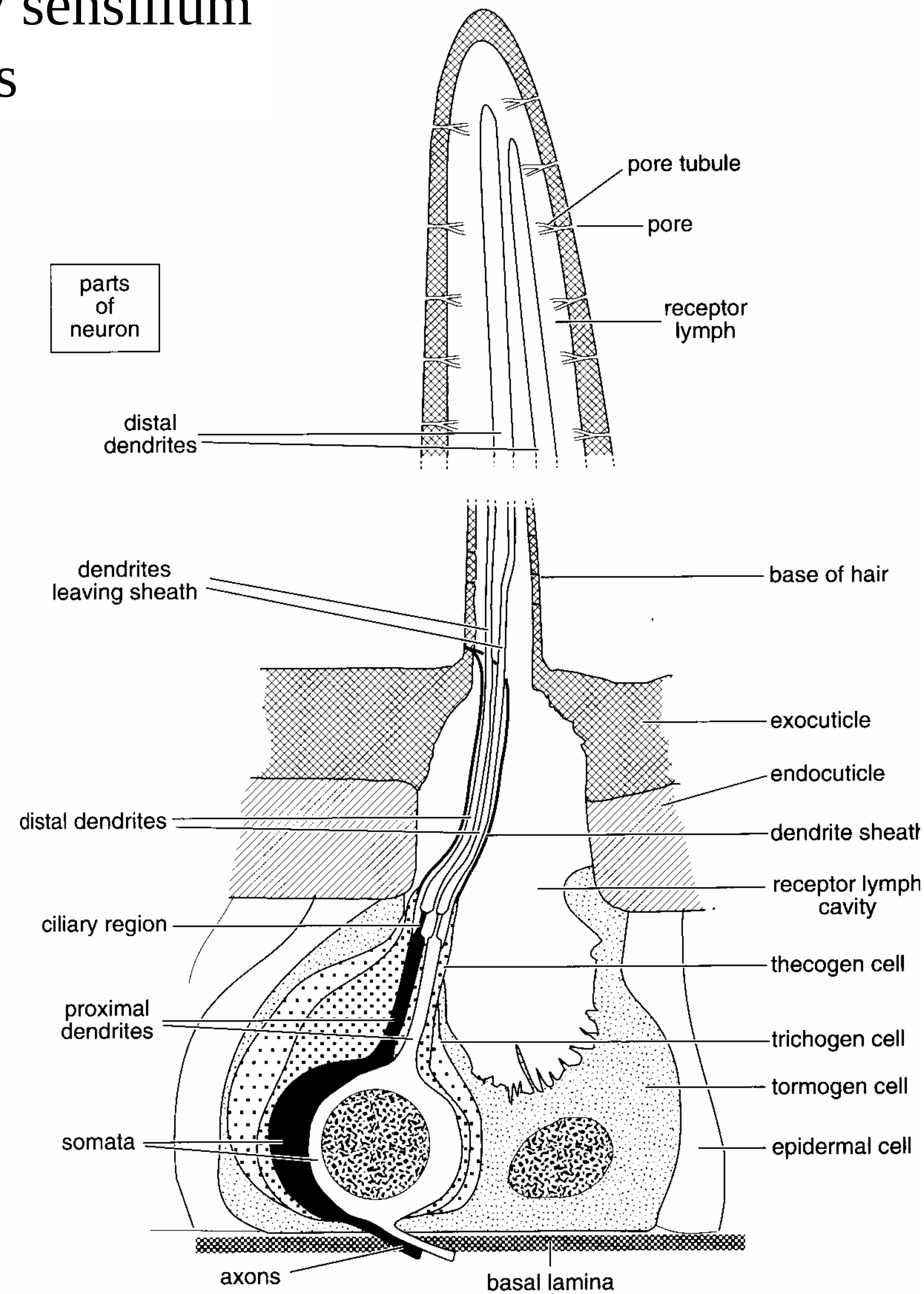
2. Usually consist of a blend of different chemical species, unlike bombykol (thought to initially exist as a single molecule but now is more complex). Unfortunately, this set the stage for research in this area and researchers were looking to identify pheromones on this single-chemical model

3. Usually active in extremely small concentrations

PERCEIVING PHEROMONES USING VARIOUS TECHNIQUES

1. Whole animal bioassays
   a. Fabre's early work
   b. Tethering or caging insect
2. Bioassays using just glands or gland extracts
3. Bioassays using flight mills and moving floors
4. Electrophysiological techniques
   a. EAG's-recordings from a group of nerve cells
   b. Implanted electrode recording from individual cell

Note that this SEM of an olfactory sensillum clearly shows that it is multiporous



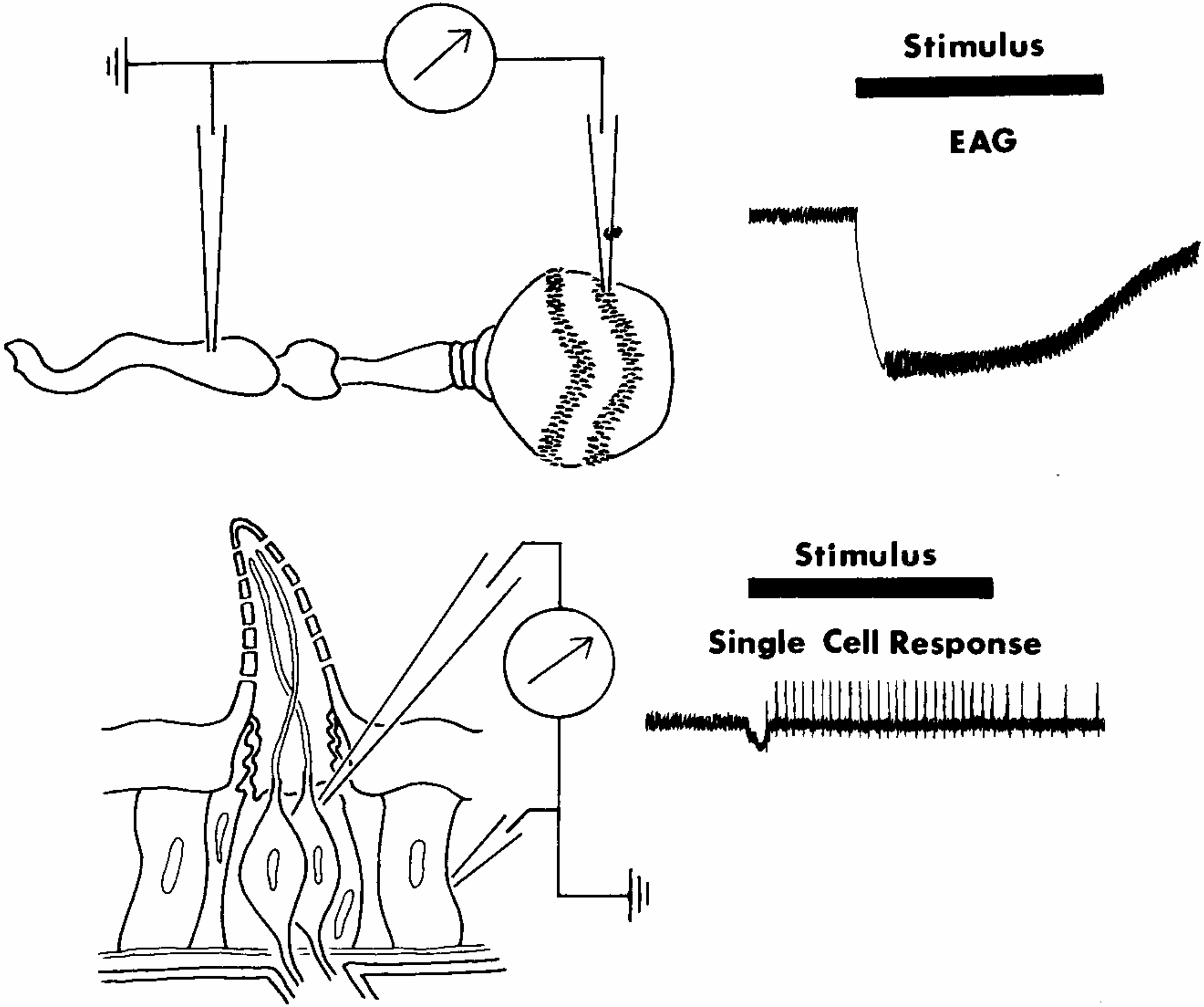
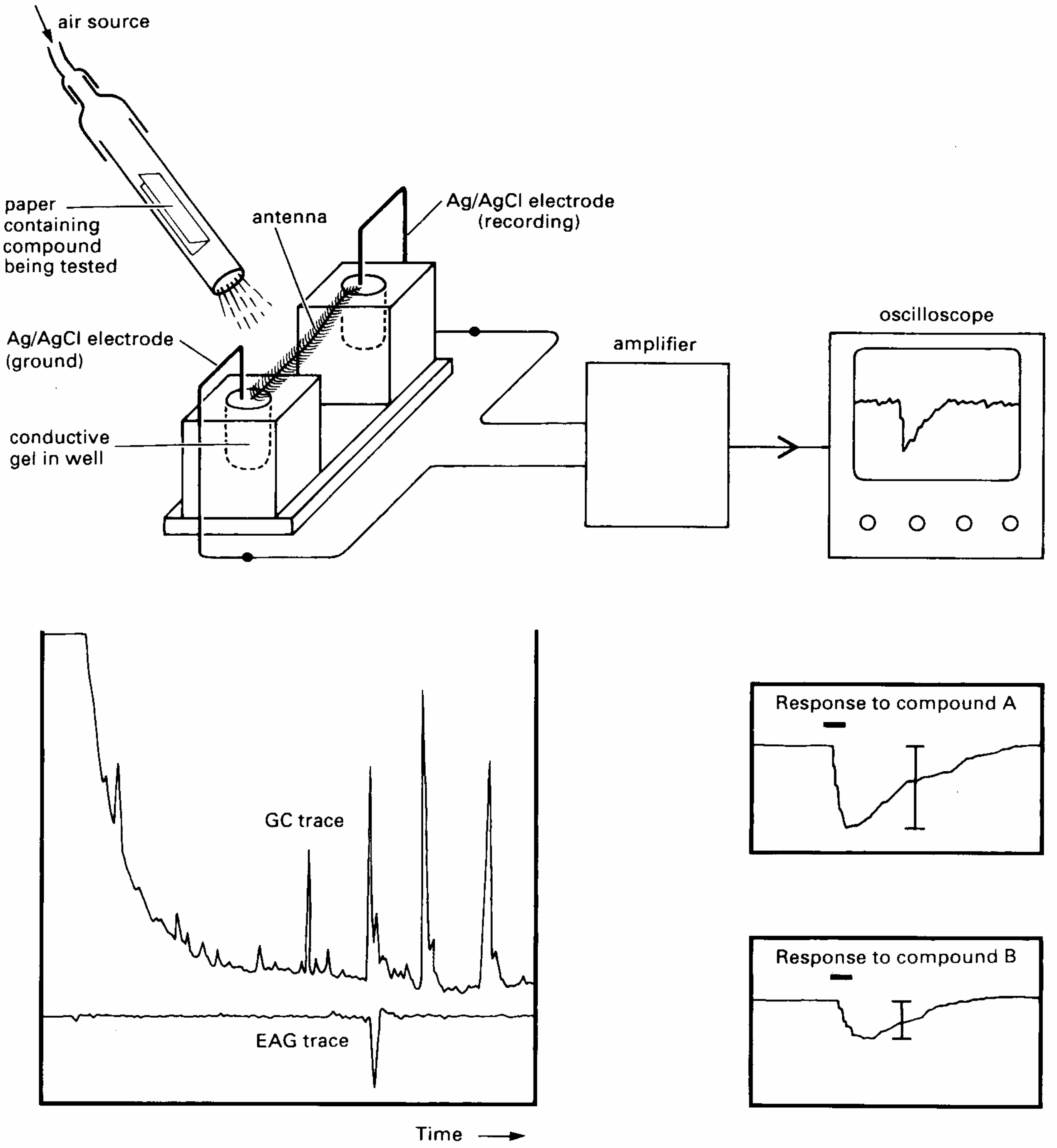


Fig. 3.1. Schematic diagram of the electroantennogram (EAG) and single cell recording techniques.

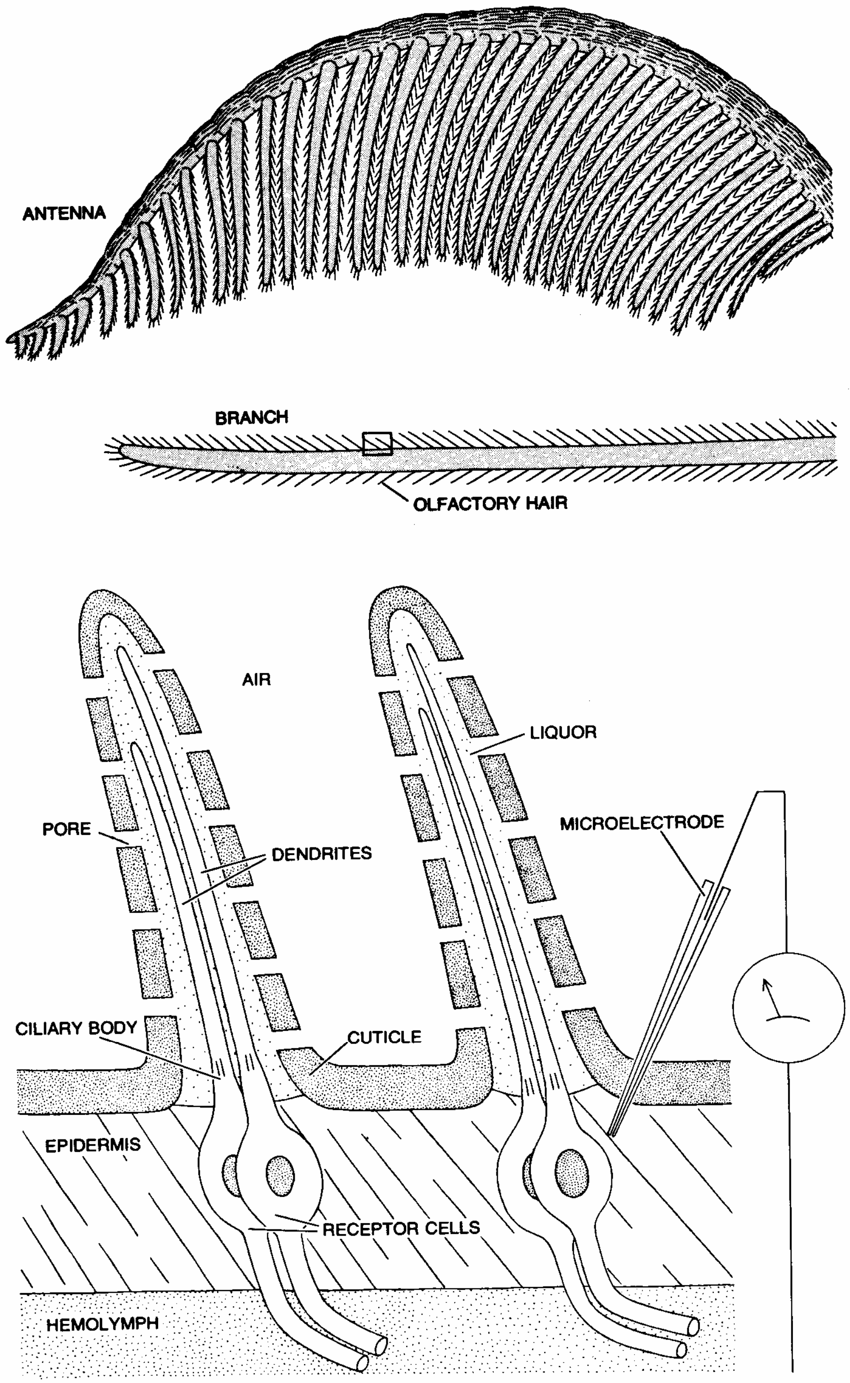
Evaluating the olfactory antennal response to various chemicals using the EAG (electroantennogram) technique.

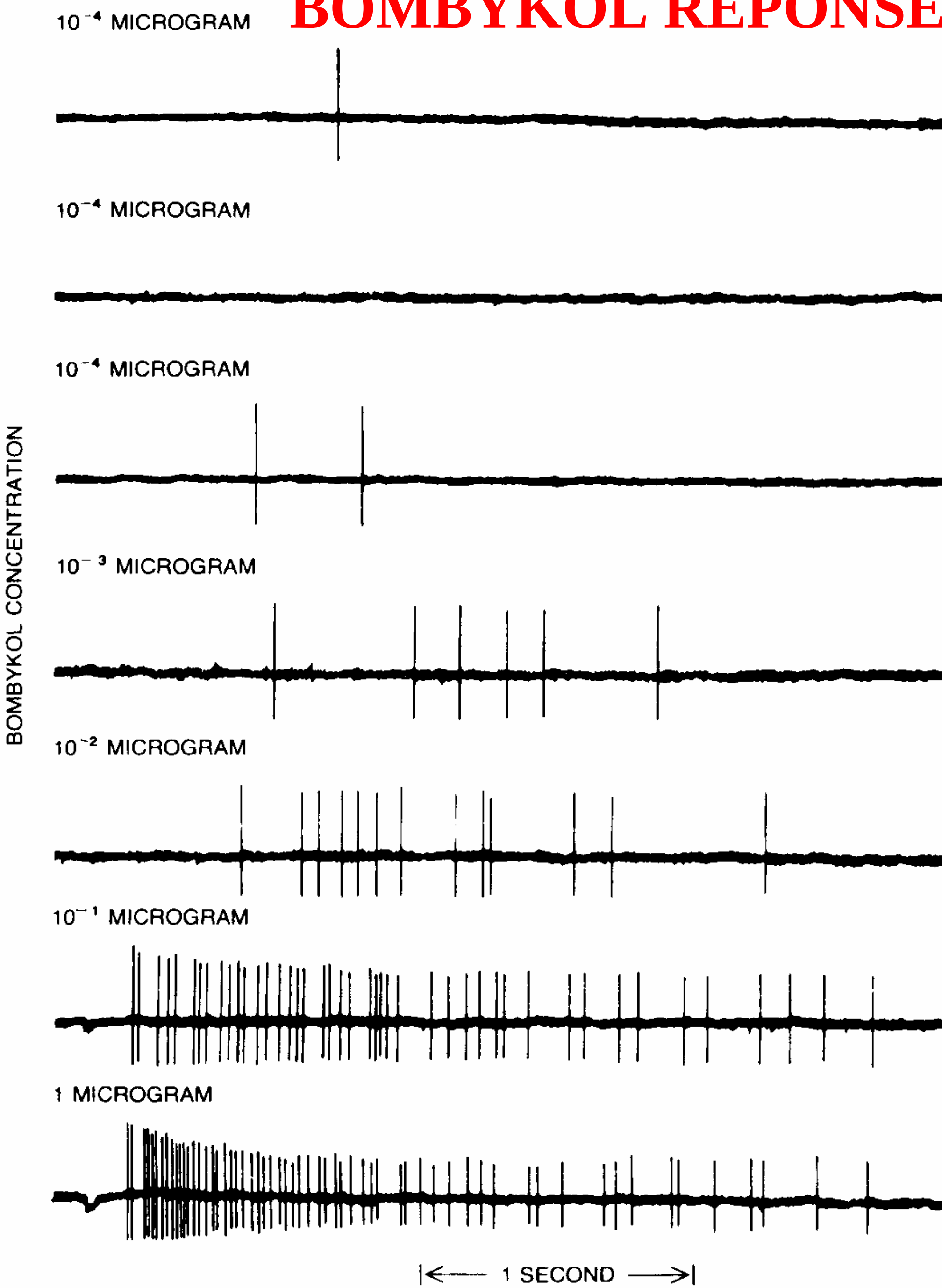
Both EAG and single cell recording are now hooked up with flow-over the sensillum with air associated with a GC or gas chromatographic setup. Thus, one can correlate chemical species identifications with the antennal response.

Box 4.2 The electroantennogram



BOMBYKOL REPONSE



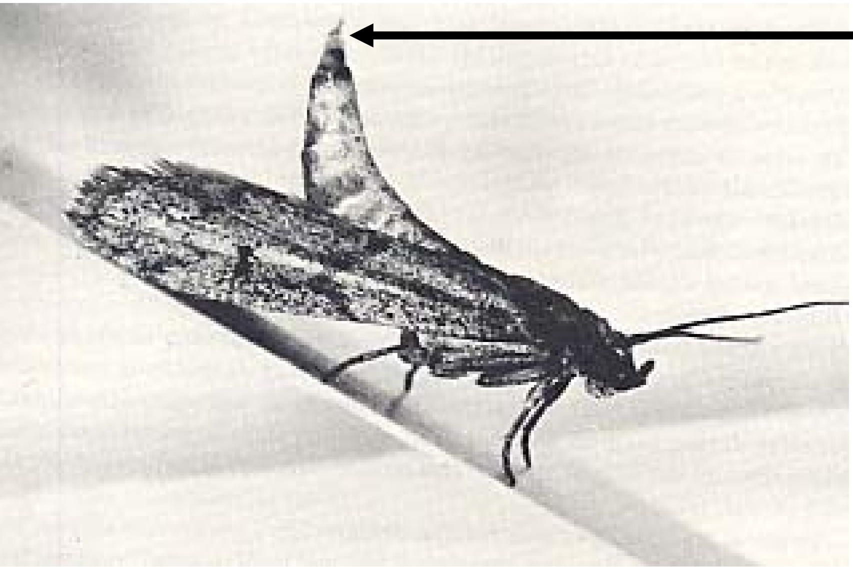


RELEASER PHEROMONES

1. **Sex pheromones**-Why were these studied first and most extensively?
2. Aggregation pheromones
3. Alarm pheromones
4. Trail pheromones
5. Dispersal pheromones
6. Spacing pheromones

Behavioral evidence of sex pheromone release & its attractancy

1. Female moths assume a calling behavior posture (see below) where they extrude or expose the pheromone gland and pulse the abdomen



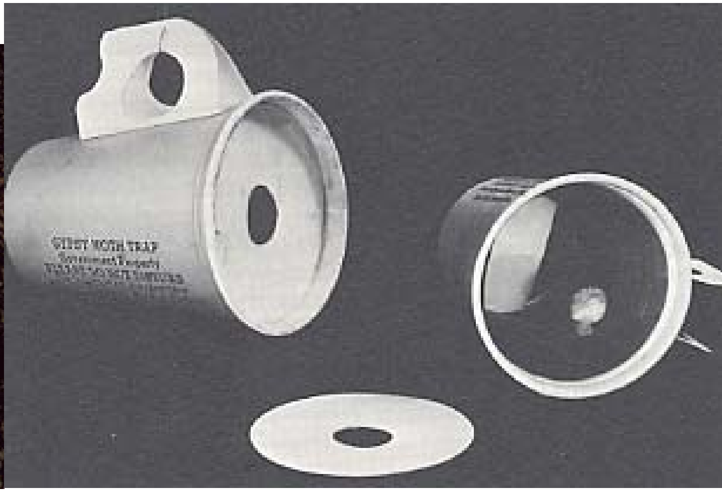
Female calling with extruded pheromone gland

Male gypsy moth showing feathery antennae

2. Attraction of males to calling females. Male moths often have feathery antenna while females usually don’t. Sexual dimorphism often gives a clue as to structure complimenting function (see photo above). The structure of the male’s antenna facilitates collection of odorant molecules and also they contain many more sensilla to detect the low concentrations of pheromone

Various photos of gypsy moth, traps and damage

Endocrine role of the brain 1st recognized by Kopec in 1917 using the gypsy moth



Excellent website for lots of photos of gypsy moth life cycles and control

http://www.forestryimages.org/browse/detail.cfm?imgnum=1247237

Male gypsy moths (below) respond to the female sex pheromone by fluttering their wings, increasing their excitement level and eventually flying to the source of the pheromone. Photos to the right show the female's abdomen extended as if calling (fig. 1) and the other figures showing the convoluted pheromone glands (black arrows).

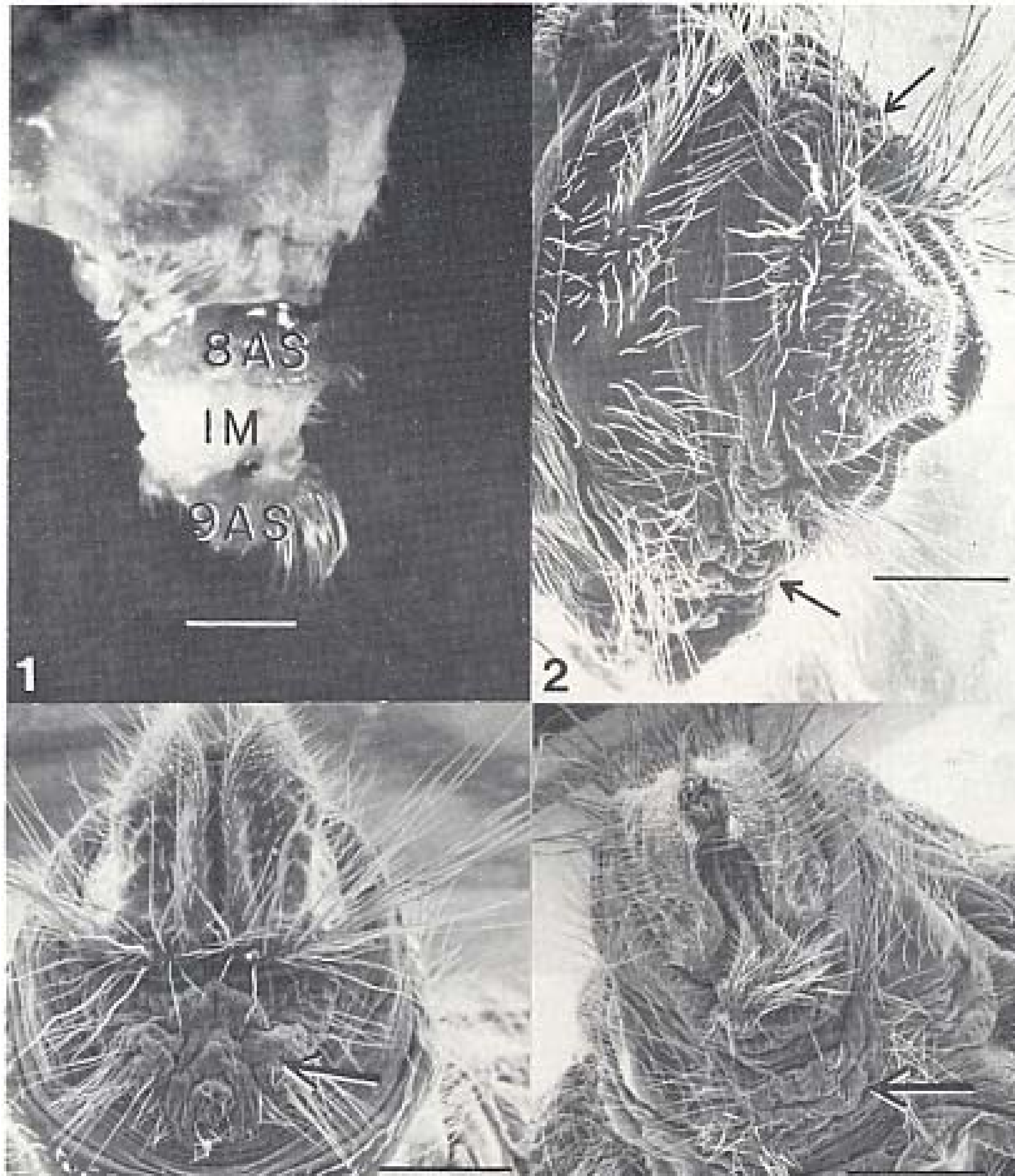
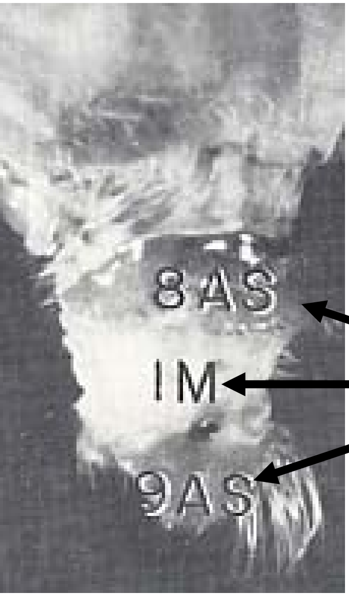


Table 1. Gypsy moth wing fanning bioassay for female sex pheromone of tissue extract*

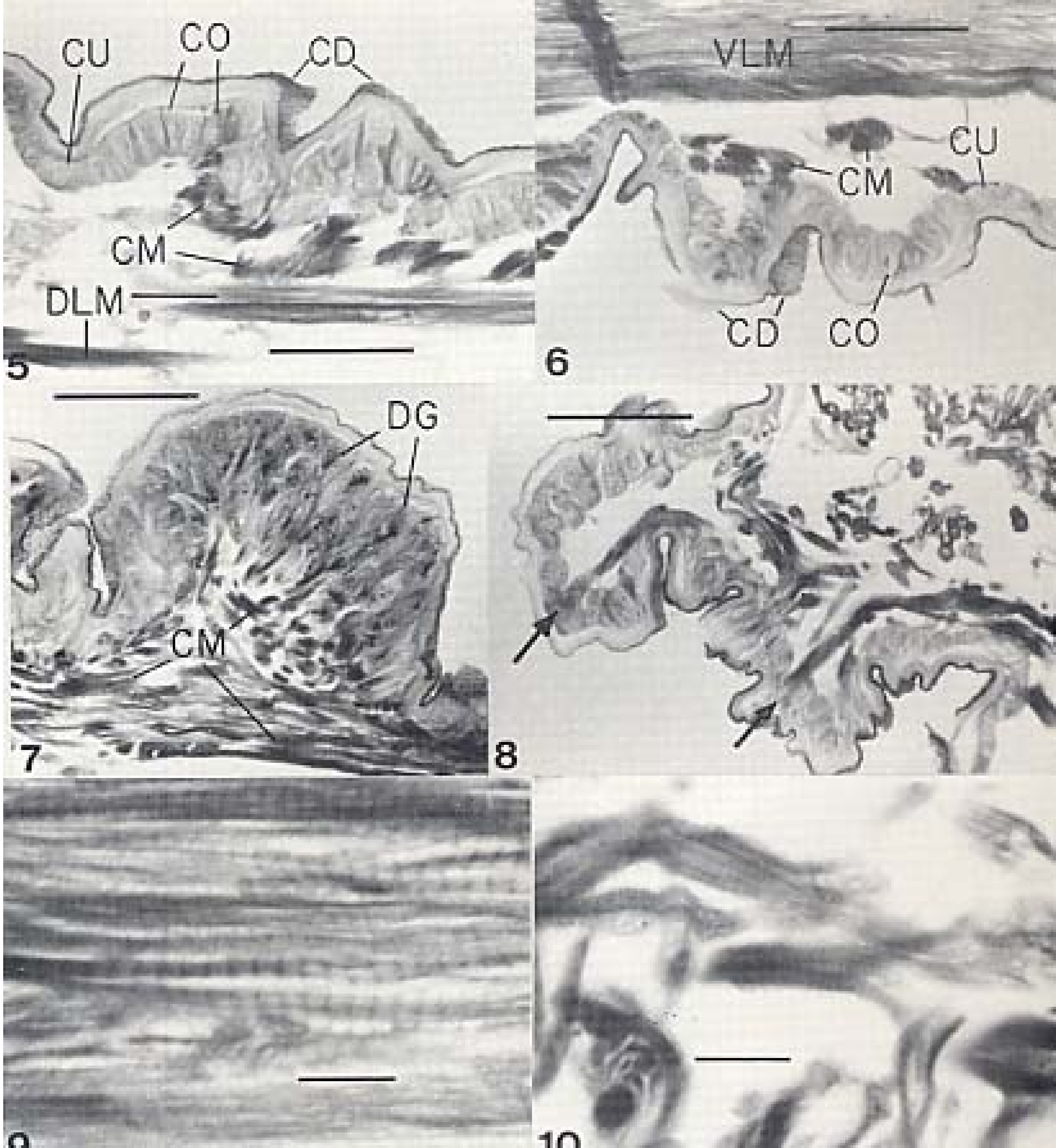


Treatment	Wing fanning response positive No.	(%)	negative No.	(%)
Blank control	0	(0)	124	(100)
Solvent control	0	(0)	24	(100)
Calling female	31	(89)	4	(11)
8th abdominal segment	0	(0)	20	(100)
Intersegmental membrane	12	(60)	8	(4)
9th abdominal segment	2	(10)	18	(90)
Entire terminal abdominal segments	9	(90)	1	(10)
Dorsal half of terminal segments	13	(62)	8	(38)
Ventral half of terminal segments	15	(75) .	5	(25)

* Tissue extracts were prepared from virgin females 24–48 hr post-emergence.

Hollander, Yin and Schwalbe. 1982. Location, morphology and histology of sex pheromone glands of the female gypsy moth, *Lymantria dispar* (L.). Jour. Insect Physiol. 28: 513-518.

Histology of the pheromone gland in female gypsy moth. Sections taken through the convolutions of the interseg-mental area between abdominal segments 8+9. See previous slide. Note the CU=cuboidal cells of the gland; CO-columnar cells of the gland; CD=cuticular domes of the gland. VLM and CM are muscles associated with the gland and are involved in gland extrusion. Figs. 9 and 10 show the striated muscles involved in the calling behavior posture and gland extrusion.



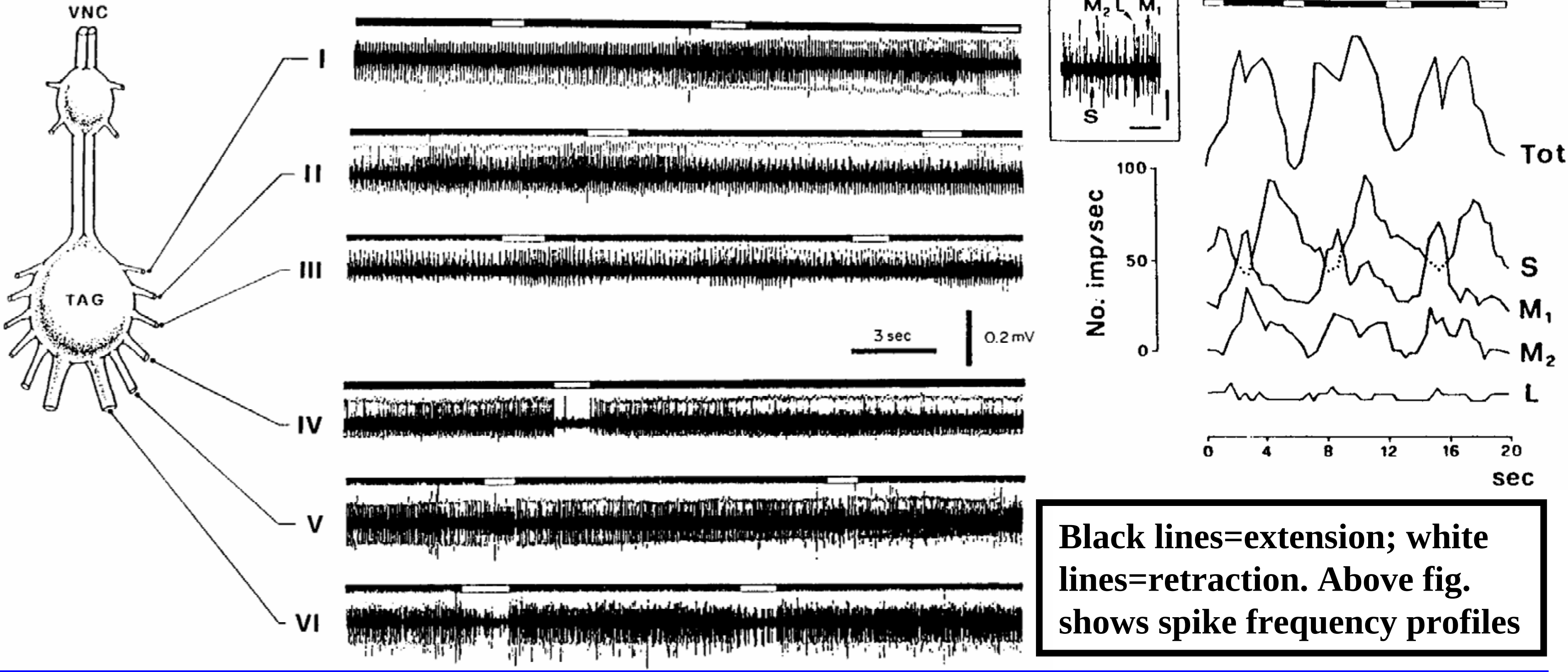
WHAT CONTROLS CALLING BEHAVIOR IN GYPSY MOTH?

Table 1. Effect of various surgical procedures on calling behaviour and pheromone release by the adult female *Lymantria dispar*

Treatment	No. bioassayed	% Calling	% Pheromone* release
A. Section of circumoesophageal connectives (CEC) in pupae			
Unoperated control	33 (3)†	100.0[a]	90.9[a]
Sham on CEC	25 (8)	88.0[b]	80.0[a]
CEC cut	22 (11)	86.4[b]	2.3[b]
B. Removal of corpora allata from last instar larvae			
Unoperated control	43 (5)	100.0[a]	89.5[a]
Sham operated	23 (4)	91.3[a]	86.9[a]
Corpora allata removed	27 (1)	96.3[a]	87.0[a]
C. Removal of corpora cardiaca–corpora allata complex (CC–CA) from last instar larvae			
Unoperated control	43 (5)	100.0[a]	89.5[a]
Sham operated	26 (8)	100.0[a]	78.8[a,b]
CC–CA removed	23 (19)	95.7[a]	67.4[b]
D. Ovariectomy in the last-instar larvae			
Unoperated control	35 (4)	94.3[a]	90.0[a]
Sham/clamp and wax	23 (1)	95.5[a]	71.7[b]
Ovariectomy/clamp and wax	24 (2)	79.2[a]	75.0[b]
Sham/glue	23 (2)	91.3[a]	78.3[a,b]
Ovariectomy/glue	23 (5)	95.6[a]	76.1[b]

What does this suggest?

Hollander, A. and C.-M. Yin 1985. Lack of humoral control of calling and pheromone release by brain, corpora cardiaca, corpora allata and ovaries of the female gypsy moth, *Lymantria dispar* (L.). J. Insect Physiol. 31: 159-163.



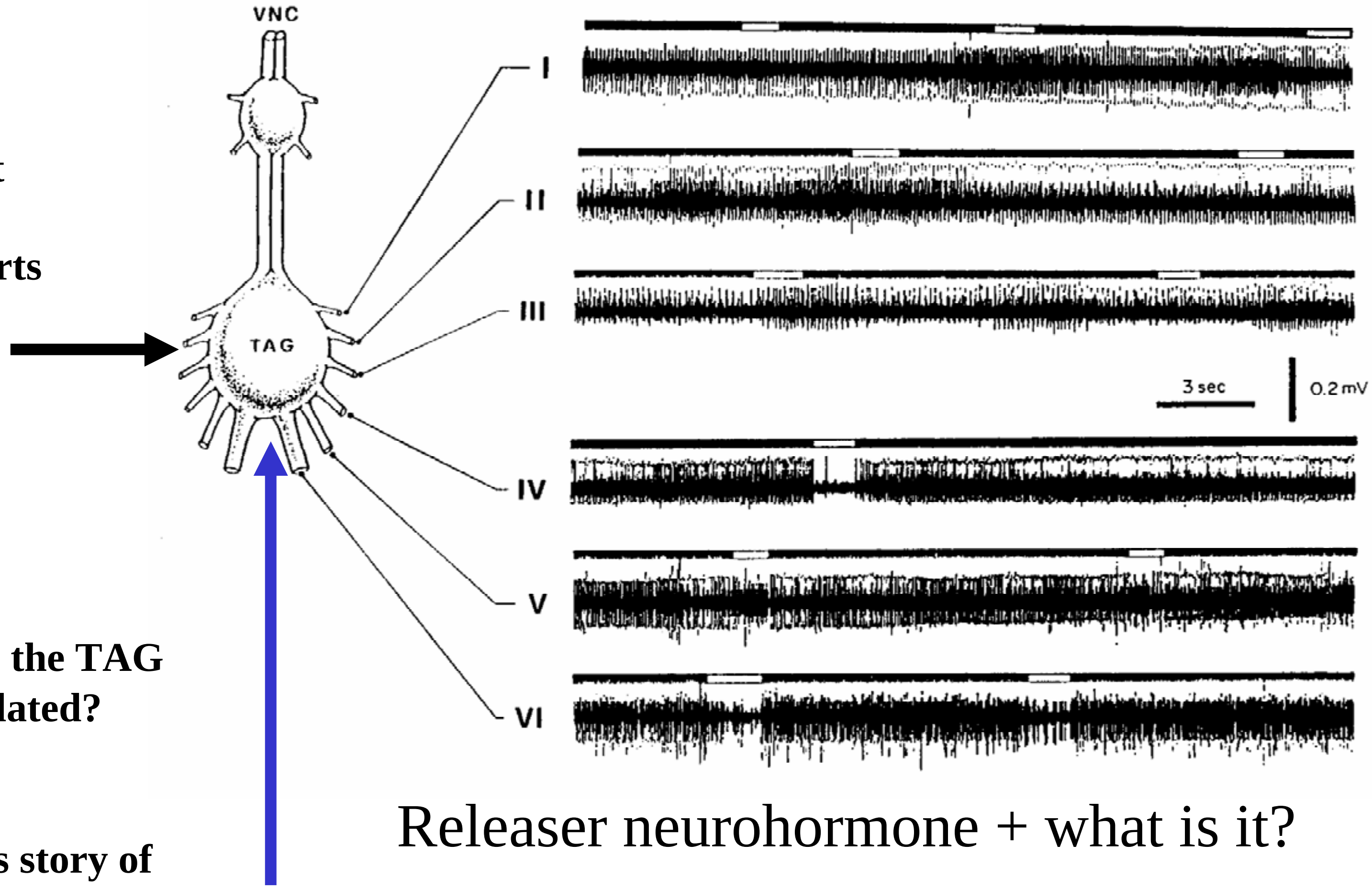
Black lines=extension; white lines=retraction. Above fig. shows spike frequency profiles

1. Once calling started, cutting the VNC anterior to the terminal abdominal ganglion did not stop the signals from the ganglion going out to the ovipositor for extension and retraction associated with calling nor did it stop calling.
2. Suggests that there is a preprogrammed motor output for this behavior in the TAG
3. Electrophysiological recordnings from the nerves of the TAG demonstrate that they, especially V and VI, are involved in extension and retraction of the ovipositor.

Crnjar, Angioy, Pietra, Yin, Liscia, and Barbarossa. 1988. Control mechanims of calling behaviour in *Lymantria dispar*: an electrophysiological investigation on the role of the terminal abdominal ganglion. J. Insect Physiol. 34:1087-1091.

Once calling starts, if one cuts VNC anterior to the TAG the calling continues but go back two slides and see what happens if you cut the CEC before calling starts

Calling continues but pheromone release stops



What do we call how the TAG functions once stimulated?

Releaser neurohormone + what is it?

Remember Truman's story of eclosion behavior. What is missing in this story?

PBAN acts on terminal abdominal ganglion to activate nervous stimulation of the pheromone glands

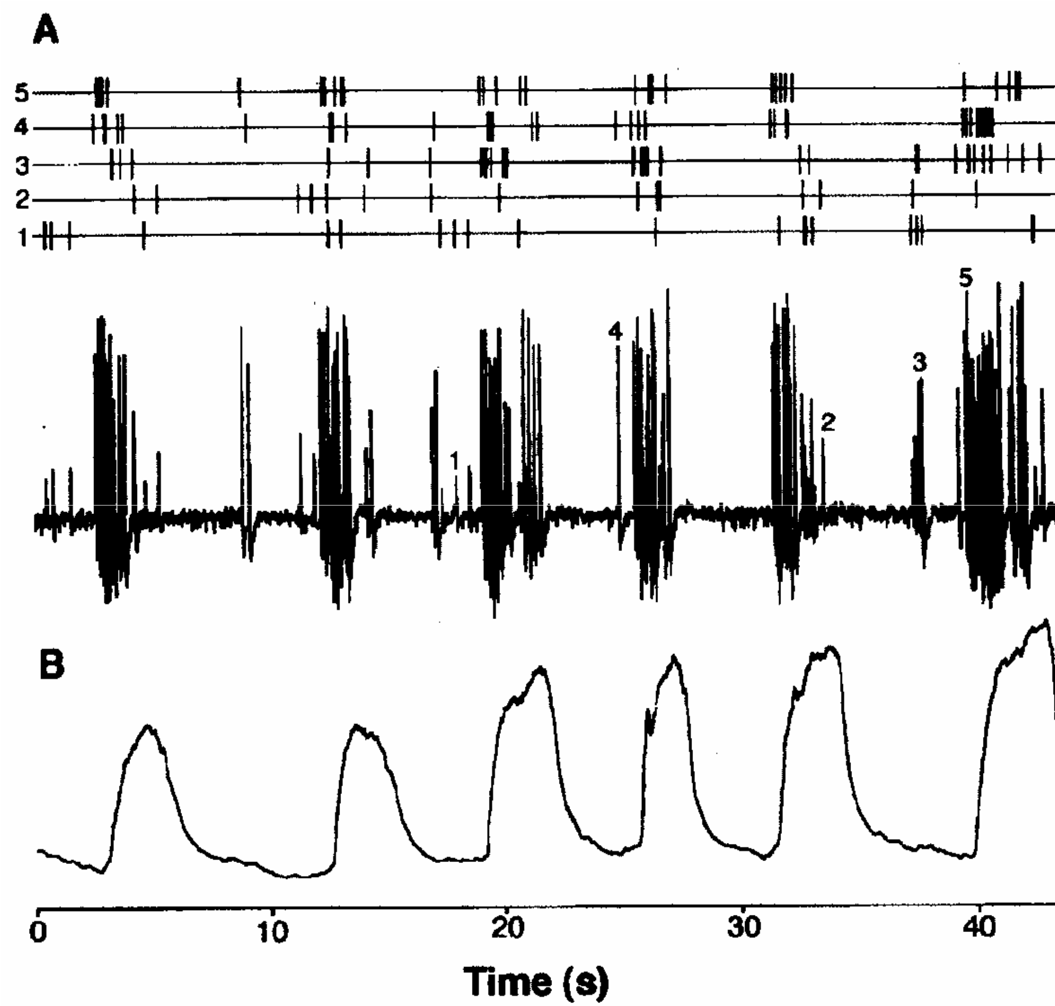
The PBAN nsc are located in the suboesophageal ganglion

The PBAN neurosecretory cells are located in the suboesophageal ganglion

1. If neurosecretory cells produce action potentials and these have been correlated with the nsc release, then more studies should focus on this aspect of control.

NSC firing

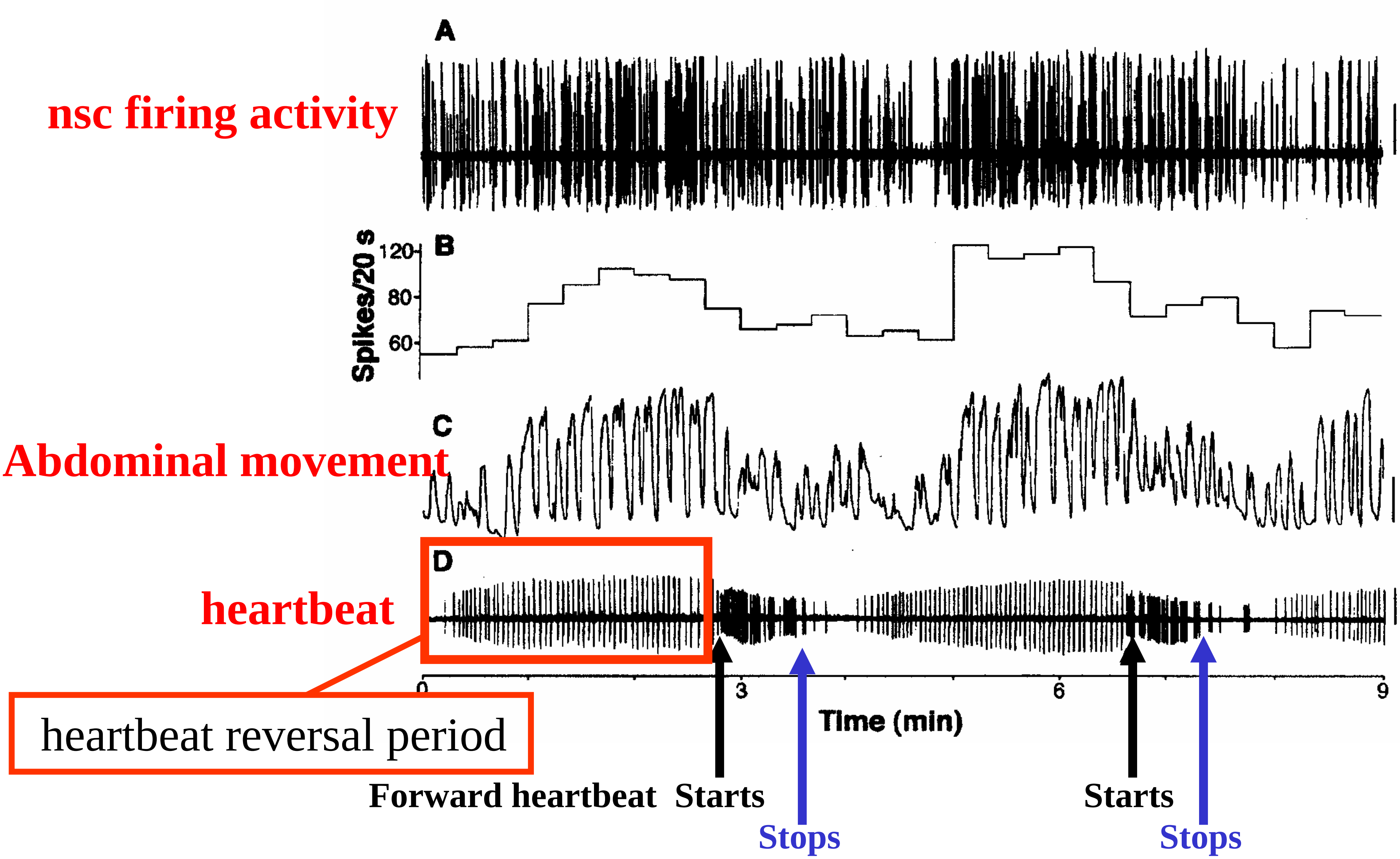
Synchronized bursting activities of nsc (A) with calling behavior in virgin *Bombyx mori* as measured by rising movement of the abdomen (see B).



Abdominal movement

Ichikawa, T. 1998. Activity patterns of neurosecretory cells releasing pheromonotropic neuropeptides in the moth *Bombyx mori*. Proc. Natl. Acad. Scie. 95: 4055-4060.

Coordination of nsc firing activity (A) with abdominal movements (C) and with the electrocardiogram (D)



HEARTBEAT REVERSAL

Ichikawa suggests that the heartbeat reversal is not involved in the movement of hemolymph into the abdomen but more importantly it is involved in the delivery of the neurosecretory material from its site of release in the head area to an area close to the target organs in the abdomen.

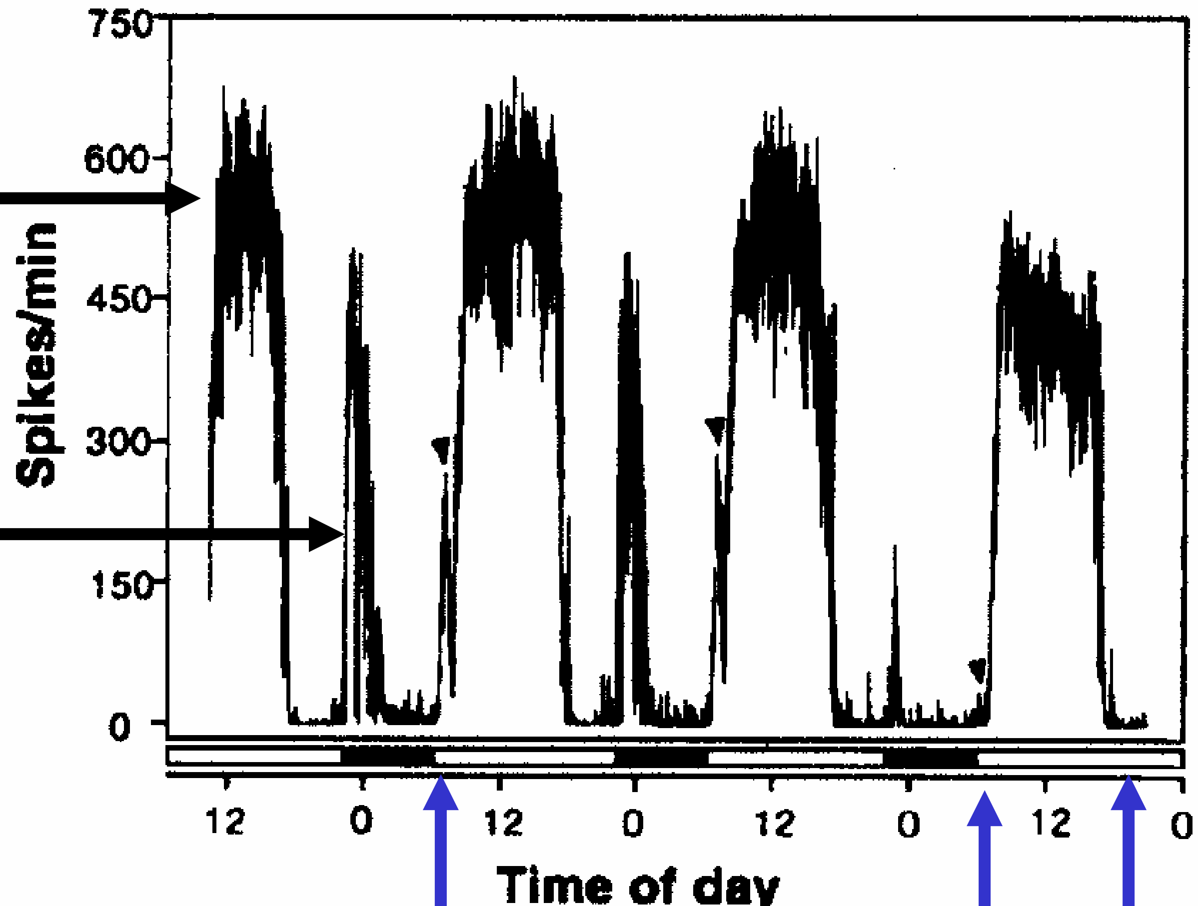
Injecting methylene blue into the heart he demonstrated that the heartbeat reversal delivered the dye to the vicinity of the pheromone gland.

PHEROMONOTROPIC NEUROPEPTIDES

1. Recorded over a long-term
2. Recordings of nsc showed diel firing pattern with light/dark

1st component
Lights on-1 or 2 h after get
Avg. rapid firing=8.9 h

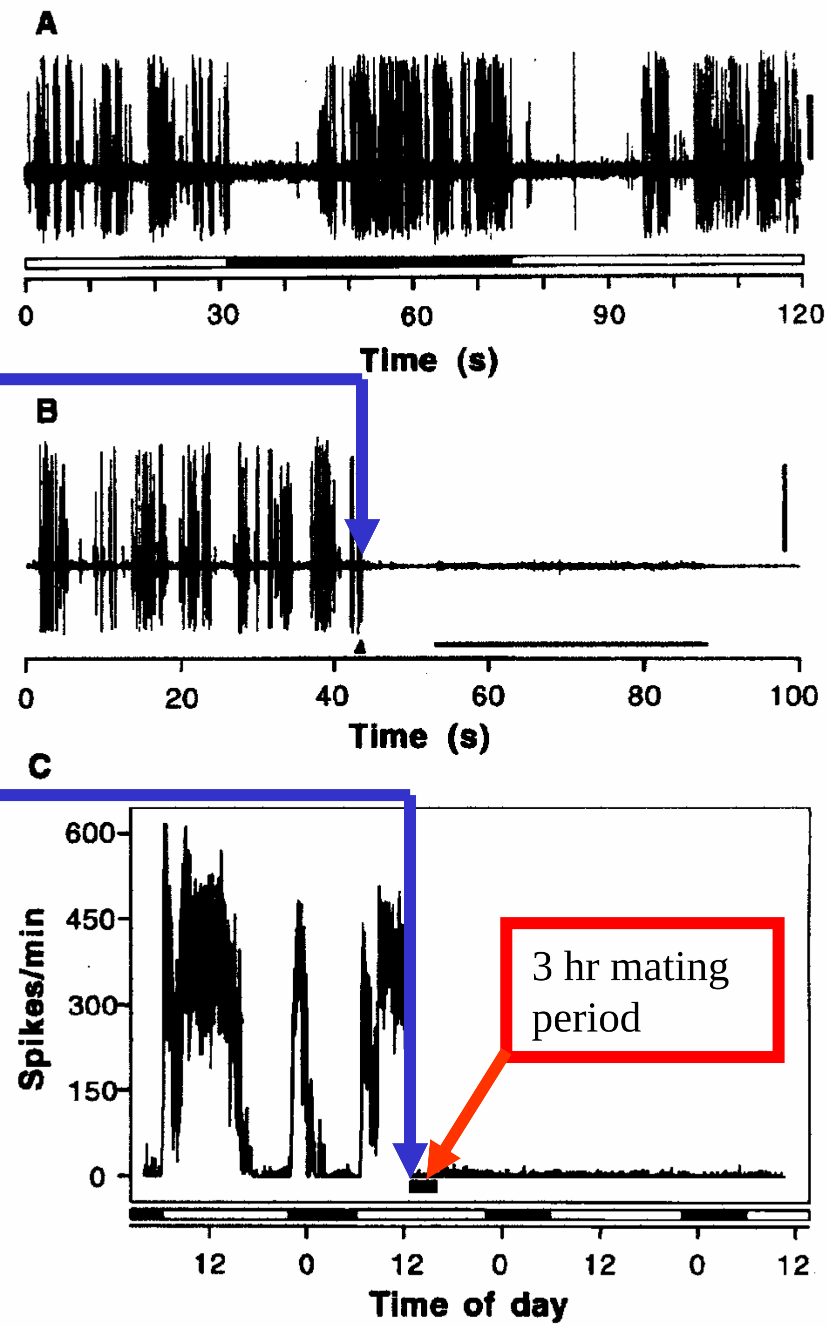
2nd component
Lights off to dark



daylight

10-14 hrs

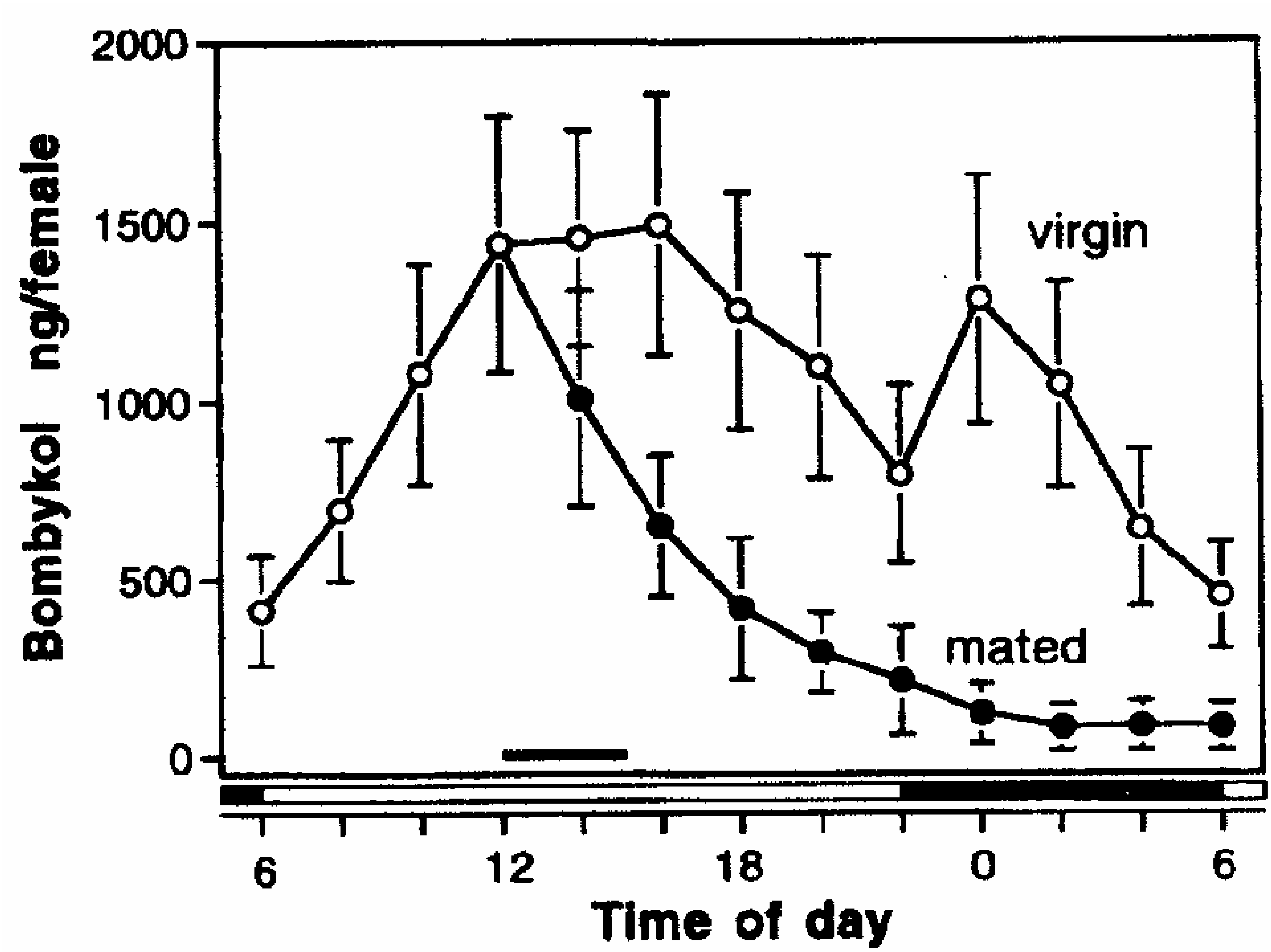
Sensitivity of nsc to stimuli (light) and mating



Inactivity in firing pattern due to active male touching the tip of female's abdomen

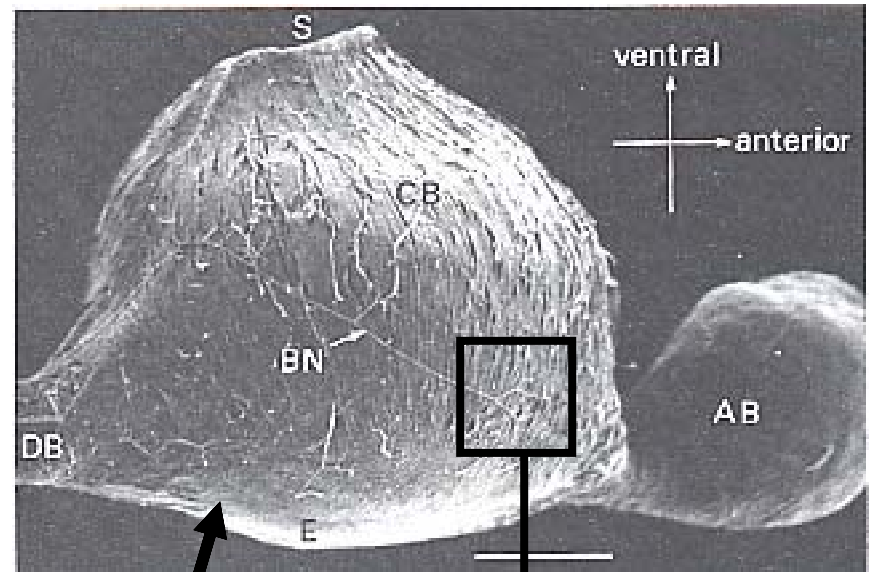
Inactivity in firing pattern before and after a 3-h mating period (shown as dark bar above time bar)

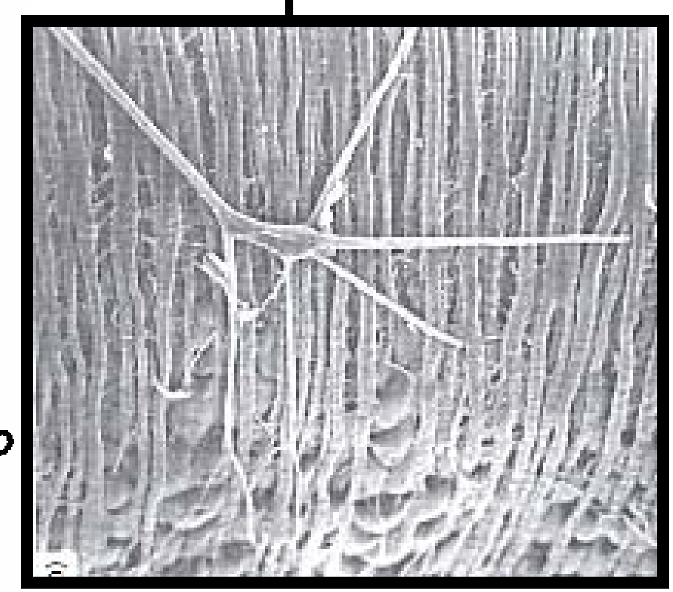
Once mating occurs, the production of *Bombykol* is shut down



How might mating suppress the activity of the pheromonotropic cells in the brain of moths?

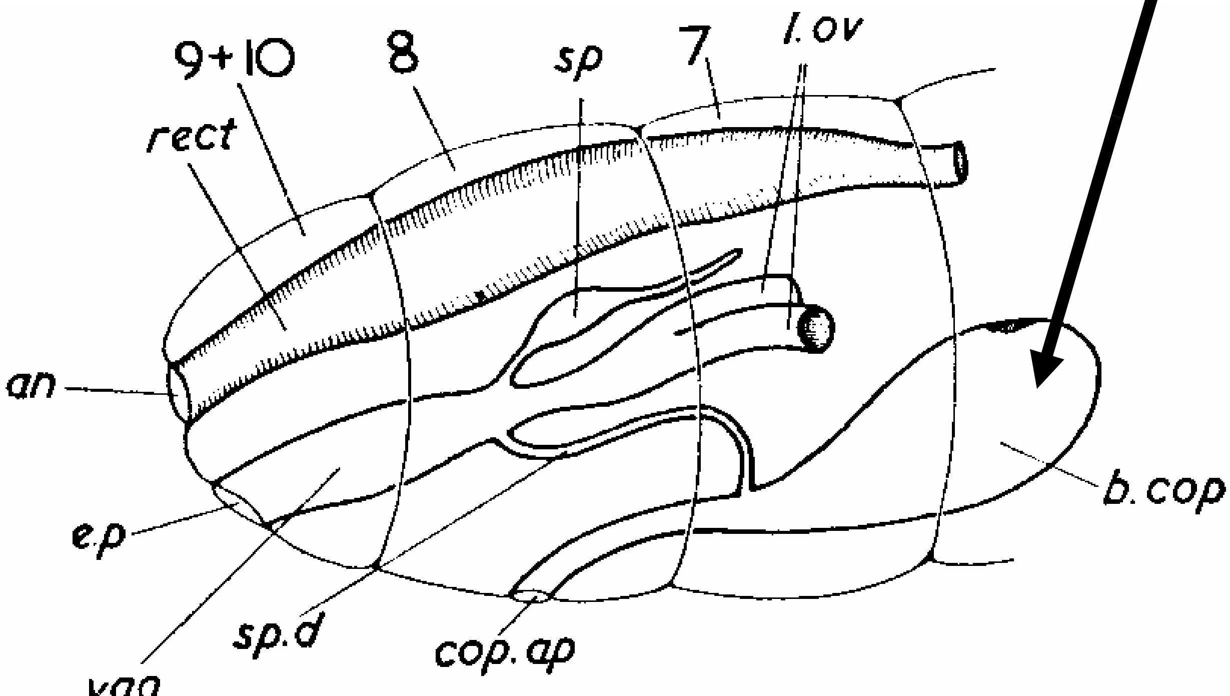
Stretch receptor on the internal (hemolymph side of the bursa copulatrix of adult *Pieris*. Note the musculature of the bursa and the multibranched stretch receptor. As the bursa enlarges, the stretch receptor (BN) that is anchored at each point to the bursa (inside the box) becomes stretched.





What is deposited in the bursa copulatrix during mating?

A spermatophore



SUMMARY OF ICHIKAWA'S PAPER ON NEUROSECRETORY CELLS RELEASING PHEROMONOTROPIC NEUROPEPTIDES

A. These cells show 3 types of rhythmic changes in firing activity

1. Bursting activity with intervals of several seconds were synchronized with rhythmic abdominal motions for calling behavior

2. Slow fluctuation in firing activity over a period of several minutes depended on cyclic alternations of the flow of hemolymph. Heart reversal may deliver neuropeptides to gland

3. Their electrical activity displayed a diel rhythm that related to light/dark cycles of the environment and sex pheromone titers in the gland

B. Inhibition of the firing activity can be caused by tactile or light stimulus and a long-term inhibition by mating

Two major events in sex pheromone communication by the female

Synthesis of pheromone

Calling behavior

Controlled by:

Hormone

a. JH in some cockroaches + *Tenebrio*

Neuropeptides

b. Brain-neuropeptide PBAN- pheromone biosynthesis activating neuropeptides in Leps. SOG in *Heliothis* + *Bombyx*

Octopamine mimics action of PBAN

Circadian rhythm

a.

Neural

Removal of brain + endocrine glands showed that in gypsy moth control of both calling and pheromone release are under neural control

a. Pheromone gland is only sensitive to PBAN stimulation at night in *Heliothis zea*

Physiology should be the study of the interactions and integration of how the different systems function and often coordinate to solve a particular physiological, metabolical, and/or behavioral problem

CONTROL OF CALLING BEHAVIOR IN MOTHS

Systems studied and other topics involved:

1. **Integumentary**-epidermal cells become pheromone producing glands and produce a special cuticle for pheromone release
2. **Muscular**-muscle sets involved in extension and retraction (pulsing) of abdomen involved in calling behavior and pheromone release
3. **Circulatory**-hemolymph pressure increase probably involved in the extension of abdomen
4. **Nervous + neuroendocrine**-nervous control with preprogrammed motor output in the TAG involved in activation of muscles involved in extrusion and retraction of ovipositor in calling behavior. Neural control of circadian rhythm involved in when the pheromone gland is sensitive to PBAN stimulation. Biogenic amine octopamine involved in pathway leading to calling behavior/PBAN pathway.

PRIMER PHEROMONES-don't have an immediate effect on the receiver but prime its system (usually the nervous and endocrine) such that in time the primer pheromone will have an effect and change behavior, morphology, etc.They are usually found in social insects or insects that aggregate.

1. Locust primer pheromone (cuticular hydrocarbon) acting via the antenna and producing a shift from the solitary to the gregarious phase

2. Honeybee colonies. Queen produces a multicomponent primer pheromone from her mandibular glands. Major component is 9-oxo-2-decenoic acid. Acts on the endocrine system of the workers to suppress JH production. If the queen is removed, the JH inhibiting queen primer pheromone is removed. Developing larva are fed royal jelly and the absence of the queen substance and something in the royal jelly puts these larvae on the path of higher JH development and potential queens
3. Similar queen primer pheromone found in fire ants, *Solenopsis invicta*

AGE GRADING FLIES IN THE WILD

- PROBLEM-Seldom do we know the **chronological age** of the insect we are working on in the field. Sometimes we know the **physiological age,** usually based on reproductive conditions.
- In an attempt to better understand both chronological and physiological age in the field, several techniques have now been put to practice.
- Hayes, E. J., and R. Wall. Age-grading adult insects a review of techniques: Physiol. Entomol. 1999, 24 1–10.

REFERENCES AND TECHNIQUES USED FOR CHRONOLOGICAL AGE GRADING

A. Pteridine levels in the head using near-infrared spectroscopy (NIRS) to determine the chronological age

1. Chronological Age-Grading of House Flies by Using Near-Infrared Spectroscopy
Joel Perez-Mendoza,[a, e] Floyd E. Dowell,[e] Alberto B. Broce,[b, e] James E. Throne,[e] Robert A. Wirtz,[c, e] Feng Xie,[d, e] Jeffrey A. Fabrick,[e] and James E. Baker[e]. ***Journal of Medical Entomology:*** **Vol. 39, No. 3, pp. 499–508**. IS ONLINE

B. Counting daily growth layers of cuticle in the thoracic apodemes

C. Physiological age determination has also been accomplished by examining the state of ovarian development and parity status for insects of medical and veterinary importance