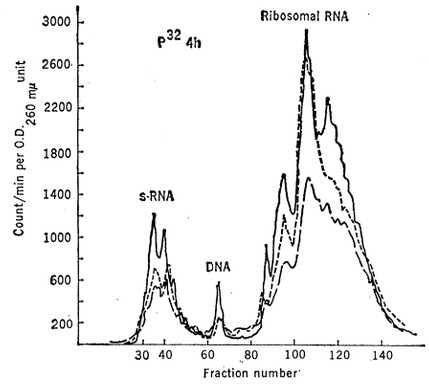

Fig. 2. Effect of dormin on nucleic acid synthesis of *Lemna*. Fractionation of pulse-labeled nucleic acids on MAK columns. Solid line, *Lemna* cultured in Hoagland medium only; dash line, after 1 day in dormin at 1 ppm; broken line, after 3 days of dormin at 1 ppm.

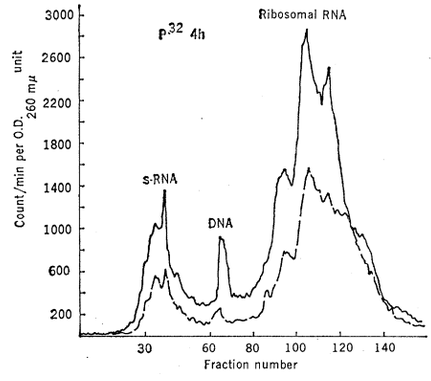

Fig. 3. Restoration of dormin-inhibited nucleic acid synthesis by benzyladenine. Fractionation of pulse-labeled nucleic acids on MAK columns. Solid line, *Lemna* cultured for 7 days in nutrient which contained 1 ppm dormin and to which was then added 100 parts per billion (10^9) of benzyladenine, for 3 more days of culture. Broken line, dormin at 1 ppm for 3 days (drawn here for comparison).

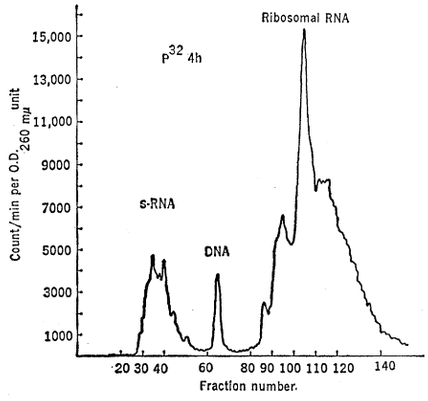

Fig. 4. Nucleic acid fractions separated from *Lemna* treated with 100 parts per billion (10^9) of benzyladenine for 3 days. Note that the scale on the ordinate differs from that of Figs. 2 and 3.

chemical structures of dormin and benzyladenine are so different from each other that its seems unlikely that their antagonism in the *Lemna* system could be due to competitive inhibition of an enzyme system. Such considerations attracted us to Monod's scheme of allosteric enzyme inhibition and activation (*14*, his figure 6) as a working hypothesis. Further work will be necessary to establish whether such an allosteric inhibition of DNA polymerase, for example, is indeed functioning in *Lemna*. Our observations on *Lemna* appear to be compatible with the Monod model. Dormin as the inhibitor would lock some of the enzyme in its *inactive* conformation. This would cause a shift of equilibrium in this direction, leaving only a little of the enzyme in its active conformation, thus leaving only a small residual capacity for DNA synthesis. The structure of dormin would make one suspect that it fixes itself to its site of action by a two-point hydrogen bond attachment. Such a loose bonding would be easily reversible. With dormin detached, the enzyme could then resume its normal state of equilibrium. Now cytokinin could lock the enzyme in its *active* conformation, thereby increasing DNA synthesis.

Because in our *Lemna* system there is an antagonism between dormin and cytokinin one should not think that cytokinin is the only possible antagonist for dormin. Thus, at the MSU/AEC Plant Research Laboratory (*15*), an antagonism involving dormin and gibberellin has been found in the aleurone system of barley. Here dormin inhibits the gibberellin-controlled amylase production, and gibberellin will overcome this inhibition only when the inhibition does not exceed a critical level, as we found in *Lemna* for dormin and cytokinin. It could be, then, that activation can be achieved by a number of promotive hormones, which differ in different systems, and which may function as activators in the sense of Monod's model. These conclusions suggest that it may be profitable to look at other systems such as cell elongation in the lentil epicotyl (*16*) in which it was found that gibberellin activates DNA synthesis.

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Mosquitoes: Female Monogamy Induced by Male Accessory Gland Substance

Abstract. *Male accessory glands were implanted in virgin females of* Aedes aegypti. *When exposed to males, females copulated readily but were not inseminated; they remained sterile for life. Extract from one male could sterilize more than 64 females. The active principle may be a protein or peptide. Intraspecific transplant prevented insemination in 12 species, including* Aedes, Anopheles, *and* Culex; *interspecific transplant gave partial protection.*

Frequent, repeated copulation can be observed in laboratory colonies of numerous species of mosquitoes. It is often assumed that a single female can be inseminated by several males. However, experiments with genetically marked males of *Aedes aegypti* have shown that females of this species are usually inseminated only once (*1*). Copulation may take place many times, but a female mated only once is refractory to subsequent insemination for life. Multiple insemination (*2*) can take place only when several males copulate with a female in a short period of time. In some *Culex* (*3*) and *Anopheles* (*4*), genetic-marker experi-

ments have shown that multiple insemination is infrequent.

The agent which stimulates this reaction in females comes from male accessory glands and is passed to the female in seminal fluid. To establish this point for *Aedes aegypti*, I dissected various male tissues in saline (*5*) and implanted them in the thorax of virgin females (Table 1). After a 24-hour recovery period, these females were placed in a cage with a surplus of virgin males, where they remained an additional 24 hours. Females were then dissected and examined for sperm in the spermathecae (the sole storage organ for sperm in mosquitoes). At least 85 percent of control females were inseminated, compared to 0 percent for females with implanted male accessory glands.

This "sterilizing" effect lasts for the entire life of the female. In another experiment, glands were implanted in females less than 1 hour old. Each week for 10 weeks, these females received a blood meal and were placed with a new batch of males 1 to 2 weeks old. At the end of 10 weeks, when 188 females with gland implants were dissected, none was inseminated. In the control with gut implants, 155 of 157 females were inseminated. Some females were isolated individually with five males. Repeated blood meals were provided, and each female was placed with five fresh males after each oviposition. Each of five females with gland implants produced six separate egg batches, with total egg production of 357, 314, 292, 275, and 258. None of these eggs hatched. Five females with gut implants also produced six separate batches, giving 327, 302, 300, 249, and 246 eggs. More than 90 percent of the eggs from each batch were hatchable.

Apparently, several hours are required before the sterilizing effect of accessory gland material is accomplished. When females with gland implants were placed with males immediately after operation, 26 percent were inseminated (Table 1). Five percent of females that were allowed 4 hours for recovery were inseminated, while those given longer periods had complete protection from insemination. For more precise information on time of action, implanted females were force-mated (*6*) at various times after operation. In this method, anesthetized females are pushed into contact with the genitalia of decapitated males, and copulation results. The technique was not satisfactory for these experiments; about 25 percent of females tested 72 hours after gland implantation were inseminated by force-mating compared to 0 percent inseminated by normal mating.

Table 1. Effect of transplantation of various tissues into the thorax of virgin females on subsequent insemination in *Aedes aegypti* (ROCK strain).

Time after implant (females placed with males) (hr)	Females		
	Virgins implanted (No.)	Examined* (No.)	Inseminated (%)
No tissue (sham-operated)			
24	30	28	86
Testis			
24	40	36	89
Male gut			
24	75	68	85
Male gland			
24	100	86	0
16	45	43	0
8	30	26	0
4	25	22	5
0	42	42	26

* Treated females kept with a surplus of males for 24 hours and then dissected and examined for presence of sperm in spermathecae.

The mechanism of action of the accessory gland substance in females has not yet been determined. Behavioral factors may be involved. The sound in flight of females with gland implants is fully attractive to males, but such females sometimes avoid copulation by lowering the abdomen to the surface or otherwise preventing the male from assuming the coital position. Males placed with once-mated females often go through the preliminary steps of coitus but fail to ejaculate (*7*). In normal coitus in *A. aegypti*, male paraprocts are inserted between the genital lips, opening them and facilitating insertion of the aedeagus (*8, 9*). Perhaps the accessory gland stimulates the female to hold the vaginal lips closed. If so, the lips might be breached occasionally by the mechanical pressure of forced mating but not by the male in the normal act of mating. Certainly, force-mating is much more readily accomplished with virgins than with once-mated females (*9*). We found that males, force-mated to females with gland implants, seemed to have difficulty in inserting the aedeagus, and external ejaculation was fairly frequent. On the other hand, Spielman *et al.* (*10*) suggest that semen is introduced into the female in second matings but is subsequently expelled due to cuticular changes in the female genital atrium induced by accessory gland substance.

The effect of male accessory gland substance was similar in *Culex pipiens, Anopheles quadrimaculatus*, and ten species of *Aedes* (*A. aegypti, albopictus, atropalpus, mascarensis, polynesiensis, scutellaris, sierrensis, togoi, triseriatus*, and *vittatus*). For each species 40 virgin females received glands from homologous males, and 40 control females received male gut. After 24 hours, operated females were exposed to homologous males for 96 hours. Insemination in the controls with gut implants varied from 70 percent for *Anopheles* to 100 percent for *C. pipiens*. No insemination occurred in any female with gland implants among the 12 species tested. These results seem to indicate that female monogamy is widespread in mosquitoes.

Heterologous transplants between species gave some protection from insemination, but the effect was less pronounced. For example, 40 females of *A. aegypti* gave the indicated percentage of insemination when implanted with male glands of these species: *A. atropalpus*, 15; *A. triseriatus*, 33; *Culex pipiens*, 50; *A. scutellaris*, 63; and *Drosophila melanogaster*, 30. With females of *A. triseriatus*, glands of *A. aegypti* gave 8 percent, and those of *A. atropalpus* gave 10 percent. In females of *Culex pipiens*, glands of *A. albopictus* gave 10 percent, and those of *A. aegypti* gave 65 percent. Thus, there is no apparent correlation between phylogenetic position and amount of protection. Even glands of *Drosophila* gave protection in *A. aegypti*, although multiple insemination is common in *D. melanogaster* (*11*). Other insects may serve as a source of material which will prevent insemination in mosquitoes. Female monogamy in the housefly has been reported (*12*); in this species, females lose sexual receptivity after injection or implantation of material from the male ejaculatory duct.

Extraction, purification, and identification of the active principle in male accessory glands of *A. aegypti* are being investigated. Preparation of stock solutions is as follows: (i) obtain 250 males, preferably virgin and over 4 days old; (ii) remove terminalia and abdominal segment VIII of all males and place in 1 ml of saline; (iii) sonicate for 1 minute; (iv) centrifuge for 30 minutes at 40,000*g* and discard residue; and (v) freeze supernatant for storage.

Assay procedures to determine activity in *A. aegypti* are as follows: (i) anesthetize 20 virgin females; (ii) inject into the thorax 1 μl of solution; (iii) allow 24 hours for recovery, and

Table 2. Assay of male accessory gland activity in *Aedes aegypti* by injection of females with serial dilutions of various gland preparations.

Dilution injected (1 μl per female)*	Females inseminated from treated preparation (%)†			
	With solution from males fed on apple		With solution from males fed on sugar	
	Unfrozen	Frozen and thawed ten times	Unfrozen	Frozen and thawed ten times
Full	0	0	0	0
1/2	0	0	0	0
1/4	0	0	0	10
1/8	0	60	0	50
1/16	0	55	15	70
1/32	35	100	50	95
1/64	45	90	90	85
1/128	90	95	85	100

* Stock solution from 250 male terminalia in 1 ml of saline. Injection of 1 μl gave a dose equivalent to one-fourth of a pair of male glands. † Twenty females injected for each dilution, placed with males 24 hours later, and examined for insemination 48 hours after injection.

then place females with 40 males; and (iv) in 48 hours after injection, dissect females and examine spermathecae for insemination. Control females injected with saline generally show 85 to 95 percent insemination after 24 hours with males.

Twofold serial dilutions were used to determine activity of stock solutions by titration (Table 2). Glands taken from males fed on slices of canned apple gave slightly more activity than those from males fed on dry sugar cubes. Activity of the stock solution is unaffected by freezing but is somewhat reduced by freezing and thawing ten times. The first column of results in Table 2 is typical of many other assays of stock solution. Each male apparently contains enough active principle to sterilize at least 64 females. The initial stock solution contained glands from 250 males in 1 ml of saline. Each female received 1 μl, a dose equivalent to one-half gland or one-fourth of a pair of glands. Thus, the gland material from one male was distributed among four females. The stock solution could be diluted 16 times and yet retain enough activity for complete prevention of insemination.

The stock solution of male accessory gland substance from *A. aegypti* is clear and colorless. Activity is retained indefinitely while the solution is frozen at −20°C and is reduced only slightly by repeated freezing and thawing. This activity was preserved for 12 days at 5°C and for 2 days at 25°C, but was then destroyed, probably by bacterial action; activity was destroyed by 5 minutes at 50°C but was not affected at 40°C. The active principle is nondialyzable and is readily precipitated with 50 percent ammonium sulfate. These data are consistent with the hypothesis that the substance is a protein or polypeptide.

The physiology of the female mosquito is changed markedly by mating and reception of seminal fluid. For *Aedes aegypti*, females receiving male accessory gland substance show increased oviposition (*13*) and modified biting behavior (*14*) as well as monogamy. One might consider the substance to be a pheromone (*15*), in view of the fact that it is secreted to the outside of one individual and releases a specific action in a second individual. This substance might be useful in insect control, provided that methods could be found to sterilize young females by spraying or by baits for feeding.

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Life Cycle and Variation of Prototheca wickerhamii

Abstract. Prototheca wickerhamii *is a yeastlike organism that resembles the green alga* Chlorella. *Nuclear division in coordination with cytoplasmic cleavage gives rise to uninucleate cytoplasmic segments, each of which acquires a delicate cell wall and develops into an autospore. The autospores in this species are spherical; but in a variant that presumably arose as a result of spontaneous mutation, the cytoplasmic cleavage is irregular, and the resultant autospores are ovoid to bacillary. When these variant autospores grow, they swell and round up before the nuclear division begins, producing spherical cells like those seen in wild-type cultures. In view of the fact that species concept in the genus is based on size and shape of cells, the variation limits in these morphological characteristics have significant bearing on species classification.*

The genus *Prototheca* comprises a group of unicellular organisms whose phylogeny and taxonomic position are still a matter of debate and controversy. Krüger (*1*), who created the genus in 1894 and described it as a fungus, was the first to recognize its close resemblance to certain unicellular green algae which subsequently prompted Chodat (*2*) to describe it as an achloric strain of *Chlorella*. Experimental evidence for Chodat's view was presented by Butler (*3*) who obtained by irradiation white (lacking chlorophyll) mutants of *Chlorella pyrenoidosa* which, in his opinion, could be assigned to *Prototheca*. However, the white mutants of *Chlorella* are "autotrophic," in contrast to the thiamine-deficient species of *Prototheca* which are heterotrophic "like certain fungi and bacteria" (*4*).

Although the number of species included in the genus is relatively small, a precise species concept is not yet known, and the number of species is not definite. Based on morphological characteristics alone, five species have been recognized (*5*), each requiring thiamine for growth. A new species, *P. segbwema* (*6*), was isolated from a case of human protothecosis. I studied the development of a spontaneous variant of *P. wickerhamii* and its bearing on the species concept in the genus.

Prototheca wickerhamii was established by Tubaki and Soneda (*5*) to