

verse is true; gregarious adults are larger than isolated ones (1).

Crowded adults of *M. differentialis* show color changes associated with sexual maturation that do not occur in isolated adults; Uvarov (1) considers these changes also to be a characteristic of phase polymorphism. Newly molted crowded adults are bright yellow, with a black chevron pattern on the hind femur and scattered spots of black pigment on the pronotum and dorsal surface of the abdomen. After approximately 2 weeks, under our conditions, copulation occurs and the cuticle darkens to olive green. The ventral surface of the abdomen, however, may remain yellow for several weeks longer. Adults live approximately 4 months; the integument darkens throughout adult life, passing from olive green, through brown, to a dark brown that is almost black in some individuals. Isolated adults are more variable in color and do not show the sequence of color changes mentioned. Newly molted adults may be bright yellow, dark green, or brown, and most individuals darken somewhat during adult life. Some females develop a pink tinge to the hind femurs when they become older.

At least so far we have failed to produce crowded adults with relatively longer wings; nor have we observed typically gregarious behavior in either nymphs or adults. Temperature may be a factor in wing length since *M. sanguinipes* does develop relatively longer wings when raised at high temperatures (10). Regarding behavior, although we have seen no differences between isolated and crowded animals, one should note that, in locusts, phase differences often appear only after several generations. Since we have raised *M. differentialis* through only one generation, final assessment of possible phase-specific behavior is still premature.

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Poisoning with DDT: Effect on Reproductive Performance of Artemia

Abstract. *Brine shrimp die within 5 days after being given doses of above 10⁻⁵ parts of DDT per million. At this and lower doses, shrimp deposited broods at a normal frequency, and cysts predominated. The number of cysts that emerged and the survival of larvae to adulthood from broods of treated parents exceeded those from controls. Therefore lethality based on induced dominant or sex-linked recessive gene mutation was not evident. Decreased fecundity is due to maternal debility.*

Using populations of the brine shrimp, *Artemia salina*, treated with DDT, we have investigated whether chlorinated hydrocarbon insecticides have a direct influence upon fecundity and fertility of an aquatic organism. This branchiopod, one of the most primitive of living Crustacea, is a primary consumer in the food chain in saline waters. We have successfully cultured this animal in large numbers in our laboratory for some time, and it has been useful in studies of hereditary damage following exposure to radioisotopes (1).

As standard procedure, we culture the animals in gallon jars with 3 liters of filtered sea water supplemented with 50 g of NaCl per liter. The cultures are fed 1 ml of yeast suspension daily. Populations numbering up to 300 adults are easily maintained. To analyze reproductive performance, we transfer at least ten newly clasped adult pairs from the mass culture. Each pair is placed in its own quart jar of sea water supplemented with NaCl and is fed several drops of yeast daily. Zygotes are removed and counted at the time of feeding. Viviparously produced

Table 1. Observations on populations of *Artemia* after the addition of DDT to the cultures. Tremors are recorded as present (+) or absent (—).

Parts DDT per million	Knock-down hour	Tremors	Adults dead (%) at					Adults in first generation after treatment (No.)
			24 hr	48 hr	3 day	5 day	21 day	
10	½	—	17.0	100.0				
10 ⁻¹	1	—	19.8	95.5	98.0	100.0		
10 ⁻²	0	+	11.8	19.7	82.9	100.0		
10 ⁻³	0	+	0	9.6	95.0	100.0		
10 ⁻⁴	0	+	0	2.1	85.8	100.0		
10 ⁻⁵	0	+	0	0	0	0	71.7	125
10 ⁻⁶	0	—	0	0	0	0	39.2	221
Acetone control	0	—	0	0	0	0	0	256

Table 2. Average life span and reproductive performance of adult pairs transferred from the treated population to individual quart jars.

Parts DDT per million	Survival (days)		Frequency of broods (days)	Broods (No.)	Zygotes encysted (%)	Cyst emergence (%)	Zygotes per brood (No.)	Survival to adult (%)	No. males/No. females
	Males	Females							
Experimental animals									
10 ⁻⁵	35.6	34.0	2.9	9.6	51.4	51.9	108.5	81.2	0.85
10 ⁻⁶	34.2	36.2	2.9	7.6	17.9	49.4	130.4	74.3	0.86
Controls in acetone									
0	37.6	35.0	2.9	8.4	12.5	45.5	155.0	60.8	0.85
Stock 3									
0	43.0	44.0	3.0	10.3	11.6	25.0	157.2	59.5	0.85

young are transferred as a group to a separate quart jar. Cysts are filtered, dried, and resuspended in sea water so that emergence may be determined.

Eight 3-liter subcultures of our diploid amphigonic stock No. 3 were begun on the same day. After 3 weeks each jar had a population of 250 ± 5 adult shrimp. One jar received only acetone and served as a control. The other seven jars each received 1 ml of one of a series of dilutions of DDT in acetone. Geigy's 99.9 percent *p,p'*-DDT was used.

That knockdown occurs within an hour after 0.1 part of DDT per million was added to the culture (Table 1) is consistent with the Beltsville bioassay procedure (2). In lower concentrations of DDT, spasmodic swimming at irregular rates was punctuated by jerky flexing of the body axis. Settling to the bottom was gradual. Immobilization and death proceeded for a period of days.

Within 5 days all adults in the jar containing 10^{-4} parts of DDT per million were dead, although a few small larvae persisted. These, too, died before reaching maturity, and except for those given the two lowest doses, populations became extinct within 3 weeks. Even in solutions with 10^{-5} parts per million a majority of adults died within 3 weeks or before the next generation had matured. The first generation produced after treatment was noticeably smaller, while that produced by the controls experienced no difficulty in maintaining population size.

Table 2 characterizes the mated pairs of adults and their reproductive performance. A summary of data on mated pairs from stock 3 is included for comparison because exposure to the organic solvent, acetone, decreases survival time and shortens the opportunity to deposit broods. Mean survival times for pairs removed from the treated mass cultures approach those of acetone controls. With standard errors of 1 to 2 days, differences between the means are not statistically significant. The frequency of brood deposit was unaltered and more broods were deposited by animals given the higher dose of DDT. Furthermore, proportionately more of the zygotes from treated adults were deposited as cysts. Presumably this is a characteristic response to environmental stress because it has also occurred in experiments with radioisotopes (1).

Among the various aspects of reproductive performance tabulated, only the

average number of zygotes per brood shows a decrease correlated with exposure to a toxic agent. However, 108.5 ± 27.8 is not impressively different from 155.0 ± 25.9 when the variability reflected in the associated standard errors is considered. Nevertheless, young shrimp evidently profited from the relaxed crowding because more larvae survived to adulthood in the smaller broods. Such compensating tendencies are reflected in a trend toward achieving the top carrying capacity of the mass culture. More than 250 shrimp are maturing in the second generation after treatment, and the populations appear to be on the way to numerical recovery.

A decrease in the number of zygotes can be accounted for by somatic debility of the mothers. Induced dominant lethality must be discounted since embryos and larvae have good viability. Furthermore, sex ratios favoring females, the heterogametic sex, are nor-

mal and reveal no induced recessive sex-linked lethality. Therefore we have no evidence of direct damage to cells of the germ line. The only studies of this kind on animals other than insects have been with birds. There, too, poor reproduction can be accounted for by the poor condition of the adult females (3); however, the fact that the eggs incorporate DDT further complicates the matter.

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Antiandrogen Implanted in Brain Stimulates Male Reproductive System

Abstract. *Chronic intrahypothalamic implantation of cyproterone, an antiandrogen, in male rats resulted in specific stimulation of testes, seminal vesicles, and prostates. Implantation of cholesterol-filled or empty tubes in the median eminence in controls was ineffective. We conclude that decreases in the amount of testosterone reaching specific receptors in the median eminence or in nearby regions activate a mechanism that produces increased gonadotropin secretion.*

It is well known that a negative-feedback relation exists between the gonadal steroids and the pituitary gonadotropic hormones, such that the circulating level of steroids tends to be inversely related to the gonadotropin output (1). It has been shown that increased local concentration of estrogen (2) or androgen (3) in the medial basal hypothalamus (median-eminence region), by implantation of small amounts of the crystalline hormones, results in inhibition of gonadotropin output in both sexes. Since, in those studies, similar implantation in the pituitary had little or no inhibitory effect, it was concluded that steroid-sensitive structures in the hypothalamus respond to increased circulating levels of steroids by initiating a signal that results in decreased gonadotropin secretion, the pituitary itself being insensitive (4). The converse experiment—production of regional decreases in steroid concentration in the brain or pituitary—is much more difficult to perform, and there is no direct

evidence of the site of receptors for reduced circulating steroid levels.

The recent discovery of a potent and specific antiandrogenic compound suggested an experimental approach to this problem. It is reported that cyproterone, a synthetic steroid (5), blocks the action of testosterone on the reproductive systems of rats and other species and shows no progestational, androgenic, or estrogenic activities (6). The finding that cyproterone prevents differentiation of the male reproductive system by fetal or neonatal androgen (6) suggests that it inhibits the action of testosterone on the brain as well as on its peripheral target organs, since it is generally accepted that this early effect of androgen is exerted on the central nervous system—probably on the hypothalamus (7). We reasoned that, if this conclusion is correct, intrahypothalamic implantation of crystalline cyproterone should prevent the action of testosterone on the area surrounding the implant. Such prevention would permit testing of the