These results argue that the oak emanation causes the female to release her sex pheromone, which is then responsible for the sexual activation of the male (*3*). This interpretation is further supported by the failure of antennectomized males to mate under any circumstances (*4*).

It seemed altogether likely that the female antennae were the receptor organs for the oak emanation (*5*). This proposition was tested by removing the antennae and placing these females with normal males in the presence of oak leaves. No mating occurred when the female antennae were cut off 24 hours before the beginning of the assay. Despite this fact, a substantial number of females mated when the antennae were cut off 0 to 6 hours before the beginning of the assay. This puzzling result was finally clarified by the finding that mating of females soon after antennectomy did not require the presence of oak leaves. Apparently, for a period up to 6 hours after antennectomy, the cut ends of the antennal nerves, including the sensory fibers serving the oak receptors, generate impulses which trigger the secretion of the female sex pheromone. This interpretation is supported by the additional finding that the presence of antennectomized females often caused normal males to mate with normal females in the complete absence of oak leaves. And, finally, we may note that all of these phenomena vanished when the cut ends of the antennal nerves were killed by the application of a drop of 95 percent ethanol.

As a first step in a study of the oak factor itself, extracts were prepared by blending successive batches of leaves from the red oak in water, methanol, ethanol, acetone, benzene, and petroleum ether. The aqueous and alcoholic extracts proved highly active when tested in the biological assay, whereas the acetone, benzene, and petroleum ether fractions were inactive. Therefore, the active material is a highly polar substance. In further tests of the aqueous extract, the biological activity was not diminished by freezing and thawing, lyophilization, or exposure to 100°C for 10 minutes in a sealed tube. The aqueous extract is normally *p*H 5, and retains its activity over the range 4 to 8.

As far as we are aware, the oak factor constitutes a finding not previously described in the literature pertaining to attractants, pheromones, and kindred substances (*3*, *6*). The phenomenon as a whole reveals an unsuspected link in the system of chemical signaling between male, female, and host plant.

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References and Notes

1. Previous workers have noted the failure of polyphemus moths to mate under laboratory conditions and have routinely mated females out-of-doors: P. Rau, *Can. Entomol.* **56**, 271 (1924); R. W. Dawson, in *Culture Methods for Invertebrate Animals*, J. G. Needham, Ed. (Dover, New York, 1937); D. Ludwig and J. M. Anderson, *Ecology* **23**, 259 (1942).
2. This experiment was encouraged by the finding of Ludwig and Anderson (p. 260) that the reluctance of polyphemus to mate "was partially overcome by hanging cages containing the moths in the foliage of trees upon which the larvae commonly feed."
3. For pertinent reviews of insect sex attractants see: P. Karlson and A. Butenandt, *Ann. Rev. Entomol.* **4**, 39 (1959); M. Jacobson, *Insect Sex Attractants* (Wiley, New York, 1965); M. Jacobson, *Ann. Rev. Entomol.* **11**, 403 (1966). The role of female sex pheromones in the mating of saturniids has been described by: A. G. Mayer, *Psyche* **9**, 15 (1900); C. G. Soule, *ibid.* **9**, 224 (1901); P. Rau and N. Rau, *Trans. Acad. Sci. St. Louis* **26**, 83 (1929); W. R. Sweadner, *Ann. Carnegie Mus.* **25**, 163 (1937); B. Götz, *Experentia* **7**, 406 (1951).
4. The reception of the female sex attractant by the male antennae has been documented by V. L. Kellogg, *Biol. Bull.* **12**, 152 (1907); P. Rau, *Trans. Acad. Sci. St. Louis* **20**, 275 (1911); D. Schneider, *Ann. Rev. Entomol.* **9**, 103 (1965).
5. Electrophysiological studies have established that the male antennae contain receptor cells which respond to the female pheromone. The female antennae of the Saturniidae do not respond to the pheromone. D. Schneider, *Experentia* **13**, 89 (1957); *J. Insect Physiol.* **8**, 15 (1962); ———, V. Lacher, K. E. Kaissling, *Z. Vergl. Physiol.* **48**, 632 (1964); J. Boeckh, K. E. Kaissling, D. Schneider, *Cold Spring Harbor Symp. Quant. Biol.* **30**, 263 (1965).
6. V. Dethier, *Chemical Insect Attractants and Repellants* (Blakiston, Philadelphia, 1947); *Can. Entomol.* **91**, 554 (1958); G. Fraenkel, *Science* **129**, 1466 (1959); A. J. Thorsteinson, *Ann. Rev. Entomol.* **5**, 193 (1960).
7. Supported in part by NSF grant GB-3232. We thank E. A. Seling and Lorraine Baber for technical assistance.

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Phase Polymorphism in the Grasshopper Melanoplus differentialis

Abstract. *Individuals of* Melanoplus differentialis *raised in isolation or in a crowded environment show conspicuous morphological differences indicating phase polymorphism. Isolated nymphs are pale brown at low humidities and green at high; crowded ones show extensive black pigmentation. Isolated adults are larger than crowded, while crowded adults show maturational color changes that are not present in the isolated. No behavioral differences have been noted in the one generation.*

Since phase polymorphism, resulting from population density, was first described over 40 years ago, it has been extensively studied in Old World locusts, especially of the genera *Locusta, Normadacris,* and *Schistocerca* (Orthorptera, Acrididae) (*1*). Yet the general subject of phases in grasshoppers and locusts has received comparatively little attention in North America: the only study conclusively demonstrating distinct phases resulting from isolation or crowding of individuals seems to be that by Faure (*2*) of the lesser migratory grasshopper *Melanoplus sanguinipes,* although there is evidence that such phases may occur in other forms as well (*3*). Faure predicted that phases would be found in other North American grasshoppers also. We have now substantiated that prediction by producing morphological phases in *M. differentialis* (Orthoptera, Acrididae, Catantopinae) raised under controlled conditions of isolation or crowding. The occurrence of distinct forms in *M. differentialis* is interesting because, although when in large numbers it has been recorded as a crop pest (*4*), we find no records of its migrating with or without swarming. Most other polymorphic species undertake migratory flights, especially as swarms of the gregarious phase.

The grasshoppers used by us were the offspring of field-caught females that had deposited egg pods in the laboratory. Diapause was broken by immersion of the eggs in mineral oil (*5*), and the resulting hatchlings were reared in one of three ways: For crowded conditions, approximately 50 hoppers were placed in a 14 by 14 by 14 cm wooden cage having a wire-screen back and top and a removable glass front. For isolated rearing, a single hopper was placed in a cage of this type, while some isolates were reared in jars approximately 8 cm in diameter and 15 cm high. With damp cotton continually present, the relative humidity was 70 to 90 percent as opposed to 10 to 20 percent in the cages. The photoperiod was 16 hours of light and 8 hours of darkness; temperature 23°C. Cages or jars were arranged around a 60-watt light bulb that provided radiant heat for 8 hours

daily. Cracked corn was constantly available and fresh oat seedlings were provided daily; food was always abundant. Animals in jars were not supplied with corn, which became moldy at high humidities; this factor had no obvious effect on the results.

First-instar hoppers are uniformly colored, with a small amount of black pigment scattered through the integument, giving them a gray appearance. Coloration of the second to sixth instars depends on rearing conditions, although in a few instances the effects of rearing did not appear before the third instar. Hoppers reared isolated in a brown box were a uniform pale brown, with a slight black chevron pattern on the hind femur and a faint black horizontal stripe on the pronotum; some abdomens were speckled with black. Crowded hoppers, on the other hand, showed the same brown background color, but with much greater amounts of dispersed black pigment; the pronotum had a conspicuous black horizontal stripe, the wing buds were dark, and the abdomen was mottled with black; thus the crowded hopper was conspicuously the darker (Fig. 1). Occurrence of a melanic pattern in artificially crowded hoppers seems to be fairly common (*1*), even in species that show no other apparent effects of density (*6*).

Of the 15 nymphs raised isolated but at high humidities, eight (53.3 percent) became green in the fourth or fifth instar, with a small amount of black pigment similar in distribution to that of low-humidity isolates raised in boxes. The remaining 46.7 percent also had very little black pigment and were pale-fawn or creamy-white in color—not brown. A control group, reared in similar jars on an identical diet but at low relative humidity (30 to 40 percent), all became creamy white.

Table 1. Means of four measurements (mm) taken from isolated and crowded adult *M. differentialis*. All differences between phases are statistically significant at $p < .001$ or better.

Sex	Head width (*C*)	Length		
		Wing (*E*)	Femur (*F*)	Pronotum (*P*)
		Crowded		
Male	5.00	25.03	16.09	6.69
Female	5.83	28.47	19.29	7.86
		Isolated		
Male	5.41	28.07	18.01	7.54
Female	6.78	32.78	22.88	9.69

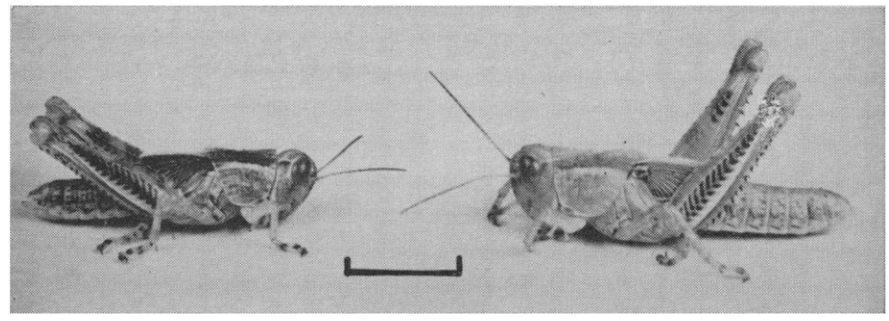

Fig. 1. Crowded (left) and isolated sixth-instar female nymphs of *M. differentialis*. Note that the crowded individual is smaller and darker. Scale, 1 cm.

An essential feature of phase polymorphism is that isolated nymphs adjust their color to the surroundings whereas crowded nymphs do not (*1*). The isolated nymphs reared at low humidity in our experiments did tend to match the background (brown in a brown box and creamy-white or pale-fawn in a glass jar), while crowded ones were much darker. Our results also show that green coloration is produced at high relative humidities—above about 70 percent. Similarly, in two of the best-known polymorphic genera, *Locusta* and *Schistocerca*, high humidity makes hoppers become green (*7*). Faure (*2*) reported that 9 percent of isolated hoppers of *M. sanguinipes* given succulent food became green; he reared no controls.

Both green and fawn hoppers of *M. differentialis* collected in the field are in appearance typical of those raised isolated in the laboratory. Along a damp, marshy, river bank more than 95 percent of nymphs were green, whereas along a roadside bordering a small upland meadow the population was approximately 70 percent green and 30 percent fawn. Both green and fawn forms were brought into the laboratory and raised crowded in jars; after two molts, all developed the black pigment and brown background of typical crowded hoppers.

A further characteristic of isolated locusts is an extra nymphal instar. In the present instance, of a total of 43 nymphs raised in isolation at either low or high humidity, 15 had an extra stage between the fourth and fifth instars (wing-bud eversion first occurs in the fifth instar). The fact that 12 of the 15 were females could partly account for the particularly large size of isolated adult females. Extra instars have been reported in other species of *Melanoplus* (*8*), but in *M. differentialis* the phenomenon appears to be correlated with phase since crowded hoppers showed no extra stages.

Adult isolates are conspicuously larger than crowded specimens in all respects. For direct morphological comparisons, head width (*C*), pronotum length (*P*), elytron length (*E*), and hind-femur length (*F*) were measured by Dirsh's (*9*) standard procedures (Table 1). The distribution of *F* is shown in Fig. 2. Because all parameters varied in the same direction, ratios such as *E*:*F*, often used to distinguish locust phases, were not useful to us. Only isolated adults reared at low humidity were used for these measurements because the smaller jars used for high humidity may have affected size. The strong sexual dimorphism associated with phase change in *Locusta* and *Schistocerca* was not present, but the phase effect is somewhat more pronounced in females of *M. differentialis* than in males. There seem to be no previous records of isolated individuals of both sexes showing an increase in size over crowded individuals. In *Dociostaurus* and *Locustana*, for example, the con-

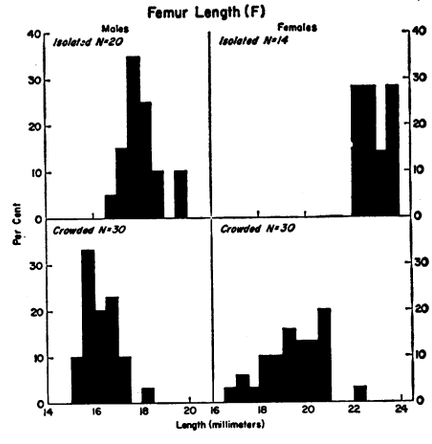

Fig. 2. Distribution of measurements of femur length (*F*) in isolated and crowded adult *M. differentialis*.

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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References and Notes

1. B. Uvarov, *Grasshoppers and Locusts* (Cambridge Univ. Press, London, 1966), vol. 1.
2. J. C. Faure, *J. Econ. Entomol.* **26**, 706 (1933).
3. N. C. Criddle, *Can. Entomol.* **65**, 97 (1933); J. M. Grayson, "Studies of some factors influencing coloration of the grasshopper, *Melanoplus bivittatus* Say," thesis, Iowa State College, Ames, 1941); A. B. Gurney, *Proc. Entomol. Soc. Wash.* **51**, 267 (1949); C. Wakeland, *Tech. Bull. U.S. Dept. Agr. 1167* (1958); O. L. Barnes, *J. Econ. Entomol.* **53**, 721 (1960).
4. J. R. Parker, *J. Econ. Entomol.* **26**, 102 (1933); R. C. Froeschner, *Iowa State Coll. J. Sci.* **29**, 163 (1954).
5. E. H. Slifer, *J. Exp. Zool.* **138**, 259 (1958); *Nature* **184**, 1424 (1959).
6. H. B. Johnston, *Bull. Entomol. Res.* **23**, 49 (1932); L. G. Duck, *J. Kansas Entomol. Soc.* **17**, 105 (1944).
7. D. L. Gunn and P. Hunter-Jones, *Anti-Locust Bull. 12* (1952); P. Hunter-Jones, *Entomol. Monogr. Mag.* **98**, 89 (1962).
8. R. L. Shotwell, *Tech. Bull. U.S. Dept. Agr. 774* (1941).
9. V. M. Dirsh, *Anti-Locust Bull. 16* (1953).
10. C. H. Brett, *Tech. Bull. Oklahoma Agr. Exp. Sta. T-26* (1947).
11. Supported by NSF grant GB-2949 to H.D.

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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