analyzed by means of a 400-channel pulse-height analyzer (Technical Measurements Corp., model 401C) coupled to a NaI(T1) scintillation crystal (7.5 by 7.5 cm) and photomultiplier. Crystal and photomultiplier were mounted in a steel cave with walls 27.5 cm thick.

The resulting spectra, shown in Fig. 2, demonstrate that sodium, as activated ^{24}Na, was present in the washed hair. Parallel tests on hair that had not been treated with ^{22}Na, but had been subjected to similar washing procedures, showed that approximately 40 percent of the original sodium, in terms of counts per minute, remain in the hair even after the extended period of washing. These findings suggest that part of the sodium associated with hair is a loosely held contaminant which can be washed out. The remaining more tightly held fraction does not wash out with water alone. Furthermore, the removable fraction comes out with relative ease and quickness, while the fixed fraction remains at about the same levels during extended washing, which suggests that there is a qualitative difference involved in the association rather than a simple quantitative difference dependent on, perhaps, depth of the sodium molecule within the hair matrix.

Although the details are beyond the scope of this report, it is pertinent to point out that certain other trace elements behaved in a manner similar to that of sodium, while still others did not. For example, bromine and nickel were completely removed by the washing procedure described above; manganese, copper, and zinc were not, which suggests that, among other factors, ionic radius and charge may be involved in the control of the distribution of trace elements in hair. These patterns are the subject of continuing studies that will be reported more fully at a later date (*4*).

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References and Notes

1. L. C. Bate and Frank F. Dyer, *Nucleonics* **23**, 10 (1965).
2. R. E. Jervis, A. K. Perkons, W. D. MacKintosh, M. F. Kerr, *Proc. Int. Conf. Modern Trends Activation Analysis*, College Station, Texas (1961).
3. G. S. Kennington and C. F. T. Ching, *Science* **151**, 1085 (1966).
4. G. S. Kennington, in preparation.
5. Supported by grant from Associated Rocky Mountain Universities, Inc. and by cooperation of the National Reactor Testing Station, Idaho Falls, Idaho. The advice and encouragement of C. W. Sill and D. G. Olson and the assistance of C. F. T. Ching are particularly appreciated.

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Volatile Principle from Oak Leaves: Role in Sex Life of the Polyphemus Moth

Abstract. *An emanation from oak leaves is necessary for the mating of polyphemus moths under laboratory conditions. This requirement can be satisfied by placing the moths in the presence of oak leaves or aqueous or alcoholic extracts prepared from oak leaves. The active principle is a volatile, heat-stable, polar material which has been partially purified. The oak emanation acts on the female and not on the male, and the sensory receptors are located on the female antennae. The reception of the oak emanation is prerequisite for the female's release of her sex pheromone, which in turn, is necessary for the sexual activation of the male.*

We have long been puzzled by the sex life of the polyphemus moth (*Antheraea polyphemus*). For example, when caged out-of-doors during the proper season, virgin females have routinely attracted the male polyphemus from afar. Yet under laboratory conditions we have (until recently) failed to obtain a single mating among hundreds of these moths when the sexes were placed together in small cages, or in large cages, or even in the Harvard gymnasium (*1*). The solution of this paradox proves to be far from trivial.

A pair of polyphemus moths was placed overnight in a laboratory cage containing red oak seedlings (*Quercus rubra*) (*2*). The next morning the pair was mating. But so also was another pair of polyphemus in a nearby cage not containing any plant material! This was the first indication that oak—a favorite food plant of the polyphemus silkworm—produces an emanation prerequisite for the sexual activation of these moths.

To explore the phenomenon in further detail, we standardized the biological assay in the following manner. Each evening, one female and two males, or two females and three males, were placed together in a cage and stored overnight in a darkened laboratory ventilated by a ceiling fan. If mating occurred, the moths ordinarily remained paired until the following evening. If mating did not occur, the sexes were removed to separate cages and stored apart during the day. Unmated females were tested on two successive nights; males on three successive nights. All unmated individuals were then placed together in one large cage apart from oak leaves.

In control experiments (Table 1), no matings occurred when 30 normal females were tested in the absence of oak leaves. Likewise, none of the older moths mated in the large cage, despite the high density of the population and the frequent collisions between the sexes. By contrast, 12 (33 percent) of the females mated in cages containing oak leaves; 10 (29 percent) mated when oak leaves were only in the vicinity of the cage.

The experiment was repeated with maple, birch, chestnut, horse chestnut, elm, hickory, and beech leaves—all of which are known to be acceptable food plants of polyphemus silkworms. No matings occurred among the 24 females subjected to this treatment.

Does the oak emanation act on the male or the female moth or on both? Answer to this question was sought by separately exposing males and females to oak leaves for specific periods. Then, each of the moths thus treated was caged in separate rooms with untreated moths of the opposite sex in the absence of oak leaves.

The results of these experiments showed that the oak emanation acts solely on the female. In no case did prior exposure of the male provoke mating (Table 1). By contrast this preliminary treatment of females for 4 to 6 hours led to subsequent successful mating in a substantial number of cases. In additional experiments not recorded in Table 1, we found that this preliminary treatment of the females was effective only when the treated females were promptly placed with males at the proper time of day. Thus, a delay of only 30 minutes negated the effects of 24 hours of treatment.

Table 1. Effect of oak leaves on the mating of polyphemus moths.

Treatment	Females assayed (No.)	Mating (%)
Control (no leaves)	30	0
Red oak, in cage	36	33
Red oak, in vicinity	35	29
Prior exposure of males to oak leaves for:		
0.5 hr	2*	0
4 hr	4*	0
6 hr	2*	0
Prior exposure of females to oak leaves for:		
0.5–1 hr	4	0
2–3 hr	5	0
4–5 hr	6	33
6 hr	4	50

*Number of males tested, with equal numbers of females.

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 s→ 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