Table 1.—Percent negative responses of passalus beetles exposed to various light intensities of different angles. Blanks indicate intensities at which no tests were run.

Ft-c of light	Experiment: Horizontal light	Overhead 45° angle	Overhead 90° angle
3.5	—	0	—
6.0	0	—	—
7.0	0	—	—
8.7	100[a]	30	50
10.0		—	56
13.0		34	50
17.4		37	—
20.0		—	50
27.0		56	—
35.0		76	90
52.5		80	—
70.0		100[a]	100[a]

[a] All tests above this light intensity gave 100% negative responses.

intensities of 11 ft-c and above the beetles moved away from or out of the light regardless of initial position.

The response of the beetles to light coming from a 45° overhead angle showed an increasingly higher proportion of negative responses with increasing light intensities. In these tests, to remove any possible conditioning effect, the beetles were numbered and each was tested at one intensity before being tested at the next higher intensity. During these tests it was noted that each beetle had its own threshold value for negative response. Although this value varied from beetle to beetle, once a beetle responded negatively to a given light intensity it responded negatively to all intensities above that value. Increased light intensities resulted in a slightly higher temperature at the point of beetle release than occurred in the other experiments, but this temperature variation did not exceed 1° C and was not considered sufficient to have any significant effect upon the results.

In experiment no. 3 the percent of negative response at 8.7, 10.0, 13.0, and 20.0 ft-c of light (Table 1) shows a random distribution of the beetles in the box with no statistically significant difference in the number of beetles in the light or dark area. At these intensities the beetles could not orient to the overhead light, i.e., move directly away, and the light was apparently not bright enough to cause continuous movement. Above these intensities the level of light was apparently such that the beetles continued to move until they found the darkened area of the box. Locating the darkened area was probably more by chance than by orientation to the light. The beetles undoubtedly remained in the darkened area of the box at the higher light intensities as the result of a response on the part of the beetle.

Passalus beetles may be attracted to extremely dim light. Beetles that escaped or were released in a darkened room which had a single spot of incandescent light entering at the bottom of the door were always found at the spot where the light was entering. This light intensity was much too low to record with the light meter used and was visible only after several minutes in the darkness.

In our experiments using red, blue, and ultraviolet lights, beetle response appeared to be controlled by intensity and not by color of the light. These tests were discontinued after this fact became evident.

These experiments indicate that the passalus beetle responds negatively at much lower light intensities to horizontal light than to light coming from a 45° or 90° angle overhead. This sensitivity may be due partly to an inability to orient and move away from overhead light and, therefore, to tolerate this light until the intensity reaches a level (about 35 ft-c in this study) which will cause the beetle to continue moving until it finds a suitably darkened area. The light sensitivity level varies from beetle to beetle but appears to be generally constant for a given beetle.

Its natural habitat of tunnels in decaying logs would suggest that the passalus beetle is adapted to darkness or dim light. The sensitivity to low intensities of horizontal light would be of great value to the species in enabling it to leave a log at times of reduced light such as late evening, early morning, or at night, and it is probably at such times that the beetle moves to new logs. Beetles caught in bright light would move away from the light and continue moving, if not obstructed or trapped, until a suitable environment was found.

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Chemical Releasers of Social Behavior—IV. The Hindgut as the Source of the Odor Trail Pheromone in the Neotropical Army Ant Genus *Eciton*[1]

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Among the legionary or army ants in the Dorylinae, foraging workers lay continuous exploratory trails during emigrations and raids. Especially during emigrations these odor trails become very concentrated since they are being reinforced by the innumerable individuals in the massive columns. The most highly developed of the Neotropical genera, *Eciton*, lays persistent trails which may be followed even if encountered weeks after the trail was originally generated (Schneirla and Brown 1950). Although rainfall does not rapidly destroy these old trails, they appear to last longer during dry seasons (Schneirla and Brown 1950). These facts indicate that the *Eciton* odor trail pheromone is a substance which is relatively photostable, thermostable, and water-insoluble, and which possesses a low vapor pressure. With the exception of a recent investigation on the primitive doryline genus, *Neivamyrmex*, in which artificial trails were successfully prepared from recta and midguts (Watkins 1964), nothing is known about the source of army ant trail pheromones. The present experiments were carried out to establish the glandular origin of the odor-trail pheromone of *E. hamatum* (F.). The ants were collected along the Ucayali River at Buenos Aires, 25 km south of Pucallpa, Peru.

MATERIALS AND METHODS

It was observed that workers of *E. hamatum* foraging on trees frequently touched the tips of their gasters to the substrate as they moved on the odor trails. This behavior is consistent with the trail-laying behavior of ants in

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other subfamilies, in which abdominal organs are employed to dispense the trail pheromone. Consequently, the abdominal organs capable of secreting products into the external environment were dissected out of major and media workers and were employed to lay artificial trails. These organs included the poison gland (plus vesicle) and accessory gland of the sting, the hindgut, and the ovaries. All dissections were performed in water and each organ was rinsed twice in fresh water.

Artificial trails were prepared by placing the tissue sources on the ends of sharpened cork tips and smearing them on 6 in.-diameter circular trails which had been drawn on 8½×11 in. papers. Subsequently, 20 *Eciton* workers were introduced into the center of the circle and a positive response was recorded if an ant traveled around the entire circle after encountering it.

RESULTS

The hindgut of *Eciton* is clearly the source of the odor trail pheromone (Table 1). However, it still is not known whether the pheromone is produced by special cells in the hindgut or is a product of digestion formed more anteriorly in the digestive tract. No specialized glandular tissue was apparent in the hindgut, but the presence of glandular cells cannot be ruled out until a detailed histological examination is made. That products associated with digestion may possibly serve as the ultimate source of this pheromone is indicated by the fact that trails prepared from the empty hindgut of *Eciton* workers which had been starved for 48 hours were either devoid of odor trail activity or were very weakly active.

The properties of the artificial *Eciton* trails are consistent with what is known of these trails when they are laid in the field. The apparent stability and low vapor pressure of the pheromone were indicated by the fact that ants strongly followed these trails 72 hours after they were prepared. These trail-treated papers were stored at a temperature which often exceeded 90°F. The water-insolubility of the pheromone was demonstrated by the fact that trail-treated papers could be soaked in water and after being allowed to dry, the artificial trails were still potent releasers of trail following.

DISCUSSION

Although none of the ant odor trail pheromones have been chemically characterized, the glandular sources of these important releasers have now been identified for some genera in most of the major subfamilies. The versatility of the Formicidae is evident from the fact that these Hymenoptera also employ the accessory gland (Wilson 1959), poison gland (Moser and Blum 1963), and in 1 subfamily, the Dolichoderinae, a unique ventral gland (Wilson and Pavan 1959) to generate their potent odor trails. Interestingly, the highly developed Formicinae, like the more primitive Dorylinae, employ the hindgut to secrete their odor trail pheromones (Blum and Wilson 1964). Although the formicine odor trails are recruitment rather than exploratory trails such as the dorylines produce, the former nevertheless are capable of being built up into highly persistent "trunk routes." If the Dorylinae and Formicinae are indeed employing digestive products as the source of their odor trail pheromones, they have selected a readily available source of chemicals which is admirably suited to the laying of persistent trails.

Table 1.—Response of *Eciton hamatum* workers to artificial trails prepared from 4 abdominal organs.

Organ	No. replications	No. workers responding
Accessory gland	10	0
Poison gland (plus vesicle)	10	1
Hind gut	10	186
Ovary	10	0
Control (water)	10	0

ACKNOWLEDGMENTS

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Preparation of House Fly Chromosomes[1]

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Over the years the adult house fly, *Musca domestica* L., has shown itself to be an excellent experimental animal in various fields of entomological research. Since some research is of a cytological nature, a need exists for additional techniques for studying this insect. Recently, French et al. (1962) published a method for preparing chromosomes from the brains of mosquito larvae and from the ovaries and testes of mosquito pupae. Oster and Balaban (1963) published a method of preparing somatic chromosomes from the cerebral ganglion of *Drosophila* larvae and also demonstrated that this method would work on the cerebral ganglion of house fly larvae. By modifying these procedures we developed a technique that enabled us to obtain consistently good chromosome preparations from the gonads of adult male house flies.

METHODS

Gonads of adult male house flies are dissected out in a modified Ringer's solution.[2] Gonads are then transferred to a cold (about 40°F) 1.0% solution of hypotonic sodium citrate (1 g sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) in 100 ml of double glass distilled water) (Oster and Balaban 1963) for 10 min, after which all extraneous attached tissue is removed.

Next, gonads are placed in a drop of cold (about 8°F) modified Carnoy's fixative (1 part 95% ethyl alcohol and 1 part 45% acetic acid) (French et al. 1962) for 10 to 15 min. The gonads are removed from the fixative and placed in a small drop of 45% acetic acid on a clean mic-

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[2] Modified Ringer's solution formulated by dissolving 0.1 g KCl, 0.0135 g $CaCl_2$, 0.0120 g $NaHCO_3$, and 0.75 g NaCl in sufficient distilled water to make 100 ml of solution.

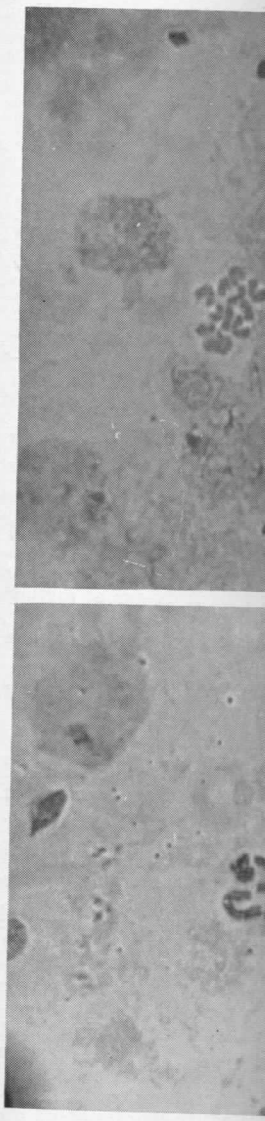

FIG. 1.—Chromosomes pre
adult male house flies (*Mus*

roscope slide. The tissue is
which has been treated by
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changes of 95% ethyl alcoh
dried with a piece of lint-fr
between 2 pieces of filter pa
with gentle but firm thumb
of the coverslip. This is a
exercised to avoid movement
slip is then firmly tapped th
times over the area of the t
ately heavy metal rod (we
diamond-tipped marking pen
removed and the slide is plac
a minimum of 30 min.

While the slide is still o
coverslip is removed by ins
razor blade under the edge.
prevent the adherence of the
slide is then placed in 95% et

[3] General Electric SC-87.

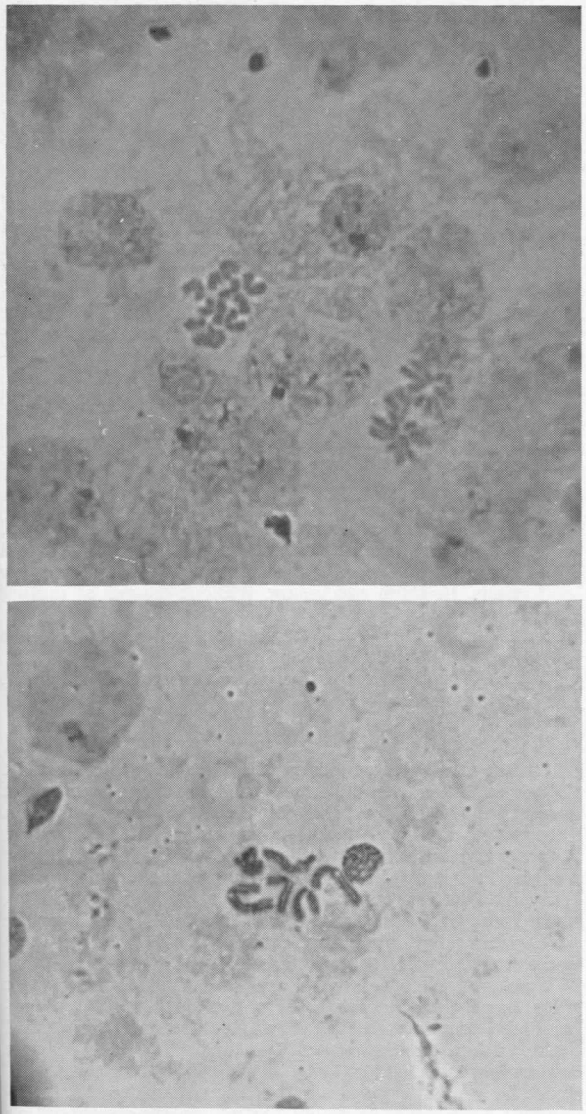

FIG. 1.—Chromosomes prepared from the testes of two adult male house flies (*Musca domestica* L.) (1400×)

roscope slide. The tissue is then covered with a coverslip which has been treated by immersion in a silicone solution,[3] washed in running tapwater and then in several changes of 95% ethyl alcohol to remove excess acid, and dried with a piece of lint-free cloth. The slide is placed between 2 pieces of filter paper and the tissue is squashed with gentle but firm thumb pressure applied over the area of the coverslip. This is a vital step and care should be exercised to avoid movement of the coverslip. The coverslip is then firmly tapped through the filter paper several times over the area of the tissue with a smooth, moderately heavy metal rod (we used the rounded end of a diamond-tipped marking pencil.) Then the filter paper is removed and the slide is placed on a piece of Dry Ice® for a minimum of 30 min.

While the slide is still on the Dry Ice, the siliconed coverslip is removed by inserting a chilled single-edged razor blade under the edge. The coating of silicone is to prevent the adherence of the tissue to the coverslip. The slide is then placed in 95% ethyl alcohol for 5 min.

[3] General Electric SC-87.

Finally, the slide is removed from the alcohol, the excess alcohol allowed to drain off, and a drop of orcein-fast green stain (Oster and Balaban 1963) placed on the slide over the area of the tissue while the slide is still damp. A new siliconed coverslip, rinsed in absolute ethyl alcohol to remove any lint, is placed over the drop of stain. A piece of filter paper is placed over the slide and all excess stain is removed by gently pressing and rolling the thumb over the area of the coverslip. The chromosomes are now ready to be examined by phase microscopy.

Staining of chromosomes at this stage gives excellent results but even better results can be obtained by overnight staining at a freezing temperature. If slides will be used only as temporary mounts and for only a few weeks, coverslips are ringed with a warm balsam-paraffin sealant (Breland 1961). However, if permanent mounts are desired the slide is placed on Dry Ice for 1 hr or longer following the overnight staining and then in 95% ethyl alcohol for 5 min. While in the alcohol, the coverslip slides off and the excess stain is removed. After the slide is removed from the 95% alcohol and allowed to drain, it is placed in absolute ethyl alcohol for 1 min. Upon removal from the alcohol, the excess alcohol is allowed to drain off and a drop of euparal is placed on the slide. A siliconed coverslip rinsed in absolute alcohol is mounted on the slide and the excess euparal is removed with a piece of filter paper. The mount should be allowed to dry 24 hr prior to use.

Consistently good slide preparations of house fly chromosomes were obtained quickly and easily by the above described method. Fig. 1 shows the type of preparation obtained.

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The Phospholipids of Various Subcellular Fractions from the Larvae of the Blow Fly, *Phormia regina*[1]

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The phospholipids of *Phormia regina* (Meigen) are unusual in the high proportion of ethanolamine-containing lipids, while those containing choline contribute to a relatively small extent (Bieber et al. 1961). The phospholipids of a related insect, *Musca domestica* L., are essentially the same as those of *P. regina* (Crone and Bridges 1963). In view of the involvement of phospholipids in mitochondrial and other membranes (Green 1963), it seemed pertinent to determine whether the phospholipids containing ethanolamine were associated with a particular subcellular fraction.

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