# DETECTION OF A PHEROMONE RELEASED BY THE FEMALES OF *CULICOIDES NUBECULOSUS* (DIPTERA, CERATOPOGONIDAE) ATTRACTING THE MALES AND STIMULATING COPULATION

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ABSTRACT. The authors give evidence of a pheromone released by the females of *Culicoides nubeculosus* which actively stimulates mating and its action cannot be mistaken with factors such as sight or sound. When males were given the opportunity to choose their way in a T tube, the authors observed that this pheromone attracted the males to its source.

RÉSUMÉ. Les auteurs démontrent l'existence d'une phéromone émise par les femelles de *C. nubeculosus*. Cette phéromone stimule activement la copulation et son action ne peut pas être confondue avec des facteurs, tels que le son ou la vue.

Par l'observation des mâles ayant la possibilité de choisir leur déplacement dans un dispositif en T, les auteurs mettent également en évidence l'effet attractif de cette phéromone sur les mâles.

In a study of the sexual activity of *Culicoides nubeculosus* (Meigen) according to age (Kremer et al. unpublished) we showed that males and females have their maximum activity on the 2nd day of imaginal life. Therefore we attempted to detect a pheromone in insects of that age.

## DETECTION OF A PHEROMONE STIMULATING MATING IN *C. NUBECULOSUS*.

MATERIAL AND METHODS. MATERIAL:—4 glass flasks (F1, F2, F3, F4) equipped with 2 tubes (air inlet and outlet); diameter of the tubes small enough to prevent passage of the insects.—Air generator.—Teflon tubing adapted to the tubes.—Screw clamp to adjust the airflow rate.

Tests were made in an insectary: 27° C; 70 to 80% relative humidity and constant lighting. The insects were reared in our laboratory according to the method of Boorman (1974).

EXPERIMENTAL DESIGN (see figure 1). In order to have a control for each experiment, the air flow coming from the air generator was divided in two by a Y connection. From the Y each tube led successively into a 1st flask, then into a 2nd, and from this via a tube, into a water container, in order to give an estimate of the airflow rate by counting the bubbles.

METHODS. After the technique of Barrett (1977), flasks F1 and F3 are used for the eventual production of pheromone, one being active, the other empty as a control. Flasks F2 and F4 receive the airflow coming from the preceding flasks and are the points where the effects of an eventual pheromone can be detected. The effects are quantified by counting the matings.

UNIT. Number of matings/male/hour = NMH. The NMH is less in the experimental device used for the detection of pheromone than in normal rearing conditions (Kremer et al.). This reduction may be due to a higher level of illumination during the tests, or due to the glass container itself (the rearing is normally done in paraffined cardboard).

The rearing of *C. nubeculosus* and the phenomenon of mating often being variable, it was not possible in this preliminary study to quantify the data of our observations, nor statistically to confirm these otherwise evident conclusions.

## EXPERIMENTS.

EXPERIMENT SHOWING A PHEROMONE AND ELIMINATING THE ACTION OF DIRECT

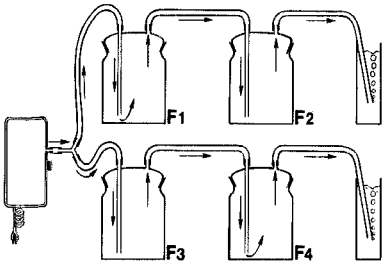


Fig. 1. Setting used to test pheromone from live *Culicoides nubeculosus* females.

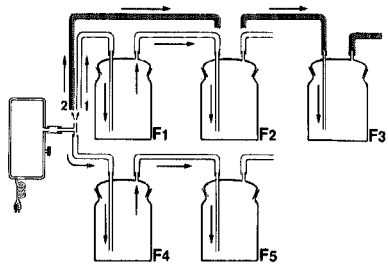


Fig. 2. Setting used to eliminate sound and sight in the first test.

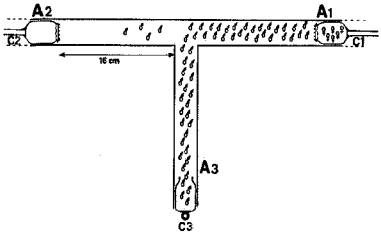


Fig. 3. T shaped glass tube showing the female attraction for the males.

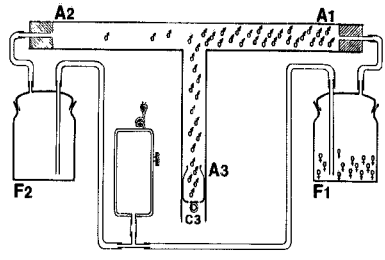


Fig. 4. Elimination of sound and sight in attraction test.

CONTACT BETWEEN MALES AND FEMALES. About 100 two-day old females were placed in flask F1; F3 was empty. Thirty (24 to 48 hr old) males were placed in each of F2 and F4. The airflow was turned on and the NMH in F2 and F4 were calculated (number of matings between males).

| NMH Observ. | 1 | 2 | 3 | 4 | 5 | 6 | Means |
|---|---|---|---|---|---|---|---|
| Experiment | 5.6 | 5.6 | 5.2 | 5.2 | 10.8 | 14.8 | 7.8 |
| Control | 2 | 1.6 | 1.6 | 1.6 | 1.2 | 2.4 | 1.7 |

The NMH were calculated with the following variations:

*—1 and 2:* normal experiment, the airflow being established for 2 min.

*—3:* blind test.

*—4:* connections between flasks inverted (F2 = control, F4 = experiment).

*—5 and 6:* the airflow being continuous.

RESULTS. The number of matings between males was four times higher in the experimental flask than in the control.

EXPERIMENT WITH MALES AND FEMALES. Same experiment as before, but in F2 and F4, 16 males and 16 females were introduced. On 7 different trials, in F2 (experiment) the NMH were 12, 7.7, 12, 8.6, 9.4, 10.5, 16.8 (means 11) and in F4 (control) they were 2.5, 1.7, 1.6, 1.7, 3.4, 1.5, 3.2 (means 2.2).

*Results.* The existence of a volatile pheromone released by the female of *C. nubeculosus* was confirmed. Under its influence, the mating activity of the males was increased 6 times.

EXPERIMENT TO DETECT A PHEROMONE RELEASED BY MALES. a) The females of F1 were replaced by males, and F3 remained empty. In F2 and F4, 15 males and 15 females were introduced. From 12 different observations, NMH data from F2 were: 4.8, 4, 4, 3.2, 4, 4.3, 4.3, 1.7, 3.4, 6, 3.2, 6.5 (means 4.45); while in F3 (con-

trol): 4, 3.2, 4.8, 1.6, 4, 4.3, 2.5, 4.3, 4.3, 5.1, 3.2, 4 (means 3.78).

*Results.* No evidence of an effect could be observed. It was therefore impossible to detect a pheromone produced by the males of this species in this experiment.

b) Many females were introduced in F1, many males in F3 and males and females in the observations flasks F2 and F4. The data from 6 observations are: NMH in F2 (experiment): 2.72, 18.8, 15.2, 10.4, 12, 15.2 (means 16.13); and in F4 (control): 6.4, 3.2, 6.4, 5.6, 2.4, 4 (means 4.6).

*Results.* From the results of experiments a and b, the NMH are not clearly increased under the influence of the presence of males in the pheromone-production flask. We cannot therefore detect the existence of a pheromone produced by the males.

4. EXPERIMENTS ELIMINATING FACTORS LIKE SOUND AND SIGHT, THAT MIGHT INTERFERE. The setting of the experiment was according to figure 2. A reservoir flask (F2) was inserted between the experimental flask F3 and the test females (F1). Air was allowed to flow for 5 min in the system (F1 and F2) and F4 + F5, then the connection between F1 and F2 was released, and established between F2 and F3. Airflow was then switched from F2 to F3. F2 was supposed to act as a reservoir of pheromone. The NMH were counted in F3 (experiment) and F5 (control). The following data have been calculated in 4 different experiments, where the copulations were counted at regular intervals:

| NMH Experiment | | | | NMH Control | | | |
|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| 13.2 | 12.8 | 7.2 | 12.8 | 2.4 | 2.4 | 2.4 | 4.8 |
| 12 | 8 | 6.4 | 10.4 | 2.4 | 4 | 3.2 | 4 |
| 9.6 | 11.2 | 5.6 | 8.8 | 3.6 | 4.8 | 4 | 3.2 |
| 12 | 12.8 | 9.6 | 13.6 | 4 | 5.6 | 4.8 | 2.4 |
| 13.2 | 8.8 | 7.2 | 13.6 | 4.8 | 3.2 | 4 | 4 |
| 19.2 | — | 7.2 | 8.8 | 8.4 | — | 3.2 | 4.8 |
| *Means* | | | | | | | |
| 13.2 | 10.7 | 7.2 | 11.3 | 4.6 | 4 | 3.6 | 3.9 |
| *General means* | | | | | | | |
| 10.6 | | | | 4 | | | |

It is obvious that the accumulated pheromone provoked an increase of the mating of the males in this experiment, in F3. This allows us to conclude that the stimulating product released by the females has a volatile chemical nature.

*Conclusion.* It seems evident that the females produce a stimulus that induces mating in males. The nature of this stimulus is not sound nor sight. We can therefore conclude the existence, in *C. nubeculosus,* of a volatile pheromone released by the females and affecting frequency.

## EVIDENCE OF AN ATTRACTIVE PHEROMONE RELEASED BY THE FEMALES OF *C. NUBECULOSUS*

MATERIAL AND METHODS. *Material:*—T shaped glass tube (length of arms = 20 cm, internal diameter = 1.5 cm); arms: A1, A2, A3.—3 glass cylinders (C1, C2, C3) closed on one end with nylon mesh. One of the cylinders has small openings to allow equilization of pressure.—sliding fittings.

The experimental conditions were the same as in the first tests.

*Experimental Design* (see figure 3).

*Methods:* Males are introduced in A3 of the T tube after $CO_2$ anesthesia.

EXPERIMENTS. 1) *Evidence of attraction of the males by the females, eliminating possible effects of lighting.* In C1, 50 females were introduced, C2 remained empty. About 70 males were released in C3. The distance between the cross of the T and each cylinder was 16 cm. The males migrating in each side were counted in 4 observations. The results are given for the 4 experiments: the 1st number indicates the males counted on the side where the females were, the 2nd number on the empty side. For the 3rd and 4th tests, the position of the females was reversed in the T.

| | | | | |
|---|---|---|---|---|
| After 5 minutes: | 17/4 | 16/5 | 32/12 | 19/13 |
| After 10 minutes: | 45/15 | 40/7 | 38/13 | 42/14 |

(The remaining males stayed in A3)

2) *Placing the cylinder closer.* Same experiment but placing the cylinders 5 cm from the middle. The phenomenon is not so clear-cut, the pheromone seeming to disperse rapidly in the small space between the 3 cylinders.

3) *Non-evidence of attractive effect of males to the females.* In C1, 50 males were introduced; C2 remained empty, and about 60 virgin females were released in C3. The females migrating in each side were counted, and the experiment was reversed.

| Number of females on each side | Time in min 1 | 2 | 3 | 4 | 10 | 15 |
|---|---|---|---|---|---|---|
| Males / Ø C1 / C2 | 3/3 | 4/4 | 7/8 | 10/12 | 24/25 | 24/26 |
| Ø / Males C1 / C2 | 2/0 | 2/2 | 4/6 | 8/13 | 16/12 | 23/22 |

*Results.* The females dispersed themselves in the 2 sides of the tube; apparently there is no attractive pheromone released by the males.

4) *Elimination of possible role of sound and sight* (See figure 4). We adapted to this setting the flasks used in part one. Two day-old virgin females were placed in F1, F2 remaining empty. The airflow going through F1 was directed via tubing into A1 of T tube, an equal airflow going through F2 into A2. The airflow was set for 3 min; then the tubings were removed, eliminating contact with the females. About 70 males were then released in A3.

Four observations were made, the number of males in A1 and A2 noted according to time, as indicated in table 3. Males remaining in or returning to A3 were not counted.

The results were the same as in the other experiments, the majority of the males heading always toward the side where the air had been in contact with the females. This confirms that sound and sight have no influence.

## CONCLUSION

We can confirm the release of a pheromone by females of *C. nubeculosus.* This pheromone not only induces mating but, as could be predicted, also attracted the males. The detection of this pheromone released by the females of ceratopogonid midges is quite surprising, although this had already been reported by Lang and Foster (1976) for mosquitoes, and Kliewer et al. (1966) demonstrated that a sex attractant is produced in the female *Culiseta inornata.* These results are otherwise not in correspondence with those of other ceratopogonid workers (Linley and Carlson 1978). The implications and consequences of the knowledge of pheromones are numerous, in the field of ethology as well as in physiology and ecology. The variations of the production of this pheromone in regard to the age of the females and the effects on other species are being investigated.

## ACKNOWLEDGMENT

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| Number of males going toward A1 and A2 | Time in min | | 1 | 2 | 3 | 4 | 5 | 7 | 10 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|
| *Experiment* | *A1* | *A2* | | | | | | | | |
| Exp. 1 | Ø | Females | 0/2 | 2/3 | 4/10 | 6/15 | 7/25 | 7/24 | 8/28 | 12/41 |
| Exp. 2 | Ø | Females | 1/0 | 2/4 | 4/6 | 4/11 | 5/18 | 9/22 | 18/32 | 20/28 |
| Exp. 3 | Females | Ø | 2/0 | 3/5 | 9/4 | 11/5 | 13/10 | 25/15 | 44/16 | 45/16 |
| Exp. 4 | Females | Ø | 3/1 | 4/1 | 8/4 | 15/6 | 18/5 | 24/6 | 30/14 | 35/15 |

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# ISOLATION OF A VIABLE HOMOZYGOUS TRANSLOCATION STRAIN IN *ANOPHELES CULICIFACIES*

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ABSTRACT. A viable homozygous translocation strain has been isolated in *An. culicifacies* using genetic and cytogenetic techniques. The strain has been outcrossed to the standard Sattoki strain and has been successfully reisolated in a wild type background.

During the course of an experiment to induce, isolate and characterize chromosomal aberrations in *Anopheles culicifacies* (Baker et al. 1978), an attempt was made to isolate homozygous strains of the induced aberrations. Initially a true-breeding line of a pericentric inversion on the X chromosome, *In(X)1*, was isolated (Baker et al. 1978). Repeated attempts to isolate other homozygous aberration lines were unsuccessful although in one complex aberration line, *In(3L)T(2R,3L)1*, a few homozygous females were detected cytologically (Baker et al. 1978). In mitotic configurations, this aberration is seen as an unequal exchange between chromosomes 2R (long arm) and 3 with the longer segment from 2R translocated to 3. In the ovarian nurse cell polytene chromosomes almost all of 2R (break point:19C) has been exchanged with a large part of 3L (break point:39A; see the ovarian polytene map of Saifuddin et al. 1978). Therefore, centromere 3 of the translocated chromosome is included in the longest chromosome of the complement. In addition the translocated segment of 3L included a small paracentric inversion between 41A and 42A (for mitotic and polytene configurations see Baker et al. 1978). This paper reports the successful isolation of a homozygous strain from *In(3L)T(2R,3L)1* herein designated as *T-1*.

## MATERIALS AND METHODS

To facilitate isolation of *T-1*, two eye color mutants, rose eye (*re*, chromosome 1; Sakai et al. 1977) and maroon eye (*ma*, chromosome 2; Sakai et al. 1979) were used. All crosses were done in a rose eye