

die engere systematische Verwandtschaft berücksichtigt. Einen Hinweis auf die Rangstufe der einzelnen Formen gibt uns der Vergleich des Winkels, der von der Schläfenlappenbasis mit der Bodenebene gebildet wird (Figur 5): *Eubalaena australis*, + 36°; *Sibbaldus musculus*, + 22°; *Megaptera novaeangliae*, + 11°; *Balaenoptera borealis*, - 10°. Der Winkel ist Ausdruck des Rotationsgrades des Schläfenlappenpoles um den insulären Hilus (Temporalisation). Die Rotation ist beim Seiwal am stärksten. Der Seiwal hat auch den niedrigsten Hypothalamusquotienten (Hypothalamuslänge:Grosshirnlänge) und somit, auch wegen dem relativ geringerem Körpergewicht, die höchste Zerebralisationsstufe (PILLERI⁶).

Summary. Macroscopic anatomical investigations on the brain of the Blue Whale (*Sibbaldus musculus* Linnaeus, total brain weight without dura: 6500 g), show

that this species of cetacea has a degree of cerebralization between that of the primitive Southern Right Whale (*Eubalaena australis* Desmoulins) and that of the Humpback Whale (*Megaptera novaeangliae* Borowski). Of the Balaenopteridae examined to date, the Blue Whale has the lowest cerebralization.

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21. Juli 1966.*

⁶ G. PILLERI, J. Hirnforsch., im Druck.

STUDIORUM PROGRESSUS

Location of the Sex Pheromone in the American Cockroach, *Periplaneta americana* (L.)

The sex attractant of the American cockroach, *Periplaneta americana* (L.), may be collected on filter papers exposed to virgin females¹. Although such papers trigger the full courtship display (fluttering or outspread wings and extended abdomen) in males tested in groups, they have failed to evoke the complete display in single males¹⁻³. Conversely, the single male readily performs the full display in the presence of an 'attractive' (pheromone producing) female. In an effort to obtain a defined pheromone source as a basis for further study we investigated the site of production of the sex attractant.

During this work we found that some males removed from groups were so highly sensitive that they even performed the full display towards impregnated papers. These males retained their high sensitivity for several hours in isolation and were used as a basis for the tests.

Method. A series of 10 individual male cockroaches (3-5 weeks old) was assembled daily from 16 groups of 12 males by selecting those that responded first with the complete display when stimulated with an attractive female. Each of the 10 males was kept in a glass jar during the assay, after which the males were returned to the groups at random; thus, a series of 10 males was selected daily from 192 males.

The specimen to be assayed was held by forceps and brought between the antennae of each male at a distance of 3-5 cm from the head. It was held there for 10 sec, or less when display occurred. A response was judged to be positive when the full display occurred. Intervals of at least 3 min elapsed between tests with the same male. Contact between the antennae of the male and the assay specimen was avoided by following his movements closely. The forceps were checked with groups of males before use and were washed in hexane when contaminated. The day-night rhythm was changed before adult molt to perform the assays during the first 4 h of the dark period. All tests were made in reduced light at 22-26°C.

An attractive female was used as a control specimen between every 2 assays to indicate whether any of the

males had lost his readiness to respond. When this occurred he was replaced; over a test period of 4 h, 2-5 of the 10 males usually required replacement.

The test specimens, of both sexes, consisted of last instar nymphs, adult virgins (1-33 days old), and mated individuals (20 days old, mated 22 h before testing, and 3-11 months old with the date of mating unknown). The intact specimen, its severed head, and the remainder of the body were assayed. The sequence of tests with parts of the body was random. In addition, a dummy - the head of a dried male extracted with hexane - was used as one control, and the heads of mature virgin females were taken as another control in tests with heads of nymphs.

When 9 or 10 identical responses were obtained from a series of 10 tests with the intact specimen or its parts, the test series was not repeated. When fewer than 9 identical responses occurred, the test series was repeated once or twice with the same series of 10 males, and the mean % response was calculated. The assays with each specimen, its head and the remainder of the body were replicated 7 times by using 7 specimens. To reduce correlations, only one specimen of the same age and sex was assayed the same day. The arc sin \sqrt{P} transformation, where P = mean % response, was applied to all mean responses, and after statistical analysis, the appropriate means and confidence limits were transformed back to percentages.

Results. The response of the males to the test specimens is shown in Figure 1. The difference in the effectiveness of the females or their parts showed a significant dependence on age and mating ($P \sim 0.01$). In contrast, age and mating caused no significant difference in the effectiveness of males or their parts ($P \sim 0.05$). The display of males, to males or their parts, was of particular interest (Figure 2).

¹ L. M. ROTH and E. R. WILLIS, Am. Midl. Nat. 47, 66 (1952).

² D. R. A. WHARTON, G. L. MILLER, and M. L. WHARTON, J. gen. Physiol. 37, 461 (1954).

³ Own observation.

The strength of the excitation, judged by the frequency of wing fluttering and the degree of wing spreading, could not be measured except by subjective observation. We noticed independently that the response to the severed head was nearly as strong as the response to the whole insect and much stronger than the response to the remainder of the body. (This observation is indicated in Figure 1 by the use of bold signs for the intact specimen and the head, and light symbols for the headless body.)

The males hesitated for a fraction of a second before they displayed when parts of an attractive specimen (the head or the remainder of the body) were offered. Therefore, shape may influence the response, but to a minor degree. A full display released by the dummy (extracted head of a male) was seen 3 times in 7×10 tests; however, this occurred significantly less than in tests with heads of 35 female nymphs ($P < 0.01$) and 35 male nymphs ($P < 0.01$).

In additional experiments, without statistical treatment, the head and remainder of the body were further dissected. Assays with parts of strongly attractive heads showed no effect for the brain and glandular tissues, a positive effect for the antennae and the integument of the frons, and a weak effect for the remainder of the head. Assays with parts of the remainder of the body showed

that the thorax was as effective as the abdomen, and that the tip of the abdomen was less effective than the entire abdomen.

Discussion. Only 2 instances are known in which a head gland furnishes a sex attractant, the mandibular glands of male aculeate Hymenoptera⁴⁻⁷ and the queen of the honey bee, *Apis mellifica* L.^{8,9}. In the American cockroach, we could find no correlation between a ducted gland and the sex pheromone; on the contrary, pieces of integument from the frons triggered the complete display. Therefore, the sex attractant is probably produced in differentiated areas of the integument located mainly on the head and to a much smaller extent on the remainder of the body. Secretion of sex pheromone by the integument occurs in *Prodenia litura* (F.) [= *Spodoptera litura* (F.)] and *Bombyx mori* (L.); in these moths, the sex

⁴ A. HAAS, *Naturwissenschaften* 39, 484 (1952).

⁵ B. KULLENBERG, *Zool. Bidr. Uppsala* 31, 253 (1956).

⁶ G. STEIN, *Naturwissenschaften* 50, 305 (1963).

⁷ G. STEIN, *Biol. Zbl.* 82, 343 (1963).

⁸ N. E. GARY, *Science* 136, 773 (1962).

⁹ J. PAIN and M. F. RUTTNER, *C. r. Acad. Sci., Paris* 256, 512 (1963).

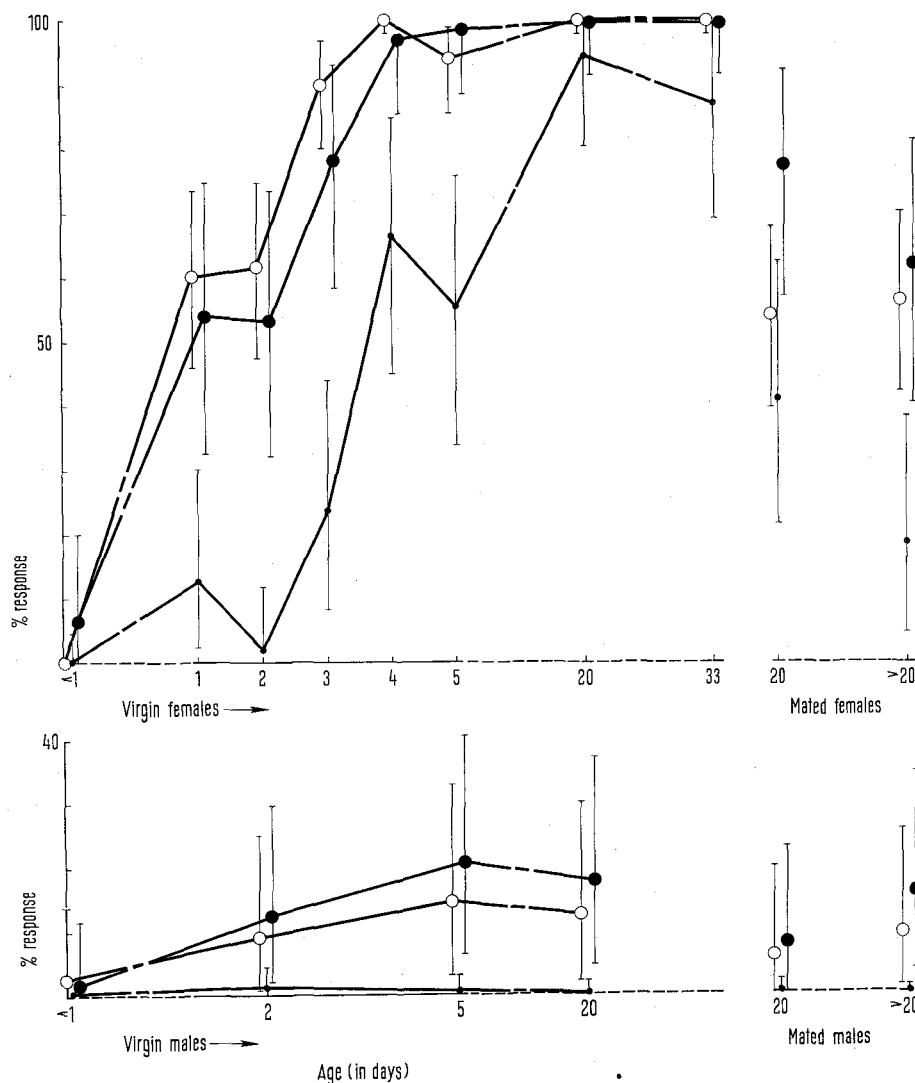


Fig. 1. Stimulation-response curves and 95% confidence limits derived from the complete courtship display of selected single male in response to specimens of both sexes and their parts. Each trial (○ insect, ● its severed head, and ◐ remainder of the body) was assayed with a series of 10 males and replicated 7 times. Each mean is based on at least 7×10 tests or up to 210 tests in the case of repetitions. (< 1) last instar nymph, (> 20) specimens 3–11 months old.

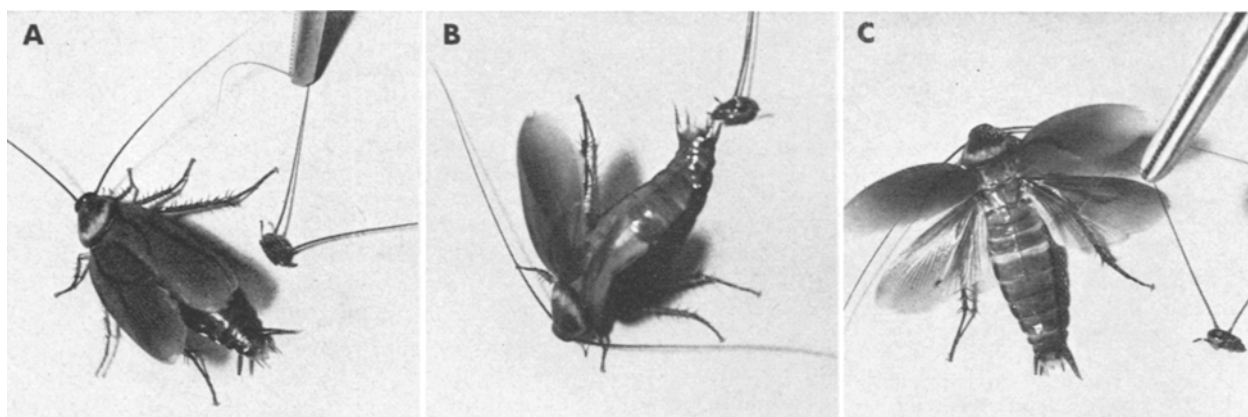


Fig. 2. Phases of the courtship display of a male American cockroach stimulated by a head of a female held between his antennae. (A) initial response, the male orientates his abdomen toward the head and raises his wings slightly; (B and C) two forms of the complete display, (B) fluttering wings and arched abdomen, (C) extended abdomen and motionless outspread wings. The photographs were taken on a vertical surface.

attractant is produced by modified epidermal cells of abdominal sacs and diffuses through the layers of the cuticle to the outside¹⁰⁻¹². Similarly, the trail pheromone of the termite *Zootermopsis nevadensis* (Hagen) also diffuses through differentiated epidermal cells to the outside^{13,14}.

The sexual excitement elicited by males may explain why single males taken from groups display more readily than males kept isolated, and why males in groups sometimes display without apparent stimulus¹. These males are exposed to the pheromone of other males and maintain a higher level of sexual excitement. ROTH and DATEO¹⁵ found that extracts of males of 9 species of cockroaches (*Periplaneta americana* included) released sexual behavior in females of the cockroach *Nauphoeta cinerea* (Olivier); but these extracts were not assayed with males. The weak effectiveness of *Periplaneta americana* nymphs of both sexes as a releaser of the courtship display was not without precedent since male and female nymphs of the German cockroach, *Blattella germanica* (L.), release the courtship display in adult males of the same species¹.

The pheromone of the American cockroach is not a sex attractant in the strict sense – an attractant and aphrodisiac produced by only one sex – but is a sexual stimulus released by both sexes. In low concentrations it may also be a social pheromone that attracts members of the same or possibly several species of cockroaches.¹⁶

Zusammenfassung. Das Vorkommen von Sexualpheromon wurde an dem Werbeverhalten von selektierten einzelnen Männchen getestet. Das Pheromon kommt in larvalen und adulten Insekten beiderlei Geschlechts vor, wird aber von geschlechtsreifen Weibchen in grösseren Mengen produziert. Es kommt hauptsächlich im Kopf vor und wird wahrscheinlich an Orten modifizierter Körperdecke abgesondert.

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¹⁰ S. M. HAMMAD and H. J. JARCZYK, Bull. Soc. ent. Egypte 42, 253 (1958).

¹¹ R. A. STEINBRECHT and D. SCHNEIDER, Naturwissenschaften 51, 41 (1964).

¹² R. A. STEINBRECHT, Z. Zellforsch. mikrosk. Anat. 64, 227 (1964).

¹³ A. M. STUART, Proc. zool. Soc. London 143, 43 (1964).

¹⁴ P. SATIR and A. M. STUART, J. Cell Biol. 24, 277 (1965).

¹⁵ L. M. ROTH and G. P. DATEO, J. Insect Physiol. 12, 255 (1966).

¹⁶ The statistical analyses and interpretations by Dr. W. F. KWOLEK, Biometrical Services, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois, are gratefully acknowledged.

PRO EXPERIMENTIS

Eine neue Methode zum schnellen Einfrieren von Geweben

Für Versuche, einzelne morphologische Zell- und Gewebestandteile aus gefriergetrockneten Gewebepulvern ohne Verwendung von Flüssigkeiten zu gewinnen, wurden größere Mengen Gewebepulver benötigt. Die Hauptschwierigkeit bestand für uns im einwandfreien Einfrieren größerer Gewebemengen. Vor kurzem veröffentlichten wir eine Methode¹, die eine hohe Einfriereschwindigkeit erlaubt. Da diese Methode für unsere Zwecke zu umständlich war, suchten wir nach einem anderen Weg. 1952 ga-

ben wir ein Verfahren² an, bei dem das Gewebe gemeinsam mit fester Kohlensäure in einem Starmix zerkleinert wird. Wir fanden nun, daß sich die Kohlensäure unter gewissen Bedingungen durch flüssigen Stickstoff ersetzen läßt.

Das Prinzip ist folgendes: Ein Schlagmesser rotiert mit hoher Geschwindigkeit (20000 U/min) in flüssigen Stick-

¹ M. BEHRENS, W. NEU und R. THALACKER, Experientia 22, 265 (1966).

² M. BEHRENS, Hoppe-Seyler's Z. physiol. Chem. 291, 245 (1952).