Entomology Papers from Other Sources        Entomology Collections, Miscellaneous

1978

# Sex Pheromone of the Tsetse Fly: Isolation, Identification, and Synthesis of Contact Aphrodisiacs

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to surrender. These four cases have been treated as suicide, because the murderer could have avoided death had he so desired. Cases like these, in which the victim precipitates his own death, have long been considered by some researchers to be a type of suicidal behavior. See, for example, M. Wolfgang, in *Suicidal Behaviors*, H. Resnik, Ed. (Little, Brown, Boston, 1968), pp. 90–104.

6. Two of these stories (Kline and Bray) occurred at almost the same time. In accord with previous procedure (2) they were treated as one to avoid problems of statistical dependence. Information on television network evening news coverage was provided by *Vanderbilt Television News Archives* (Joint University Libraries, Nashville, periodic issues). Weekend stories before July 1970 were excluded from the analysis, because until then weekend broadcasts were not recorded in the archives.

7. The particular 2-week period studied was chosen so as to make Fig. 1 comparable with earlier results for automobiles (2). The graph of airplane accidents after murder-suicide stories resembles that of automobile accidents after suicide stories. In both, there is a primary peak on day 3 after the publicized death and a secondary peak on day 8. This secondary peak is at present unexplained.

8. An alternative approach to testing statistical significance would be to use one or another variant of the *t*-test to determine whether the number of crashes on day 3 is significantly larger than the number to be expected from an analysis of the crashes in the other 13 days studied. This approach is probably not valid, because the data do not meet the assumptions of the *t*-test. For the reader who wishes, nonetheless, to use the *t*-test, the following are the number of multifatality crashes from 2 days before to day 11 after the story: 7, 8, 12, 6, 10, 19, 9, 10, 13, 12, 14, 15, 12, 9.

9. *Ayer Directory of Publications* (Ayer, Philadelphia, yearly volumes).

10. The top nine states with respect to ownership of operative U.S. civil aircraft are (in order) California, Texas, Ohio, Illinois, Florida, Michigan, New York, Pennsylvania, and Washington [Department of Transportation, *Census of U.S. Civil Aircraft 1970–1971* (Government Printing Office, Washington, D.C., 1971), table 7].

11. The nine newspapers examined are *Los Angeles Times*, *Dallas News*, *Cleveland Plain Dealer*, *Chicago Tribune*, *Miami Herald*, *Detroit News*, *New York Times*, *Philadelphia Bulletin*, and *Seattle Press Intelligencer*. For three states (Texas, New York, and Washington) the largest newspaper was not easily available, and the second largest paper was used (9).

12. This significance level is valid only under the assumption that the joint distribution of the two variables is bivariate normal. I do not know whether this assumption holds for these data. The assumption is not required for assessing the significance of the Spearman $\rho$ ($\rho = .707$, $P < .005$, one-tailed correlation, corrected for ties, for the data in Table 1).

13. Because of the failure of recording equipment and other factors, the Vanderbilt Television News Archives does not have information on the total number of networks covering the stories about Chegwin, Kline, or McLachlan. Consequently, these stories were excluded in the calculation of the correlation between the television publicity devoted to a story and the number of crashes after that story. The correlation with television publicity may be weak because the networks almost never reported the murder-suicides as lead stories. In contrast, the newspapers studied did treat the murder-suicides under examination as lead stories and carried them, very visibly, on page 1.

14. For these stories, the partial correlation between newspaper coverage and the number of multifatality crashes (correcting for television coverage) is .683. Single-fatality crashes should not be triggered by murder-suicide stories; hence, the fluctuation of single-fatality crashes after a story should not be correlated with the amount of newspaper publicity devoted to that story. This prediction is consistent with the data ($r = -.050$).

15. Because television publicity seems to be nonsignificantly related to plane crashes, television news coverage was ignored in this analysis, and stories receiving no newspaper coverage (only television publicity) were excluded from the analysis.

16. P. Hoel, *Introduction to Mathematical Statistics* (Wiley, New York, 1965), pp. 116–117; F. Mosteller, R. E. K. Rourke, G. B. Thomas, *Probability with Statistical Applications* (Addi-

son-Wesley, Reading, Mass., 1970), table 3-3. In this analysis, $N = 121$ rather than 156 as in the binomial analysis, because the present analysis omits crashes associated with five stories publicized only on television and three missing planes that crashed in unknown locations. One would prefer to code crashes by "place of take-off" rather than by "place of crash" but this is not possible because the publication describing the crashes studied (3) often does not provide information on place of take-off.

17. The day-3 peak might be argued to result from a fortuitous association between a day-of-the-week cycle in murder-suicide stories and a day-of-the-week cycle in multifatality plane crashes. For example, if most murder-suicides occurred on Wednesday and most plane crashes occurred on Saturday, crashes would peak 3 days after murder-suicides, even if murder-suicides had no effect on plane crashes. However: (i) If the day-3 peak were due to a day-of-the-week cycle in plane crashes, the peak in crashes on day 3 should be followed by an equally large peak 1 week later (on day 10), 2 weeks later, and so on. There is no evidence of a peak on a day 10. In fact the number of crashes on this day (12) was almost precisely equal to 11.14, the number to be expected if crashes are uniformly distributed from day $-2$ to day $+11$. (ii) More generally, if the "day-of-the-week" argument were correct,

there should be a strong, positive correlation $r_{x,x+7}$ between the number of multifatality crashes on day x and the number of multifatality crashes on day $x + 7$. If this argument is not correct, then $r_{x,x+7}$ should be approximately 0. For the data displayed in (8) $r_{x,x+7} = -.0206$. This does not support the "day-of-the-week" argument. (iii) If the "day-of-the-week" argument were correct, there would be no causal connection between publicized murder-suicides and plane crashes; hence, there should be no correlation between the amount of publicity given to a murder-suicide story and the number of crashes thereafter. In addition, there should be no correlation between the location of the publicity devoted to the murder-suicide and the location of the plane crashes occurring just afterward. Both of these predictions are inconsistent with the data.

18. I thank M. Murphy, city editor of the *Los Angeles Times*; M. Pritchett and J. Pilkington, Vanderbilt Television News Archives; J. Coolman and J. Cruse, University Library, University of California, San Diego; H. Field, R. Kuever, S. Ostroff, and S. Newcomb for helping to collect and analyze data; and B. Berger, F. Davis, M. Davis, J. Gusfield, C. Mukerji, and J. Wiseman for comments and criticisms.

16 December 1977; revised 28 April 1978

# Sex Pheromone of the Tsetse Fly: Isolation, Identification, and Synthesis of Contact Aphrodisiacs

Abstract. *Sex pheromones isolated from the cuticle of the female tsetse fly,* Glossina morsitans morsitans *Westwood, release mating behavior in the male fly at ultrashort range or upon contact with baited decoys. Three active components were identified as 15,19-dimethylheptatriacontane, 17,21-dimethylheptatriacontane, and 15,19,23-trimethylheptatriacontane. Chemical and biological comparisons show that the natural and synthetic compounds are identical.*

The tsetse fly *Glossina morsitans morsitans*, a carrier of trypanosomes, is the major vector of Rhodesian sleeping sickness. The use of domesticated animals across tropical Africa is limited to areas free of this fly owing to transmission of trypanosomes in animals and the resulting disease, nagana (1). We now report the isolation, identification, and synthesis of sex recognition pheromone components (2) produced by the female of this species of tsetse fly. Three compounds were isolated from female flies, and all of these independently cause release of mating attempts by the male at ultrashort range or upon contact. These three compounds are 15,19-dimethylheptatriacontane (1), 17,21-dimethylheptatriacontane (2), and 15,19,23-trimethylheptatriacontane (3).

A sex recognition pheromone that stimulates males on contact was demonstrated in *G. morsitans* and was shown to be associated with the nonpolar cuticular lipid extract of adult female flies (2). Adult males initiated sexual behavior but did not persist in attempts to mount and copulate with live or dead male flies or with females thoroughly extracted with solvent. Live or dead females gave rise to male sexual behavior subjectively described as (i) mounting, (ii) attempting to

orient to the copulating position, and (iii) flexing of genitalia with attempted engagement of the genitalia in 76 to 79 percent of the tests. Live males showed these strong stimulant activity responses when presented with a black shoelace knot (pseudofly) treated with extracts of dead females. Copulatory attempts began apparently only after contact with the pseudofly (2). These observations were consistent with those of previous workers who were unable to demonstrate a sex attractant operating at long range and who observed that "the male does not seem to identify the female fly until within 1–2 cm" (3). However, these workers (3) were not able to determine how the male differentiates between another male or another species and noted "the apparent absence of a mating stimulus other than visual" (3).

Since the initial identification of an attractant of the male housefly, *Musca domestica* L. (4), that causes aggregation of males and females in the field (5), male mating stimulants have been identified in several species of muscoid flies. A series of 1,5-dimethyl paraffins was reported in extracts of female flies of *Stomoxys calcitrans* (L.) (6), while *Fannia canicularis* (L.) and *M. domestica* (7) have male stimulatory compounds identified as

long-chain branched hydrocarbons, and *Musca autumnalis* DeGreer (8) females have long-chain olefins that stimulate males. Long-chain hydrocarbons have been described (2) and a major unidentified 42-carbon branched paraffin was reported in *G. morsitans* (9).

Initial biological tests were made with a mature (> 7 days old) male *G. morsitans* in a stoppered glass tube (2.5 by 7 cm) and a black shoelace knot treated with natural materials or synthetic compounds. For quantitation of active materials, a nonadsorptive decoy was used; the decoy consisted of a frosted glass bead (6.5 to 7.5 mm in diameter) glued to a steel pin with epoxy resin adhesive and spotted with hexane solutions of candidate compounds. The tube was moved and tapped by hand to induce the fly to make contact with the decoy because males did not readily strike at stationary active decoys. If no sexual activity was forthcoming after three contacts, the score was recorded as nil. The technique proved satisfactory for assessing male mating responses to increasing amounts of the test compounds. Either 10 or 25 males were tested against each quantity of material, with progressive scoring as follows: arresting of male movement on the pseudofly (+), characteristic copulatory movements with orientation to the copulating position (+ +), and full mating response with flexing and attempted engagement of the genitalia (+ + +) (2).

Active extracts were obtained from mature (> 2 days old) female flies (from the colony at Bristol) that were immobilized at 4°C and rinsed with diethyl ether (1 ml per fly); the ether washing was evaporated to dryness at reduced pressure. The residue was reconstituted in hexane for testing and separation (Table 1). A concentrate obtained from several hundred flies was eluted from a column (2 by 45 cm) of activated silica gel (60 to 200 mesh, Baker) with hexane containing

increasing quantities of ether. The active fraction, which contained only hydrocarbons, was eluted with hexane; the more polar fractions were inactive. These hydrocarbons were then eluted from a column of silica gel impregnated with silver nitrate (10); only the first hexane fraction, which contained paraffins, was active. Gas chromatography (GC) (11) showed the presence of 22- to 44-carbon hydrocarbons, which were separated by preparative GC (12) for bioassay. Full activity was associated only with compounds that eluted in two peaks at KI 3750 and 3770 [Kovats indices 3700 and 3800 (13) refer to unbranched paraffin standards having 37 and 38 carbons]. The mass spectrum of the peak at KI 3750 showed major fragments at $m/e$ 224, 225, 252, 253, 280, 295, 323, 351, and 533 (M − 15). Only the fragments having an even number of carbons show doublets with the even mass predominating (that is, $m/e$ 224 > 225 for 16 carbons) (Fig. 1, left). These are caused by cleavage internal to the methyl branch closest to each end (14) and are consistent with two long-chain dimethyl-branched paraffins with 37 carbon backbones. Two structures account for all of the observed fragments; symmetrical 17,21-dimethylheptatriacontane (1) and 15,19-dimethylheptatriacontane (2). The mass spectrum of the peak at KI 3770 showed major fragments at $m/e$ 224, 225, 295, 365, and 547 (M − 15), the first two being a doublet caused by cleavage internal to a methyl branch (Fig. 1, right). All fragments are consistent with one symmetrical structure, 15,19,23-trimethylheptatriacontane (3). The compounds were present at 1.2, 1.2, and 4.2 μg per female, respectively, and constituted 37 percent of the paraffins present per female or 9.4 percent of the total hydrocarbons.

Extracts of mature virgin male flies were taken through the same scheme. Both GC and GC-MS (mass spectrosco-

py) analysis showed that 1, 2, and 3 were present, but in much reduced quantities (0.09, 0.09, and 0.23 μg per male).

Compound 1 was obtained from another source (6, 15). Compound 2 was synthesized by the addition of methyl cyclopropyl ketone to the Grignard formed from 1-bromohexadecane, and when the resulting cyclopropylhexadecylmethylcarbinol was stirred overnight with 48 percent HBr, the formation of 1-bromo-4-methyl-3-eicosene occurred via the Julia rearrangement (16). The bromide and triphenylphosphine were heated overnight in acetonitrile to form the Wittig salt. The salt was dissolved in tetrahydrofuran cooled to 5°C; *n*-butyl lithium was then added. After 30 minutes, the red solution was stirred at −30°C during addition of 2-octadecanone in tetrahydrofuran. The solvent was removed, the residue was dissolved in hexane, the hexane solution was washed with water, dried, and eluted from a column of silica gel (2 by 45 cm) with hexane to give a mixture of *cis*- and *trans*-dimethyl diolefins. The olefins were hydrogenated at atmospheric pressure over 5 percent palladium catalyst, and subsequent GC analysis showed the disappearance of olefin peaks at KI 3710 to 3770 and appearance of one peak at KI 3750.

Compound 3 was synthesized by the addition of methyl cyclopropyl ketone to the Grignard compound formed from 1-bromotetradecane, and the resulting cyclopropylmethyltetradecylcarbinol was stirred overnight with 48 percent HBr to form 1-bromo-4-methyl-3-octadecene via the Julia rearrangement. The bromide was distilled (148° to 150°C at 0.1 mm), and converted to the magnesium Grignard in ether, which was then reacted with a half-molar equivalent of ethyl acetate to form a tertiary alcohol. Treatment of the alcohol under reflux with *p*-toluenesulfonic acid in toluene yielded four major trimethyl *cis*- and *trans*-tri-
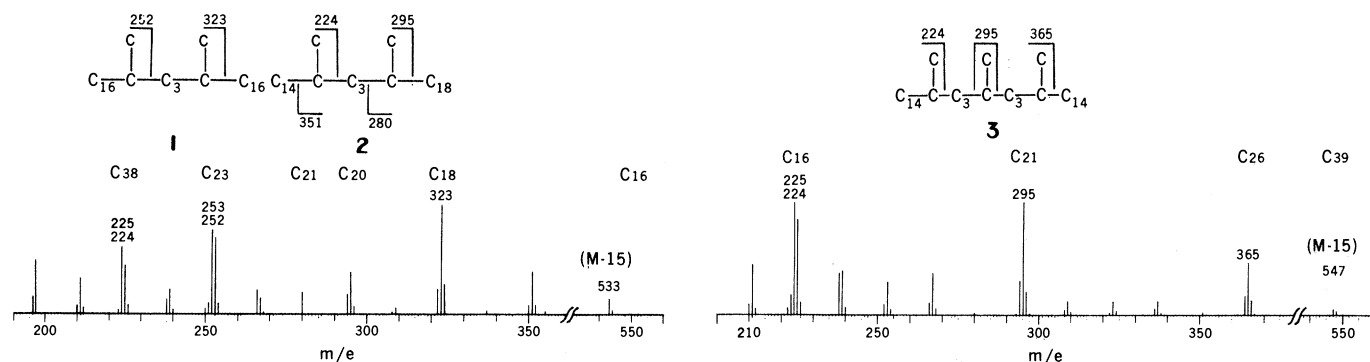


Fig. 1. Mass spectra of cuticular paraffins extracted from mature virgin female *Glossina morsitans*. (Left) 17,21-Dimethylheptatriacontane and 15,19-dimethylheptatriacontane coeluting at KI 3750; (right) 15,19,23-trimethylheptatriacontane eluting at KI 3770.

olefins that were eluted from a silica gel column with hexane. These olefins were then hydrogenated, and GC analysis showed the disappearance of olefin peaks from KI 3725 to 3820 and the formation of one peak (3) with KI 3770. Mass spectra and GC retention times of the natural and synthetic 1, 2, and 3 were coincident and appeared identical in all respects (14).

Probit analysis of behavioral data for 3 showed that the threshold of copulatory attempts (+++) was exceeded in 50 percent of the population (ED$_{50}$) at an estimated dosage of 13.3 $\mu$g (fiducial limits at 0.05 were 5.3 to 24.8 $\mu$g, Student's $t$-test for five doses). A linear response was obtained within the range of doses tested (4 to 100 $\mu$g), with ED$_{10}$ at 1.9 $\mu$g, and ED$_{95}$ at 153.1 $\mu$g. Data for 1 and 2 were not subjected to probit analysis, but comparison of scores at dosages of 20 $\mu$g show that 3 released responses four times more often than 1, and 14

times more than 2. The addition of both synthetic 1 and 2 to 3 did not cause an increase in the intensity or duration of sexual activity. Of other synthetics tested, only 13,17,21-trimethylheptatriacontane had some activity. Decoys that were untreated or had been cleaned by Soxhlet extraction with chloroform never released sexual activity, as test males would perch quietly on or near them. But when sexual behavior was released, excited males ardently grasped active decoys, held tenaciously with extended legs (++), and often had to be forcibly separated from the sex object—especially when the hypopygium was extended to grasp the object (+++). Lower scores with increased amounts of 3 appeared due to the greasy layer of material on the bead, as flies had difficulty maintaining their hold. This decline in response was not seen when an absorptive decoy such as a knot was used. Corks, silicone rubber decoys, and freshly killed

male flies were treated with synthetic compounds for comparison purposes, which showed that responses could be obtained with equivalent doses regardless of the material and color of decoy used. However, it was necessary for the chemical to be presented on a surface of the correct configuration, that is, having a size and shape similar to that of a female fly.

The visual component of attraction to a mate is important in the tsetse fly, but sex recognition on contact is probably mediated through the tarsal chemoreceptors described by Lewis (17). Sexual behavior of Glossina males is thus determined by the presence of these stimulants, once potential mates are in close proximity. These are the least volatile and most stable pheromones yet reported; a natural sample retained full activity despite fractionation and six trips across the Atlantic Ocean, as did a treated shoelace knot which had been left exposed on a bench top for a month.

Compounds 1, 2, and 3, and possibly similar compounds in other tsetse species, may prove useful in rearing experiments by promoting fecundity in otherwise reluctant colonized flies, or in arresting male flies lured to traps, decoys, or animals in the field.

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Table 1. Hierarchy of mating responses of adult male G. morsitans to pseudoflies receiving various treatments; +++, full mating response with flexing of genitalia; ++, characteristic copulatory movements; +, arresting of movement. Percentages in all tests add to 100 percent; the column for no response is omitted; eq, equivalent.

| Material tested | Amount | Percentage of flies responding | | |
|---|---|---|---|---|
| | | +++ | ++ | + |
| *Female natural products** | | | | |
| Crude extract | 1 fly eq* | 50 | 0 | 0 |
| | 2 fly eq | 95 | 0 | 0 |
| | 100 fly eq | 100 | 0 | 0 |
| Hydrocarbons | 40 fly eq | 100 | 0 | 0 |
| Paraffins | 6.5 fly eq | 100 | 0 | 0 |
| | 8.5 fly eq | 100 | 0 | 0 |
| | 50 fly eq | 100 | 0 | 0 |
| Preparatory GC fractions | | | | |
| KI 3500–3735 | 4 $\mu$g, 15 $\mu$g | 0 | 0 | 0 |
| KI 3740–3755 | 4 $\mu$g | 0 | 0 | 0 |
| | 15 $\mu$g | 0 | 0 | 40 |
| KI 3760–3780 | 4 $\mu$g | 60 | 20 | 20 |
| | 16 $\mu$g | 80 | 20 | 0 |
| *Synthetic‡* | | | | |
| Compound 1† | 4 $\mu$g | 4 | 36 | 16 |
| | 10 $\mu$g | 8 | 44 | 4 |
| | 20 $\mu$g | 12 | 16 | 24 |
| | 40 $\mu$g | 8 | 48 | 12 |
| | 100 $\mu$g | 8 | 28 | 28 |
| | 200 $\mu$g | 60 | 8 | 0 |
| Compound 2† | 4 $\mu$g | 0 | 0 | 0 |
| | 10 $\mu$g | 0 | 0 | 16 |
| | 20 $\mu$g | 4 | 4 | 12 |
| | 40 $\mu$g | 12 | 12 | 24 |
| | 100 $\mu$g | 12 | 12 | 36 |
| | 200 $\mu$g | 15 | 12 | 32 |
| Compound 3† | 4 $\mu$g | 24 | 24 | 16 |
| | 10 $\mu$g | 40 | 36 | 4 |
| | 20 $\mu$g | 56 | 28 | 4 |
| | 40 $\mu$g | 80 | 16 | 0 |
| | 100 $\mu$g | 92 | 4 | 4 |
| | 200 $\mu$g | 92 | 8 | 0 |
| | 400 $\mu$g | 84 | 16 | 0 |
| 13,17,21-(CH$_3$)$_3$C$_{37}$H$_{73}$* | 1000 $\mu$g | 40 | 60 | 0 |

*Tested on shoelace pseudoflies (measured as fly equivalents) and hence subject to errors due to absorption.    †Tested quantitatively using glass spheres.    ‡$n$-Paraffins of 34 to 44 carbons were inactive at 1000 $\mu$g each.

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10. With a column of Adsorbosil-CABN, 100 to 140 mesh, 25 percent silver nitrate on silica gel (Applied Science Laboratories, State College, Pa.), paraffins, monoolefins, and polyolefins were eluted with 0, 1, and 10 percent ether in hexane, respectively.

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11. Varian model 2100 instrument, with FID detector and a glass column (1.8 m by 2 mm, inside diameter) containing 3 percent SE-30 on 120 to 140 mesh Gas-Chrom Q at 275°C.
12. Varian model 90-P instrument, equipped with thermal conductivity detector and a 5 percent SE-30 column was temperature programmed from 150° to 280°C.
13. E. Kovats, Adv. Chromatogr. 1, 229 (1966). Authentic straight-chain hydrocarbons were used (Analabs, Inc., North Haven, Conn.).
14. The position and number of methyl branches were determined for simultaneously eluting isomers [D. R. Nelson, D. R. Sukkestad, R. G. Zaylskie, J. Lipid Res. 13, 413 (1972)] with a Varian MAT CH5 instrument equipped with a glass column (3 m by 2 mm, inside diameter) containing 3 percent OV-101 held at 275°C and a membrane separator. Dimethyl-substituted paraffins eluted on a nonpolar GC column half-way between n-paraffins of the same and the next higher number of carbons. Any one of the optical isomers of these compounds could be more active than the racemic synthetic compounds used in bioassays.
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28 December 1977; revised 3 April 1978
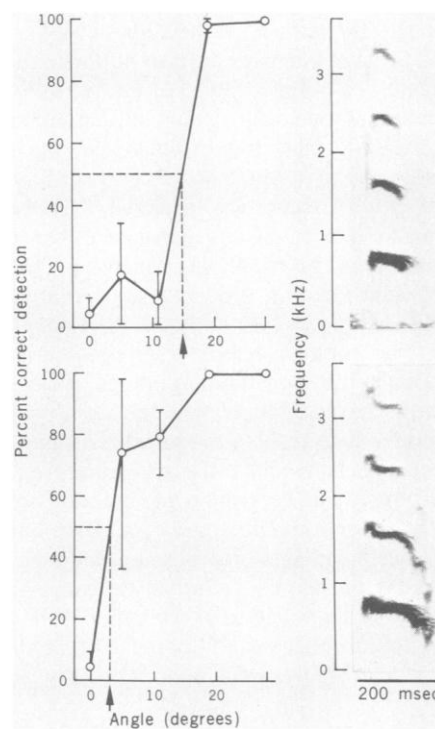
# Localization of Primate Calls by Old World Monkeys

Abstract. Monkeys (Macaca) were trained by operant conditioning techniques to report the minimum detectable change in location of a sound in space, and were tested with a series of recorded coo or clear call vocalizations. Acuity of localization varied from approximately 4° to 15° and was a function of the magnitude of the change in pitch (frequency modulation) of the different clear calls.

Several lines of evidence suggest that an evolutionary premium has been placed on the ability of animals to locate the origin of sound in space (1). The location of other animals may be revealed by acoustic signals and as a consequence sound localization is likely to play a fundamental role in a variety of biologically critical events including predator avoidance, the capture of prey, territory delineation, and the location of kin, competitors, and prospective mates. While the biological importance of auditory localization has long been recognized, only recently has localization in species other than man, or of signals other than tones or clicks, been seriously considered (2). Both field and quasi-field observations have shown that under certain conditions individuals of many species may approach, and hence must localize, vocalizing members of their species (3). Indeed, certain vocalizations may function to reveal the location of the vocalizer, yet the acuity with which they are localized or the design features which enhance localization are poorly understood.

This is the first report of an ongoing series of experiments which assess through psychophysical techniques the locatability of biologically relevant signals in Old World monkeys of the genus Macaca. The data presented here (i) show that different macaque coo vocalizations (approximately equal in duration and loudness) vary substantially in the accuracy with which they may be located in space and (ii) demonstrate that the degree of modulation in frequency of the call's fundamental (change in pitch)

is a critical design feature for auditory localization.

The macaques employ an extensive and varied vocal repertoire (4, 5). Macaque coos or clear calls, a family of harmonically structured musical sounding

calls, are of particular interest because recent field observations have shown that different vocal subtypes are emitted in restricted social contexts and may function selectively and precisely in one of a variety of situations including sexual solicitation, contact initiation, position marking, and dispersal (5). The data which follow suggest that acoustic differences in this class of vocalizations also so influence the ability of conspecifics to determine the location of the vocalizer.

One rhesus (Macaca mulatta) and two pig-tailed macaques (M. nemestrina) were trained by operant conditioning procedures to report when they detected a change in the horizontal position (azimuth) of a sound in space. The monkeys, positioned in a primate chair in an anechoic chamber, learned to place their hand on a response disk. Contact with the disk produced a recorded macaque vocalization (about 50 dB above the monkey's threshold, 140 to 205 msec in duration) which was repeated once per second from the standard speaker located at zero degrees azimuth (that is, straight ahead). Following a variable number of presentations from the standard speaker the call was delivered from
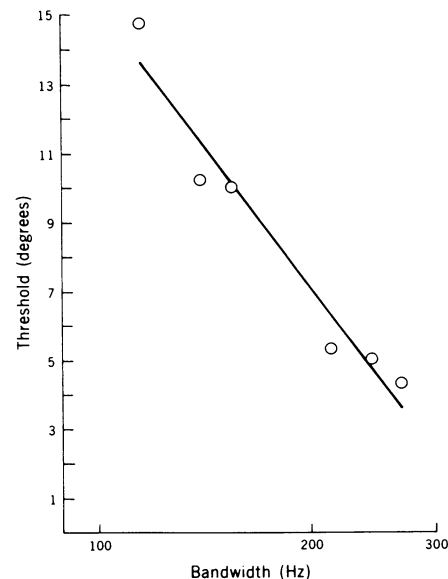


Fig. 1 (left). Psychophysical functions for the locatability of macaque clear calls. The top panel presents detection for a hard-to-locate call; the bottom panel presents localization for an easy-to-locate call. Sonagrams of each call are displayed to the right, and were constructed by a Kay 7029 A sonograph equipped with a 6076 C scale magnifier. Each function was constructed from approximately 10,000 trials presented to the three monkeys in eight experimental sessions. The data are averaged for the three monkeys; the range in performance between subjects is represented by the vertical bars. Threshold, the 50 percent detection locus, is denoted by the arrowheads.    Fig. 2 (right). The localization threshold of six clear calls. Threshold, averaged across monkeys, is plotted as a function of the range of frequency modulation or effective bandwidth of the call. The diagonal line is the least squares regression of threshold on call bandwidth and satisfies the equation: threshold = − 27 log[call bandwidth] + 69. The correlation coefficient between threshold and the bandwidth of the call is − .98.