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Analysis and identification of specialized sounds possibly used by the Caribbean fruit fly, Anastrepha suspensa (Loew), for communication purposes

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To the Graduate Council:

I am submitting herewith a dissertation written by J. C. Webb entitled "Analysis and identification of specialized sounds possibly used by the Caribbean fruit fly, Anastrepha suspensa (Loew), for communication purposes." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Biosystems Engineering.

John J. McDow, Major Professor

We have read this dissertation and recommend its acceptance:

John I. Sewell, Walter L. Green, Smith Worley

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

November 16, 1973

To the Graduate Council:

I am submitting herewith a dissertation written by J. C. Webb entitled "Analysis and Identification of Specialized Sounds Possibly Used by the Caribbean Fruit Fly, *Anastrepha suspensa* (Loew), for Communication Purposes." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Agricultural Engineering.

John J. McDow
Major Professor

We have read this dissertation
and recommend its acceptance:

Walter L. Green

John I. Sewell

Smith D. Worley, Jr

Accepted for the Council:

Hilton A. Smith
Vice Chancellor for
Graduate Studies and Research

ANALYSIS AND IDENTIFICATION OF SPECIALIZED SOUNDS POSSIBLY USED BY THE CARIBBEAN FRUIT FLY, *ANASTREPHA SUSPENSA* (LOEW), FOR COMMUNICATION PURPOSES

A Dissertation
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

by
J. C. Webb
December 1973

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ABSTRACT

Investigations were made to isolate, analyze, and identify the specialized sound produced by the male Caribbean fruit fly, Anastrepha suspensa (Loew), before pair formation and to determine if this sound is used as one mode of communication in the fly's sexual behavior. Three other sounds were identified and related to specific behavioral activities. These experimental data were to provide information on one of several factors that is needed to develop effective control methods for this and other closely related insect species.

These sounds were recorded with the flies located in an anechoic chamber. Calibrated condenser microphones were used as the transducers to detect these sounds, to convert them to electrical signals, and to transfer them to instrumentation located outside of the anechoic chamber. After the sound pressure level was recorded, the data were stored on magnetic tape for later analysis and identification. The data were analyzed for frequency, waveform, and pulsed information. A power spectral density or a frequency spectrum was calculated and plotted for each sound signal on a real-time spectrum analyzer and an x-y recorder. The power spectral density and the frequency spectrums were compared for each sound signal for similarities and differences.

A bioassay test system was designed and constructed to measure the response of both the males and the females to the reproduced calling sound alone or in combination with pheromone extract. The test insects were allowed to move toward the treatment, away from the treatment, or to remain in the entrance cell.

Four sounds from the male flies were isolated and identified and each related to a specific behavioral activity; these activities are flight, aggression, premating, and calling. Each of these sounds was found to contain different information and each has its own distinct frequency signature. The calling sound was the only one to contain pulsed information. The pulse duration ranged from 0.04 to 21 seconds, with 60 percent of the pulse durations less than 0.50 seconds and 85 percent less than 0.75 seconds.

The males did not respond to other calling males or to the reproduced calling sound either alone or in combination with pheromone extract. The females, eight to thirteen days old, responded to calling males, pheromone extract, and reproduced calling sound plus pheromone extract, but did not respond to sound alone. The response of the younger flies, six to eight days old, was significantly greater to sound plus pheromone extract than to pheromone extract alone.

The techniques developed in the research for obtaining frequency signatures of the acoustical properties for specific insect activities show promise of becoming a very important tool for measuring insect quality. Such a tool would be a true asset to research scientist as a measure of quality control for both laboratory reared and mass reared insects. These frequency signatures could also be useful as a taxonomic tool in identifying other species of Tephritid fruit flies.

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LIST OF SYMBOLS AND ABBREVIATIONS

a c	Alternating current
B_r	Resolution
B&K	Bruel and Kjaer
dB	Decibel
d c	Direct current
ft	Feet
F_r	Frequency range
F	Fahrenheit
H_z	Hertz (cycles per seconds)
hr	Hour
in	Inch
K	Number of lines = 400
kHz	kilohertz
log	$\log_{10}$
min	Minute
msec	Milliseconds
ml	Milliliter
PSD	Power Spectral Density
PSL	Processed signal length
RMS	Root Mean Square
RH	Relative humidity
sec	Second
SPL	Sound pressure level

usec	Microseconds
uv	Ultraviolet
v	Volts

CHAPTER I

STATEMENT OF THE PROBLEM

Man has always had problems with insects. As far back as 1400 B.C., the Egyptian writer wrote, "Worms have destroyed half of the wheat, and the hippopatami have eaten the rest; there are swarms of rats in the fields, and the grasshoppers alight there" (83). North America has been especially hard-hit by losses due to insects. This is caused in part by the cultivation of many crops not indigenous to this country. Losses of crops, stored products, domestic products, and other commodities were estimated in 1951 at 10.2 billion dollars (83). Another 5.0 billion loss was estimated for illness and deaths due to insect-borne diseases. These losses were in addition to any insect control program cost.

Insecticides have assumed a major role in the total area of insect control. However, because of the concern of the harmful effects many of these chemicals have on man and animal, the use of some of these insecticides has been restricted in recent years. Therefore, new methods of insect control are being sought.

Emphasis is now being placed on non-chemical control methods, which are mainly based upon physical, genetic, and biological principles. Improved cultural practices and the use of insect resistant varieties have already proven to be of great value. Biological methods, including the propagation and distribution of parasites and natural predators, are receiving increasing attention.

Another method of control that has been used to some degree of success is the sterile male release technique, which involves rearing large numbers of insects, sterilizing and releasing the males to mate with females in the natural population (89). This method was used successfully to eradicate the screwworm fly in the Southeast; and the potential of this method depends on the mating habits of the insect, their geographical distribution, and other factors.

Considerable study has been given to the use of electromagnetic radiation to control insect populations. One application is the use of "light traps," usually employing an ultraviolet or visible source, to attract and trap the insects. Light traps have given an encouraging degree of control in a few cases. The primary weakness of this method is that the capture does not usually constitute a significant portion of the population.

The buzz of insects has drawn the attention of man since prehistoric times (27). Many phenomena of nature were reviewed by Aristotle who also discussed the possible origin of the hum emitted by insects. Later, following his ideas, numerous scholars of natural history have described a variety of organs in the insect body which were thought to produce sound. There is a new interest in the possible involvement of sound in the communication process of insects. In the past fifty years, many of the acoustic receptors and sound producing mechanisms have been determined. Some of these receptors are used by insects to detect the presence of predators, such as insectivorous bats; some are used for communication purposes, but the purpose for many of these receptors is unknown. The frequency response has been determined for some of these receptors, and it ranges from a few hertz to over 250 kilohertz.

While a considerable amount of research has been done on insect sounds, most of the work was concentrated in the area of insect predator detection and insect communication by stridulation. Little information is available on sound and its interaction on the mating process of insects of major economic importance. Many of these insects produce a specialized sound sometime during their mating period, and many researchers believe that these specialized sounds play a role in attracting mates. Dahm (36) indicates that an interaction exists between the sound that is produced and pheromone released to enhance attractance of the lesser wax moth during the mating process. Therefore, it is important that these specialized sounds be isolated and identified so that their role as an attractant may be determined.

The potential of electromagnetic radiation and sound in insect control probably will be strongly influenced by the progress made in understanding their communication system. The basic nature of these responses and the biological mechanisms involved are usually not well understood. Some recent theories of insect communication may provide guidance in conducting research into new areas as well as giving better direction in the previously researched areas (30).

With the present and anticipated restrictions on the use of pesticides, information is needed on new attractants that can be used in integrated control programs. Therefore, a better understanding of the insects' communication systems will be of great interest in this respect.

The Tephritidae fruit flies are one of the most important families of insects known. These pests are responsible for large economic losses in many countries around the world. There are over 4,000 species of fruit flies; some of the more common species are the oriental fruit fly, the melon fly, the Mediterranean fruit fly, and the Caribbean fruit fly.

The Caribbean fruit fly, Anastrepha suspensa (Loew), is a native of the West Indies, but the adults have been trapped in central and southern Florida since 1965 and as far north as Jacksonville, Florida, in recent years. This species is not yet a serious pest in Florida, but it is already known to infest many tropical and subtropical fruits including common guava, surinam cherry, peach, loquat, rose apple, and tropical almond (68). The potential danger of this insect is that it is not a native of the United States, and it is now in the process of establishing itself geographically and selecting the host plants best suited for its survival. Many insects that have been introduced into this country have gone through this same process and have become a very serious pest to agricultural and forestry products. It is therefore essential to begin collecting basic information of the Caribbean fruit fly before control methods are needed. This procedure has not always been followed in the past and millions of dollars have been wasted on crash insect control programs that have not worked, primarily because a bank of basic information was not available. A limited amount of information is known about this potentially harmful insect, and one of the major goals of this research is to obtain basic information that will aid in developing control methods when they are needed.

I. Problem

Many insects have receptors that receive various types of information that regulate their behavioral responses. This information can be transferred in the form of light, odor, sound, electromagnetic radiation, etc. These factors usually do not function independently but interact with each other to obtain a given behavioral response. However, it is important to determine the characteristics of each factor involved to better understand its role in a given behavioral response. This research is to be concerned with the information that is transferred from one insect to another by means of sound.

Nation (68) has shown that the male Caribbean fruit fly produces a sex pheromone (odor) that aids in the attraction of female flies; however, it has not been identified and reproduced. He and others believe this sex pheromone is released by the male at the same time that a specialized sound is produced. If this is true, there could be an interaction between the sound and the sex pheromone in attracting mates. There is also a possibility that the males and females are in visual contact with each other at the time the sound is produced and the sex pheromone is released. Therefore, it is possible that odor, sound, and vision are present simultaneously. These factors, singly or in combination, could be used by the male to attract the female fly for mating purposes. Information is needed on all three factors before any one of them can be fully evaluated.

All insects produce sounds that are incidental to ordinary movements, such as flight, chewing, muscle tones, and many others. In addition to these, some insects make specialized sounds normally used to communicate with other insects of the same species. The male Caribbean fruit fly and the male of many other species of tephritid fruit flies have been observed to produce two specialized sounds. One of these sounds is produced by the male fly after the female has been attracted for mating, and it will be referred to in this report as the premating sound. This sound is of general interest; therefore, the physical characteristics of this sound will be determined.

The other specialized sound, the one of interest in this research, is made by the male fruit fly before pair formation. A similarly produced sound is generated by other species of tephritids such as the Mediterranean fruit fly. Much speculation exists as to the purpose of this sound and its functions in communication with other insects of the same species. It has been proposed that this sound may play an important role in forming aggregations of males, in establishing male territorality, in attracting females, or used as a species recognition cue (68). A better understanding of this sound and the basic mechanisms involved could provide insight into basic insect communications and could also lead to techniques useful in the management of this and related species of tephritid fruit flies. Therefore, the problem in this research will be to isolate and identify this calling sound produced by male Caribbean fruit flies so that entomologists may use this information to determine the role of this sound as an attractant that might lead to a control method for this and related insects.

II. Objectives

The objectives of this study were:

1. To develop techniques and procedures for recording and analyzing the sounds produced by the Caribbean fruit fly.
2. To determine the characteristics of flight sounds as related to age and sex.
3. To determine the characteristics of those sounds that are incidental to ordinary movement.
4. To isolate and characterize the specialized sound (calling sound) that is produced by the male Caribbean fruit fly before pair formation.
5. To determine the response of both the males and females to the reproduced calling sound under laboratory conditions.

CHAPTER II

REVIEW OF LITERATURE

Many insects are capable of producing as well as detecting sound. The use of sounds by insects for communication purposes implies that insects produce special sounds, receive these sounds through sense-organs, and react to them with appropriate behavioral patterns. Frings (45, 46) stated that all insects produce sounds that are incidental to ordinary movements, such as flight tones and chewing sounds. In addition to these, some of the following specialized methods of sound production are known: tapping the substrate; explosive explusion of material through a small orifice; snapping of a prosternal spine from a cavity in the mesosternum; buzzing, whinning or piping produced when the body is vibrated without flight; rhythmic snapping of tymbals or tymbal-like organs; stridulation; explusion of air over a specialized vibrating membrane; and wing beat sounds (45). The morphology of many of the ordinary sound producing organs are known (45). It is also known that many insects use sound as one mode of communication.

A considerable amount of literature is available on the sound production and sound reception of insects. In 1960, Frings and Frings (46) published a bibliography containing 1,752 references on the sound production and sound reception of insects. Most of the studies in this area have been conducted since this time; therefore, the reference material has increased many times.

The type of sound receptors and sound producing mechanisms used by the Caribbean fruit fly is unknown. Therefore, this literature review includes information of many known sound receptors, sound producing mechanisms, and uses of sound by insects for communication purposes.

I. Sound Receptors

Most bats have a very sophisticated navigation system. This system operates on the echo-ranging principle, and the sensing mechanisms are highly selective. The bat has the ability to discriminate echoes of its own vocalizations reflected from a mosquito or moth when the background noise is 2,000 times stronger than the level of the echo. With this ability, insectivorous bats are predators of many night flying insects. Scientists have observed that many of these insects can detect the sound generated by these bats and take evasive action (46). As a result of these observations, a considerable amount of work has been done on the sound receptors and behavioral responses of many of these insects. Busnel (27) has described several receptors, and they range from tympanic membranes to simple hair-like structures. He also states that all receptors can be classified as either pressure or displacement transducers (88). Belton (18) in his study of the physiology of sound reception in insects, has grouped insect sense organs into four main groups: hair sensilla, subgenual organs, tympanic organs, and Johnston's organ. The frequency response of these organs has been reported from a few Hz to as many as 200 kHz (79, 114).

Physiology

The corn earworm, Heliothis zea (Boddie), is a serious pest on corn, cotton, tomatoes, and many other crops. Because it is a serious pest and because it attempts to avoid the bat, extensive studies have been made of its sound receptors. The corn earworn has two sound receptors, one on each side of the thorax. Roeder (77) has described these receptors as tympanic membranes which contain two active sense cells, an A-1 and an A-2 cell. The A-1 cell was more sensitive than the A-2 cell. As the tympanum of the moth was stimulated with sound, the two sense cells became active. When no sound was applied to the insect, the A-1 cell generated a random (potential) spike, but as the sound pressure level was increased, the number of spikes increased and became periodic (1). Several possible types of information could be transmitted to the moth from a bat. With a tympanum on each side, the moth could detect the general direction and relative distance from the sound source. With the two sense cells at different sensitivities, the moth could get some idea of the distance between the bat and himself. Other information could be included in the number of spikes generated by each cell and the spacing between each spike.

Roeder (81) analyzed the different responses from the tympanic organ of noctuid moths and reported that intensity differences of ultrasonic pulses are encoded by the spike discharge of the acoustic cells in four different ways; number of spikes, inter-spike interval, number of acoustic cells responding, and spike generation time. He stated that the most obvious effect of increasing intensity is shown by the increase in the number of spikes per stimulus. As the intensity of the stimulus was increased, the interval between the spikes decreased. Roeder theorized that moths could detect the sounds emitted by such predators as bats, decode the sound, and take evasive action.

Roeder (76) also studied the tympanic receptors of the forage looper, Caemurgina erechtea (Cramer), and found that the A-1 cell was about 10 times more sensitive than the A-2 cell. He also determined that the forage looper could detect frequences up to 80 kHz. By using a tympanic nerve preparation, Roeder determined that this moth could detect bat cries up to 40 meters.

Roeder (78) also reported on the interneurons of the thoracic nerve cord activated by tympanic nerve fibers in noctuid moths. He stated that many noctuid moths turn in free flight and assume a course away from the source of faint ultrasonic pulses. This avoidance behavior appeared to be steered primarily by a difference in signals delivered by one acoustic A-1 sense cell in each tympanic organ. The A fibers distributed signals ipsilaterally within the three thoracic ganglia. They were connected with the relay neurons that conveyed the different signal with minor transformation to other ipsi- and contralateral regions of the meso- and prothoracic ganglia and to the ipsilateral cervical connectives. The posterior medial part of the mesothoracic ganglion appeared to be a site for various types of integration of the different signals. This appeared to take the form of discarding certain parameters of the stimulus and retaining others. Ultrasonic frequency was discarded and the neural signal was generated by the A cells. Their signal was in turn transformed by pulse-marker neurons so that pulse intensity and duration were discarded; leaving only pulse repetition rate in the pulse-marker response. Relay neurons, A fibers, and pulse-marker units responded unilaterally, that is, only to the stimulation of one tympanum. Other neural units summated signals from right and left tympanums, while still others responded with a tonic discharge phased by the repetition rate of the stimulus.

It has long been known that a decapitated insect might live and continue to show complex behavior for many days; whereas, similar treatment of an animal would cause immobility and death in a few minutes. Roeder (78) has investigated the brain and the central nervous system of certain insects in an attempt to find some answers as to why this occurs. He indicated that the life support centers are concentrated in the vertebrate brain; whereas, in insects they are distributed in other parts of the body. While both have different histories, the insect nervous system and the vertebrate nervous system depend on the same neural principles. The acoustic signal from the tympanic organ was relayed to the brain where the neurons discharge a potential, and this acoustic signal was capable of acting as a steering signal. In view of the above, the life support centers in insects are distributed. Frings (42) has shown that at least some insects have duplex sound receptors. He measured the response of some insects when exposed to sound up to 40 kHz, and then destroyed the tympanic organ to determine if the insect would continue to respond. The insects did continue to respond but at much lower frequencies, and no response was recorded at higher frequencies. Both Roeder and Frings speculated that even moths with tympanic organs had at least two pairs of acoustic receptors. The tympanic organs would be the primary receptors and at least one secondary set would be some other place in the body. Frings speculated that there may be a galaxy of sound receptors which enables the insect to react to its total acoustic environment.

Most Lepidoptera are equipped with at least one type of sound receptor, a tympanic organ, and possibly others. However, only a limited amount of research has been done on the possibility of sound producing mechanisms that might be used for communication purpose between the sexes of this group of insects. Kay (50) and Agee(5) have detected a high frequency sound generated by the bollworm moth. This sound is believed to be generated by the wing tips while the moth is in flight. The sound is in the general nature of high frequency clicks composed of rapidly damped oscillations of 50 kHz burst every 25 msec. The sound pressure level of those clicks was measured at 98.5 dB at 1.5 cm from the insect. The purpose of the sound is not known, but Kay speculated that it may be used in insect communication and may specifically be used by the moth for signaling for the purpose of assembling or for locating a mate.

Callahan (29) compared the conjunctive and tympanic membrane of the corn earworm with those of the cabbage looper, Trichoplusia ni (Hubner), by using a scanning electron microscope. Organs of both moths were covered with micronodules. The corn earworm micronodules were elongated and of about the same diameter. The cabbage looper micronodules were unevenly shaped and connected by lateral ridges. Callahan speculated that these micronodules possibly contribute to filtering or attenuating specific frequencies involved in the auditory behavior of each species.

Agee (2) reported the response of the sense cell of the bollworm and tobacco bud moth, H. virescens (Fabricius), to ultrasound. The A-1 cell responded to pulses of ultrasound ranging in frequency from 10 kHz to 100 kHz, but it was most sensitive to frequencies between 18 and 25 kHz. Free flying moths of both species responded with similar evasive behavior when they were stimulated with pulses of ultrasound at frequencies of 21 to 22 kHz. When electrophysiological techniques were used to determine the acoustic sensitivity of the European corn borer, Ostrinia nubilalis (Hubner), Agee (3) reported the tympanic organ was found to be served by two acoustic A cells and one nonacoustic B cell. One A cell was more sensitive than the other A cell. The B cell had a spike rate from 50 to 100 Hz. The tympanic organ detected frequencies of 25 to 60 kHz. Pulse trains composed of frequencies of 25 kHz caused more moths to show the avoidance response than trains of 20, 30, or 50 kHz at sound levels of 80 to 90 dB.

Miller (60, 61) studied the physiological responses of the green lacewings (Neuroptera chrysopidae), to ultrasound and found that the tympanic organs functioned as ultrasonic receptors. Sensory responses to sound occurred at frequencies from 13 to 120 kHz and at pulse rates from 1 to 150 pulses per second. The tympanic organ of the green lacewing appeared to be about 20 dB less sensitive than the tympanic organ of noctuid moths.

Behavior

Agee (4, 7) also reported the response of the bollworm moth to ultrasound while the moth was resting, feeding, courting, mating, or ovipositing. When the bollworm moths were confined in a large cage, 63 percent did not react to the pulsed sound while they were resting, feeding, mating, or ovipositing. However, about 50 percent of those in the midst of the courtship display ceased these activities and assumed a resting posture. When the moths were exposed to several pulses of ultrasound, many of those reacting became habituated to the sound within two minutes or less and then continued their sexual activities irrespective of stimulation. A few did not become habituated to the sound stimuli.

Agee and Webb (1, 6, 103) studied the response of free-flying bollworm moths and ten other species of tympanate moths to pulsed ultrasounds. These moths made evasive maneuvers, turn-aways, loops, and dives to the ground when they were exposed to pulsed ultrasound; the looping was the more successful method of evading pursuing bats. Agee reported that not all moths exposed to pulsed ultrasound made evasive responses. A check of the acoustic sensitivity of the two sound-detecting cells in the tympanum of some nonresponding moths showed that the acoustic cells were responding to the stimuli; hence, the pulsed ultrasound had been detected. He speculated that habituation at the second or higher order neutral levels probably explains the behavior of the nonresponders.

The directional sensitivity of the tympanums of noctuid moths was studied by Payne, Shorey, and Roeder (72, 73, 87). In the presence of high-intensity pulses, the moths' responses took the form of diving and looping, but since these responses generally bring the moth to the ground, there was no clear directional reaction to the sound source. However, it was consistently noted (17, 43, 80, 82) that moths flying at greater distances from the sound source would turn and fly away from the source. This turning away was observed only if the moth encountered a series of low-intensity ultrasonic pulses. It is likely that at such intensities only one nerve cell in each tympanum was stimulated. It also implies that moths with this sensory equipment can extract directional information from sound waves in all planes.

Treat (93, 94) studied the responses of some Lepidoptera to frequencies up to 24 kHz. The responses were measured in terms of insect activity such as diving, swerving, turning, etc. Many of the day flying, as well as many of the night flying insects, responded in some way to the ultrasound and were often seen to make swerving or diving movements at the approach of bats; and in many instances they appeared to escape capture by such maneuvers. These evasive actions were initiated by an auditory mechanism responsive to the bat's ultrasonic pulses. Treat (92) also reported that electrical responses were recorded from the main lateral nerve trunks of the mesothoracic ganglia in noctuid moths of five common species when the tympanic organs were acoustically stimulated with frequencies between 3 and 120 kHz.

II. Stridulation and Insect Communication

Insects are said to stridulate when they produce sound by rubbing together special bodily structures. These stridulatory sounds are produced in discrete sound patterns usually in the form of pulses and pulse trains. The tooth impact frequency of the scraper is synchronized with the resonant frequency of the file, thus producing one sound wave per tooth impact (44). As the tooth on the scraper strikes the tooth on the file, a damped oscillation occurs that decays exponentially before the next chain of events. This sequence of events results in a pulse train where each pulse could have a different frequency depending upon the resonant frequency of the tooth. The duration between pulses depends upon the motion of the wing or other moving organs and the spacing of the teeth on both the file and the scraper. These events result in a very complex sound pattern. It is believed that this sound is used for communication purposes; and if this is true, then the male, female, or both must be equipped with some type of sound receptor.

Stridulatory organs are wide spread among insects. Eastop (37) has found a stridulatory surface on the abdomen of certain aphids that earlier field observers had reported as making sounds. Leston (56) reported that in the Pentatomodea super-family alone at least six different types of stridulatory apparatus are known.

Alexander (9) stated that the number and variety of insects which produce sounds with specialized apparatus exceed those of all other living organisms combined; but only a few of these, the crickets, katydids, grasshoppers, and cicadas, make noises loud enough to be noticeable to man. These are what Alexander (8) calls the singing insects and their ancestors may well have been the first organisms on earth to communicate through sound waves transmitted in air. Alexander gave some different ways that he believes insects produce sound: by stridulation; by striking body parts together or against a substrate; by vibrating some body part such as the wings; by vibrating drum-like membranes called tybols; or by forcibly ejecting air or fluid. The method of sound production by Orthoptera (crickets, katydids, and locusts) is primarily by stridulation, but other methods may be used (52). These insects ordinarily stridulate either by rubbing together specialized surfaces or by stroking with the hind legs. Crickets, cicadas, and grasshoppers have similar sound producing mechanisms which consist of two organs commonly called the file and the scraper (35). The file is normally found on some part of the body in a stationary position; whereas the scraper is normally found on a moving part such as the wings, legs, etc. Sound is produced when the scraper and the file are rubbed together. With the introduction of the scanning electron microscope in recent years, these organs, such as the ones described by Lawson (55), are being examined in great detail.

Much of the sound made by Orthoptera is audible and can be heard at some distance, and many researchers have studied it at various angles of approach. Walker (102) has studied these sounds from a taxonomic standpoint as well as how they are used in male to female communications. Walker reported that males of most crickets, tettigoniids, and cicadas, and of some short-horned grasshoppers produce sounds frequently or continuously for many minutes or hours daily which function in bringing the sexes together. In most species the female is attracted to the male sound. However, it has a potential disadvantage of advertising the location of the sound-producing male to many predators. Walker (101) studied ten species of crickets and found morphological differences based on the sound each produced. He also found a direct correlation between wing stroke and temperature. The wing stroke increased as the temperature increased. When Walker (100) and Neilson (70) studied crickets, they found that the stridulation rate was dependent upon temperature. The average pulse rate within a chirp was also temperature dependent; and chirp duration, as well as the chirp interval, shortened or lengthened with increasing or decreasing temperature. Walker also showed that these sounds were produced by the male as a calling song for the female. The sexually responsive females responded to the calling song by approaching the stridulating male or by producing an answering sound which directs the male in approaching the female. Differences in pulse rates, normally corresponding to wing-stroke, are the basis of initial species discrimination by the females of some species.

Walker (99) studied the sound produced by six species of crickets that occur in the United States. Each species had a distinctive calling song even though they may have different seasonal life histories and may overlap in geographic distribution and habitat. He (96, 97, 98) has also described a method of determining the pattern of wing movement of crickets and katydids during stridulation by means of high speed photography. The variations in forewing movement that produce this complex sound in crickets and katydids are not well understood. Perhaps this photographic method can help in correlating the movements of the wings with the sound that is produced.

Ewing (39) classified the sound produced by crickets into three classes: calling, aggression, and courtship songs. The calling song was the most common and consisted of a series of chirps containing two or three pulses given at regular intervals. It was emitted spontaneously by a sexually mature male in his territory. The aggression song was exhibited by males in the presence of other sexually mature males. It was composed of short pulse trains, apparently identical with those used in calling, but averaged a greater number of pulses. The courtship song was quite different from the others, the component pulses of sound were shorter and were emitted at a much lower intensity; and the sequences were long, always forming a pulse train. Interspersed among the weak sounds were single occasional loud pulses. The significant sounds appeared in a sequence of major units with each corresponding to a single wing closure. Ewing theorized the pulses vary in amplitude through three causes: as a result of differences in the force with which the two wings press against each other; as a result of differences in the velocity of the wing stroke; and as a result of different extents of opening of the wings prior to the closing stroke.

Reid (75) studied the sound producing mechanism of the 17-year cicada. He found that these sounds were produced by sequentially buckling a series of stiff ribs embedded in a flexible tymbal. Each such collapse excited a damped oscillation in a resonant cavity. By this means, the cavity was excited 10 to 12 times per muscle contraction, which permits a normal muscle to perform a task requiring very rapid repetitive activity. Lewis (57) studied the high frequency produced and the action potentials generated by several species of crickets, and he found that the tympanic nerve was stimulated when exposed to these high frequencies.

Hubner and others (10, 49, 71) reported on the central nervous system control of sound production in crickets. When a male cricket sings, he raises the tegmina and rubs them together causing a file on one tegmen to move against a scraper on the other. The song pattern produced reflects a pattern of wing movement. Each song is different and each requires different wing movements. Hubner identified 14 pairs of muscles that are involved during stridulation or sound production. He also stated that three parts of the central nervous system controls the starting and stopping of singing and the type of song. Sound production and associated behavior were not detected if any of these three structures were destroyed (20).

Morris (65) recorded and analyzed the sound from *Metrioptera sphagnorum*. There were two stridulation modes corresponding to two intensity levels. Characteristic frequency spectrograms were associated with each mode. In one mode audio frequencies were emphasized; the other mode was dominated by high frequencies.

The literature on crickets and cicadas are numbered in the thousands. It has been clearly demonstrated that these insects communicate by sound with their mate during the mating process. Each species has a distinctive communication signal and a given species can be identified by its sound pattern. Since crickets and cicadas are of no great economic importance, no attempt has been made to use their mating calls to attract and destroy the insects.

Extensive studies on the stridulatory sounds of European Orthoptera have been made by Broughton (26), Busnel (27), Busnel and Chauasse (28), Evans (38), Haskel (48), and others. It has been established that grasshoppers and locusts communicate acoustically. The communicative behavior among grasshoppers consists of a great variety of acoustical and visual patterns (58) between the sexes and among members of the same sex. These signals, which are expressed in the form of stridulations and vibrations by the male or as the waving-gesture by both sexes, characterize either the premating and mating state or the state of disturbance and defense, depending on the circumstances that elicit these expressions. Loher (58) recorded and analyzed the songs of *C. pacifica* and reported that these sounds were emitted in pulses with some frequencies as high as 40 kHz.

Young (118) studied the songs of three species of grasshoppers and reported that the songs are divided into four distinct types: the calling song, the rivalry song, the agreement song, and the precourtship song. The duration of the chirps and the frequency within chirps were analyzed and found to be different in each song.

The acoustic communication in Frimerotropis agrestis, a species of desert grasshoppers, was studied by Willey (115, 116, 117). He reported that in addition to the sounds produced by stridulation, a visual stimulus was also provided. This stimulus was produced by the male grasshopper when the legs passed above the body and exposed the coral-red inner surface of the femora. The acoustical communication system of this species provides communication between male-to-male, male-to-female, female-to-male, predator-to-insect, and insect-to-predator. The communication of these insects is quite complex and is encoded in a series of pulses, pulse-trains, and carrier frequencies. In addition to the sound communication signals of this species, there are other sound signals which could not be directly correlated with activity. There was a flutter-rasp sound produced by a rapid oscillation of the femur in contact with the tegmen. The effective sound produced was a broad-spectrum noise produced about 20 times per second. There was also a buzz and flutter-squeal sound made by the males that has no certain communications function. The buzz lasted one to two seconds with a pulse frequency of about 110-120 per second. The males also produced a clicking sound just before attempting to copulate. The activity associated with this click was not determined.

The sound frequency spectrum of the long-horned grasshopper was investigated by Morris (64). His study revealed that the pulses were composed mainly of frequencies extending from 28 to 60 kHz. However, there were also some audible frequencies. Suga (90) also reported ultrasonic frequencies produced by four species of neotropical grasshoppers. These frequencies consisted of mainly two frequency bands, one was from 22 to 24 kHz and the other 40 to 60 kHz. He also reported that the optimum sensitivity of the tympanic organs of these two species was between 20 and 25 kHz. Blondheim (22) reported that in addition to the stridulation sounds produced by two species of grasshoppers, a sound was made by substrate drumming. This could indicate that grasshoppers have more than one type of sound receptors.

Michelson (59) made a rather in-depth study of the physiology of the locusts' tympanum. The frequency sensitivity of single cells in the isolated tympanum was studied under controlled acoustical conditions. The four anatomical groups studied differed as to frequency sensitivity, and it was concluded that fairly accurate information about sound frequency reaches the control nervous system. Michelson's (59) next study was frequency discrimination based upon resonances in the tympanum. The expected resonance frequencies of the tympal membrane were calculated from its dimensions, mass, and compliance. It was determined that the thin part of the tympanic membrane could vibrate independently of the entire tympanum. Therefore, receptor cells attached to different areas on the membrane may receive different modes of vibrations. The receptor cells, also, may fail to respond to some modes if their area of attachment is at a nodal circle of these modes at resonance. The third study of

Michelson (59) was the acoustical properties of the intact tympanum, and he determined that the sensitivity of the intact tympanum to low frequencies depended on the fat content of the insect. The internal tissues seem to act as an acoustic low pass filter; therefore, the high frequencies were heavily damped.

Some beetles are known to produce sounds. Kogan (53) has studied some of the leaf beetles that are known to produce sound and found that they are equipped with stridulatory organs. The geometry of these organs is different from those of crickets, cicadas, and grasshoppers; and the pulse repetition rate is higher. Rudinsky (84) studied the acoustic communication between the male and female douglas-fir beetle. The male beetle was found to produce two distinct sounds. One was found to be a stress sound, but the other was correlated with attraction behavior. Rudinsky believed that acoustic communication among beetles is more important than heretofore believed.

Finn (40) and Ruppel (86) identified the stridulatory organ in the long-horned and cereal leaf beetles. These organs were very similar to those described by Kogan in the leaf beetle. Sound producing mechanisms and sound receptors have also been identified on the cockroach (41).

Songs of Acridadae are generally broad band noises with a maximum at 4 to 8 kHz and intensities of 30 to 40 dB at 10 to 30 cm (48). Songs of Tettigoniidae are also wide-band noises but have higher frequencies up to 100 kHz with the maximum at 8 to 15 kHz and intensities of 45 to 75 dB (28). Songs of Gryllidae have relatively pure tones at frequencies from 2 to 6 kHz and intensities from 40 to 60 dB.

Many other insects are known either to generate or detect sounds. In stored products alone about 50 families of beetles are known to produce sounds (42). Also, about 40 families of Lepidoptera have been reported as having sound producing organs.

III. Tephritid Fruit Flies

Fruit flies belong to the Tephritidae family of insects. This family includes approximately 4,000 species distributed throughout the temperate, subtropical, and tropical areas of the world (34); and they annually take a heavy toll of fruits and vegetables in these areas. Barriers to trade in fresh food commodities; difficult quarantine and and regulatory problems; prevention of development of desirable crops; and costly survey, control, and eradication programs are some of the undesirable by-products of these insects.

The body length of individual fruit flies varies from 1 to 20 mm (34); and most species' wings are yellow, brown, black stripes or spots, or a combination of both. The females of most species insert their eggs in living, healthy plant tissue, especially in ripening or ripe fruits and vegetables. After the egg hatches, the larvae feed and grow within the fruit; and after the fruit falls to the ground, pupation takes place in the soil. The adult emerges from the soil and after a few days attains sexual maturity; then mating occurs and a new cycle begins (107). The average life of an adult is from 30 to 40 days. Several species of fruit flies are closely related in their host plant selection, life history, and mating habits. Some of the related species are: the Caribbean fruit fly (113), the Mexican fruit fly (112), the Mediterranean fruit fly (111), the melon fly (110), the Oriental fruit fly (109), olive fruit fly (108), Anastrepha interrupta (104), Anastrepha nigrifascia (105), Queensland fruit fly (106), and others.

A considerable amount of literature is available on many species of fruit flies with respect to their ecological, biological, and morphological characteristics. In 1914, Back (13) reported on the life history of the melon fly. In 1916, he studied the effects of temperature on the pupae (14) and the adult (15) Mediterranean fruit flies. In 1939, McBride (67) reported on the response of the Mediterranean fruit fly to its environmental factors; and in 1970, Chambers (31) reviewed the recent research on the Mediterranean fruit fly. The biology of 14 species of related fruit flies was reported by Christenson (34).

While the tephritid fruit flies have been studied for some 60 years, very little information is available on their sensory mechanisms. During this literature review, relatively little information was found on any of the major sensory receptors such as vision, olfactory, sound, or any other receptors that the fly might possess. However, it is assumed that this insect contains several types of sensory mechanisms. The input to these receptors should control the behavioral responses and the basic functions of the insect. As a result of this lack of information, the modes of communication from male-to-female, female-to-male, male-to-male, and female-to-female is not well understood. However, Nation (69) reported that visual, auditory, and chemical stimuli are important to successful mating in several tephritid fruit flies.

There is evidence that sound may be used as one mode of communication in several species of tephritid fruit flies. The exact purpose of these sounds is unknown, but it has been proposed that they may play a role in forming aggregations of males, in male territoriality, or in attracting mates; or they may be used as a species recognition cue. In 1952, Myers (66) reported that male Queensland fruit flies produced a high-pitched sound which he considered to be a mating call. In 1953, Monro (62) reported on his investigation of the sound from this fruit fly. While he did not prove how this sound was produced or what its purpose was, his observations indicated that the sound resulted from stridulation in which the wings were drawn along two rows of large bristles. His findings did show that these bristles were only on the male Queensland fruit fly and absent on the female.

Myers (66) also reported that light intensity was an important factor in the mating activity of the Queensland fruit fly. Under natural conditions, mating behavior is stimulated by lower light levels and normally occurs between sundown and dark. Under laboratory conditions, he found that when this dusk period was omitted no mating occurred. He stated that when this dusk period begins the males always initiated the sexual activity by beginning to call. The calls were heard as rather high flute-like sounds. The calls stimulated the females, and in many cases they were seen to walk from one end of the cage to the other in a straight line toward the calling male.

Similar sounds have been reported in other tephritid fruit flies. Keiser (51) reported that these sounds were observed from the Oriental fruit flies, the melon flies, and the Mediterranean fruit flies. These sounds were observed at the same time of day, between sundown and dark.

The Caribbean fruit fly is a native of several Caribbean Islands and was first reported in Florida in 1932. This insect was not reported again until 1965 (23) when large numbers were reported in Miami Springs near the Miami International Airport. The fly has been found as far north as Jacksonville along the East Coast, and it is known to infest several central and south Florida counties. It has a wide range of host plants infesting many tropical and subtropical fruits and causing damage to peaches (95). At present, no control methods are known for this fly. It is relatively new in this country; therefore very little information is available relating to its behavior (16, 74). However, the literature of other tephritid fruit flies can be of some value in studying the Caribbean fruit fly.

Nation (69) studied the courtship and mating behavior of the Caribbean fruit flies and showed that the male flies produce a sex pheromone that attracts the female flies. However, he reported that visual and auditory stimulation are also important in these mating events. The mature male emits the pheromone by enlarging the pleural region of some abdominal segments to form a small pouch on each side of the abdomen. This puffing action is usually accomplished by a rapid fanning action of the wings. Two distinct wing movements, slow and rapid, have been observed. In the slow wing movements, the male may move one wing at a time or both simultaneously to a position at right angles to the body axis. He raises and lowers them in this position to form the slow wing movement. This slow action may frequently be interrupted by rapid wing fanning. This rapid burst of wing fanning constitutes the high-pitched sound that has been reported in many of the tephritid fruit flies. Nation observed caged Caribbean fruit flies and found that a female would fly or walk toward the fanning male, and as the female approached the male, the fanning would cease. He also observed that in crowded cages the fanning was sometimes omitted. He speculated that at close range other stimuli, perhaps largely visual, are probably important in mate recognition and acceptance. He interpreted the wing fanning and puffing behavior of males as serving to attract females from some distance away.

CHAPTER III

EQUIPMENT AND FACILITIES

The equipment and facilities used in this research were located in the Insect Attractants and Basic Biology Research Laboratory of the United States Department of Agriculture at the University of Florida, Gainesville, Florida.

I. Insect Cages

The fruit flies used in this research were shipped to the Gainesville Laboratory in the pupal stage by Dr. R. M. Baronowski located at the Sub-Tropical Experimental Station in Homestead, Florida. After the flies arrived at the laboratory, they were removed from the shipping containers and placed in rearing cages.

Once the flies had emerged into adults, the females and males were separated and placed in separate holding cages. The rearing and holding cages were constructed of aluminum frames with outside dimensions of 12 x 12 x 12 inches. The bottom, top, and two sides were covered with copper screen to assure adequate ventilation. One end of the cage contained a removable glass panel to facilitate cleaning. The other end contained a sheetmetal panel with a 3-inch diameter opening in the center. A cheesecloth sleeve was attached to this opening to gain entrance to the cage without allowing flies to escape (Figure 1).

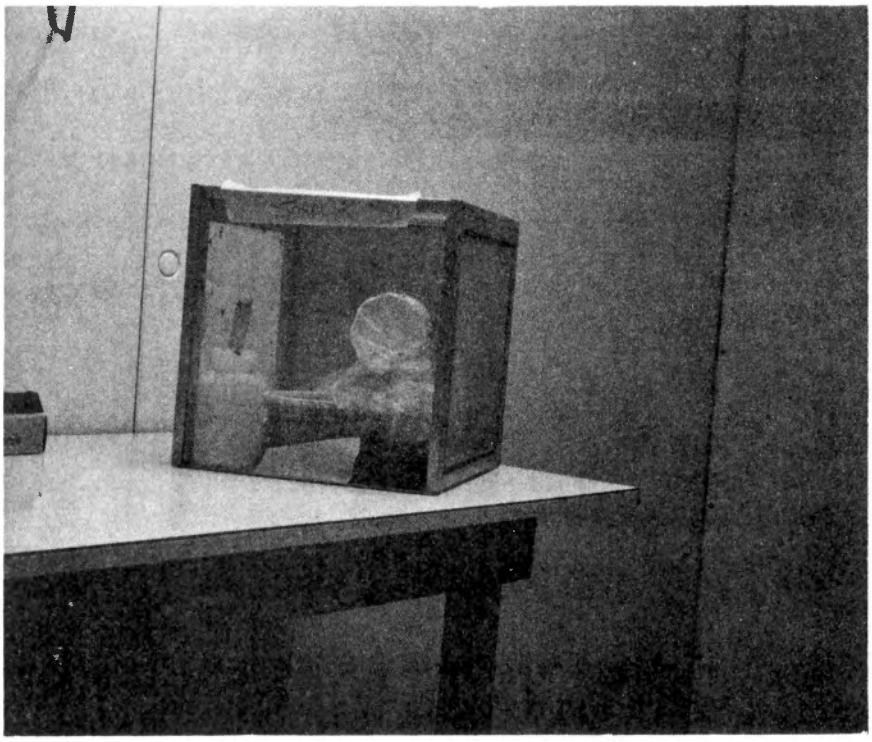

Figure 1. Rearing and holding cage.

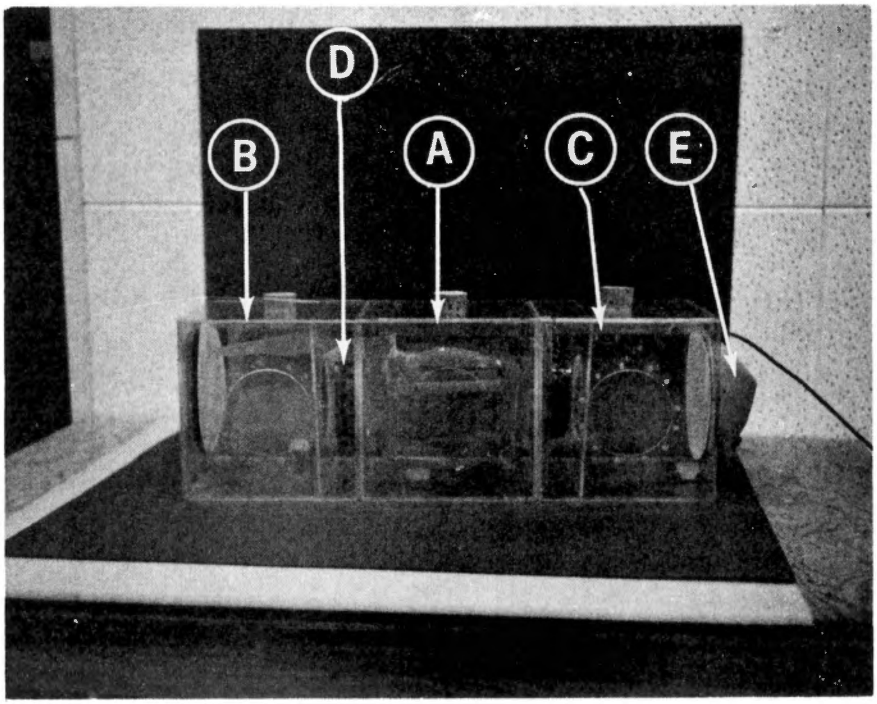

Figure 3. The bioassay plexiglass test unit: (A) Release cell, (B) Control cell, (C) Treatment cell, (D) Fly trap, and (E) Speaker.

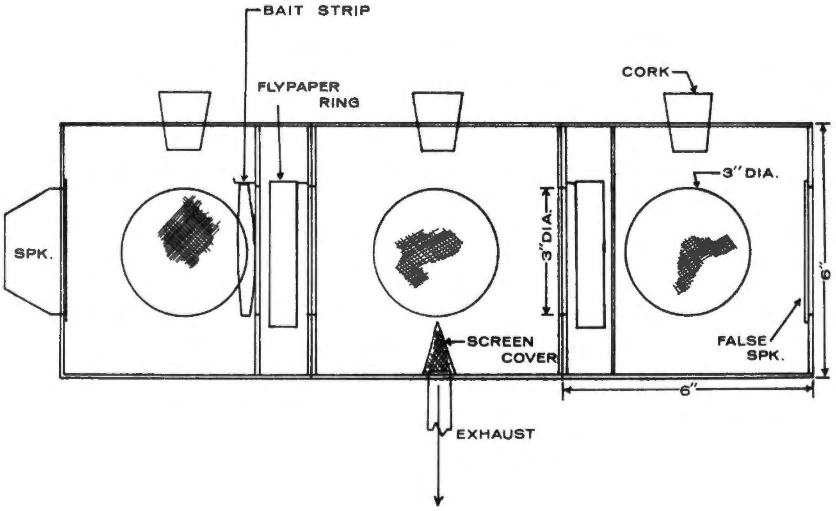

Figure 4. Side view of bioassay test unit.

on the flypaper or trapped in the bottom of the trap cell. Based on observations, it was estimated that less than 1 percent of the flies entering the trap cell escaped.

Ventilation System

The airflow was controlled in each test unit. A hole was drilled in the bottom of each of the center cells and a 5/8-inch outside diameter plastic hose was inserted into the hole. This hose was connected to a plenum chamber located under the test units. A 2-inch flexible hose was attached to the exit of the plenum chamber and extended through a utility opening from the anechoic chamber. This hose was then connected to the suction side of a shop type vacuum cleaner which was located inside an exhaust hood (Figure 5). All exhaust gases were then carried to the outside of the building.

The air entered the test units through one of the 3-inch holes in the side of each end cell, passed through the fly trap cell, and then was carried into the center cell where it was exhausted through the plastic exhaust hose as shown in Figure 6. The velocity of the air entering the center cell from the fly trap was measured in each unit with a Taylor Model ML-62 anemometer. The average velocity was 21 ft per minute with variations among chambers of ± 3 ft per minute. Dry ice, carbon dioxide, was placed in front of the air opening in the end cell so the airflow pattern could be observed. There was direct flow of air from the end cell, through the fly trap, and into the center cell.

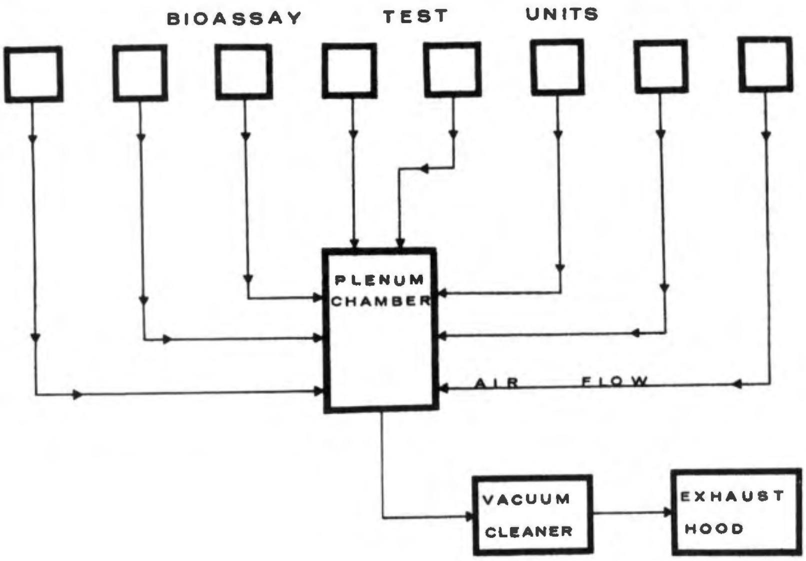

Figure 5. Air flow through the bioassay test system.

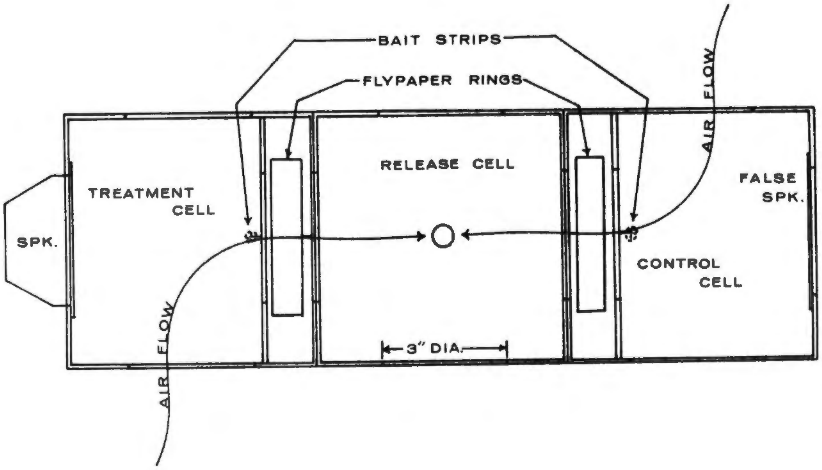

Figure 6. Air flow through each bioassay test unit.

Sound Reproduction

A 1-inch diameter speaker was installed in the end of four of the end cells. Dummy speakers were in all the other end cells. These speakers were taken from Realistic Nova Pro stereo headsets. The output waveform at 150 Hz and frequency response for each speaker are shown in Figures 7 and 8. The waveform from the speaker output showed some distortion, but were considered to be good when compared to those of other speakers.

The eight bioassay test units were located in the anechoic chamber. Each test unit was acoustically insulated with a double layer of acoustical tile (Figure 2, page 33). These tiles extended 2 inches beyond the ends of the test cages and 2 inches higher than the test cages.

Lighting

A panel of seven fluorescent lamps was installed 36 inches above the top of the test cages and extended 6 inches beyond the last test unit. A light diffusing panel was located 6 inches below the panel of lamps; this assured uniform lighting in all test units. Special lamps were used in this arrangement so that daylight conditions could be simulated. The fluorescent lamps were 15-watt, T8 Vita Lite manufactured by Luxor Lighting Company with output characteristics as shown in Figure 9. A low light level of approximately 11 lux was maintained at cage height.

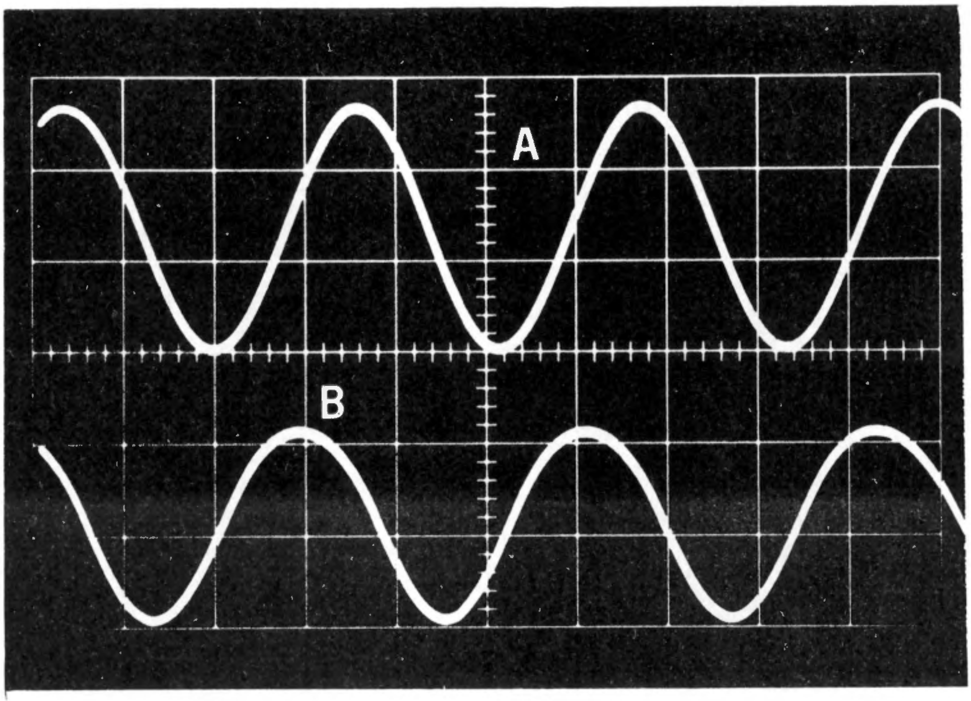

Figure 7. Waveform characteristics from speakers at 150 H_z: (A) Waveform from signal generator, (B) Same waveform as observed from speaker output.

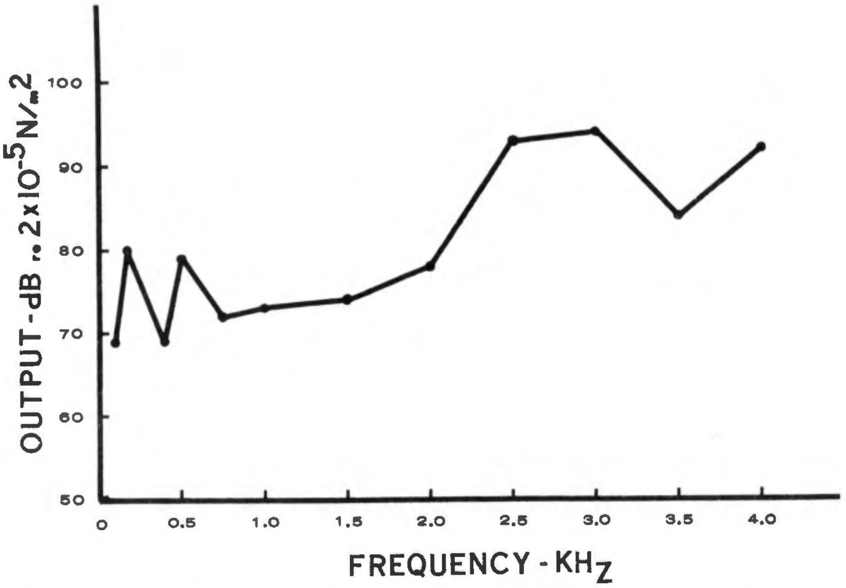

Figure 8. Average frequency response of speakers mounted in the bioassay test units.

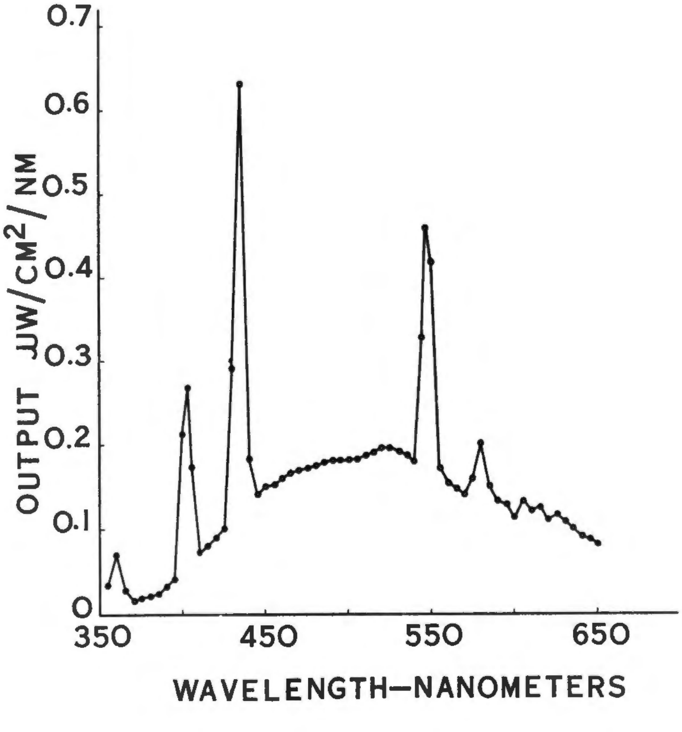

Figure 9. Output spectrum of 15-watt, T-8 Vita Lite that was used in the bioassay test.

III. Flight Mill

The flight mill used to obtain the flight data is similar to that used by Chambers and O'Connel (32), shown in Figures 10 and 11. The flight mill consisted of a plywood mounting bracket, a top and bottom support, a stainless steel wire, two magnets with Teflon bearings, and a rotating arm.

The two magnets and the rotating arm constituted the functional parts of the mill. The two magnets weighing 0.35 ounces each were suspended between the support arms by means of the stainless steel wire. The lower magnet was fixed in position and remained stationary. The upper magnet was placed with like poles facing each other, and the repelling force caused the upper magnet to float about 3/4 inch above the lower magnet. The magnets were 3/4 inch in diameter and 1/4 inch thick with a 1/4 inch diameter hole in the center.

A Teflon sleeve with a bearing surface on each end was inserted in the center hole of each magnet. The two surfaces of the sleeve were the only contacts with the wire; therefore, they were the only source of surface friction of the rotation arm.

The rotating arm consisted of a split washer attached to a 22 gauge wire. The diameter of the hole in the washer was large enough to fit over the Teflon sleeve allowing the washer to rest directly on the upper magnet. A counterweight to compensate for the weight of the fly was located on the opposite end of the wire. The distance from the stainless steel wire to the point where the fly was attached was 6-3/10 inches. This gave 39-1/3 inches of travel per arm rotation.

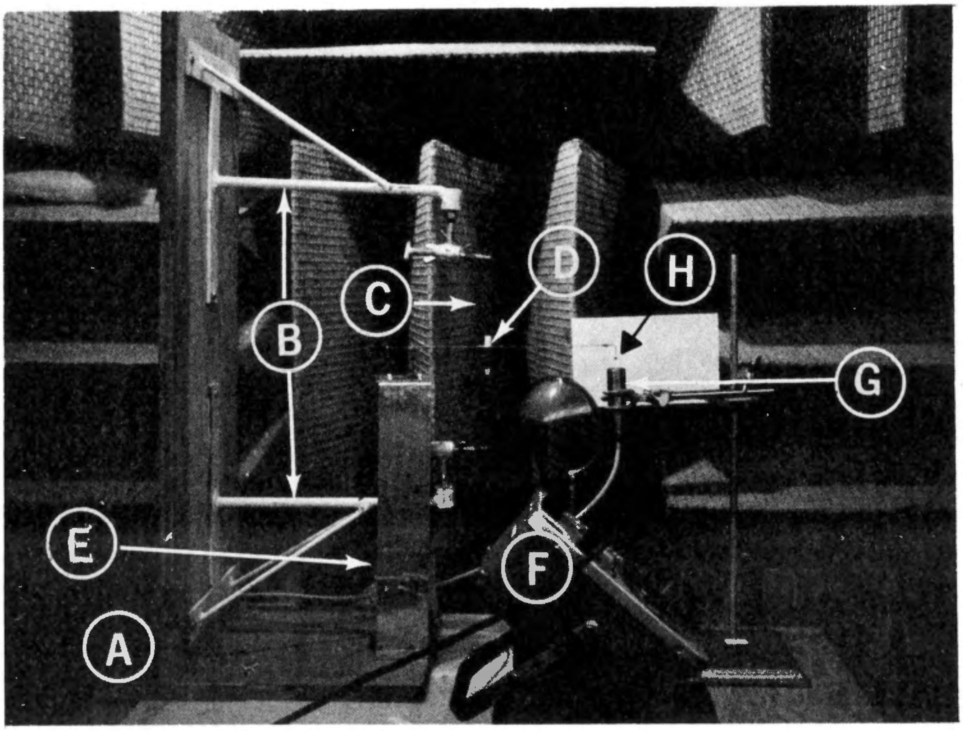

Figure 10. Flight mill details: (A) Plywood bracket, (B) Supports, (C) Stainless wire, (D) Magnets with Teflon bearings, (E) Solenoid, (F) Microphone, (G) Stroboscope, (H) Fly.

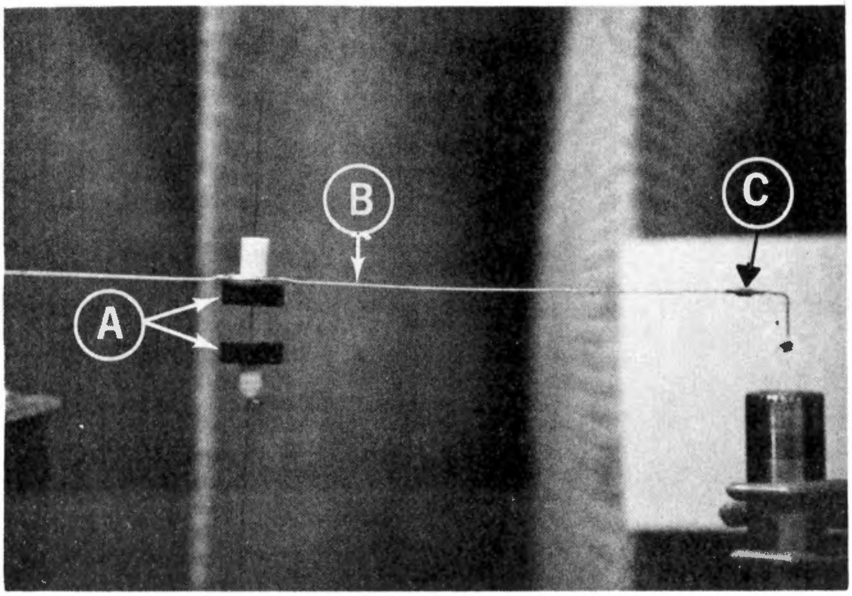

Figure 11. Close-up of (A) magnets, (B) rotating arm, and (C) sleeve.

A 6-volt dc operated solenoid was attached to the base of the plywood mounting bracket with a rod that could intersect with the rotating arm when energized. This solenoid was equipped with remote controls so it could be operated from outside the anechoic chamber. This control was used to stop or release the fly from the microphone position.

The temperature of the air near the fly location was maintained at approximately 78°F and was monitored by placing a thermocouple near the flight mill. The temperature was recorded by a Brown recorder located outside the anechoic chamber.

An incandescent and a fluorescent lamp were placed about three feet above the flight mill to assure uniform lighting during the test.

IV. Sound Laboratory

The sound laboratory is located in an isolated section of the building and consists of an anechoic chamber and an instrument room. The anechoic chamber was installed by a commercial firm during the construction of the building and is an integral part of the laboratory.

Anechoic Chamber

The outside dimension of the chamber is 16 x 16 x 12 ft, and it rests on rubber supports to reduce vibration inside the chamber. The inside dimensions as measured from wedge tip to wedge tip are 10 x 10 x 6 ft. The wedges were designed and arranged to give frequency attenuations as shown in Table I. The arrangement of the wedges are shown in Figure 12. A viewing window can be provided if needed by removing one section of wedges.

TABLE I

NOISE REDUCTION OF ANECHOIC CHAMBER

Octave Bands H_z	Noise Reduction dB
37.5 - 75	20
75 - 150	25
150 - 300	35
300 - 600	45
600 - 1200	50
1200 - 2400	55
2400 - 4800	55
4800 - 9600	50

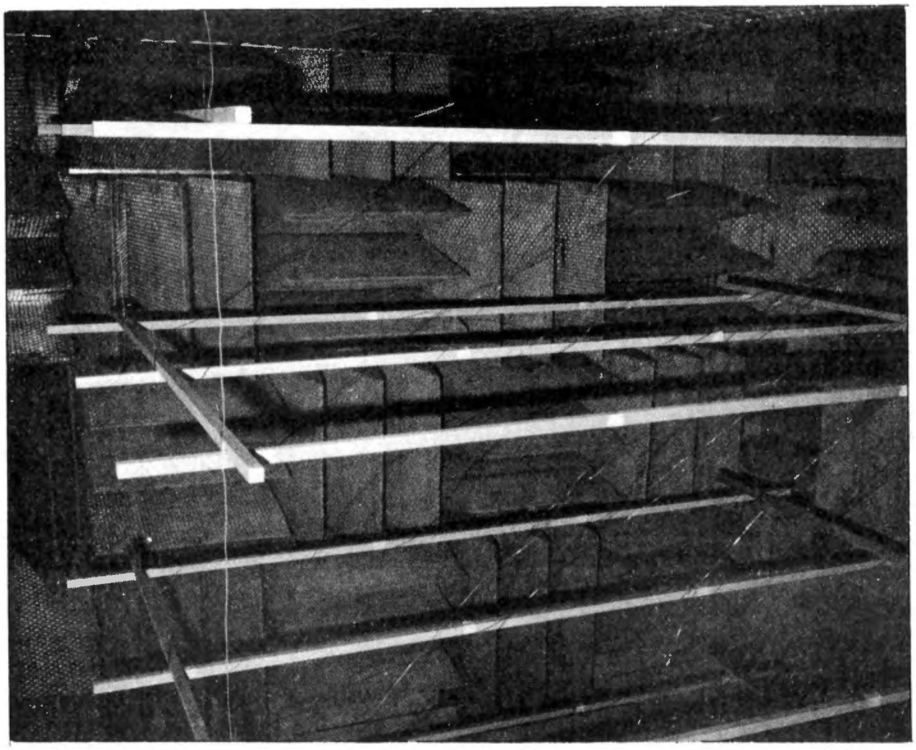

Figure 12. Placement of thermocouples for temperature measurements in the anechoic chamber. Also details of wedge arrangement.

The anechoic chamber is equipped with heating, cooling, and humidity systems that can be controlled independently of any other space in the laboratory. The air ducts entering and leaving the chamber contain acoustical insulation to attenuate any sounds that could enter the chamber from the temperature and humidity control systems. The airflow through the chamber is 110 ft/min.

The temperature gradient was determined by placing 48 evenly spaced thermocouples throughout the chamber. The temperature of all 48 thermocouples was recorded simultaneously on two 24-point recorders for a period of two weeks. The data showed that there were no major hot or cold spots inside the chamber.

Instrument Room

The instrument room is located just outside the anechoic chamber (Figure 13). Cables from the microphones or other equipment were fed through two 2-1/2-inch pipes located in the side of the anechoic chamber to the equipment in the instrument room. The walls and ceiling of the instrument room were covered with acoustical tile to reduce echoes and reflections from these surfaces.

V. Data Acquisition Instrumentation

The major components of the data acquisition system are shown schematically in Figure 14. The system is composed of a condenser microphone, a sound pressure level meter, a high-pass filter, an amplifier, an oscilloscope, and a tape recorder.

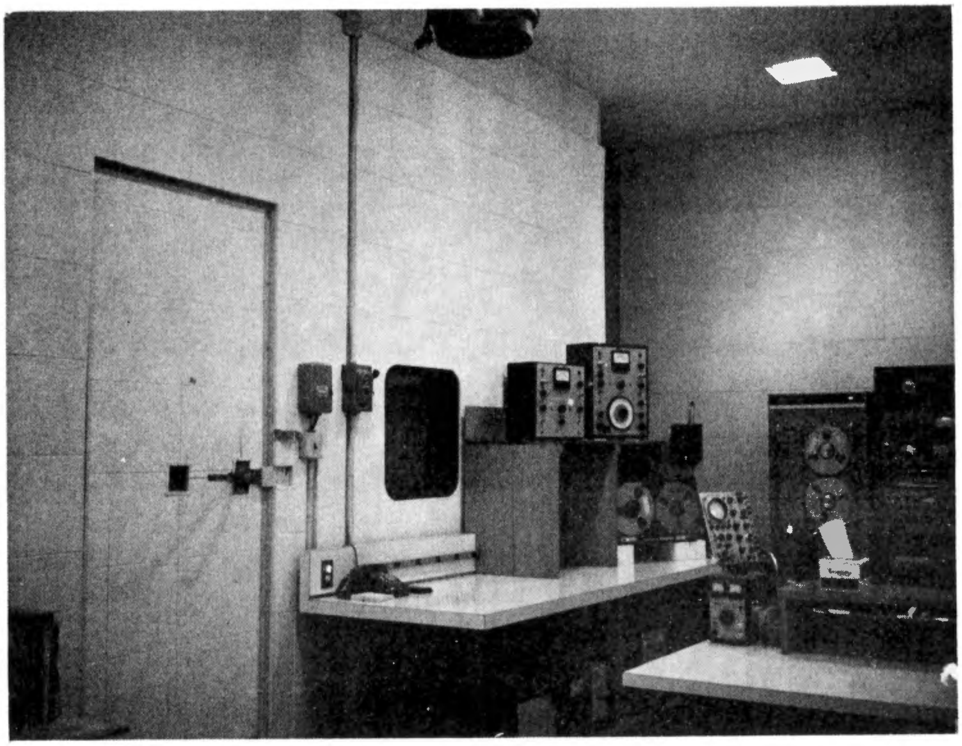

Figure 13. Instrument room and anechoic chamber showing viewing window.

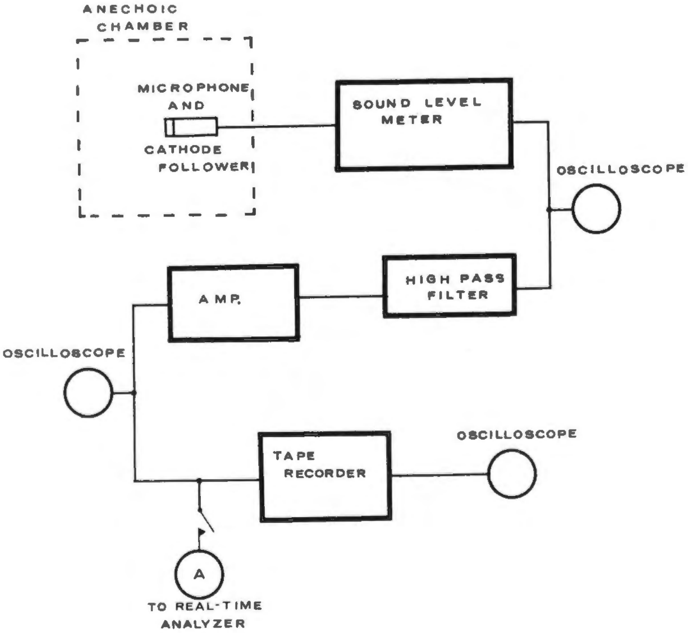

Figure 14. Major components of the data acquisition system.

Microphones

One of the most important components of any data acquisition system is the transducer. The characteristics of the condenser microphones, such as high stability, flat linear response, reasonable high sensitivity, combined with its minimal effect on the sound fields in which it is placed, makes it one of the most suitable transducers available for measuring sound pressures.

A condenser microphone consists essentially of a thin metallic diaphragm mounted in close proximity to a rigid back plate; they are electrically insulated and form the electrodes of a capacitor (Figure 15). When the microphone is exposed to a sound pressure, the diaphragm is subjected to an alternating force proportional to the pressure and the diaphragm area. The consequent movement of the diaphragm varies the capacitance, and these variations are transduced into an ac voltage component proportional to the pressure if a constant charge is maintained between the electrodes. The charge is maintained by means of a constant 200 volts dc polarization voltage, and it remains constant as long as the charging time constant of the circuit is much longer than the period of the sound pressure variations. The sensitivity of the condenser microphone is directly proportional to its diameter while the frequency response is inversely proportional to its diameter. Therefore, condenser microphones are classified according to their diameter.

The capacitance of the condenser microphone is small and any capacitance additional to the transducer capacitance loads the transducer and decreases the output voltage. The total capacitance in the circuit should not exceed the capacitance of the transducer. Therefore, it is

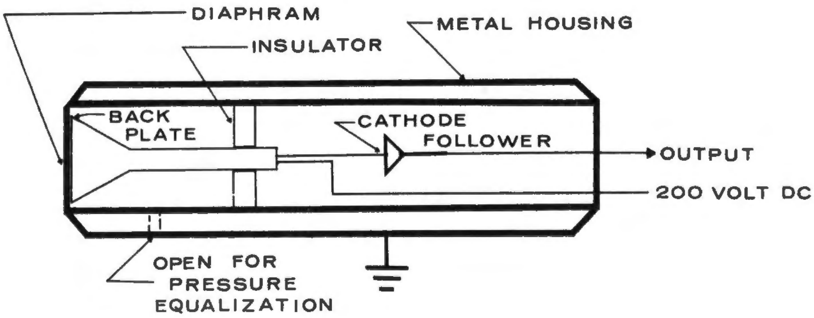

Figure 15. Condenser microphone details.

necessary to locate a cathode follower as close to the microphone as possible. The microphones used in this research have a cathode follower located in the same housing as the microphone.

Three Bruel and Kjaer condenser microphones, a 1-, a 1/2-, and a 1/4-inch, were used in this research (Figure 16). The major characteristics of these microphones are shown in Table II. Once a year these microphones were individually calibrated at the Bruel and Kjaer laboratories against a microphone calibrated at the National Bureau of Standards. A calibration chart was supplied with each microphone after each recalibration. In addition to this factory calibration, each microphone was calibrated periodically in the laboratory with a type 4220 pistonphone calibrator.

Pistonphone

The pistonphone calibrator is battery powered, lightweight, portable, and easy to use. The calibrator consists of two symmetrical pistons driven by a cam disc designed according to the law $r = a + b \sin 4a$. When rotating, the cam gives the pistons a sinusoidal movement at a frequency equal to four times the speed of rotation. The pistonphone is constructed with a cavity in one end so that the 1-inch microphone can be inserted at a precise distance into it (Figure 17). With the microphone in this position, a known volume of air exists in the cavity. Plastic inserts are provided for the calibration of the 1/2- and the 1/4-inch microphones without altering the volume of the cavity. The sound pressure level is related directly to the total displacement or volume of the piston and not related to the speed of rotation. The battery

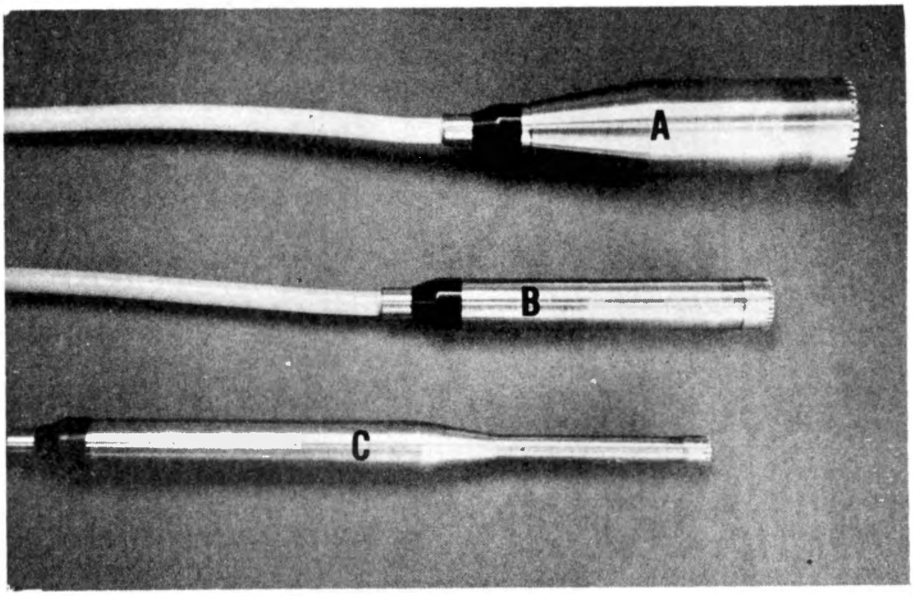

Figure 16. Condenser microphones used for sound recordings: (A) 1 inch, (B) 1/2 inch, and (C) 1/4 inch.

TABLE II

CHARACTERISTICS OF THE 1-, 1/2-, and 1/4-INCH MICROPHONES

	1-inch	1/2-inch	1/4-inch
Frequency response flat ± 3 dB	3 H_z to 20 kHz	4.5 H_z to 40 kHz	4.5 H_z to 100 kHz
Open circuit sensitivity			
mv per N/m^2	50	12.5	4
dB re 1 v per N/m^2	-26	-38	-48
Open circuit distortion limit	4 %	4 %	10 %
Correction factor dB	1	13	26
Influence of R.H. %	less than 0.1 dB		

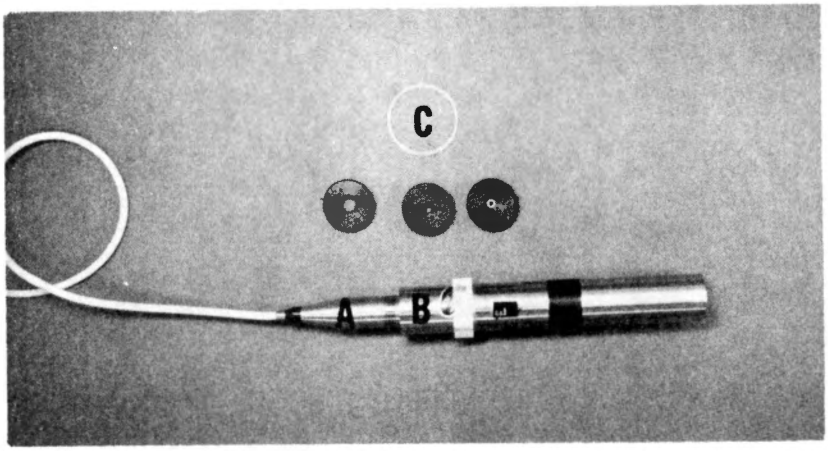

Figure 17. (A) Microphone inserted into (B) pistonphone calibrator and (C) adaptors for 1/2, 1/4, and 1/8 inch microphones.

voltage of the pistonphone is not critical with respect to sound pressure level, but the frequency is directly related to the battery voltage. The pistonphone is rated to give 124 dB re 2×10^{-5} N/m^2 ± 0.2 dB at 250 Hz ± 1 percent.

Sound Level Meter

The signal from the microphones was fed into the microphone amplifiers. Two microphone amplifiers were used, Bruel and Kjaer Models 2107 and 2604. The 2104 and 2107 microphone amplifiers have a flat frequency response up to 40 and 200 kHz, respectively. These amplifiers also supply the 200 volts dc polarization voltage to the microphones. The microphone amplifiers were also designed to be used as sound pressure level meters and the SPL could be determined directly in dB re 2×10^{-5} N/m^2. The sound pressure level was read from these two instruments after obtaining the following factors:

$$\text{Sound Pressure Level} = MR_s + RM_s + MR + CF.$$

Where:

MR_s = Meter range switch
RM_s = Range multiplier
MR = Meter reading
CF = Correct factor

Filter and Amplifier

The output from the sound pressure level meter was fed through a Spencer, Kennedy Laboratory Model 3084, low-pass, high-pass band-pass filter. This filter served to filter out any low frequency noise that might mask out the low level high frequency signals. The frequency characteristics of this filter are shown in Figure 18. Because some attenuation occurred at all frequencies through the filter, a McIntosh Power Amplifier Model MC75 with a flat frequency response from 5 Hz to 75 kHz was used on the output of the filter and adjusted to give a unity gain through the filters.

Tape Recorders

The data were taken from the output of the McIntosh Amplifier and stored on magnetic tape in analog form. Two 7-track data tape recorders (a Honeywell 5600 and a Sanborn 3900) were available for this data storage. The Honeywell recorder was equipped for both direct and FM recording and has three modes of operations: the standard mode, the extended mode, and the double extended mode. The frequency response for direct recording was flat ± 3dB from 50 Hz to 300 kHz and from dc to 40 kHz on FM. Also seven recording speeds were available: 15/16, 1-7/8, 3-3/4, 7-1/2, 15, 30, and 60 inches per second.

The recorder contained independent record and reproduce plug-in module cards for both direct and FM recording speed. An input and output card was selected for each channel and this pair of cards were calibrated to give unity gain through the recorder. The same pair of cards were used to record and reproduce all direct record data, and the same pair of FM cards were used to record and reproduce all FM data. This was done so that the amplitude on the power spectral density could be compared for all data.

The Sanborn recorder also was equipped with seven data channels. Again, the same pair of data cards were used to record and reproduce all data.

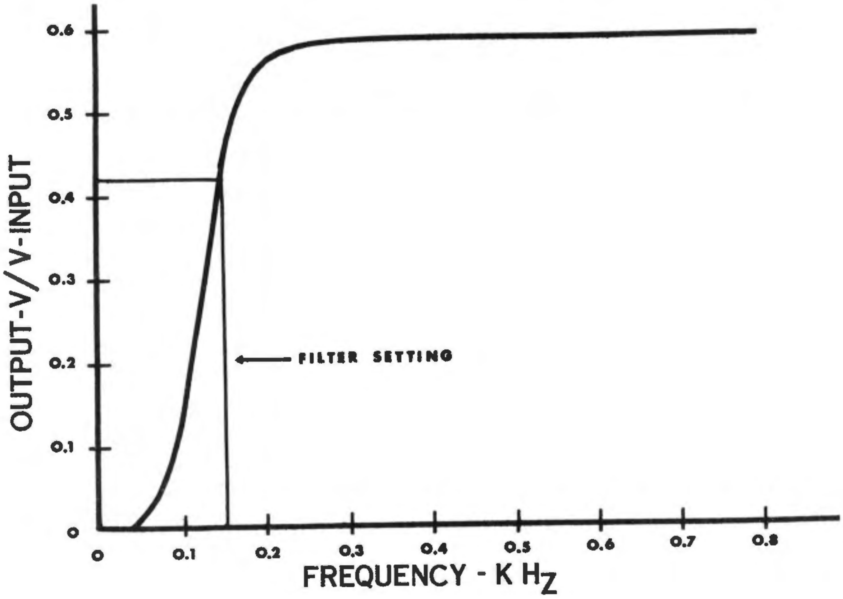

Figure 18. Characteristics of band-pass filter.

VI. Data Analysis Equipment

The major components of the data analysis system are shown in Figure 19. This system consists of a tape loop recorder, an oscillograph, oscilloscopes, a real-time analyzer, a correlator, and x-y recorder (Figure 20).

The analog data could be analyzed for amplitude and frequency content from either data stored on magnetic tape or directly from the transducer in real-time. A Signal Analysis Industries Corporation (SAICOR) real-time spectrum analyzer, Model SAI-52, was used for these analyses. For a thorough discussion of the theory of operation of this type of instrument see references 11, 12, 21, 32, and 63.

The data could be fed from either tape recorder directly into the analyzer or correlator. If time scaling was desirable, the data were fed into the Ampex bin loop recorder, Model FB 450. The bin loop recorder contained seven data channels and six record speeds. The loop could be adjusted for any given data length up to 200 ft. The electronics in this machine were compatible with the tape recorders so that data could be transferred from one to the other without loss of information.

The data on the bin loop recorder could be time scaled and viewed in analog form on a Honeywell Oscillograph, Model 2106. This graphic presentation was used to determine the type of information that was contained in the analog data. This information was then used to select more accurately the analyzer adjustment before the data were processed.

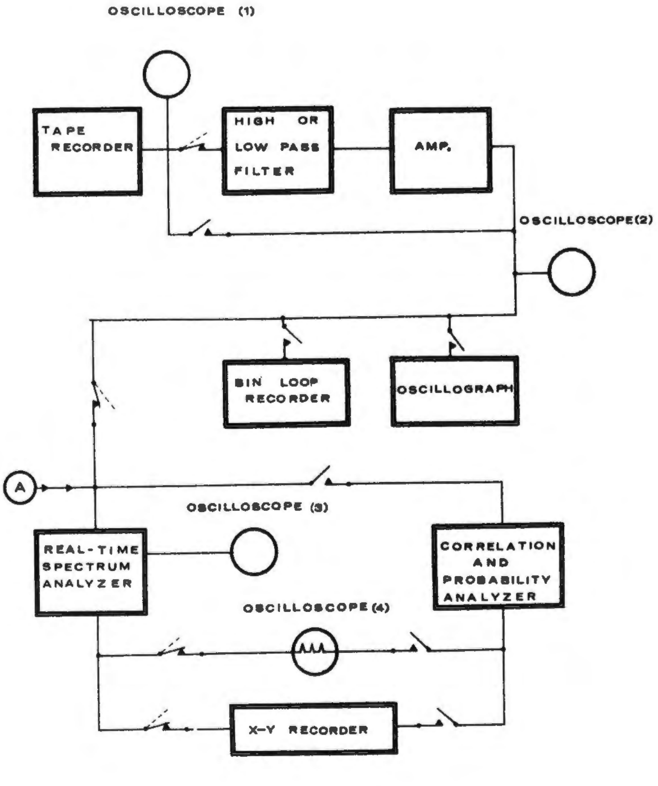

Figure 19. Major components of the data analysis system.

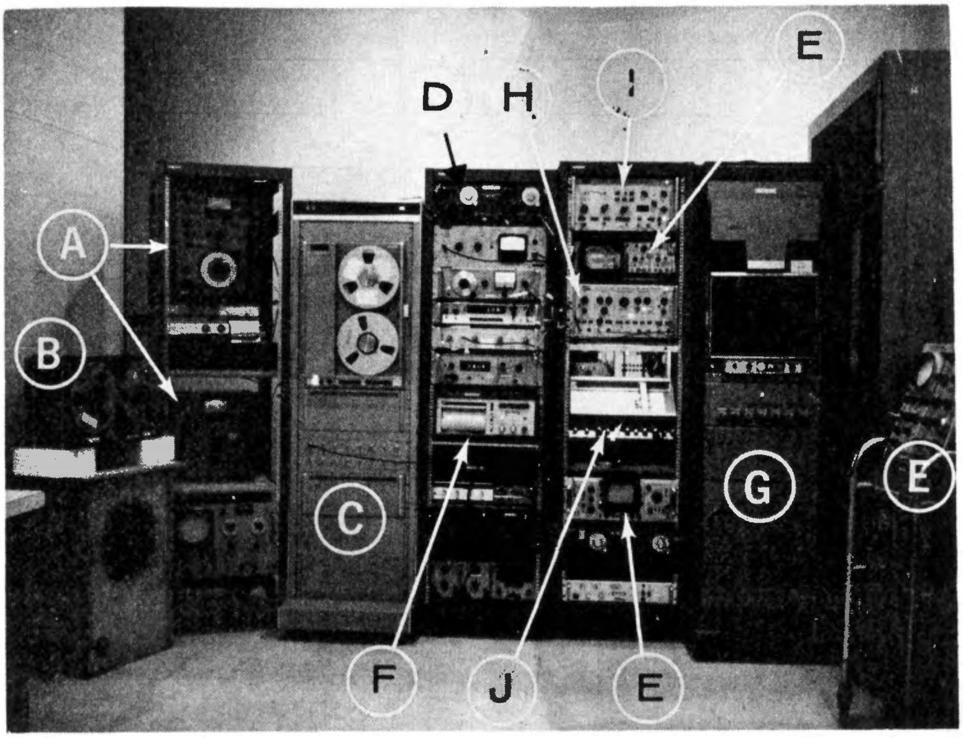

Figure 20. Data analysis system (A) Sound level meters, (B) Honeywell recorder, (C) Sanborn recorder, (D) Filter, (E) Oscilloscopes, (F) Oscillograph, (G) Bin loop, (H) Analyzer, (I) Correlator, and (J) x-y recorder.

Analyzer

The analyzer is a complex instrument and it is equipped with several functions of operations. There are six major functions as shown on the front of the instrument in Figure 21. They are the input, display, frequency range, memory, integration, and output sections.

The input section contains signal coupling, signal calibration, input signal level adjustments, and overload indicators. The display section allows the processed signal to be displayed either on an oscilloscope or on an x-y recorder. The frequency range section consists of a 10-position rotary switch for selecting the frequency range to be analyzed. The lowest range is 0.1 to 20 Hz: the next eight ranges progress in a 1-2-5 sequence up to a range of 20 kHz. The tenth range is 2.5 kHz to 1 mHz. The memory section contains two modes of operation, the manual capture and the self-capture. The memory retains the latest 1200 nine-bit words of information. The integration section integrates the function that it receives from the analyzer output. In addition, the integrator will perform a log or a log square transformation on the data and store this information for display purposes. The output section contains a calibrate control, an output overload indicator, an output gain control, and weighing functions. The weighing functions are linear, square, and log. These functions can be recorded at the output of the analyzer or they can be transferred into the integrator for time averaging.

The processed data from the analyzer were recorded on a Honeywell Model 540 x-y recorder. Both the amplitude, y-axis, and frequency,

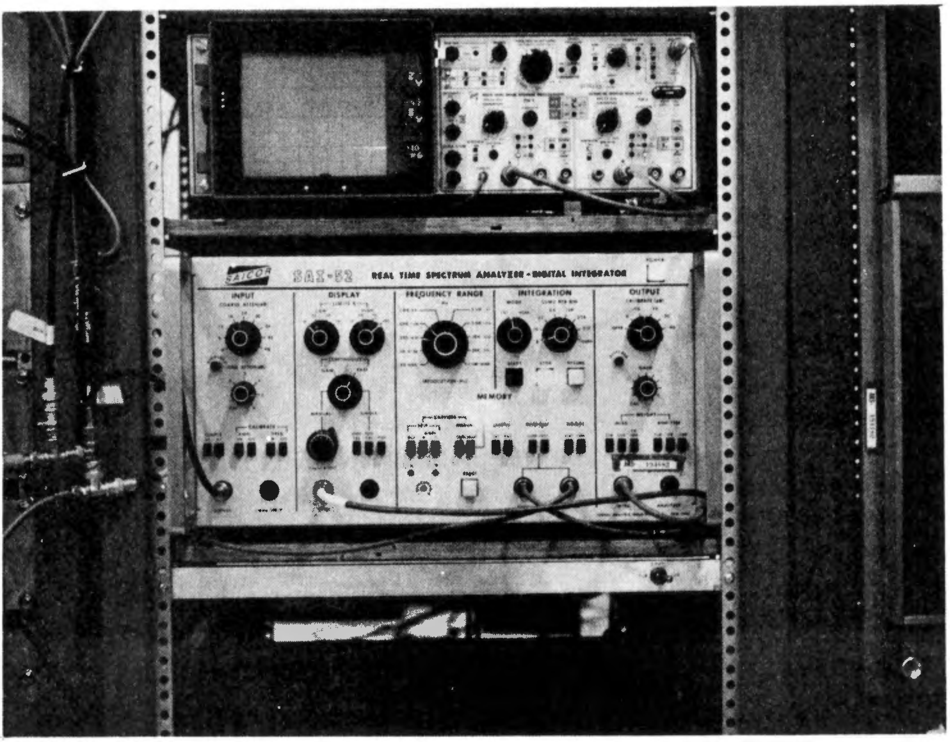

Figure 21. Real-time spectrum analyzer and oscilloscope used to display input signal and processed signal.

x-axis, were calibrated for the power spectral density measurements. Only the x-axis were calibrated for the frequency spectrum.

CHAPTER IV

EXPERIMENTAL PROCEDURES

This chapter gives a description of the techniques and procedures which were developed to record and to analyze many of the sounds that are produced by the Caribbean fruit fly. Several characteristics of flight sounds were determined and their correlation to age and to sex were investigated. The waveforms and frequency content of these flight sounds were compared with those of other sounds produced by the fruit fly. The characteristics of other sounds that are incidental to ordinary movements were also determined. In preliminary studies some high frequency signals were observed on the calling sound. Therefore, it became necessary to determine if this high frequency component existed only on the calling sound or on other sounds as well. The calling sound was isolated and characterized and those frequencies that are common to other fly movements were identified.

After the calling sound was identified this sound was reproduced to both males and females and their response to it was measured. A bioassay test system was designed and constructed for these response studies.

I. Raising and Handling of Insects

The fruit flies used were reared to the pupal stage at the Sub-Tropical Experimental Station, Homestead, Florida. The pupae, subsequently, were shipped to the Gainesville laboratory and approximately

2,000 were placed in each rearing cage where they emerged into the adult stage. The standard adult diet (sugar, water, and yeast hydrolysate) was placed in the cage so that the emerging flies would have immediate access to it. The rearing room conditions were maintained at a temperature between 70 and 80°F, a relative humidity between 40 and 50 percent, and a light level between 500 and 650 lux at cage height. Adult flies were maintained under a light and dark cycle from 0800 to 1900 and 1900 to 0800 hours, respectively.

The majority of the adults emerged within three days after initial emergence, and the emergence date was recorded so that adults of known age and sex could be used for specific tests. At the end of each day, emergent flies were sexed with an aspirator and placed in holding cages which were provided with food and water. The males were isolated in a separate holding room from the females. The room conditions were similar to that of the rearing room.

Adult flies were transferred from the holding cages as required. The flies that were used in the flight mill studies were removed from the holding cages in small plastic vials and placed in cool air until they became immobilized. A 23 gauge wire bent at a right angle was attached to the thorax of each immobilized fly by means of plastic rubber cement. Once the flies were attached to the wire, they were then placed on a holding mount where they were allowed to fully recover. After the recovery period, they were allowed to fan their wings in a stationary position for about one minute at which time a piece of paper about 1/4 inch square was placed under their feet. The flies would hold the paper by their feet and remain stationary until the paper was removed. They were then transferred into the anechoic chamber where the flight mill tests were conducted.

The flies used to record the calling and premating sounds remained in the holding cages and were transferred to the anechoic chamber as needed. These recordings were made directly from the holding cages.

II. Characteristics of Flight Sounds

Flight sounds will be defined as any sounds produced by the fly during flight. These sounds can be produced by a number of mechanisms, such as wing movements, muscle movements, etc. Therefore, the purpose of the flight sound studies was to obtain a better understanding of the signals generated by such movements and correlate them with the sounds made during calling. By eliminating incidental flight sounds, the information contained in the calling sound was easier to identify.

Experimental Design

A randomized block experimental design was used in the flight mill studies. There were 18 treatments with 15 replications: the age of the flies were considered as treatments. Data were taken on flies from 1 to 15, 20, 25, and 30 days old. Five replications were taken from each of three shipments of flies. Bartlett's test of homogeneity of the variances was used to show that the variances of all shipments of flies were from the same population 95 percent of the time.

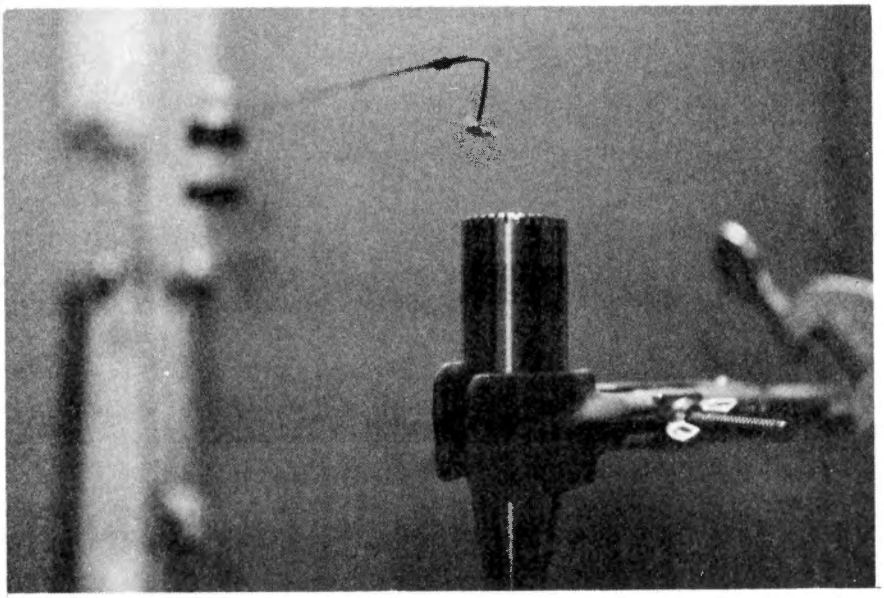

Figure 22. Position of fly over microphone.

Data Recording

The 1-inch microphone was used in the flight mill data recordings because of its greater sensitivity to the lower frequencies. The signal from the microphone was fed through the cathode follower and then into the 2604 sound pressure level meter where the sound pressure level was recorded. The signal was then fed through the high-pass filter, which filtered out all signals below 70 Hz and then through the power amplifier. At the output of the amplifier, the signal was paralleled into the spectrum analyzer for immediate analysis and into the data tape recorder for later processing. The maximum signal level at the input of the spectrum analyzer was recorded for each data run. This allowed the maximum signal level to be identified in order to later calibrate the spectrum analyzer and x-y recorder for the power spectral density plots. The frequency response of this system was flat ± 3 dB from 70 Hz to 5 kHz. The response was limited on the low side by the high-pass filter and on the high side by the tape recorder.

Data Analysis

The analog data were fed directly from the tape recorder, in real-time, into the spectrum analyzer where the power spectral density was calculated and stored in memory. The processed data were then fed from the analyzer memory into the x-y recorder where a power spectral density plot was obtained for each fly. The analyzer and x-y recorder were calibrated, as explained in Appendix B, to give a known amplitude in dB re 100 mv and a frequency in Hz for each power spectral density record. These calibrations were checked before each data run.

The data were analyzed on the 2 kHz frequency range with a resolution of 5 Hz. The processed signal length per integration was 0.2 seconds, and since 64 integrations were performed, the total processed signal length was 12.8 seconds. The 12.8 seconds of data were taken from the 20 to 32 second portion of the data record. This analog data entered the analyzer in the time domain as $f(x_t)$ and the Fourier Coefficients were then calculated in the linear mode for each of the 400 bins. The processed data $g(x_f)$, now in the frequency domain, were stored in the analyzer memory where $g(x_f)$ represented the Fourier Coefficient of the input function $f(x_t)$. This process was repeated until 64 integrations had been performed. The integrator averaged the function in each bin, squared the function, and then took the log. Therefore, the output function of each bin was as follows:

$$\text{Power spectral density} = [\frac{1}{N} \sum_{i=1}^{N} g(x_f)]^2 \log$$

where:

$$N = 64 \text{ and } g(x_f) = \text{Fourier Coefficient.}$$

The log function was included to give better identification of the low level signals by suppressing the large signals and enhancing the small ones. The amplitude, frequency, and harmonic content was obtained from each power spectral density plot.

III. Characteristics of Sounds Incidental to Ordinary Movement

There are many sounds associated with ordinary movements of insects. Such sounds include flight sounds, aggression sounds, sounds caused by walking, wing movements other than flight, cleaning and rubbing the wings with their legs. Typical signatures of these sounds were recorded and analyzed for waveform characteristics and frequency content. The high frequency content between 18 and 40 kHz was of primary interest because this frequency occurred on the calling sound. Therefore, the major purpose of analyzing these sounds was to determine if this frequency was common to all insect movements.

Data Recording

The 1/2- and 1/4-inch microphones were used to record the data from the incidental sounds. The 1/2-inch microphone has a flat frequency response to 40 kHz ± 3 dB and the 1/4 inch to 100 kHz ± 3 dB. The frequency response is much greater with these microphones than with the 1-inch; however, the sensitivity is much less. The noise level of the 1/4-inch microphone is approximately 75 dB re 2×10^{-5} N/m^2 and is primarily white noise that extends to 320 kHz. The low-level, high-frequency signals that occurred on the incidental and calling sounds ranged from 30 to 50 dB re 2×10^{-5} N/m^2. With the use of this microphone positive identification of these frequencies was difficult, even with the aid of the signal enhancement on the analyzer. Therefore, the 1/2-inch microphone was used primarily in these high frequency identifications. The 1/4-inch microphone was used to investigate for any discrete periodic frequencies that might occur above 40 kHz.

The Honeywell recorder was used to record the above data. The recorder was operated in the direct recording mode at a speed of 15 inches per second which gave a frequency response of 100 Hz to 75 kHz.

The procedures used to record the high frequency flight sound data were the same as those used in the flight mill studies except for the microphones and recorder speeds. The fly was mounted on the flight mill, and the data were recorded in static flight using the 1/2- and 1/4-inch microphones. These microphones were located in the same relative position to the fly as in the flight mill studies.

It was observed many times during this research that when a male was in the process of producing the calling sound, another male would approach and attack the calling male. These aggressive acts were accomplished by rapid wing movements and body contacts. The sounds produced by these activities were recorded and analyzed to determine if they also contained the high frequency components that occurred on the calling sound.

The aggressive sounds were recorded by placing several males in a holding cage. A male would normally select a location on the screen wire cover at the top of the cage to call. When a male was observed to begin calling, the microphone was placed about 1/2 inch above him. The microphone was left in this position until an aggressive male attacked the calling male. The same equipment was used to record these sounds as was used in all other high frequency recordings.

Sounds of other fly movements were also recorded and analyzed. These sounds were produced by such activities as walking, slow movements of the wings, and cleaning or rubbing the wings with their legs. These recordings were made by holding a microphone as close to the caged flies as possible without disturbing them. Each activity was identified on the tape as the recordings were made, and the sounds from these activities were analyzed for their high frequency content.

The high frequency components were observed to occur on the flight data, aggression sounds, incidental movements of the flies, mating sounds, and calling sounds. An investigation was made to determine if the high frequency components were associated with any particular movement or body part of the flies. Therefore, sounds were recorded and analyzed of the flies, both males and females, after certain body parts were removed. The body parts removed were: one-half of each wing, both wings, legs, abdomen, thoracic muscles, and the head. Also, one wing was removed and a sharp object was rubbed against it. These sounds were recorded by attaching the fly to a holding wire, removing the body part, and holding the fly above the microphone by the holding wire. Again the 1/2- and 1/4-inch microphones were used to record these data.

The premating sound is made by the male fly after the male mounts the female for mating but before mating takes place. The sound is made by a continuous fanning of the wings. The purpose of this sound is unknown, but its analysis was made to determine its similarity to the calling sound and to determine if the high frequency components were present.

Sound recordings were made from the holding cages that contained both males and females. The microphones were located close to a calling male, and after he had attracted a mate, the premating sounds were recorded. The 1-inch microphone was used to record the low frequency fanning action and the 1/2- and 1/4-inch microphones were used to record the high frequency signals.

Data Analysis

The high-frequency, low-amplitude signals were analyzed by filtering out the low-frequency, high-amplitude signals. All frequencies below 16 kHz were filtered out. These high frequency signals were then amplified before they were analyzed. A frequency analysis was performed on all the incidental sounds to determine if the high frequency was present on each.

IV. Characteristics of the Calling Sound

The calling sound is made by the male fly before pair formation takes place. After the male begins to make the calling sound, he will remain in a near stationary position until he either attracts a mate or moves to a new location. Therefore, it was possible to locate the microphone very close to the calling male and record this sound without disturbing the fly. The recordings were made with the fly unrestricted in a holding cage. Once the fly began to call inside the cage, the microphone was located outside the cage approximately a 1/2 inch above the fly (Figure 23). Data were taken on each fly until he moved to a new location or attracted a mate. If he attracted a mate, the premating sound was also recorded, and this allowed a direct comparison of the calling and premating sound of the same fly. Calling sounds were recorded from flies six through twenty days old.

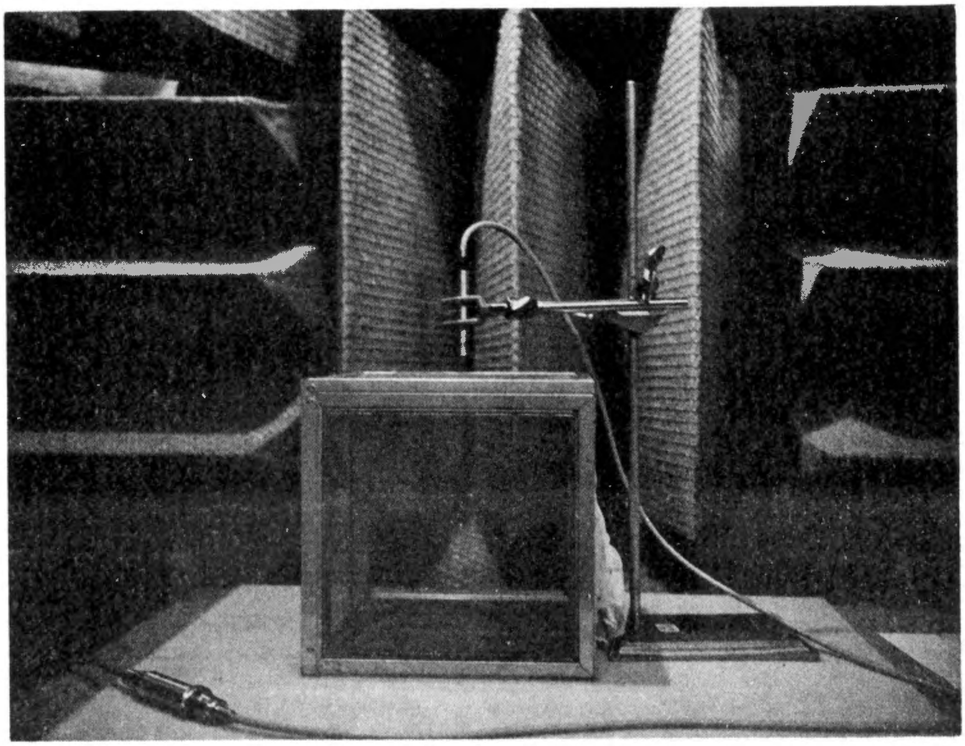

Figure 23. Microphone located over holding cage for recording calling sounds.

Most of these data were taken with the 1/2-inch microphone. However, random samples of data were also taken with the 1-inch and 1/4-inch microphones in order to better examine the very low and the very high frequency components.

The calling sounds were analyzed for waveform characteristics, frequency content, amplitude, and pulse information. The waveform characteristics and frequency content were determined and compared to those of the flight, aggression, and premating sounds.

V. Response of the Fruit Fly to Reproduced Calling Sounds

After the calling sound had been isolated and identified, tests were conducted to determine if there were any indications that this sound is used as one mode of communication between the same sex or between different sexes, either alone or in combination with sex pheromone extract. The calling sounds used in these response tests were recorded as described previously using the 1/2-inch microphone and the Honeywell tape recorder. Those sounds of good quality were dubbed onto another tape for these tests. This tape contained three hours of calling sounds of flies from six to twenty days old, and the calls were randomly placed on the tape so that no sound from the same fly was repeated for at least one hour. The waveform and sound pressure level were checked at each speaker and recalibrated, if required, before each replication.

The sex pheromone produced by males, perhaps during the wing fanning, attracts sexually matured females and has not been identified and synthesized. However, J. L. Nation of the University of Florida supplied a sufficient amount of extract containing the pheromone for these tests. The amount of pheromone contained in the extract was unknown, but it constantly resulted in good attraction, 30 to 45 percent, of mature females. The extract was stored in a refrigerator at 40°F. About 15 minutes before the tests were begun, the extract was removed from cold storage, shaken, and then 3 ml pipetted onto filter paper which had been rolled into a cylinder. The treated paper was then placed into the treatment chamber (Figure 4, page 35).

Next, the sound and air were initiated. The air flow in each unit was measured and adjusted before each replication to insure equal air flow within each cell and each test unit. Subsequently, 50 flies of the appropriate sex and age were released in the center test cell.

Three experiments arranged in a randomized block experimental design were conducted to measure the response of the fruit flies to these treatments. A sound pressure level of approximately 65, 65, and 50 dB was maintained at the entrance of the center chamber for tests 1, 2, and 3, respectively.

The first experiment using flies eight through thirteen days old was repeated 16 times and consisted of eight treatments simultaneously tested. These tests included males and females to sound, males to males, females to males, males and females to extract plus sound, and males and females to extract. Since previous data indicated age effects, the second experiment was carried out to test responsiveness of young females six to eight days old. This experiment had two treatments, sound plus extract and extract alone, and was replicated eight times. The third test was the same as the second except that the sound pressure level was reduced to 50 dB. All tests were carried out from 3:00 p.m. to 8:00 p.m.

The flies attracted and trapped onto the sticky fly trap rings were counted, and each test unit was cleaned and allowed to air for 48 hours before another replication was conducted.

The flight data were analyzed for waveform characteristics, frequency and harmonic content, sound pressure level, and flight velocity as related to the age and sex of the flies. While individual variations in waveform information occurred, the basic waveform remained constant. The basic waveform consisted of a fundamental frequency with a time period between 5 and 9 msec, which was considered to be the time for one complete cycle of the wing stroke. Contained within this time period was one or more low amplitude frequencies with a time period of 1/3 to 1/8 that of the fundamental (Figure 24). The source of these higher frequencies was not determined, but with the aid of high speed photography, the wing movements could be correlated with waveform shape. The frequency of these waveforms occurred periodically within the fundamental period; therefore, their spectral peak occurred as a multiple of the fundamental in the spectral analysis, usually in a harmonic. Typical waveforms produced by each sex at each age tested are shown in Figures 25 and 26. Also a composite power spectral density for each of these waveforms is shown in Figures 27 and 28. A frequency spectrum for the 3- and 11-day old males and females is also shown in Figures 29 and 30.

The data from these waveforms appeared to fall into two classifications: complex periodic and almost periodic data (19). The complex periodic data occurred when the flies were fanning their wings at a constant rate with no variation in the wingbeat. The almost periodic data occurred when there were variations in the wing movements. An important property of almost periodic data is that, if the phase angle is ignored, the data can be characterized by a discrete frequency

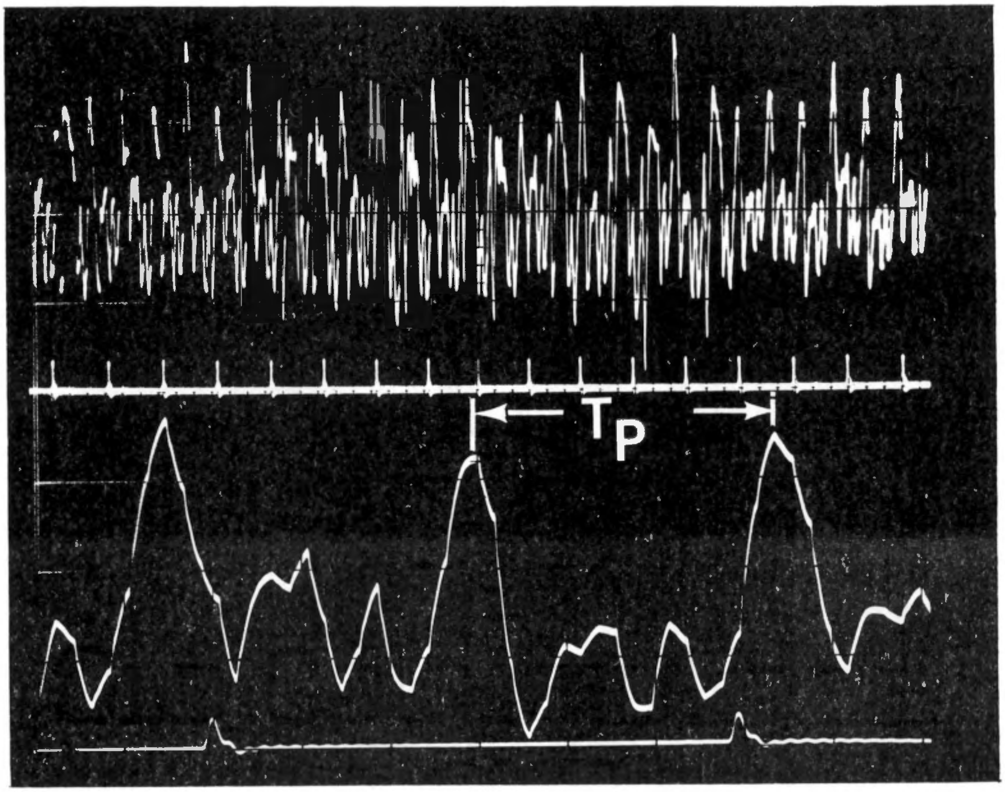

Figure 24. Waveform of flight sounds showing the fundamental period (T_p) and the secondary periods contained within T_p. Pulse marker at 10 msec.

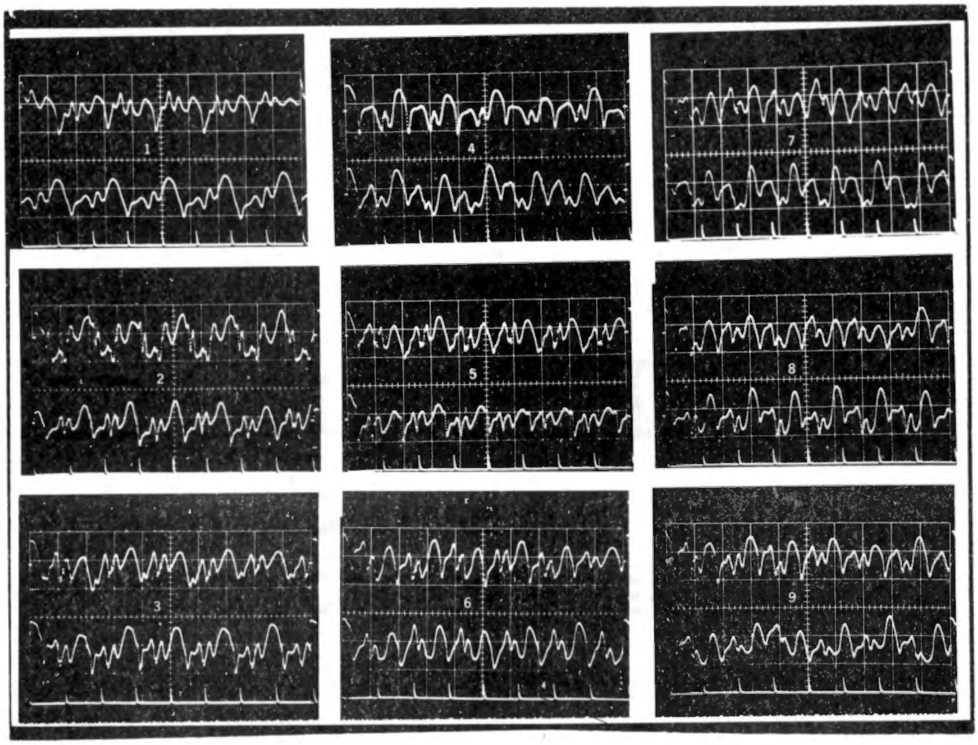

Figure 25. Waveforms of flight sounds of male (top trace) and females (bottom trace) fruit flies ages from one through nine days old. Pulse markers occur each 5 msec.

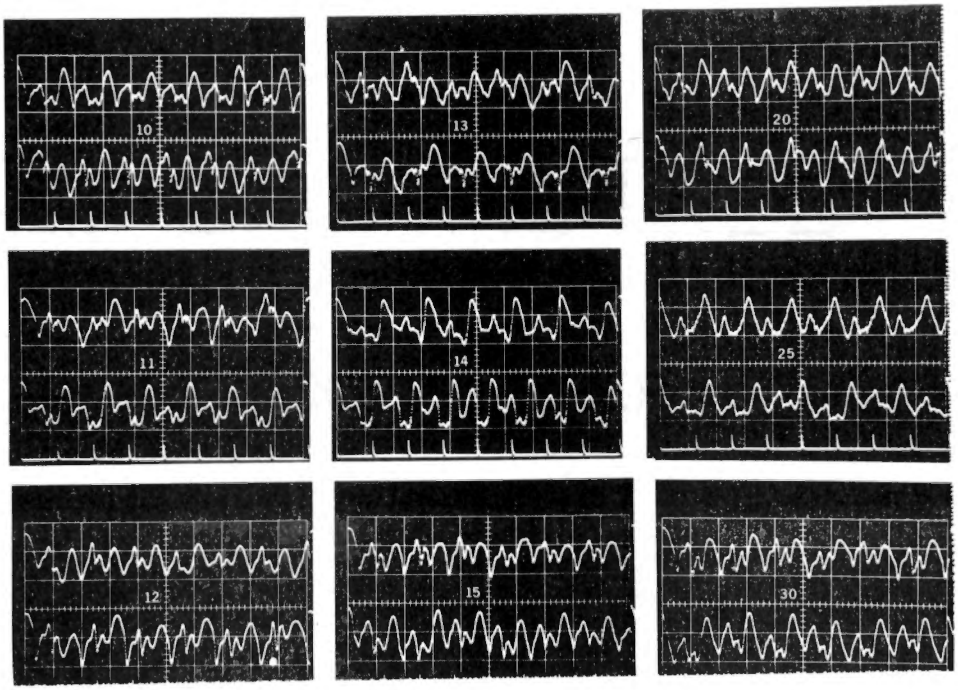

Figure 26. Waveforms of flight sounds of males (top trace) and females (bottom trace) fruit flies ages 10 through 15, 20, 25, and 30 days old. Pulse markers occur each 5 msec.

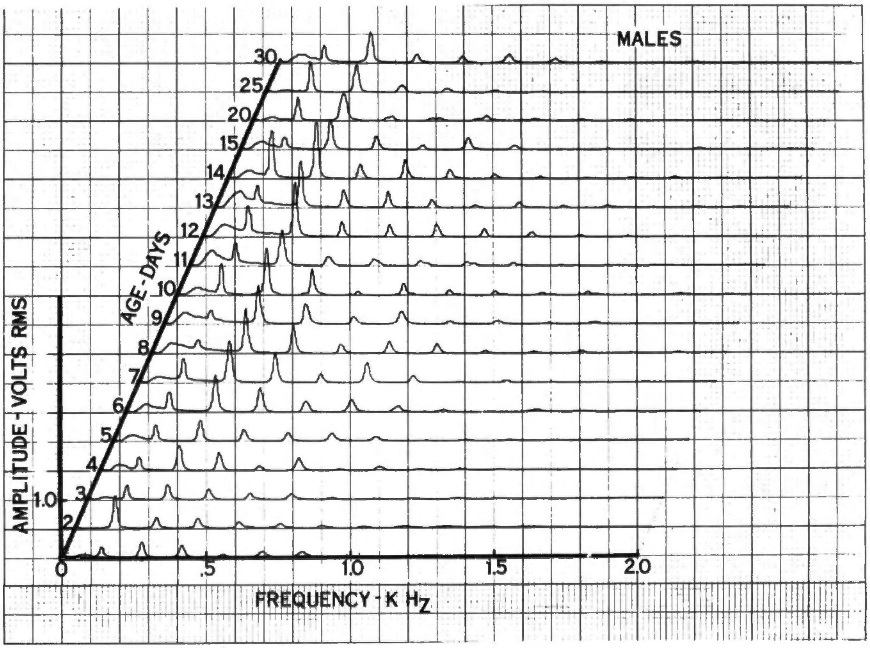

Figure 27. Power spectral density for average male flies one through thirty days old in static flight.

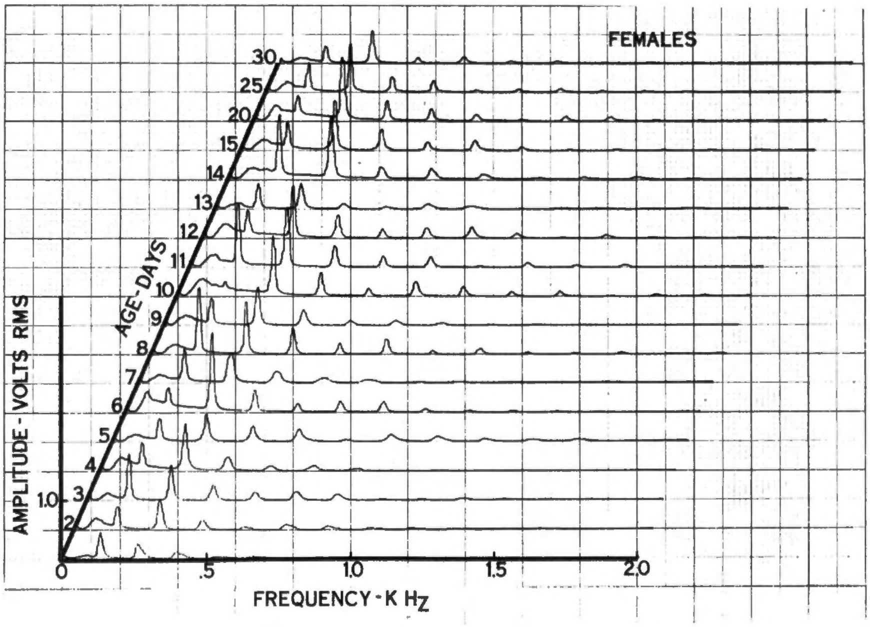

Figure 28. Power spectral density for average female flies one through thirty days old in static flight.

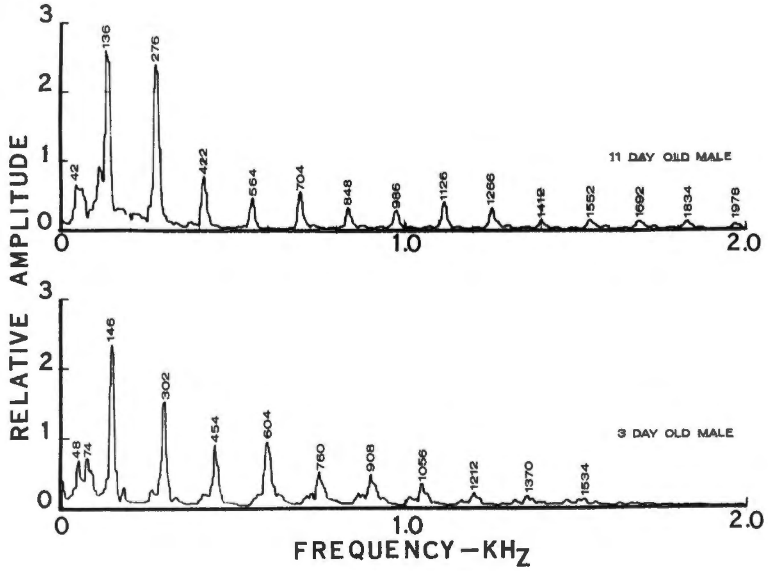

Figure 29. Frequency spectrum of three and eleven day old male flies.

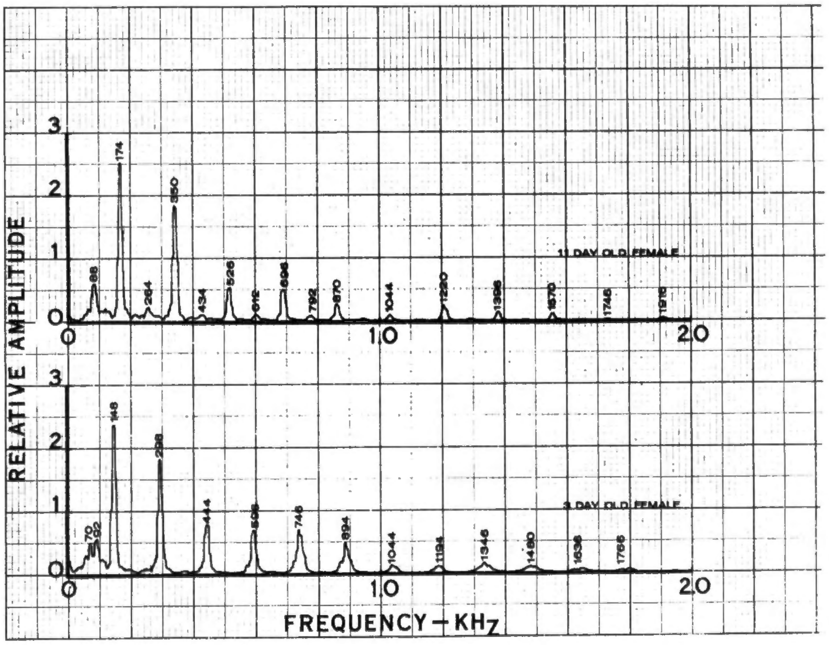

Figure 30. Frequency spectrum of three and eleven day old female flies.

spectrum similar to that for complex periodic data. Since the phase angle was lost in the time averaging process, the frequency spectrum was very similar for both the complex and the almost periodic data.

The data were considered complex when the fundamental frequency, as well as the harmonics, appeared as discrete spectral lines with the fundamental frequency being the greatest in amplitude. Data were considered almost periodic when the fundamental frequency was less in amplitude than the second harmonic and when the spectral lines in the fourth or higher harmonic contained more than one spectral peak (Figure 31). The complex periodic and almost periodic waveforms were random throughout the data with both waveforms occurring about 50 percent of the time for both sexes and all ages of the flies.

A power spectral density was calculated and plotted from the analog data of each fly in both the static and dynamic flight tests. Since it was not practical to include all 1080 power spectral density plots, the data were extracted from these plots and presented in table and graph form. The wingbeat frequency was read directly from each power spectral density plot. The sound pressure level was recorded from the sound pressure level meter in dB re 2×10^{-5} N/m^2 at the time each test was conducted. The peak power contained in the fundamental frequency was measured from each power spectral density plot in dB re 100 mv. The peak power in the harmonics was measured from each power spectral density in dB re 100 mv. Also the total number of harmonics for each power spectral density was determined from the plots. These data were tabulated and tested for statistical significance at the 5 percent probability level by the analysis of variance and the Duncan's multiple range test.

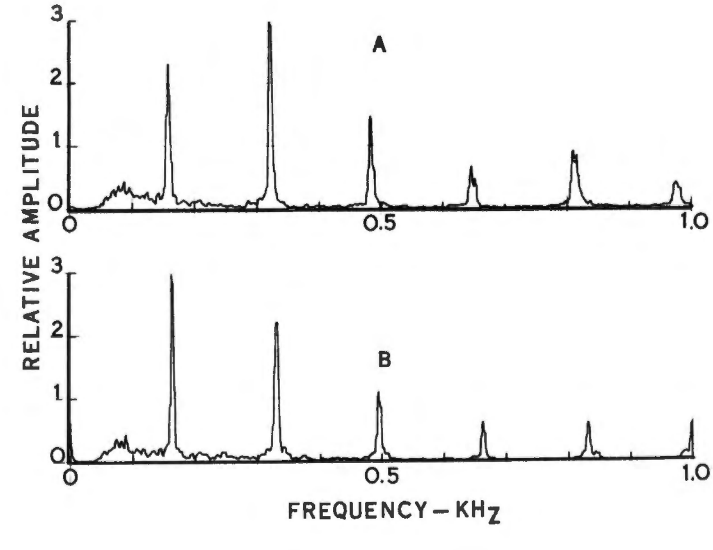

Figure 31. Almost periodic data (A) with reduced amplitude in the fundamental frequency with two peaks occurring in the 4th and 5th harmonics (B) complex periodic data.

These data are presented in Tables III, IV, and V. The standard deviation for each variable is shown in Tables VI and VII. A regression analysis was performed for each measured parameter and correlated with age. In all cases except one, the data were found to fit a third order polynomial as shown in Figures 32 through 39. The wingbeat vs velocity data were found to fit a linear regression curve with an R^2 value of 0.90 for males and 0.81 for females (Figure 36). The polynomials were of the form:

$$\hat{Y} = a + bx + cx^2 + dx^3$$

where:

$\hat{Y}$ = the estimated value of the variable that appears on the y axis of each regression curve

x = fly's age in days.

The polynomial equation for each regression curve appears with its respective curve in each figure.

The linear regression curve was of the form:

$$\hat{Y} = a + bx$$

where:

$\hat{Y}$ = the estimate of flight velocity that corresponds to a given wing beat frequency

x = wing beat frequency.

The standard deviation (Tables VI and VII) was used as a measure of the variability of flies within each age group. As expected, the variability was large in each parameter measured. The variability, in

TABLE III

SOUND PARAMETERS PRODUCED BY THE MALE CARIBBEAN FRUIT FLY DURING STATIC FLIGHT*

Age Days	Wingbeat Frequency H_z	Age Days	Sound Pressure Level dB**	Age Days	Peak Power Fundamental Frequency H_z	Age Days	Total Peak Power in Harmonics dB***	Age Days	Number of Harmonics
1	132.9 a	3	71.5 a	3	8.7 a	2	38.2 a	2	6.7 a
3	136.0 a	2	71.7 a	15	10.2 ab	1	40.1 a	1	7.1 ab
2	137.6 a	4	71.8 a	1	10.4 ab	3	45.3 a	3	7.9 abc
4	140.2 ab	30	71.8 a	4	10.8 abc	4	60.7 b	13	8.3 bc
5	146.2 bc	5	73.1 ab	2	11.4 abcd	30	61.3 b	10	9.1 cd
10	152.6 cd	13	73.3 abc	8	11.4 abcd	13	62.8 bc	30	9.1 cd
25	152.7 cd	1	73.4 abc	12	12.5 bcde	15	64.3 bcd	4	9.3 cde
7	152.9 cd	20	73.5 abc	6	12.6 bcde	10	66.6 bcd	7	9.4 def
13	154.4 cd	7	73.5 bc	9	12.9 bcde	5	67.1 bcd	8	9.7 def
30	154.6 cd	10	75.5 bc	5	13.1 bcdef	25	68.6 bcd	15	9.7 def
6	155.7 d	14	75.7 bc	7	12.1 bcdef	12	69.5 bcd	9	9.9 ef
20	155.7 d	11	75.9 c	11	13.7 cdefg	8	69.7 bcd	25	10.1 ef
15	156.8 d	15	75.9 c	13	13.9 defg	7	69.9 bcd	5	10.2 ef
11	157.5 d	6	77.0 c	25	14.1 defg	11	70.6 bcd	6	10.2 ef
12	157.7 d	12	76.0 c	10	15.1 efg	6	73.6 bcd	11	10.2 ef
9	158.1 d	8	76.1 c	14	15.7 fg	9	74.5 bcd	14	10.4 ef
8	158.4 d	9	76.2 c	30	16.5 gh	14	75.4 cd	12	10.8 ef
14	160.1 d	25	78.7 d	20	18.7 h	20	77.2 d	20	10.9 f

*Means followed by the same letter are not different at the 5 percent probability level.

**dB re 2 x 10^{-5} N/m^2.

***dB re 100 mv.

TABLE IV

SOUND PARAMETERS PRODUCED BY THE FEMALE CARIBBEAN FRUIT FLY DURING STATIC FLIGHT*

Age Days	Wingbeat Frequency H_z	Age Days	Sound Pressure Level dB**	Age Days	Peak Power Fundamental Frequency dB***	Age Days	Total Peak Power in Harmonics dB***	Age Days	Number of Harmonics
1	122.6 a	1	72.0 a	3	10.9 a	3	48.8 a	1	8.7 a
3	138.0 b	3	72.4 a	4	12.0 ab	1	51.0 ab	3	8.8 ab
2	138.3 b	30	73.4 ab	2	12.1 ab	2	57.5 abc	7	9.0 abc
4	142.7 bc	4	74.2 bc	7	12.5 ab	7	58.2 abcd	10	9.1 abc
25	146.3 bcd	13	74.5 bc	6	12.9 abc	20	60.9 abcde	15	9.1 abc
13	149.6 cde	20	74.7 bcd	5	13.3 abcd	30	61.9 abcdef	5	9.4 abcd
6	151.6 cde	5	75.5 bcde	10	13.3 abcd	4	61.9 abcdef	6	9.4 abcd
20	152.4 de	2	75.6 bcde	15	13.3 abcd	9	63.1 bcdef	2	9.5 abcd
7	152.7 de	15	76.1 cde	9	13.4 abcd	10	64.3 cdef	4	9.5 abcd
30	153.2 de	9	76.2 cde	1	13.8 abcd	15	64.7 cdef	9	9.7 abcd
15	153.9 de	10	76.5 de	20	14.0 abcd	6	65.5 cdef	8	9.9 abcd
9	154.2 de	7	76.7 de	12	14.1 bcd	5	66.6 cdef	14	10.1 abcd
5	154.3 de	8	76.9 e	25	14.1 bcd	11	69.9 cdef	20	10.2 abcd
8	155.5 def	25	76.9 ef	13	15.7 cd	25	70.3 cdef	30	10.2 abcd
11	156.5 ef	6	77.1 f	8	15.8 cd	8	72.4 def	11	10.3 bcd
12	157.4 ef	14	77.1 f	30	15.8 cd	14	72.9 def	13	10.4 cd
10	158.9 ef	12	77.1 f	11	16.3 d	13	74.3 ef	12	10.9 d
14	164.3 f	11	77.2 f	14	16.5 d	12	76.5 f	25	10.9 d

*Means followed by the same letter are not different at the 5 percent probability level.

**dB re 2 x 10^{-5} N/m^2.

***dB re 100 mb.

TABLE V

WINGBEAT FREQUENCY AND FLIGHT VELOCITY OF CARIBBEAN FRUIT FLIES*

MALES				FEMALES			
Age Days	Frequency H_z	Age Days	Velocity Ft/Sec	Age Days	Frequency H_z	Age Days	Velocity Ft/Sec
1	114.2 a	1	2.49 a	1	116.3 a	1	2.79 a
2	117.0 a	2	2.53 a	2	125.3 b	2	3.02 ab
3	127.9 b	30	2.85 ab	3	132.0 bc	30	3.08 abc
4	129.2 b	3	3.05 bc	4	132.7 c	25	3.31 bcd
5	140.9 c	4	3.05 bc	25	140.2 cd	4	3.38 bcd
6	142.5 cd	13	3.15 bcd	5	142.1 de	15	3.38 bcd
20	142.7 cd	25	3.18 cde	11	142.9 de	13	3.41 bcde
25	145.9 cde	20	3.22 cde	6	143.7 de	5	3.44 bcde
7	146.3 cde	6	3.31 cdef	13	144.3 de	12	3.48 bcde
13	146.8 cde	5	3.38 cdef	12	144.9 de	3	3.48 bcde
10	150.0 cde	15	3.38 cdef	9	145.0 de	6	3.51 cde
15	151.3 de	7	3.41 cdef	7	145.7 de	11	3.54 cde
14	151.9 de	10	3.44 cdef	30	146.3 de	9	3.61 de
11	152.1 de	11	3.44 cdef	15	148.0 de	10	3.61 de
9	154.3 e	14	3.61 def	20	148.3 de	20	3.61 de
8	154.7 e	12	3.64 ef	8	149.9 de	7	3.67 de
12	154.9 e	8	3.74 f	10	150.8 e	8	3.74 de
30	155.3 e	9	3.77 f	14	160.0 f	14	3.84 e

*Means followed by the same letter are not different at the 5 percent probability level.

TABLE VI

STANDARD DEVIATION OF THE FUNDAMENTAL WINGBEAT FREQUENCY (WBF), FLIGHT VELOCITY, AND SOUND PRESSURE LEVEL (SPL) FOR BOTH MALES AND FEMALE FLIES

	FUNDAMENTAL WBF-H_z							
	Static Flight		Dynamic Flight		Flight Velocity-ft/sec		SPL dB*	
Age-Days	Males	Females	Males	Females	Males	Females	Males	Females
1	12.5	10.9	15.1	11.9	0.50	0.63	4.3	5.1
2	13.8	8.5	8.8	11.5	0.38	0.51	4.0	2.5
3	11.4	11.5	10.8	11.2	0.65	0.69	4.9	4.6
4	16.8	9.1	12.3	10.2	0.53	0.54	4.5	1.1
5	6.6	11.8	8.1	10.6	0.50	0.57	4.2	2.3
6	10.3	9.1	9.4	9.7	0.40	0.45	2.2	2.5
7	11.0	10.2	9.0	13.2	0.54	0.60	4.7	2.3
8	9.8	11.9	10.3	8.5	0.30	0.68	2.2	2.3
9	7.6	11.0	12.4	13.0	0.55	0.48	2.4	3.6
10	13.5	10.0	14.9	11.3	0.79	0.37	5.4	2.4
11	13.7	11.0	14.3	13.5	0.59	0.53	2.5	1.9
12	9.9	13.4	8.2	16.6	0.55	0.34	3.0	2.2
13	14.8	12.1	15.8	13.5	0.60	0.60	4.8	3.1
14	11.0	12.9	7.8	18.5	0.71	0.09	2.1	2.4
15	11.6	12.9	12.8	13.6	0.50	0.64	2.3	2.9
20	12.0	12.0	21.1	10.3	0.67	0.49	3.2	2.8
25	15.2	21.0	12.0	12.9	0.59	0.55	3.2	4.1
30	17.7	12.0	14.4	11.5	0.74	0.77	4.8	3.6

*dB re 2 x 10^{-5} N/m^2.

TABLE VII

STANDARD DEVIATION OF THE AVERAGE PEAK POWER IN THE FUNDAMENTAL FREQUENCY, AVERAGE TOTAL PEAK POWER IN THE HARMONICS, AND THE AVERAGE NUMBER OF HARMONICS

Age-Days	Average Peak Power of Fundamental Frequency-dB**		Average Total Peak Power in Harmonics-dB**		Average Number of Harmonics	
	Males	Females	Males	Females	Males	Females
1	3.5	2.0	15.6	15.7	2.4	4.2
2	5.1	3.6	21.1	24.4	2.1	2.5
3	2.9	4.8	14.6	25.9	1.9	2.4
4	4.0	4.5	20.5	16.0	2.2	1.8
5	2.4	3.2	18.8	23.0	2.2	2.2
6	2.8	3.5	20.0	21.3	1.9	2.0
7	3.5	4.1	26.7	27.7	2.3	2.3
8	4.0	4.5	21.1	27.2	1.8	1.8
9	3.0	2.4	23.5	17.0	2.0	1.3
10	3.9	4.4	22.0	26.9	2.2	2.3
11	3.1	4.0	19.7	15.8	1.5	1.0
12	3.7	4.8	15.8	20.6	1.4	1.8
13	3.3	4.5	19.5	21.6	1.8	1.4
14	3.6	3.3	12.9	14.6	1.2	1.4
15	4.2	5.4	14.2	22.9	1.2	1.8
20	2.6	4.7	22.9	17.9	1.4	1.1
25	4.1	3.5	12.5	16.1	1.8	1.8
30	4.4	7.1	9.9	12.5	1.7	1.6

**dB re 100 mv.

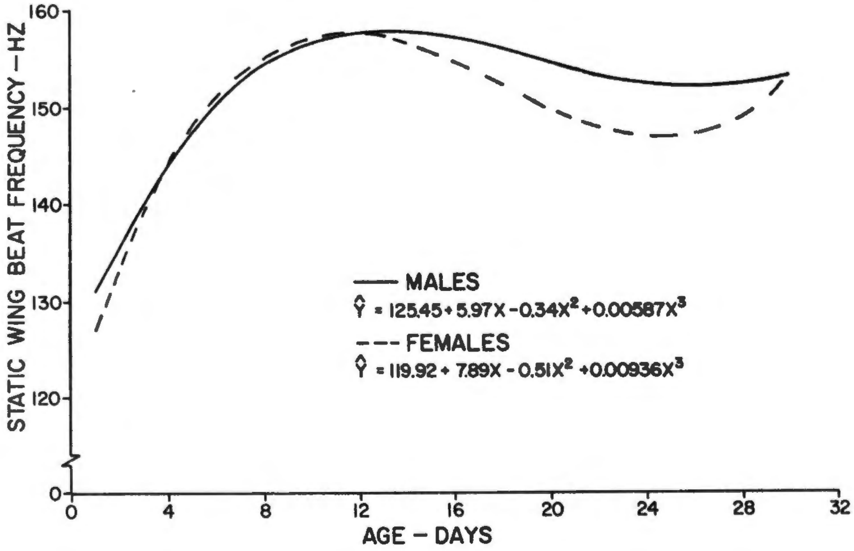

Figure 32. Wingbeat frequency of male and female flies in static flight.

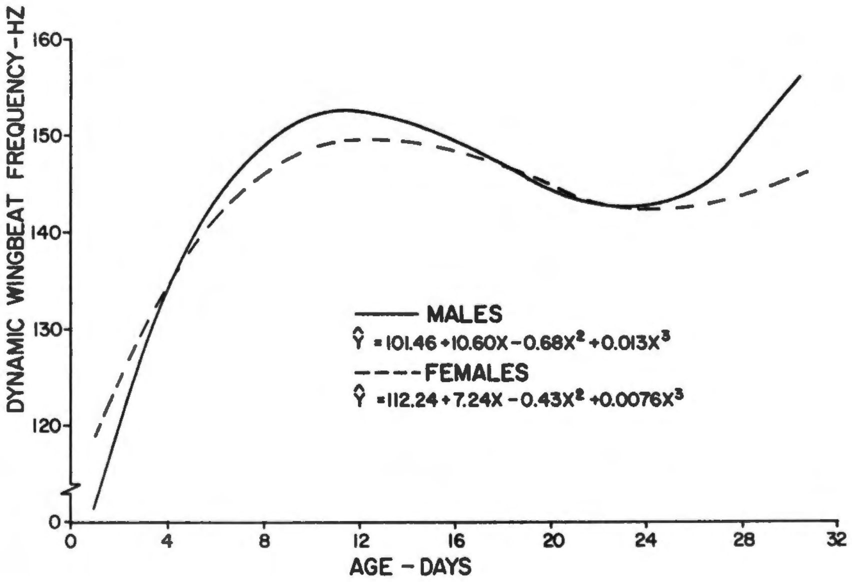

Figure 33. Wingbeat frequency of male and female flies in dynamic flight.

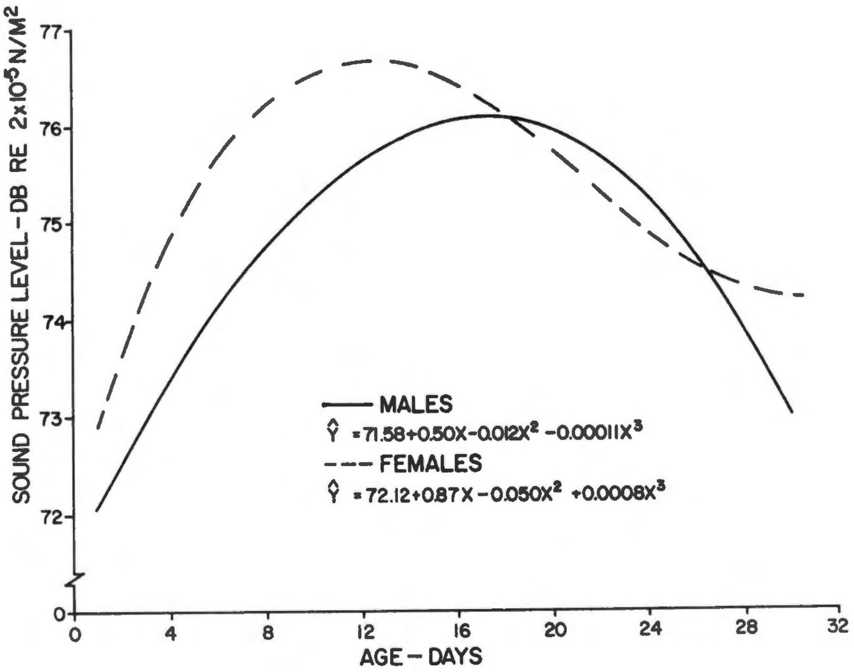

Figure 34. Sound pressure level of males and females in static flight.

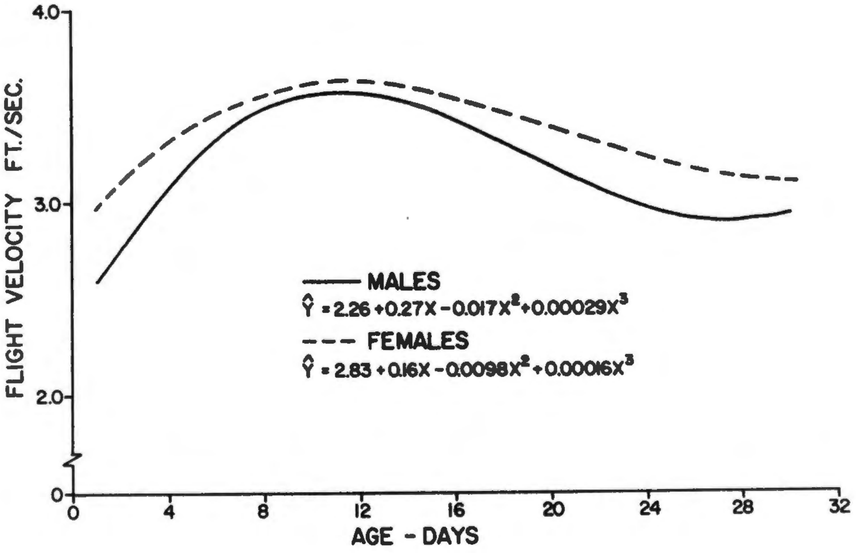

Figure 35. Flight velocity of males and females.

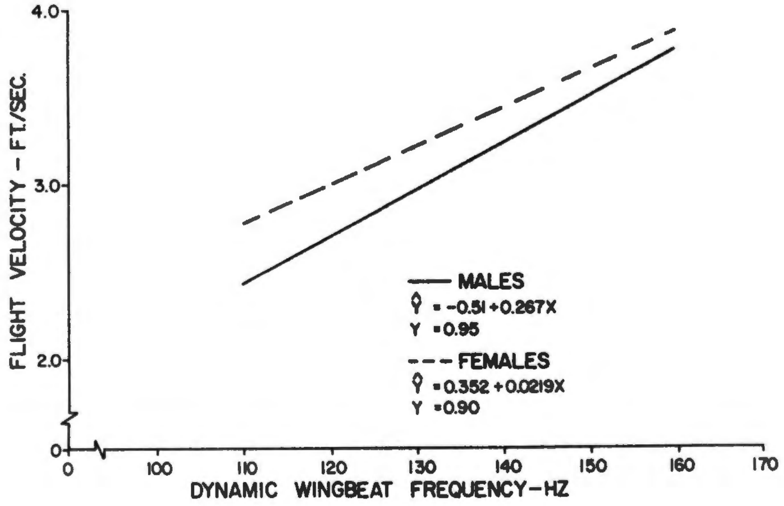

Figure 36. The relationship of velocity and wingbeat frequency of male and female flies.

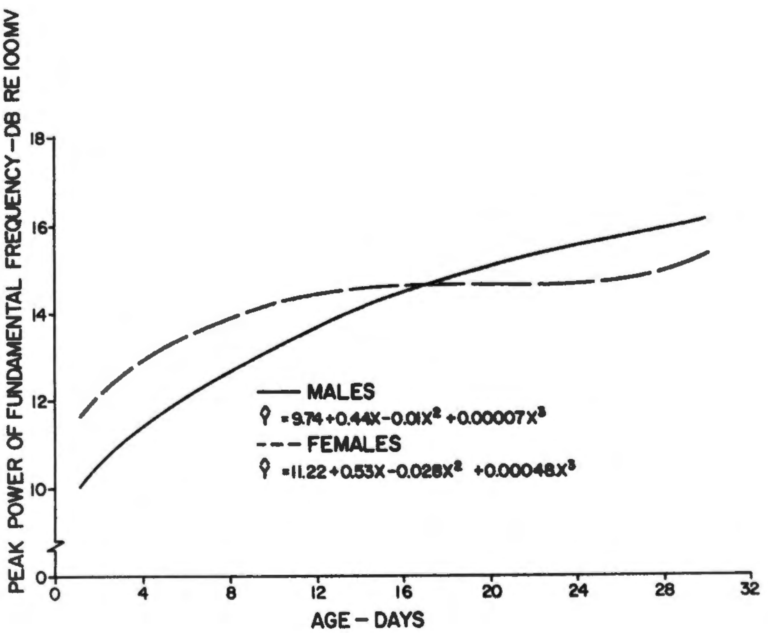

Figure 37. Average peak power of the fundamental frequency for both males and females in static flight.

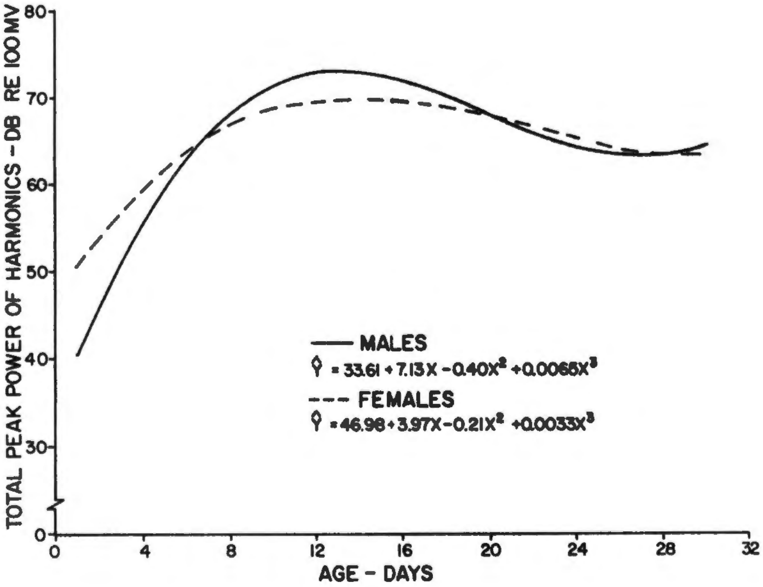

Figure 38. Average total peak power contained in the harmonics for males and females in static flight.

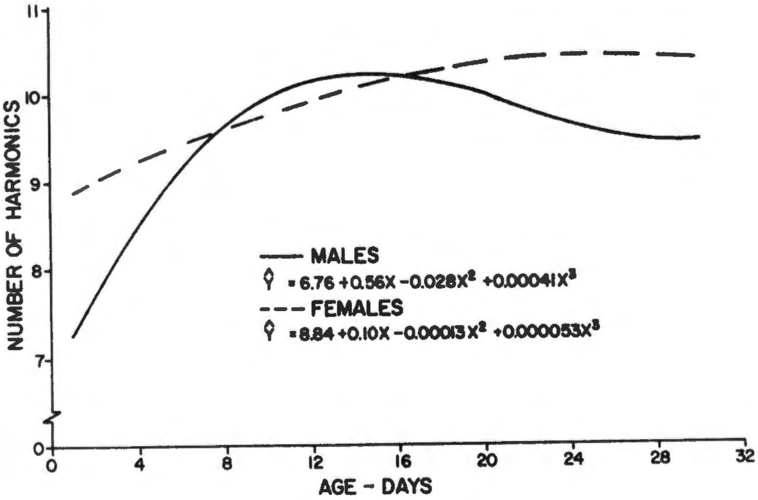

Figure 39. Average number of harmonics contained in the power spectral density of male and female flies in static flight.

general, was larger for the younger and older flies than for flies from eight to fourteen days old. However, the variability in the number of harmonics was less for the older flies than for all others. This indicated that the wing movements in flight were more uniform for the older flies than for the younger flies.

Measurements of several sound parameters produced by the wingbeat of both sexes are shown as means in Tables III, IV, and V. Generally, flies from one to four days of age were weaker than older flies. These young flies, one to four days old, recorded low values in most categories. Difficulty was experienced in recording data on these flies because only a few remained in flight for as long as 60 sec. The younger flies were also smaller than the older flies. The lowest, 133 and 123 Hz, and highest, 160 and 164 Hz, mean frequencies were recorded at one and fourteen days, respectively, for males and females. For comparisons of dynamic mean frequencies, one-day-old males and females also had the lowest frequencies, 114 and 116 Hz, but thirty-day-old males and fourteen-day-old females recorded the highest frequencies at 155 and 160 Hz, respectively.

The data were also analyzed using regression analysis, and these curves, including equations, are shown in Figures 32 through 39. These curves show that most parameters increased up to approximately 12 days for both sexes except for sound pressure level. Males reached a peak at approximately 18 days in this category. At 24 days, the wingbeat frequency decreased in dynamic flight for both sexes, but for males it increased sharply from 24 to 30 days. Differences among dynamic wingbeat frequencies were more apparent between sexes from approximately 8 to 15 and 26 to 32 days. The curves of static and dynamic wingbeat frequency were very similar for both sexes except that the wingbeat frequency was lower in the latter frequency.

The static and dynamic wingbeat frequencies were compared with a t-test, and it was found that both sexes had a higher wingbeat frequency in static flight: at ages of 1, 2, 4, 6, 14, and 20 days, wingbeat frequencies for males were higher in static flight; for females, frequencies were higher in static flight at 2, 4-6, and 9-12 days.

The differences between sexes in dyanmic flight were compared and the frequencies were found to be higher for females at two days, 125 vs 117 Hz, and higher for males at twelve days, 155 vs 145 Hz. Males and females in static flight had higher frequencies at one and five days, respectively. Also, in static wingbeat analysis, two-day-old females had a higher total peak power in harmonics than males the same age. Only two-day-old females had a higher sound pressure level than males the same age. Females at one and eight days had a higher peak power of the fundamental frequency than one- and eight-day-old males. However, males were superior to females at twenty days. Females had more peak power of the fundamental frequency than males at two and thirteen days.

The flight velocity of females was greater than that of males only at two days of age, 125 vs 117 ft/sec. Flight velocity correlated with wingbeat frequency for both sexes is shown by linear regression analysis in Figure 36, page 103. The velocity for males and females ranged from approximately 3 to 4 ft/sec, being lowest for both sexes at one to two days and highest at nine days for males and fourteen days for females.

Freshly emerged _A. suspensa_ must allow their wings to expand and their bodies to harden before any sustained flight activities can be accomplished. It was difficult to record data using one- to four-day-old flies, but when they surpassed four days of age, the flies responded well for experimentation. Sexually matured males rapidly fan their wings in courtship for the greater part of their adult life span. This fanning plus other activities within the cages by both sexes caused the wings to become frayed, broken, and completely removed in very old flies; thus, it was difficult to select a perfect specimen older than approximately thirteen days. And the reduced wing area partially accounted for the higher frequencies of older males which had wings in poorer condition than females.

Wingbeat frequencies increased up to approximately 12 days for males and females. Frequencies increased again after 24 days for both sexes, but the increase was greater for males. Wingbeat frequencies were higher in static than in dynamic flight for both sexes at most ages, and this indicated that the flies exerted more energy in the former position.

The flight sound data indicated that both sexes were similar with respect to most of the parameters studied. Also, the waveform of individual flies were similar for all ages. Based on findings of this study, the flies can be categorized into three age groups: the one- to four-day-old flies as immature, the eight- to fourteen-day-old flies as mature with good flight ability, and flies over fourteen days as mature with poor flight ability. Therefore, the most vigorous laboratory flies were found to be eight to twelve days old. This age fly was later selected for the bioassay studies.

II. Characteristics of Sounds Incidental to Ordinary Movement

All insects are known to make sounds that are incidental to ordinary movement (46). Perhaps the most common sounds in this class is the flight sounds made by all flying insects. Because of the importance of flight sounds to the behavior of the fruit fly a detailed study, as discussed in the previous section, was made. Four distinct sounds with low frequency components and one with a high frequency component were found to be produced by fruit flies. Two of these sounds were associated with mating behavior, one each with flight and aggression activities. The high frequency sound appeared to be associated with all movements of the fly.

The aggression sound is made by one male fly as he is preparing to attack another male fly. This sound is produced by a prolonged wing fanning. The frequency and waveforms produced by this sound may vary slightly from one fly to another, but the basic waveform and frequency content remain much the same, as shown in Figures 40 and 41.

The premating sound is produced by the male after he mounts the female but before mating takes place, and it appears to be used as a courtship sound. This sound is made by a continuous wing fanning with a duration of several seconds. Again individual variations occur from one fly to another, but the basic waveform and frequency content remains much the same, as shown in Figures 42 and 43.

The fundamental frequency and the sound pressure level of the aggression and premating sounds were very similar to the flight sounds;

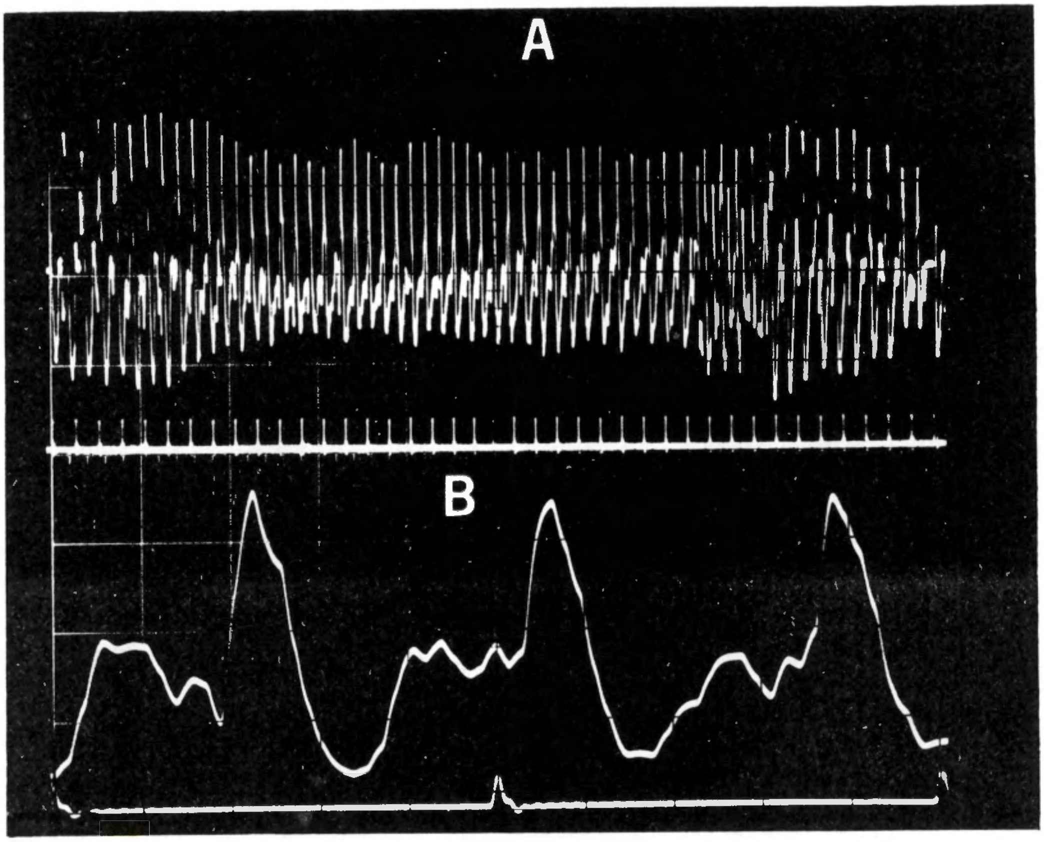

Figure 40. Aggression sound as produced by the male fruit fly: (A) Waveforms of prolonged sound, and (B) Expanded Waveform. 10 msec pulse marker.

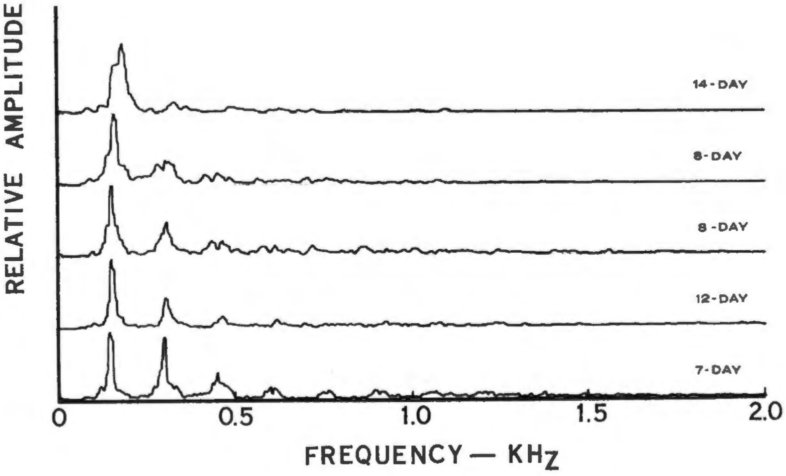

Figure 41. Frequency signature of aggression sound from several flies.

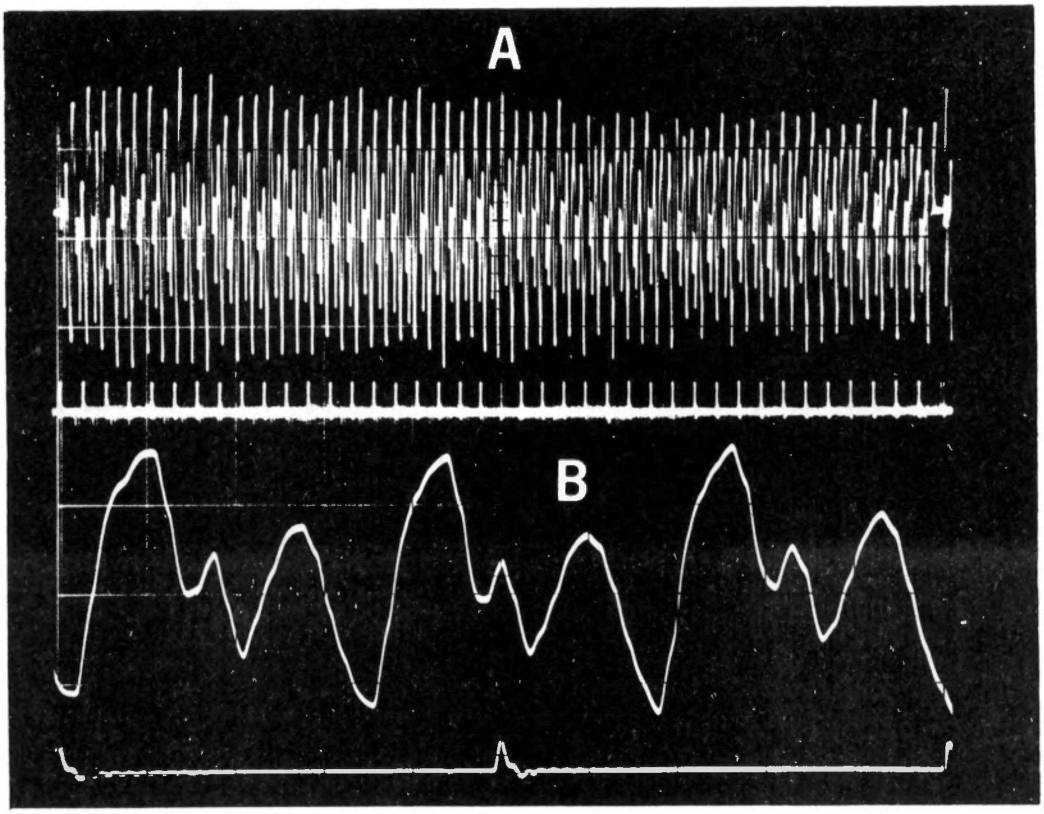

Figure 42. Premating sound produced by wing fanning of the male fruit fly: (A) prolonged fanning, (B) expanded waveform. 10 msec pulse marker.

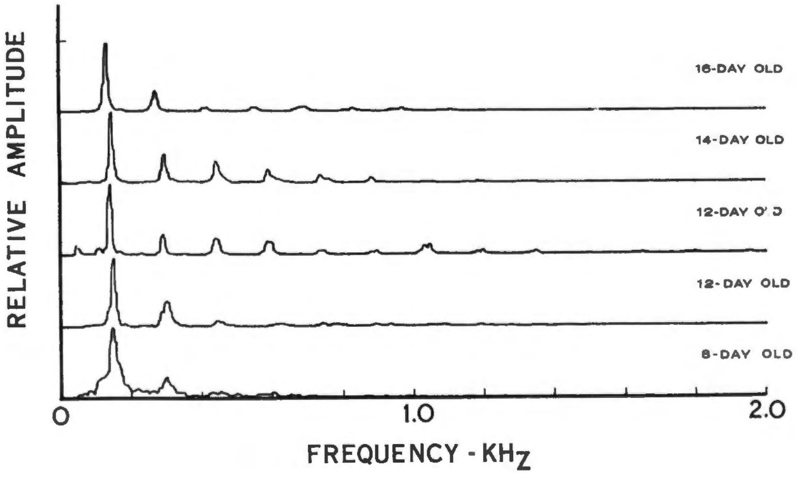

Figure 43. Frequency signature of the premating sound from several flies.

however, the waveform content was different. A frequency signature of the flight sounds of both males and females is shown in Figure 44 for comparison.

When the calling sound was examined closely, a low amplitude, high frequency signal was observed to occur at irregular intervals on the waveform. The frequency of these signals ranged from 18 to 60 kHz with the major frequencies between 20 and 35 kHz with an amplitude ranging from 50 to 100 mv. An analysis was made of the flight, aggression, and premating sounds to determine if these high frequency signals also occurred on them. It was important to determine if these signals were part of the information transferred during calling or if they were ordinary movement sounds.

The flight sounds of both the males and females were examined and were found to contain this high frequency (Figures 45 and 46). The waveforms containing this high frequency signal for the aggression, premating, and calling sounds are shown in Figures 47, 48, and 49. The high frequency signal was found to exist on each low frequency waveform.

An attempt was made to locate the source of these high frequency sounds. The method used to isolate these sounds was to remove specific body parts which were believed to be the source of these sounds.

The first body part removed was 1/2 of each wing. It was thought that the wing tips could be brushing against bristles or other objects on the thorax or abdomen; however, this did not prove to be the case. Other body parts removed completely were both wings, all legs, abdomen, halteres, thoracic muscles, and a wing was removed and a stiff object was brushed against the sclerites of the wings. When these body parts

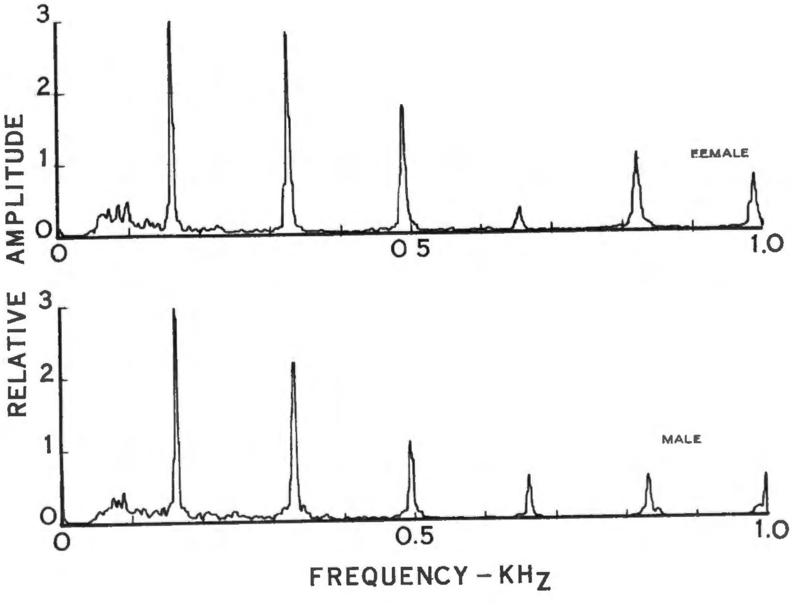

Figure 44. Frequency signature of flight sounds for males and females.

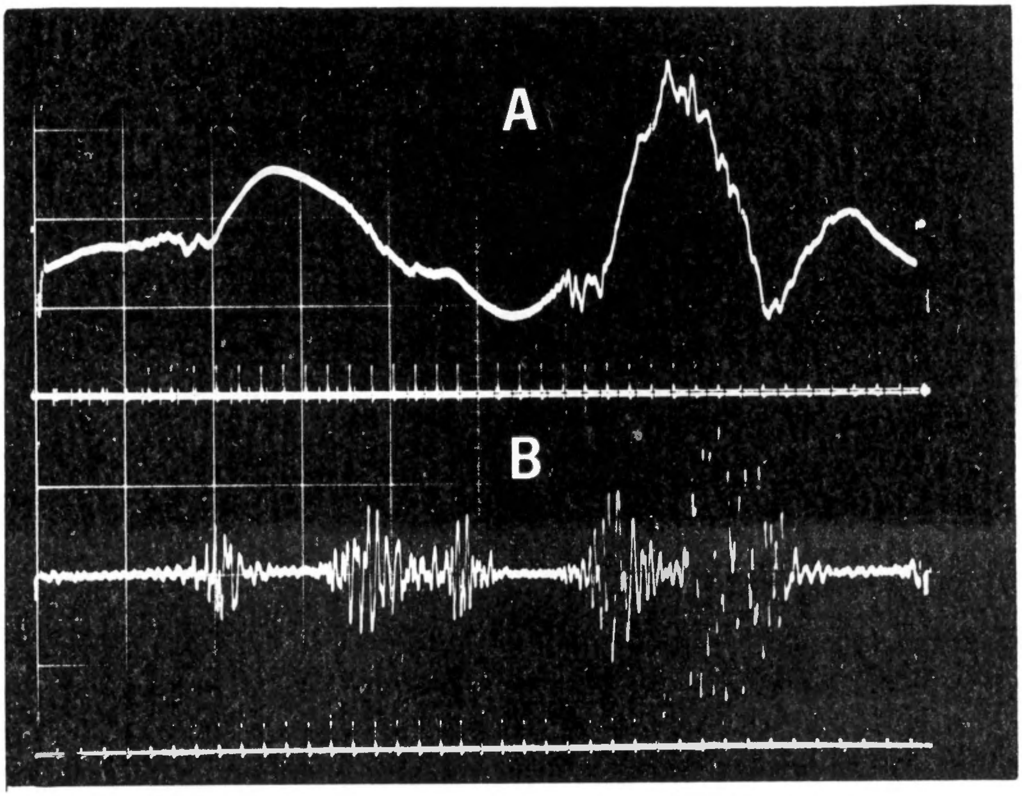

Figure 45. Flight sound of male fly: (A) Low frequency signal with high frequency component, (B) Expanded high frequency with 10 msec timing pulse.

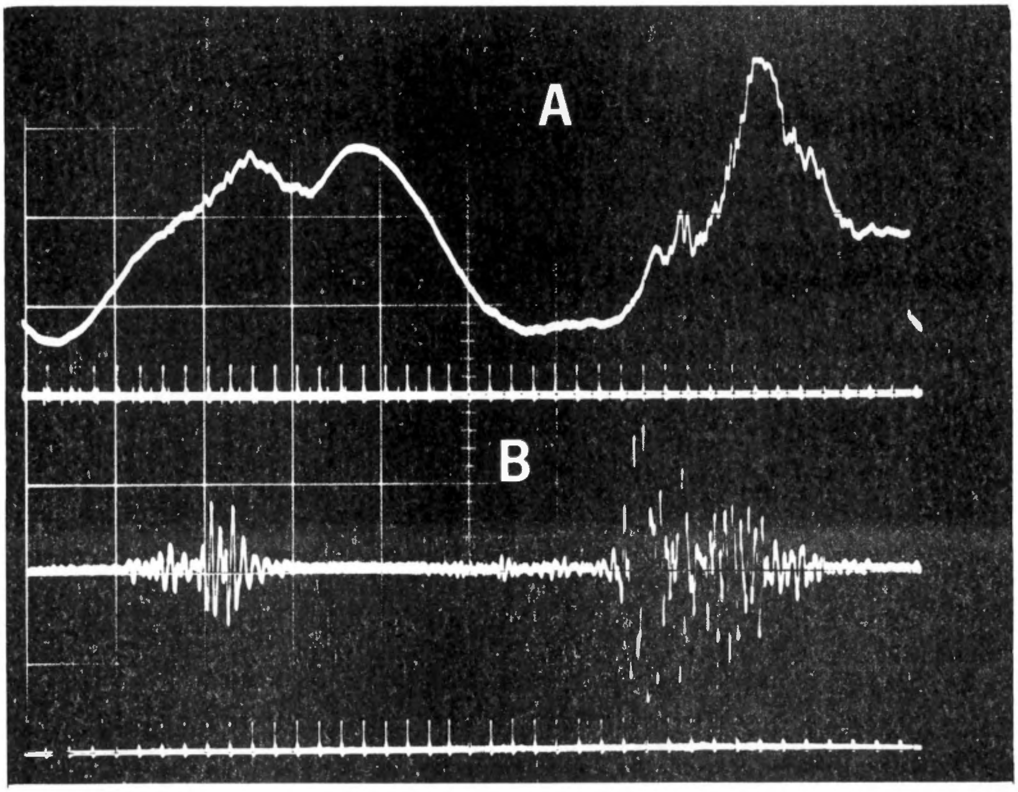

Figure 46. Flight sounds of female fly: (A) Low frequency signal with high frequency component, (B) Expanded high frequency with 10 msec timing pulse.

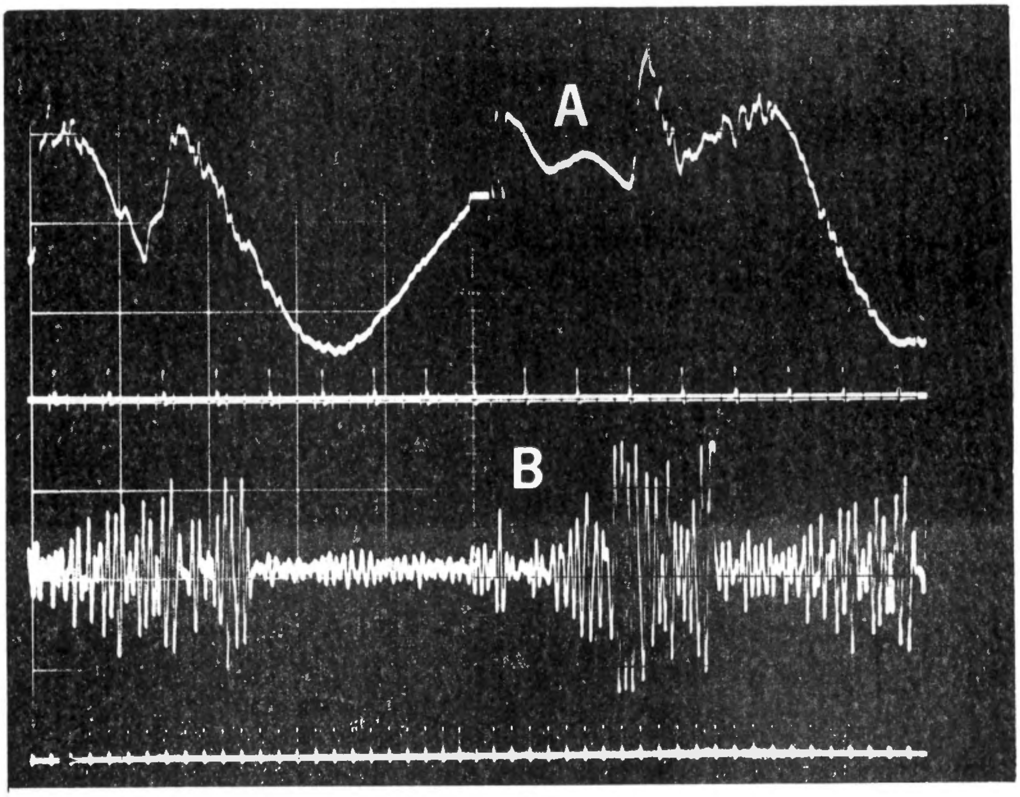

Figure 47. Aggression sound: (A) Low frequency signal with high frequency component, (B) Expanded high frequency with 10 msec timing pulse.

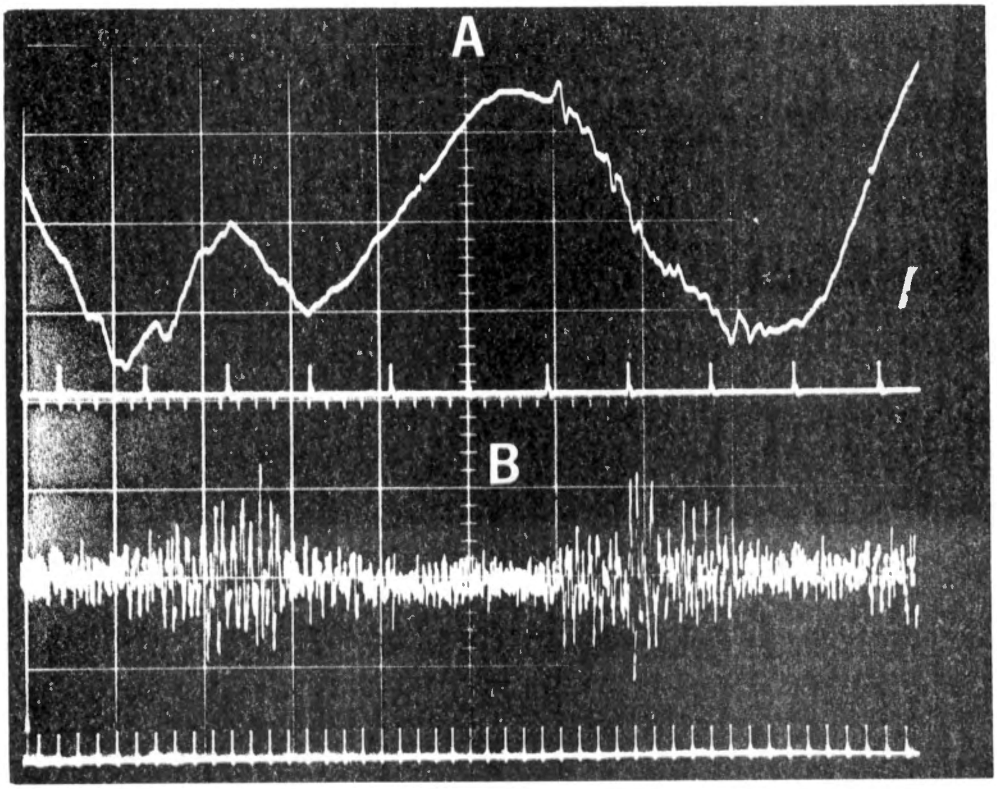

Figure 48. Premating sounds: (A) Low frequency signal with high frequency component, (B) Expanded high frequency with 10 msec timing pulse.

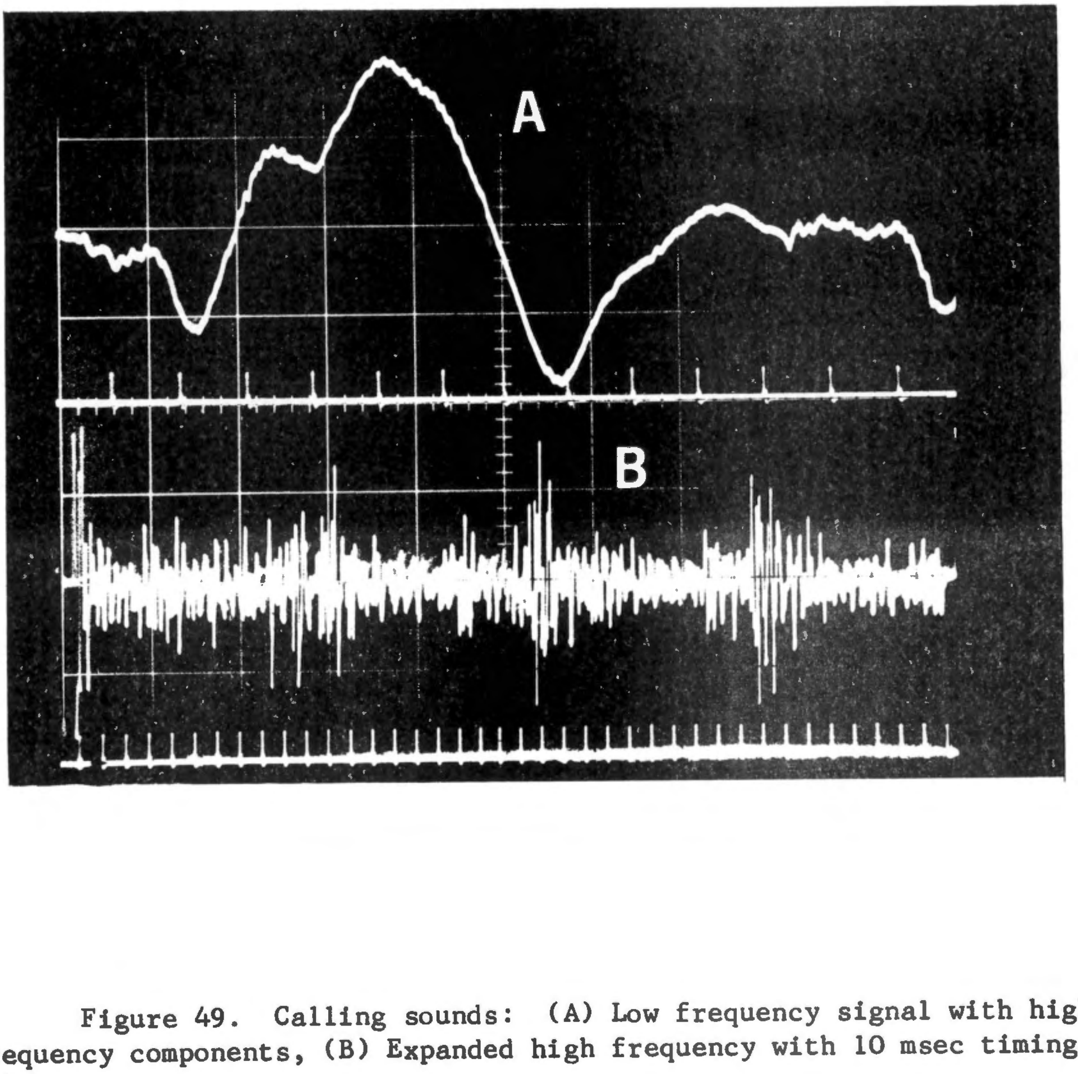

Figure 49. Calling sounds: (A) Low frequency signal with high frequency components, (B) Expanded high frequency with 10 msec timing pulse.

were removed, the high frequency was still found to exist on all waveforms. The characteristics of these signals were the same after each body part was removed as those contained on the low frequency signals: an example of the typical high frequency signal is shown in Figure 50. A frequency spectrum is shown for each condition in Figure 51, and each is identified by a number from one through thirteen; this number corresponds to the following condition: (1) a male in normal static flight, (2) the same male with 1/2 of both wings removed, (3) a female in normal static flight, (4) the same female with 1/2 of both wings removed, (5) a male in static flight with both wings removed, (6) a male in static flight with all legs removed, (7) a male in static flight with the abdomen removed, (8) a male in static flight with thoracic muscles removed, (9) a wing removed and brushed with a stiff object, (10) a male in static flight with halteres removed, (11) the high frequency on an aggression waveform, (12) the high frequency on a premating sound, and (13) the high frequency on a calling sound. Therefore, it was concluded that this high frequency was generated by parts rubbing against the sclerites which exist on all body parts. And this high frequency will not be considered as part of the communicative signal in the calling sound, but will be considered an incidental sound to ordinary movement.

III. The Calling Sound

The calling sound produced by the male Caribbean fruit fly before pair formation is a specialized sound made by very rapid, precision wing movements. Many visual observations were made of caged flies and the activities surrounding the production of this sound before data

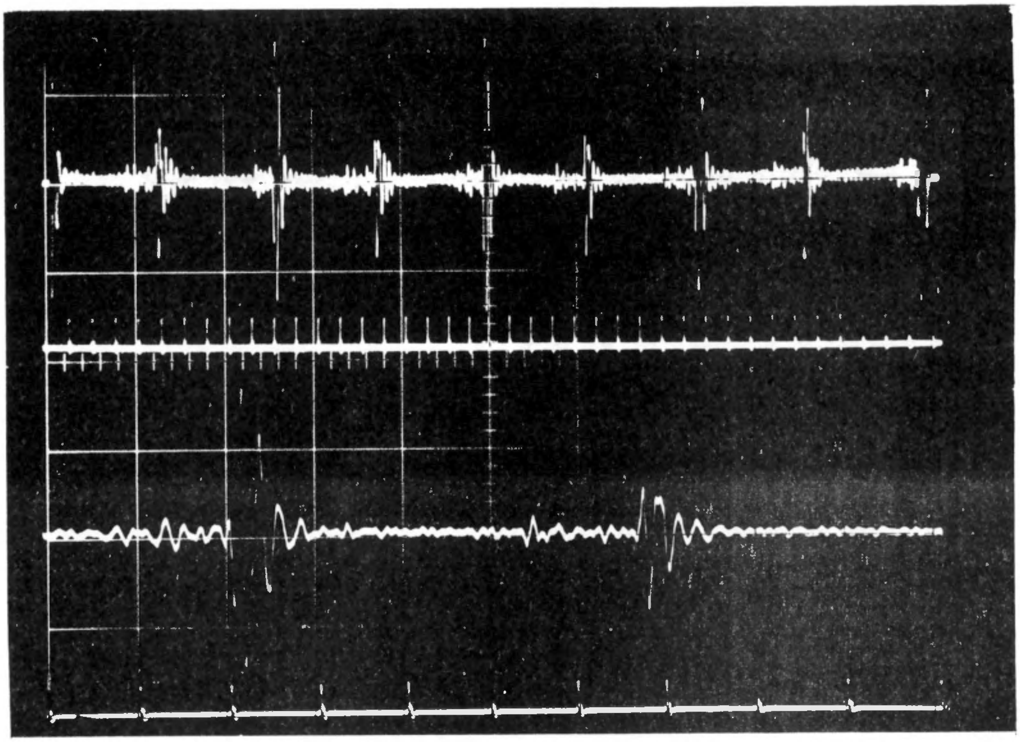

Figure 50. Typical high frequency signal contained in all fly movements. 10 msec pulse marker.

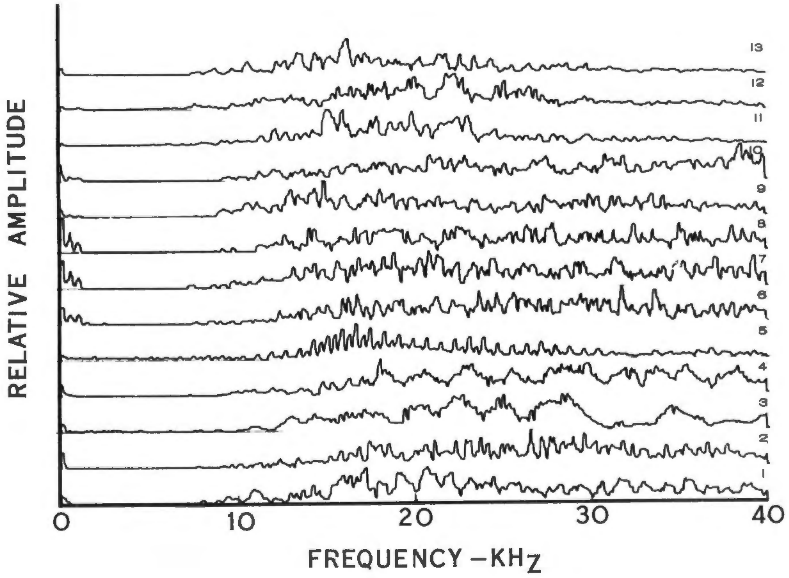

Figure 51. Frequency spectrum of high frequency signals. See test for identification of each spectrum.

recordings were begun. Several sexually mature females were placed in a holding cage; then a male was released in the cage with the females. If the male failed to produce the calling sound, no mating took place. If the male called, he would first select a location some distance from another fly and would move his metathoracic legs backward and forward over the dorsal surface of his wings. When this was completed, he would begin to call by initiating short bursts of rapid wing movements. An individual burst might last from a few msec to several seconds, and the length of the call could range from a few seconds to several minutes. The calling male would remain in the same location throughout the calling period but would turn clockwise or counter clockwise and continue to call until another male approached, until a female approached, or until he moved to a new location. Frequently, when more than one male was in the cage, another male would approach the calling male and initiate aggressive action against the calling fly. If the calling male induced the aggressive male to leave, he would continue to call from that same location; however, if the aggressive male induced the calling male to leave, the calling male would move to a new location to call and the aggressive male would begin to call at that location.

If a female approached to within 1 to 3 inches of the calling male, he would stop calling. The male would remain stationary while the female walked around and touched the male with her torso. If the female did not touch the male, no mating was observed, and the male would start calling again. If, however, the female touched the male, the male would mount the female and begin rapidly fanning his wings. This rapid wing fanning, referred to as the premating sound, would continue until the female raised her ovipositor sheath to the vertical or near vertical position for mating to begin. If the female did not raise her ovipositor sheath, the male would return to the calling location and would resume his calling. There were exceptions to the above procedures, but these were the usual.

The calling sound consists of a series of pulses within a pulse train; the length of these pulse trains depends upon the length of time a male fly remains and calls in one location. The time period of a pulse period varies from a few seconds to several minutes. An example of a typical pulse train, the pulse duration, and the pulse interval is shown in Figure 52. This pulse train usually consists of both short and long pulses. Several hundred pulse durations and pulse intervals were measured from several calling males at ages six through sixteen and twenty days old. The pulse amplitude was also measured. However, since the calling males could not be constrained, the distance between the microphone and the male could not be closely controlled. Therefore, the amplitudes could only indicate trends. The total number of pulses counted varied with each fly; however, a minimum of 300 were counted for each age. The data for the pulse duration, pulse interval, and pulse amplitude are shown and expressed as a percent of those measured in each age group in Tables VIII, IX, and X, respectively. The shortest pulse recorded was 0.04 seconds and the longest was 14.6 seconds. The youngest flies to call, six and seven days old, had short pulse durations with no long calls being recorded. In most instances, about 60 percent of the pulse durations were less than 0.5 seconds, and about 85 percent were less than 0.75 seconds. Some wild flies, which had emerged from rose apples in Homestead, Florida,

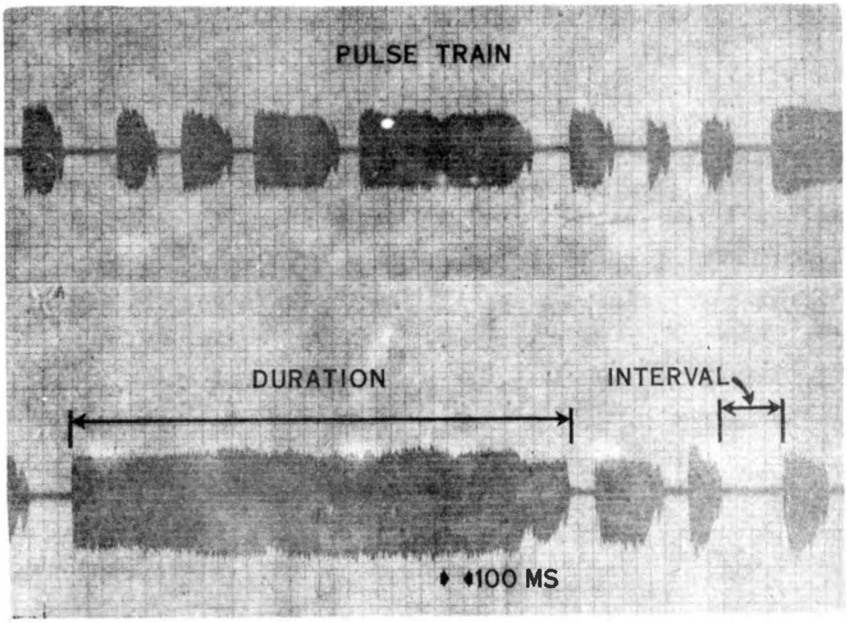

Figure 52. Typical pulse train of a calling male, showing the pulse duration and the pulse interval.

TABLE VIII

AVERAGE PULSE DURATION IN PERCENT OF THOSE MEASURED

Fly-Age Days	PULSE DURATION-SEC										Range of Pulse Duration sec	
	0 to 0.249	0.25 to 0.499	0.50 to 0.749	0.75 to 0.99	1.00 to 1.499	1.50 to 1.99	2.00 to 2.99	3.00 to 3.99	4.00 to 4.99	5.00 and Longer	Shortest	Longest
6	32.4	61.1	5.1	1.4	--	--	--	--	--	--	0.12	0.8
7	7.1	50.0	7.1	17.7	10.7	--	7.1	--	--	--	0.16	2.5
8	8.7	55.1	21.1	6.5	5.4	1.6	1.1	--	--	0.6	0.02	6.0
9	60.5	33.7	3.5	--	--	--	1.2	1.2	--	--	0.10	3.6
10	22.6	48.6	16.8	5.5	4.1	5.0	--	0.3	--	0.3	0.04	10.5
11	24.0	26.0	26.0	8.4	9.7	1.9	3.9	--	--	--	0.16	2.5
12	14.1	58.3	13.2	3.1	5.5	2.4	0.9	1.2	0.3	0.9	0.12	11.8
13	26.3	36.5	7.8	3.0	6.0	5.4	3.0	3.6	2.4	6.0	0.08	14.6
14	46.9	39.0	9.6	0.6	--	3.4	--	--	--	--	0.10	2.8
15	23.9	53.7	14.9	1.1	0.5	1.6	1.1	1.1	1.6	--	0.04	5.0
16	32.0	42.5	13.5	2.9	4.4	1.1	1.8	1.1	--	--	0.04	3.7
20	9.6	45.0	18.0	6.2	8.3	5.2	2.9	2.1	0.8	1.8	0.12	8.4
WF*	34.4	62.4	2.2	1.0	--	--	--	--	--	--	0.10	0.9

*Wild Flies.

TABLE IX

AVERAGE PULSE INTERVAL IN PERCENT OF THOSE MEASURED

Fly-Age Days	PULSE INTERVAL-SEC 0 to 0.249	0.25 to 0.499	0.50 to 0.749	0.75 to 0.99	1.00 to 1.49	1.50 to 1.99	2.00 to 2.99	3.00 to 3.99	4.00 to 4.99	5.00 to Over	Range of Pulse Duration sec Shortest	Longest
6	18.8	38.4	18.1	5.1	3.6	5.8	3.6	1.4	1.4	3.6	0.14	10.28
7	28.7	21.4	21.4	3.6	14.3	--	7.1	--	3.6	--	0.12	4.42
8	15.6	23.9	25.0	11.7	10.0	7.8	6.1	4.4	2.8	2.8	0.02	10.94
9	8.2	40.0	17.7	8.2	10.6	3.5	2.3	4.1	1.2	3.5	0.14	7.65
10	33.8	28.2	8.1	9.9	9.1	2.5	2.1	1.1	1.1	4.2	0.04	13.40
11	36.8	54.6	8.0	--	--	--	--	0.6	--	--	0.18	3.08
12	24.6	31.6	13.7	5.8	7.3	2.9	2.9	2.6	2.2	7.4	0.06	21.73
13	29.2	31.7	12.4	4.3	3.7	3.1	6.2	0.6	1.9	6.8	0.14	10.62
14	49.7	25.4	12.4	2.4	4.0	1.2	1.2	1.8	1.2	1.8	0.04	13.40
15	27.4	26.7	24.6	3.9	9.5	2.2	2.8	1.1	0.6	1.1	0.08	9.74
16	36.0	31.4	11.1	5.4	3.1	3.1	3.1	1.1	1.6	4.2	0.04	21.68
20	31.1	29.3	14.0	5.8	7.6	2.6	2.1	2.6	1.3	3.4	0.06	14.14
WF*	4.6	25.3	36.8	11.4	6.9	--	3.4	1.1	1.1	2.3	0.10	8.06

*Wild Flies.

TABLE X

AVERAGE PULSE AMPLITUDE IN PERCENT OF THOSE MEASURED

Fly-Age Days	PULSE-AMPLITUDE-PEAK VOLTS						Amplitude Range-sec	
	0 to 0.249	0.25 to 0.499	0.5 to 0.749	0.75 to 0.99	1.00 to 1.249	1.25 to 1.499	Smallest	Largest
6	--	--	11.5	44.6	36.7	7.2	0.50	1.25
7	--	--	28.6	42.9	25.0	3.6	0.50	1.35
8	1.4	7.9	72.1	22.3	17.9	--	0.01	1.24
9	--	25.9	61.8	8.2	4.7	--	0.35	1.30
10	--	26.0	28.8	38.7	6.5	--	0.30	1.10
11	--	22.7	57.0	19.6	0.6	--	--	--
12	0.6	20.2	62.3	15.6	1.3	--	0.22	1.05
13	--	13.8	32.9	39.5	12.0	1.8	0.25	1.25
14	--	0.6	49.7	8.6	1.1	--	0.45	1.25
15	--	2.2	13.7	36.3	39.0	8.8	0.45	1.50
16	--	9.6	23.9	25.5	26.8	11.2	0.45	1.50
20	--	19.5	57.0	20.3	2.6	0.52	0.35	1.26
WF*	--	20.9	65.9	13.2	--	--	0.30	0.97

*Wild Flies.

were captured and tested: the duration of their pulses were normally less than 0.5 seconds with no long pulses being recorded. The older flies had longer pulse intervals and higher amplitude signals than those of the younger flies. The sound pressure levels that were measured from 15 flies of each age averaged 73 dB at six days and 71 dB at sixteen days. No differences in sound pressure level of the calling sound were shown at any age.

An individual pulse was composed of two distinct parts, identified as A and B parts (Figure 53). In most pulses, there was a transition between the A and B parts called the C part, which consisted of one to three cycles.

The frequency contained within the A and B parts of the pulses were measured for both short and long pulses for each fly at each age. The duration of the A part was independent of the total pulse duration. The duration of part A ranged from 0.04 to 0.12 seconds and remained constant regardless of the length of the total pulse or the duration of part B. While the frequency in parts A and B varied even within the same pulse train, the fundamental frequency in part A was always lower than that in the B part. This difference ranged from 19 to 50 H_z with an average of 32 H_z for the short pulses and 35 H_z for the long pulses. The number of cycles contained in an average long and short pulse for each age fly is shown in Table XI. The number of cycles in part B varied greatly, depending on the pulse duration. The number of cycles in part A remained almost constant, varying only from five to fourteen cycles with an average of eight cycles.

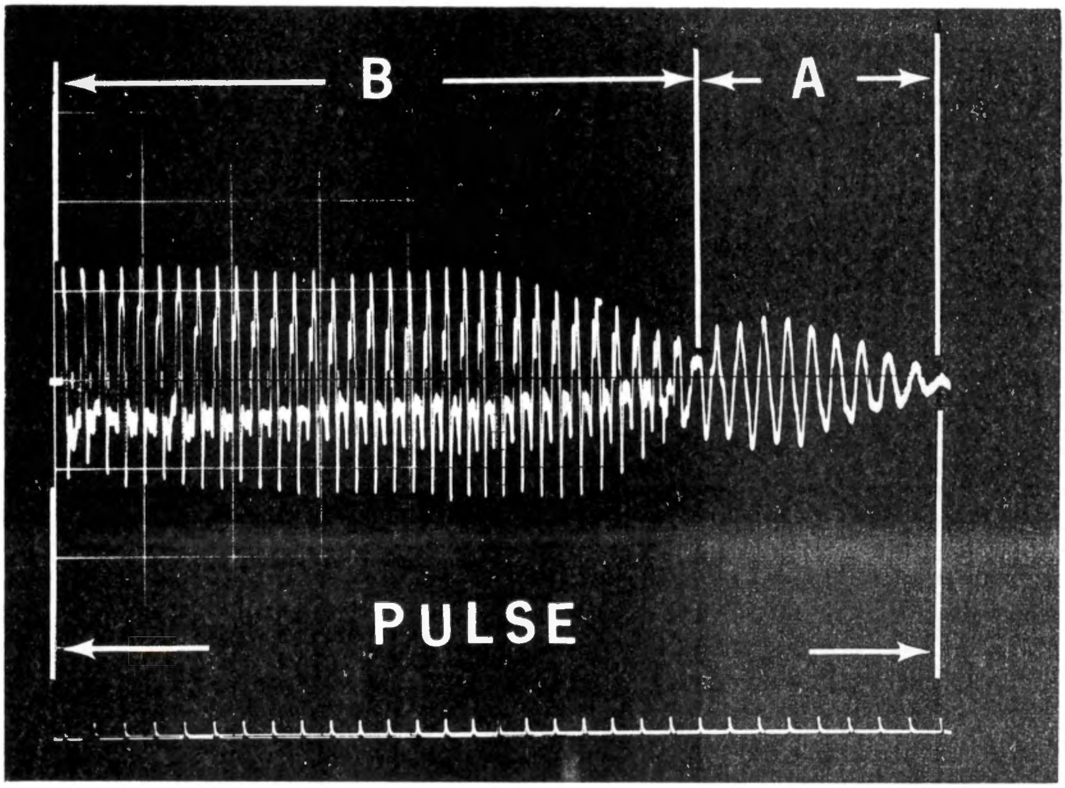

Figure 53. An individual pulse of the calling sound, showing the A and B parts.

TABLE XI

COMPARISON OF THE FREQUENCY CONTENT OF PART A AND PART B OF BOTH SHORT AND LONG PULSES

Fly-Age Days	Average Pulse Duration Sec			Frequency H_z			Number of Cycles per Pulse		
	Total	B Part	A Part	B Part	A Part	B-A	B Part	A Part	B-A
6	0.28	0.20	0.08	143	111	32	32	9	21
	0.80	0.71	0.09	153	122	31	109	11	98
7	0.32	0.28	0.04	137	111	26	39	5	34
	0.65	0.60	0.05	121	95	26	71	5	66
8	0.35	0.26	0.09	140	113	28	31	9	22
	1.46	1.38	0.08	121	101	20	223	11	212
9	0.32	0.27	0.05	135	97	38	37	8	29
	0.53	0.45	0.08	137	101	36	62	8	54
10	0.32	0.24	0.08	136	103	33	32	8	24
	0.83	0.74	0.09	141	104	37	105	8	97
11	0.23	0.15	0.08	131	103	28	20	8	12
	0.50	0.41	0.09	130	111	19	53	10	43
12	0.33	0.27	0.06	142	112	30	38	7	31
	0.61	0.49	0.12	150	113	37	73	13	60
13	0.25	0.23	0.02	150	120	30	34	7	27
	0.67	0.62	0.05	146	125	41	93	7	86

TABLE XI (continued)

Fly-Age	Average Pulse Duration Sec			Frequency H_z			Number of Cycles per Pulse		
Days	Total	B Part	A Part	B Part	A Part	B-A	B Part	A Part	B-A
14	0.32	0.26	0.06	157	116	41	40	8	32
	0.70	0.58	0.12	151	117	34	87	14	73
15	0.31	0.24	0.07	151	120	31	35	9	26
	3.96	3.86	0.10	170	120	50	654	12	642
16	0.26	0.19	0.07	149	115	34	28	8	20
	0.73	0.68	0.05	152	109	43	103	6	97
20	0.33	0.26	0.07	136	102	34	35	8	27
	1.08	0.97	0.11	156	113	43	157	9	148
WF*	0.19	0.13	0.06	119	89	30	16	6	10
	0.64	0.59	0.04	123	84	39	73	4	69

*Wild Flies.

The waveforms in part A approached a sine wave with a time period, T_a, which remained almost constant in all pulses and ages of flies (Figure 54). The information contained in part B was more complex than that in part A (Figure 55). The fundamental frequency, with a time period, T_b, ranged from 120 to 150 H_z. After the pulse started, the fundamental frequency was very constant with a constant amplitude throughout the pulse. Contained within the fundamental frequency period were some higher frequency components. The pulse amplitude was greater for the younger than for the older flies (Table X, page 130). The time periods for the various frequencies were labeled as shown in Figure 56. The time period for one cycle in the B part of the pulse was T_b. Also contained within T_b are three other distinct time periods that appeared on all pulses measured, and these periods are referred to as T_c, T_d, and T_c. The frequencies associated with these other time periods are higher in frequency content and lower in energy content than T_b. When several hundred pulses were analyzed from flies of all ages, the time periods, T_b, T_c, T_d, and T_e, were found to vary slightly in frequency and amplitude from pulse to pulse; however, they were found to be constant within a given pulse. The data obtained from a six and twelve day old laboratory fly and a wild fly, and the frequency associated with each time period are shown in Figure 57. The sharpness of the spectral lines of T_b indicated the uniformity of the fundamental frequency. The spectral line for T_a, however, was wider, indicating the frequency in this time period was more variable within each pulse than T_b. The frequency range in H_z was 90 to 125 for T_a, 120 to 150 for T_b, 240 to 300 for T_c, 260 to 310 for T_d, and 800 to 1200 for T_e. When the calling

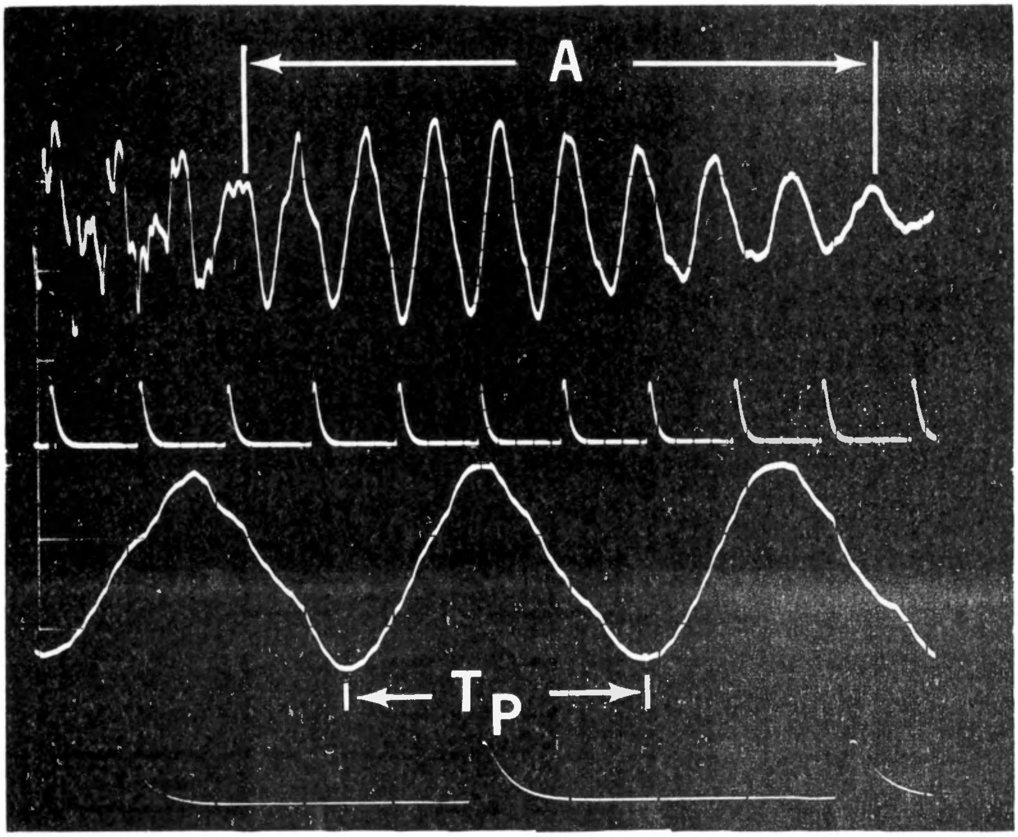

Figure 54. Part A of the calling sound: (Top) complete A part, (Bottom) expanded A part. 10 msec pulse marker.

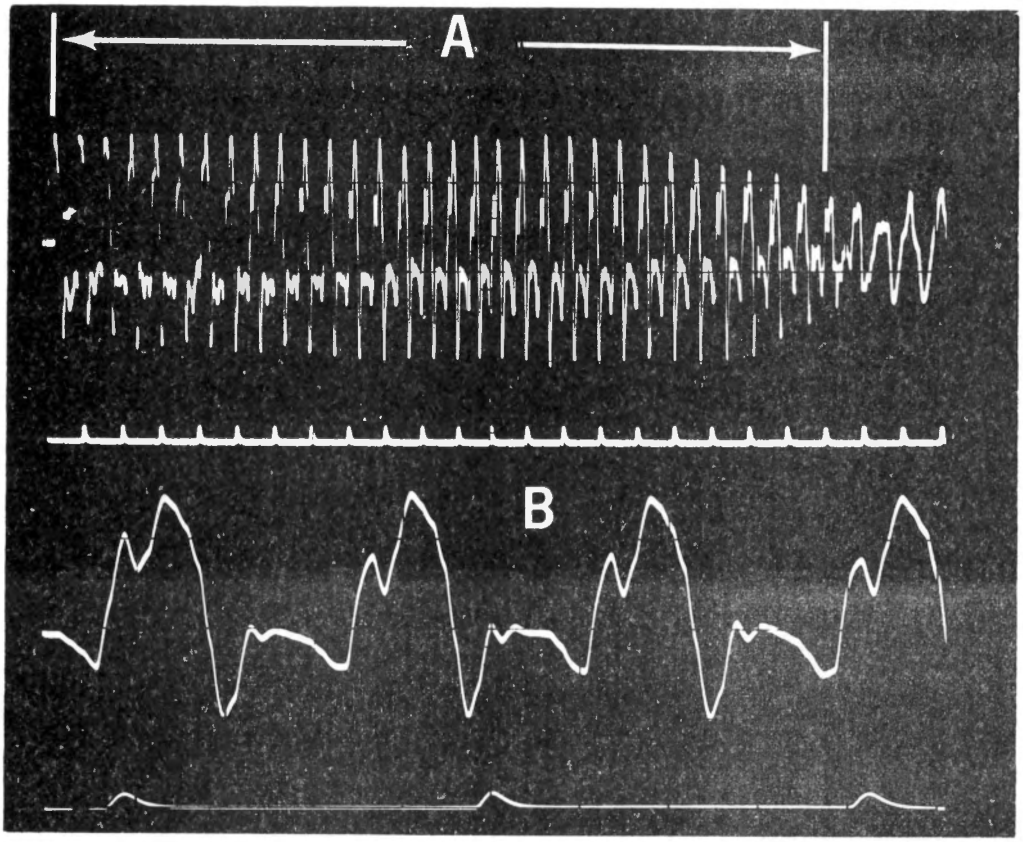

Figure 55. Part B of the calling sound: (Top) total B part, (Bottom) expanded B part. 10 msec pulse marker.

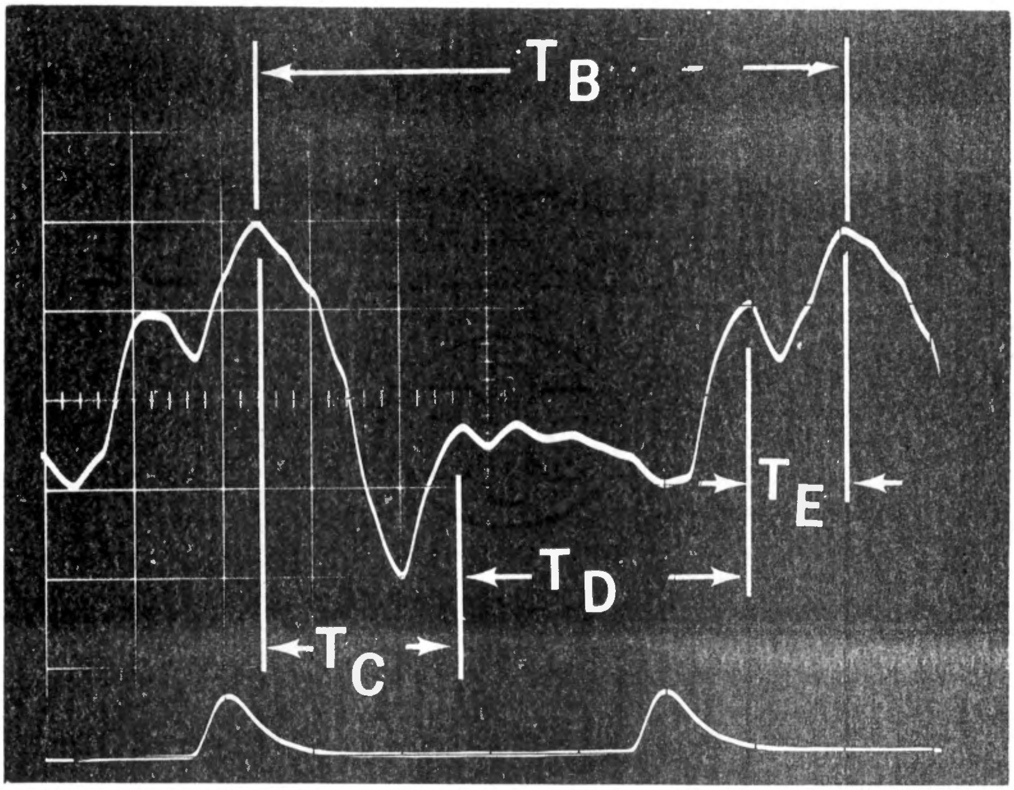

Figure 56. Information contained in one time period of T_b.

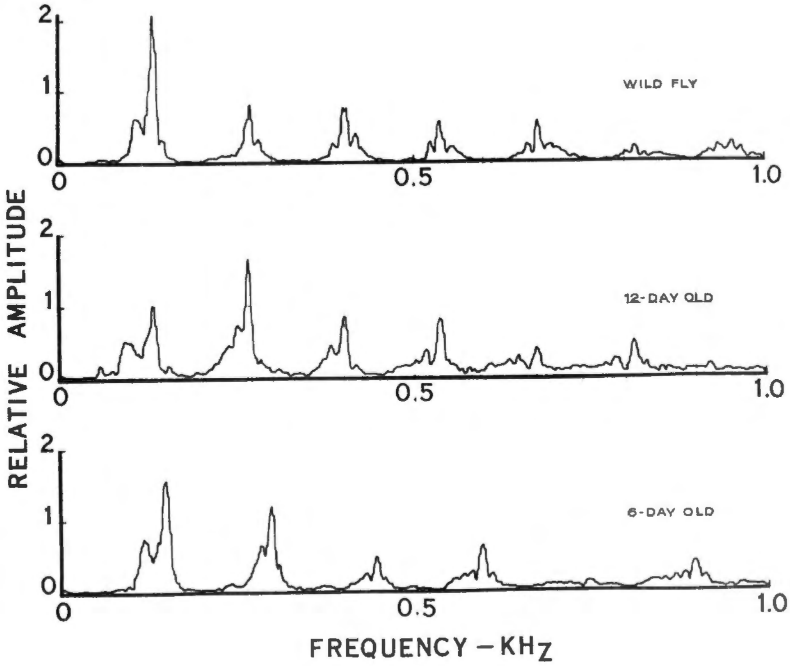

Figure 57. Frequency analysis of the calling sound of a six-day-old, twelve-day-old, and a wild fly. The time periods in Figure 56 are correlated with their respective frequencies.

sound was analyzed for males six through twenty days of age, the time periods T_b, T_c, T_d, and T_e varied in frequency and amplitude among pulses but remained constant within a given pulse. There was as much variation in frequency and amplitude of pulses within the same pulse train of a given age fly as there was between the pulses of flies of different ages. A frequency analysis for each age fly is shown in Figure 58.

A comparison of the waveform of the premating and flight sounds is shown in Figure 59. The premating, aggression, and flight sounds are produced by continuous fanning; whereas the calling sound is pulsed information. The fundamental frequency of the flight, premating, aggression, and calling sounds are very similar; however, the waveforms of each activity have a very distinct signature (Figure 60). The signature of the calling sound was shown to be different from all other sounds produced by the Caribbean fruit fly.

IV. Response of the Caribbean Fruit Fly to the Reproduced Calling Sound

The response of the fruit fly to the reproduced calling sound was measured with the use of the bioassay test units. Fifty flies were released in the center cell and they were allowed to move toward the treatment, to move away from the treatment, or to remain in the center chamber. The number of flies that moved away from the treatment were assumed to be a measure of random movement, and the random movement was assumed to be equal in both direction from the center cell. The difference between the number of flies moving away from the treatment and those going toward the treatment were assumed to be the number of

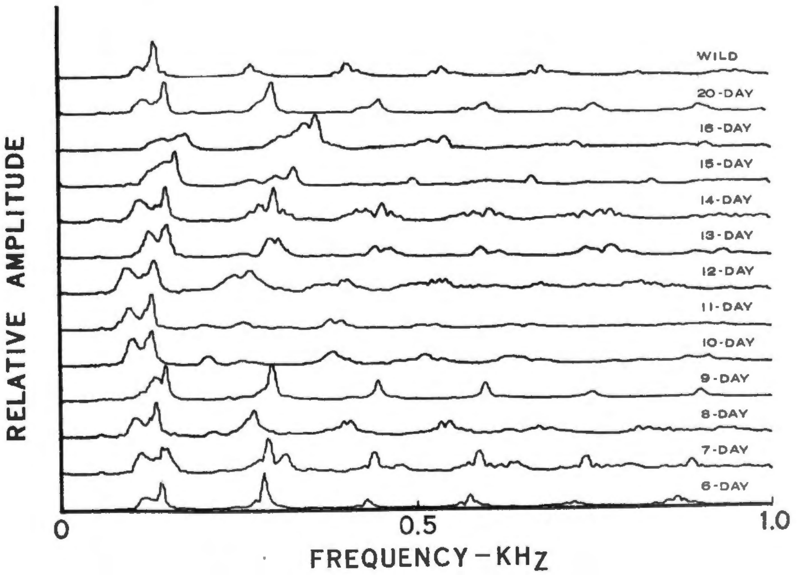

Figure 58. Frequency spectrum for the pulse information contained within a given pulse for each age fly.

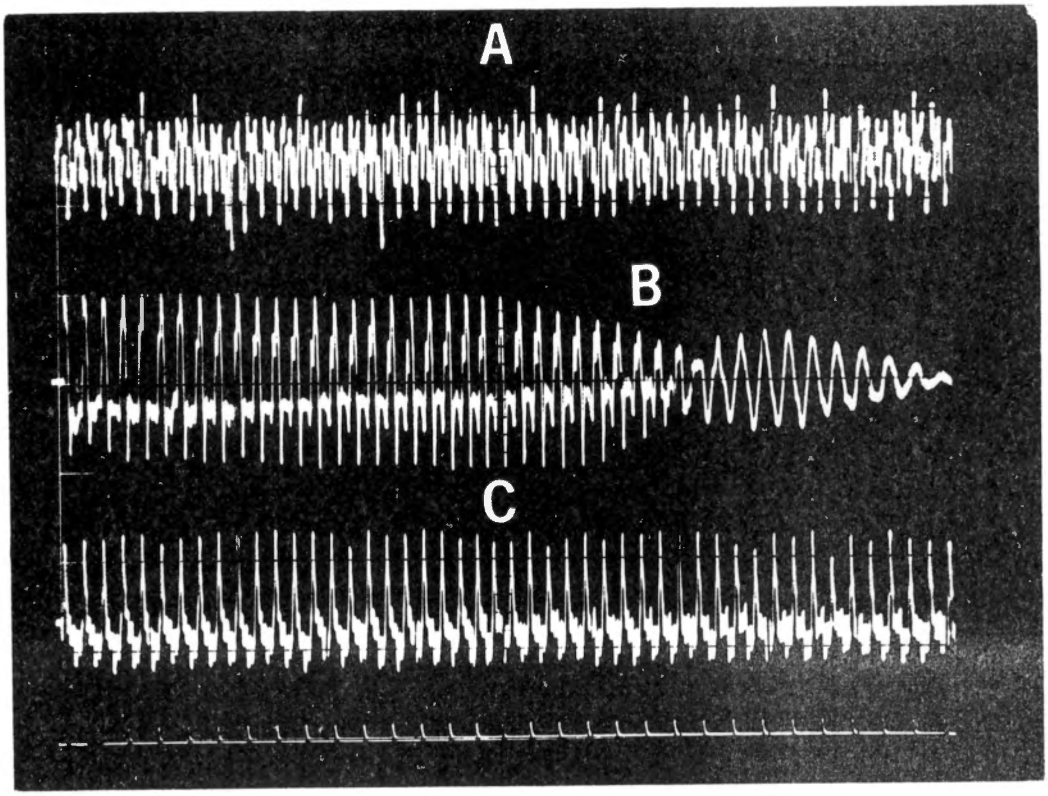

Figure 59. Example of (A) flight sounds, (B) calling sounds, and (C) premating sounds.

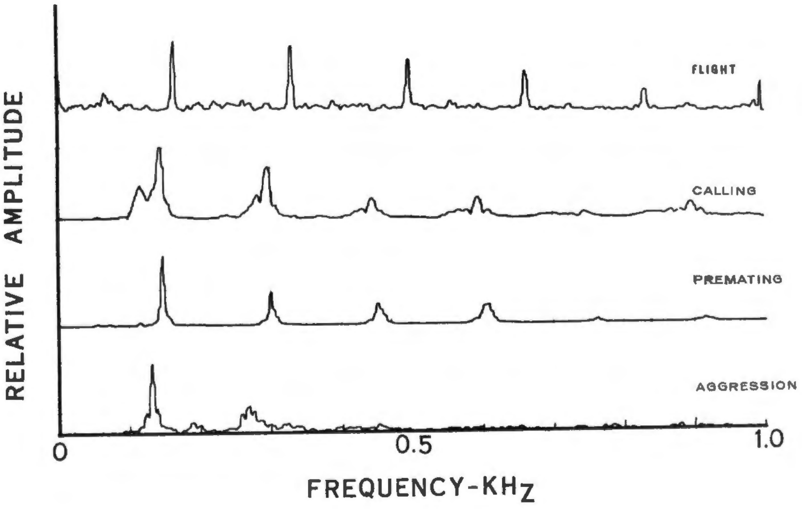

Figure 60. Signature of flight, calling, premating, and aggression sounds.

flies responding to the treatment. Three tests were conducted to determine if the calling sound is used as one mode of communication during courtship.

The first test was conducted to determine if both the females and males or only one sex responds to the calling sound. This test measured the response of the males and females to calling males, to reproduced calling sound, to pheromone extract, and to reproduced calling sound in combination with pheromone extract. The sound pressure level for this test was maintained at approximately 65 dB at the entrance to the release cell. The age of the test flies ranged from eight to thirteen days old; these ages were shown to have the best flight ability in the flight sound studies. The data were analyzed by multivariant analyses and a summary of the results is shown in Table XII.

The results show that males did not respond to sound along or to sound in combination with the pheromone extract. The females responded to calling males, to pheromone extract, and to reproduced sound plus pheromone extract, but did not respond to sound alone.

The second test was conducted to determine if the younger flies, six to eight days old, were more responsive than the older flies were to the reproduced calling sound and to the pheromone extract. The sound pressure level was maintained at 65 dB. The number of younger female flies responding to extract along was less than the number of older flies that responded. This indicated that the older flies were more dependent on the pheromone for locating a mate and that the younger flies were more dependent on sound.

TABLE XII

RESPONSE OF THE CARIBBEAN FRUIT FLY TO THE CALLING SOUND

Treatment	TEST 1 65 dB			TEST 2 65 dB			TEST 3 50 dB		
	$\bar{X}$	$Sd\bar{x}$	% Response	$\bar{X}$	$Sd\bar{x}$	% Response	$\bar{X}$	$Sd\bar{x}$	% Response
males to males	2.9 a	6.68	5.8						
males to sound	2.1 a	5.08	4.2						
males to extract	1.0 a	7.25	2.0						
males to sound + extract	1.7 a	3.98	3.7						
females to males	9.4 b	6.08	18.8						
females to sound	-0.3 a	6.06	-0.6				-0.2 a	6.39	-0.5
females to extract	8.5 b	8.64	17.0	3.2 a	6.22	6.4	2.7 a	5.19	5.4
females to sound + extract	8.7 b	6.40	16.0	5.0 a	6.12	10.0	9.9 b	6.22	19.8

*Means followed by the same letter are not different at the 5 percent probability level. Only means in the same column should be compared.

The third test was conducted to investigate the possibility that the calling sound is used as a long range communicative signal. To simulate distance effect, the sound pressure levels was reduced from 65 to 50 dB, and all other test conditions remained the same as in test 2. The response of the females to extract alone was about the same as in test 2, and no response was measured to sound alone. But there was a significant increase to sound plus the pheromone extract. This test indicated that the calling sound in combination with pheromone is used as one mode of communication between the male and female in locating a mate. This test also indicated that this sound is most likely used as a long range communication signal. The effective range of this signal was not determined in this research, but this would make an interesting study within itself.

CHAPTER VI

DISCUSSION

The flight sound studies provided valuable information of insect behavior and flight ability as related to age and sex. However, a detailed study is needed in relating specific insect activities to specific sound parameters. At the present time, there is no method available to determine insect behavior as related to insect quality. Such a method of measurement would be a true asset to research scientists in all phases of insect research and would provide them with a known quality of insects for research purposes. Some scientists have suggested that the techniques developed during this research holds promise of being adapted to provide guidelines in determining the quality of insects. No such method is now known.

The flight sound data showed that flies from one to four days old were immature, that flies from eight to fourteen days old were more vigorous and had the greatest flight ability of any age group, and that flies from fourteen to thirty days old were less vigorous and had a greater probability of having broken or frayed wings. These results agreed with both the visual observations and the sexual maturity of the flies. The fundamental frequency, sound pressure level, and flight velocity could be directly related to insect behavior. But a detailed study, perhaps with high speed photography, is needed on wing movements before the power in the fundamental frequency and factors of the harmonic content can be directly related to insect behavior.

Four sounds produced by the male flies and one produced by the female fly were isolated and identified and then were related to specific behavioral activities. The flight sounds are common to both males and females and are very similar for both sexes. The aggression, premating, and calling sounds are produced only by the male fly and only while the male fly is not in flight. In this study, the aggression sound was made by a male as he approached a calling male. The aggressive act that followed resulted in a struggle of the two males in question, and the stronger fly claimed the territory and resumed calling. The above observations led to the hypothesis that the calling sound was also used as a territorial sound. However, this hypothesis was rejected when males did not respond to calling males, the reproduced calling sound, or the reproduced calling sound plus the pheromone extract.

The purpose of the premating sound is unknown, but it appears to be used in courtship. This sound or the activity associated with this sound appears to be essential for mating because mating was not observed unless the male produced this sound. This sound does not appear to be used as an attractant because pair formation has already occurred before this sound is made. The flight, aggression, and premating sounds are produced by continuous wing fanning, and they contain only frequency and waveform information.

The calling sound not only contains frequency and waveform information, but also contains pulse information as well. During the literature review for this research, all insects, without exception, that are known to use sound as one mode of communication responded to pulsed information. This was true in both predator detection and sexual behavior. The calling sound of the Caribbean fruit fly is produced by very precised wing fanning with no evidence of striduation occurring. The male shifts the pitch of his wings to the near vertical position and produces wing strokes in the near horizontal position to produce the calling sound. Flight sounds are made with the wings in the near horizontal position and with the wing stroke about 30° to the vertical. This indicates that these sounds are used for different purposes. The fundamental frequency of all four sounds falls within the same frequency range. However, a separate and distinct frequency signature was obtained for each sound.

The response of flies from eight to thirteen days old was measured to the calling sound alone and to the calling sound plus pheromone. Male flies did not respond to the calling sound or to the pheromone. Females of all ages responded to the pheromone and to the reproduced calling sound plus pheromone, but the responses of the younger females were greater to the sound plus pheromone at 50 dB than to pheromone alone. These results show that the calling sound is used as one mode of communication and is more likely used in combination with pheromone for attracting a mate.

Most of the tephritid fruit flies produce a calling sound. Several orders of insects such as Heteroptera, Coleoptera, Deptera, Hymenoptera, etc., are known to produce and use sounds in their sexual behavior. Rudinsky (85) recently demonstrated the relationship between sound and pheromone in the mating of the Douglas Fir Beetle. The research on the calling sound of the Caribbean Fruit Fly has also shown that both sound and pheromone were important in the sexual behavior of this fly. Therefore, research is needed on the pheromone so that the relationship between sound and pheromone can be fully evaluated as an attractant for the Caribbean fruit fly. However, the results of this study show that sound plays an important role in the sexual behavior of the Caribbean fruit fly.

I. Summary and Conclusions

This research was divided into four phases. The first phase was conducted to measure the flight sound parameters produced by both the male and female fruit flies and to correlate these parameters to fly maturity and flight ability, and insect activity as related to sex and age. The second phase was conducted to measure to major sounds produced by the fruit flies and to determine those sounds that are incidental to ordinary movement and that are of a specialized nature. The third phase was conducted to isolate and identify the calling sound. The fourth phase was conducted to determine if the calling sound, either along or in combination with pheromone extract, was used as one mode of communication in the sexual behavior of the fruit flies.

These studies led to the following conclusions:

1. The acoustical properties of the flight activities showed a direct relationship with fly maturity and flight ability. These properties show promise of being a very important tool for measuring insect activity.

2. Four sounds from the male Caribbean fruit fly were identified and each was associated with a specific activity: two with mating and one each with flight and aggression.

3. The calling sound is produced in pulse form by very precise wing fanning. Each pulse is composed of two distinct parts and each part contains its own fundamental frequency. Since there was no evidence of stridulation, the calling sound is assumed to be produced only by wing fanning.

4. Young females responded to sound in combination with pheromone extract greater than to pheromone extract alone. This indicates that young females use sound in their communications to a greater degree than older females. The hypothesis that the calling sound is used as one mode of communication is accepted.

CHAPTER VII

SUGGESTIONS FOR FUTURE STUDIES

This research was considered as basic but essential to a better understanding of the basic behavior of the Caribbean fruit flies and related species. The techniques and procedures developed show promise of leading to a new and practical method for studying the behavior of many species of insects. Future research should include the following studies:

1. Information is needed to determine the specific purpose of the calling sound.
2. A study is needed to determine the relationship of sound, pheromone, and vision.
3. The sound receptors of the Caribbean fruit fly should be identified and their characteristics determined.
4. The effective range of the calling sound and the pheromone should be investigated.
5. The effects of temperature, humidity, and light on the production of and the response to the calling sound should be determined.
6. A frequency signature of the calling sound of all fruit flies of major importance is needed. This signature could be a very important from a taxonomic point of view.

7. Specific insect activities should be related to specific sound parameters. This could lead to the development of a practical method of measuring insect quality.

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APPENDIXES

APPENDIX A

Microphone Calibrations

The National Bureau of Standards uses a Western Electric type 640 AA condenser microphone, known as the "Type L," as the standard for all microphone calibrations. The Bruel and Kjaer 1-inch condenser microphone that was used in this research meets the requirements of the American National Standards Institute of the laboratory standard "Type L" microphone. This microphone was returned to the factory periodically for recalibration against a microphone which had been calibrated against the standard at the National Bureau of Standards. These microphones are durable and reliable and should maintain their calibrations for years with proper care. This Bruel and Kjaer 4145 microphone was used as the basis for the calibrations of the other two microphones used in this research.

Laboratory calibrations were made with the use of the pistonphone which had a calibrated output of 124 dB re 10^{-5} N/m^2 at 250 H_z. The 1-inch microphone was coupled to the sound pressure level meter through a cathode follower which had a gain of -1 dB. The 1-inch microphone was inserted into the pistonphone calibrator and the sound pressure level meter was then adjusted to indicate 123 dB. Therefore, a 1 dB correction factor was added to all readings taken with the 1-inch microphone.

The 1/2- and 1/4-inch microphones were then calibrated with the pistonphone and a correction factor (CF) obtained for each as follows:

$$\text{CF} = 124 \text{ dB} - \text{SPL meter reading}.$$

This calibration factor was used for each reading taken with its respective microphone. These calibrations were made once each week during the experiment, and the correction factor for each microphone remained constant throughout the tests period.

APPENDIX B

Power Spectral Density Calibrations

The spectrum analyzer is calibrated to give a 5 volt spectral line at the output when a reference voltage of 100 mv is supplied to the input. The input is also equipped with precision attenuation from 0 to 54 dB in 1 dB steps. Therefore, the analyzer output can be calibrated in dB where 0 dB is referenced to 100 mv; hence, all power spectral density calculations were made in dB re 100 mv.

Where data recordings were made, the signal level to the analyzer input was recorded for each data run, and the maximum signal level was near 30 dB. Therefore, the output of the spectrum analyzer was calibrated for a maximum output of 30 dB re 100 mv. The output of the analyzer connected to one channel of a Tektronix R 5031 oscilloscope and the x and y outputs of the analyzer were connected to the appropriate x and y inputs of the Honeywell 540 XYY' recorder. The analyzer was then programmed to calculate the power spectral density in dB re 100 mv for the following function:

$$\text{Power spectral density} = [\frac{1}{N} \sum_{i=1}^{N} g(x_f)]^2 \quad \log$$

where:

$N = 64$

$g(x_f)$ = Fourier Coefficient of the input function $f(x_t)$.

A sine wave was supplied to the input of the analyzer by a Hewlett Packard 651 test oscillator and monitored by a Hewlett Packard 3440 digital voltage meter and a Hewlett Packard 5325 A universal counter. The voltage of the test oscillator was adjusted to give a power spectral density calibration in 6 dB steps from -6 to 30 dB as calculated by the following equation:

$$n = 20 \log \frac{V_1}{V_o}$$

where:

n = dB re 100 mv

V_1 = input voltage

V_o = reference voltage of 100 mv.

The rms voltage, V_1, was calculated for each of the 6 dB steps as follows:

dB	rms voltage
-6	0.05
0	0.10
6	0.20
12	0.40
18	0.80
24	1.60
30	3.20

Therefore, the amplitude could be compared for each of the power spectral density's calculated and plotted for each fly.

VITA

J. C. Webb was born in DeKalb County, Alabama, on March 31, 1930. He attended elementary schools in this area and was graduated from Oak Hill Elementary School. In may, 1948, he was graduated from Susan Moore High School. The following August he enlisted in the United States Navy and served for four years.

In the fall of 1952, he entered the Alabama Polytechnic Institute (Auburn University) and received a Bachelor of Science degree in Agricultural Engineering in March, 1956. After graduation he was employed at Beltsville, Maryland, as a research engineer with the United States Department of Agriculture. He transferred to Knoxville, Tennessee, in 1958, at which time he began a study toward a Master of Science degree. He received his Master's degree in 1964 from The University of Tennessee. He transferred to Florence, South Carolina, in 1965. In 1968 he returned to The University of Tennessee and re-entered Graduate School. He received the Doctor of Philosophy degree with a major in Agricultural Engineering in December, 1973. He is presently employed by the United States Department of Agriculture at the Insect Attractant, Behavior, and Basic Biology Laboratory in Gainesville, Florida. He is a member of the American Society of Agricultural Engineers and the Society of Sigma Xi.

He is married to the former Earline Landers. They have two sons, Michael, 16, and Daniel, 12.