COMPARATIVE STUDIES OF COURTSHIP BEHAVIOUR IN SYMPATRIC SIBLING SPECIES OF ZAPRIONUS (DIPTERA - DROSOPHILIDAE).

A thesis submitted for the degree of Doctor of Philosophy in the University of Oxford

by
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Hilary Term, 1983.

ABSTRACT

Zaprionus tuberculatus and Z. sepsoides are two sibling species of drosophilid fly which are sympatric throughout tropical Africa. Their sexual behaviour has previously received little study.

In this thesis, the courtship behaviours of the two species are described, and the descriptions compared with previously published descriptions by other authors. Three different laboratory-bred strains of Z. sepsoides, and two of Z. tuberculatus, were studied, and their male courtship "songs" (produced by male wing vibration) were compared.

The main objects of the project were to study the mechanisms by which members of these two species identify the sex of other individuals (sexual dimorphism being slight); and the isolating mechanisms operating between the two species. Preliminary observations suggested that males could probably identify females by chemosensory cues. However, males would sometimes also court each other. Further experiments on the males' ability to distinguish sex-specific pheromones gave ambiguous results. There were no grounds to suspect that females could distinguish between the sexes by chemosensory means, and further experiments gave no evidence for such an ability.

Preliminary observations indicated that that there was an effective pre-mating reproductive barrier between male Z. sepsoides and female Z. tuberculatus, but male Z. tuberculatus were sometimes able to copulate with female Z. sepsoides. These interspecific matings resulted in infertile eggs. Further experiments seemed to confirm that the isolating mechanism between the two species is asymmetric, the onus of species recognition lying largely on Z. sepsoides. Male Z. sepsoides apparently recognise a female's species by chemosensory means, while female Z. sepsoides recognize a male's species by his "Type-2" courtship "song". There was also some evidence that female Z. sepsoides could distinguish between males of the two species by olfactory means. Z. tuberculatus were not shown to be able to distinguish reliably between the two species.

The relevance of these findings to current theories about mechanisms of speciation is discussed.

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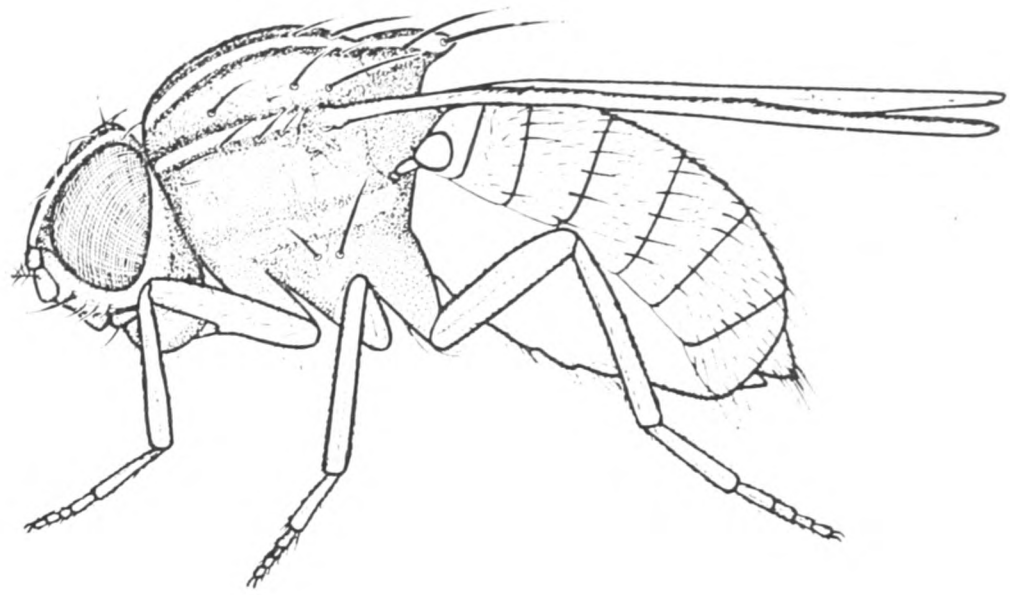


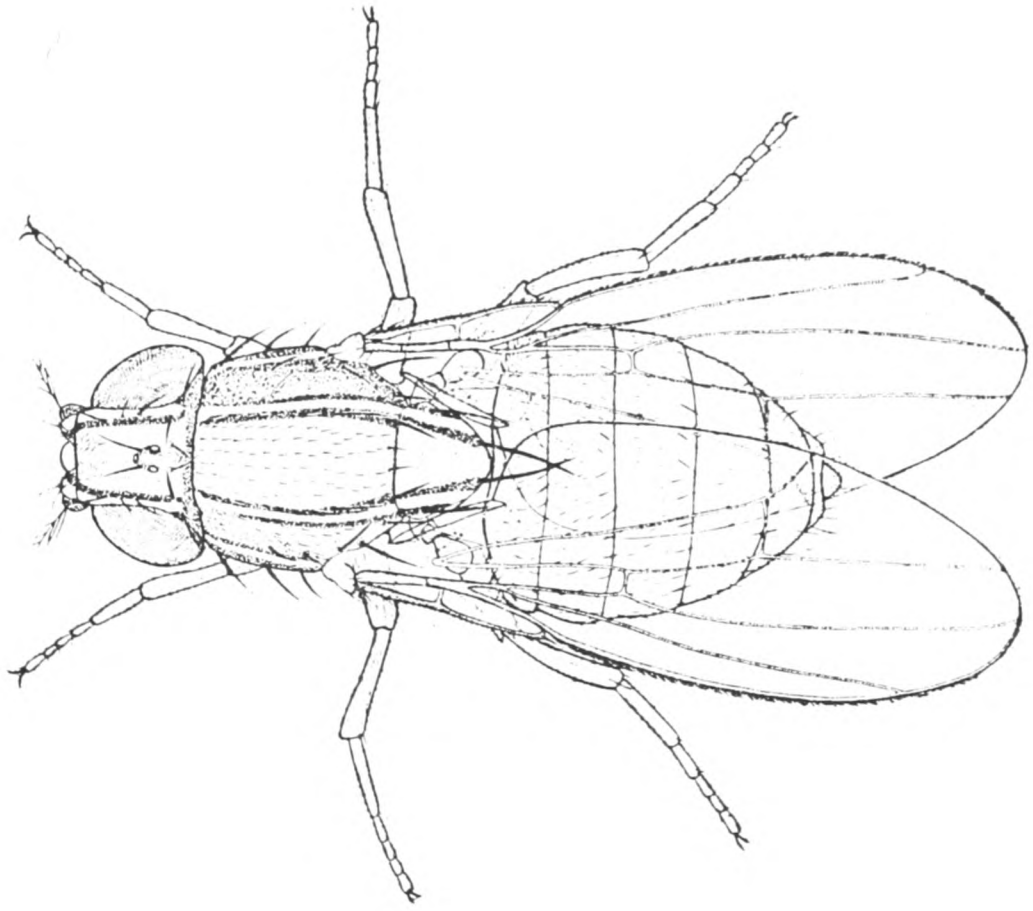
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March, 1983

"To produce a mighty book, you must choose a mighty theme. No great and enduring volume can ever be written on the flea, though many there be who have tried it." HERMANN MELVILLE - "Moby Dick" ch.CIII.

"... and the small gilded fly does lecher in my sight. Let copulation thrive." W. SHAKESPEARE - "King Lear" Act III, Sc.VI, ll.116-118.





Frontispiece: Female Zaprionus tuberculatus. Drawn by M.T. Chassagnard.

ACKNOWLEDGEMENTS

This thesis describes a project which was originally carried out between October 1978 and December 1981 in the Oxford University Department of Zoology, under the supervision of Dr. H.C. Bennet-Clark. Further work on it was carried out at Oxford in October-November, 1982, under the supervision of Dr. Marian S. Dawkins; and between December 1982 and February 1983 in Professor R. Harmsen's laboratory in the Department of Biology of the Queen's University in Kingston, Ontario, Canada.

From October 1978 to September 1981 I was receiving an SERC studentship.

I would like to thank the following people and institutions: Professor T.R.E. Southwood and the late Professor J.W.S. Pringle, Heads of the Oxford University Department of Zoology, and Dr. D.T. Canvin, Head of the Queen's University Department of Biology, for making available laboratory space and equipment. I am also grateful to all the employees of both these Departments, without whom no research would have been possible; especially Mr. David Palmer, who built the controlled-conditions room in the Oxford laboratory, and Mr. Terry Barker, who built much of the electronic equipment there.

I must thank the C.N.R.S. Laboratoire de Génétique et Biologie Évolutives, Gif-sur-Yvette, France, for providing cultures of flies which were the subjects of this study. I must also thank Dr. David Roberts and his assistants in the Oxford University Department of

Genetics, for providing me with bottles and vials containing the nutrient medium on which the flies were reared.

Dr. Henry Bennet-Clark has been known to describe my research as "largely self-propelled", as if to disclaim any part of it. In fact, I would have been unable even to begin this project without his constant help, advice and encouragement. He also tolerated my persistent begging and borrowing of various items of hardware, tools, etc., not always for scientific purposes, and patiently instructed me in their use. For all this I am most grateful.

When Dr. Marian Dawkins became my acting supervisor at Oxford when Dr. Bennet-Clark left for a year's sabbatical, she little guessed that her involvement in the project would be more than a formality. When it came to the point, she gave considerable assistance, in criticising the accounts I had written of my previous work, and in discussing the design of further experiments. I am very grateful to her for all this.

Professor Rudolf Harmsen had the major job of getting me settled into Queen's University, Kingston. He was also my effective supervisor during the closing stages of the project, and gave me much valuable advice and assistance in the design of my last two experiments. His comments and criticisms on the penultimate draft of the thesis were most constructive. For all his efforts on my behalf, I cannot thank him enough.

In addition to these people, I must also thank the following, who gave me invaluable advice on statistical matters: Dr. Colyer Dawkins, of the Oxford University Department of Forestry; Dr. Francis Marriott

of the Oxford University Department of Biomathematics; and Dr. Patrick Colgan, of the Queen's University Department of Biology.

Finally, I must especially thank Julie Beattie of the Queen's University Department of Biology for typing the whole thing out, remarkably quickly, in her spare time.

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CHAPTER 1.

Introductory Review of the General Field of Study in which a Project was Undertaken.

Contents:
1.1. General aims of the project
1.2. Courtship behaviour in Drosophilidae
1.3. Courtship behaviour and speciation
1.4. Zaprionus tuberculatus & Zaprionus sepsoides

1.1. GENERAL AIMS OF THE PROJECT.

The dipteran family Drosophilidae is well suited for a study of courtship behaviour from a functional and evolutionary viewpoint. The breeding biology of many species of Drosophila is as well known as that of any animal, and ethologists can avail themselves of the great quantity of expertise developed by geneticists in breeding, handling, and observing these insects.

The general aim of this project has been to study how a particular group of higher animals in practice meet the theoretical requirements of courtship behaviour and response to courtship, as dictated by natural selection. For this kind of a study, there is much to be said for studying an insect rather than a vertebrate. Firstly, insects have short life cycles, and many types of insects, such as the Drosophilidae, can be bred in captivity in very large numbers. This means that there is an abundance of experimental material, and large

quantities of data taken under controlled conditions can be amassed quickly. Secondly, insect nervous systems are simpler than those of vertebrates, in that for unit size they contain fewer and larger neurones, and they appear to be more "hard-wired", the circuitry being apparently both simpler and less variable between individuals. In consequence, insect behaviour, and particularly decision-making mechanisms, may be expected to be less plastic than those of vertebrates. In reality, this generally seems to be the case. This makes a basic ethological study more rewarding, because substantial progress can be made relatively easily in investigating functions of behaviour experimentally.

The requirements of courtship and response to courtship are to ensure that individuals only mate with other members of the same species, which are in a fertile condition. There will always be a strong selection pressure for unambiguity both in courtship and in response. There will also be some selection pressure for brevity. Courtship is an activity which has to be fitted in between the immediately important activities of foraging, feeding, and avoiding predators. In animals which mate repeatedly, it must not interfere with these activities to any marked extent, although it may take absolute priority over foraging and feeding in the case of animals which only mate once. If these were the only criteria, a male would have one totally unambiguous species-specific courtship signal. A female would have one totally unambiguous acceptance signal, given when receptive, in response to the right species-specific courtship signal, and not given under any other circumstances.

But in fact we observe that courtships are often more protracted and complex than this. The male's courtship commonly consists of several components, not all of which are invariably present in a successful courtship, and some of which may not be species-specific. Females do generally have an unambiguous acceptance response, which apparently invariably leads to copulation immediately. But this may not be given until a considerable time after the male has conclusively identified himself as being conspecific and ready to copulate, during which time the male has continued courtship. Males may also court unreceptive females for a considerable time, thus indicating that the female has not unambiguously and conclusively announced her unreceptivity.

The usual reason given to explain the complexity of many courtships is "Sexual selection" (Darwin, 1859). Sexual selection is said to occur when individuals of one sex (generally the male) have to compete with other members of their sex for mates. This can result in components being added to courtship because their presence indicates that the male has a trait associated with increased fitness, perhaps because of close genetic linkage to this trait. Females can thus recognise males with the enhanced fitness trait from this cue, and mate preferentially with them. The possession of this recognisable cue then becomes a fitness trait itself. It may then spread throughout the population, until it becomes universal in the species. Its absence from a male's courtship then throws doubt on the identity of the male's species. Thus the courtship comes to contain more than one obligatory species-specific component, and a

dimension of ambiguity is introduced by the possibility of a courtship with one such essential component lacking. If a female encounters courting males very frequently, and the failure to mate with one does not appreciably diminish her probable ultimate number of offspring, and if ambiguous courtships are relatively rare, a female may safely be able to ignore all ambiguous courtships. But if, as a result of Sexual selection, courtships contain a large number of separate components, some level of ambiguity may become relatively frequent, and it may become advantageous for a female to have a means of "expresssing doubt", or showing the male that his courtship is so far ambiguous. A "doubt" signal will cause the male to continue courtship of a female that does not give an "acceptance" signal for copulation. It should, therefore, be qualitatively different from the rejection signal.

There are other reasons why courtships may be complex and protracted. Sexual selection may actually favour as varied a repertoire of courtship behaviour as possible. It is sometimes suggested that a female of a given species may be sexually unreceptive until courted, and courtship has to go on for a certain length of time to make her sufficiently receptive to allow copulation. But I would suggest that this is just another way of saying that Sexual selection favours courtship of over a certain duration, since I doubt whether it has ever been shown that for any physiological mechanism of female sexual arousal there is a minimum physically possible length of time required to switch it on, such as could not possibly be reduced if there were a selection pressure to do so. Likewise, if it can be

shown that a male cannot copulate without a given minimum amount of previous sexual arousal during courtship, I would suggest that the minimum must in some way be adaptive.

A further requirement that may be made of courtship and the response to it is of sex recognition, in a species where sexual dimorphism is slight. Aggression between males is found in many species of higher animals, and if an individual's sex is not immediately apparent to other males, the first reaction of a male to an encounter with another individual may need to be compatible both with courtship and aggression, so that either behaviour may then be employed when the other individual's response discloses its sex. The initial reaction to an encounter with another individual, must, then, be a simple disclosure of sex. A female, on encountering an individual which thus shows itself to be male, may then also need to disclose her sex, regardless of her state of sexual receptivity, in order to avoid the possibility of being mistaken for a male and attacked.

Recognition cues need not be behavioral. Both sex and level of sexual activity may be communicated by chemical means (pheromones). These may be volatile, so that they give an olfactory cue, or they may be a chemotactile cue that is only detected by contact either with the individual or, possibly, with the substrate on which the individual has recently been resting. If the cue is olfactory, the initial behavioral reaction of an individual on encountering another need not be a disclosure of sex. If it is chemotactile, the first reaction to an encounter must be physical contact to receive this cue. A familiar example is in an encounter between two dogs, each smells the other's

anus. They thus ascertain each other's sex and state of sexual receptivity (this is also announced by a long-distance olfactory pheromone emitted by receptive females) and subsequent behaviour is then either aggressive or sexual.

This is a tentative theoretical construction of the functional roles of sexual behaviours based on the apparent requirements. It may serve as a starting-point for a project in which the courtship of a species is studied from functional and evolutionary viewpoints. In fact, it is worthwhile to compare the courtships of similar and closely-related species, individuals of which are likely to encounter each other in nature, in order to identify species-recognition mechanisms, since species recognition and isolation is one of the theoretical requirements of courtship. Such a project is best carried out on an animal with a moderately complex but fairly stereotyped courtship, since it is easier to assign roles to the different courtship components if they are produced according to a fairly inflexible pattern. I have already described how this requirement is better met by an insect than a vertebrate, and how the Drosophilidae are exceptionally convenient insects for ethological study.

1.2. COURTSHIPS OF DROSOPHILIDAE

The first descriptions of courtships in *Drosophila melanogaster* are given by Sturtevant (1915). Spieth (1952, 1974a) gave brief comparative descriptions of the courtships of many *Drosophila* species, and also of *Zaprionus vittiger*. Typically, male courtship contains the following components: orientation towards the female - the male

turns to face the female from a distance of less than one fly's length, and remains thus orientated throughout courtship; tapping of the female with the fore-tarsi; licking the female's genitalia with the proboscis; vibration of the wings; and mounting and copulation. The initial orientation makes it appear that visual perception plays a rôle, although it may be in response to olfactory cues. Connolly et al. (1969) showed that males track females' movements visually; but many species are known to mate readily in the dark (e.g. Manning, 1959a). Tapping and licking would apparently be ways of receiving chemotactile cues from the female. The vibrating of the wings produces the courtship "songs", which are generally species-specific (see 3.1.). The roles of visual, chemical, and acoustical components of courtship are discussed in chapters 3, 4, and 5.

In most Drosophila species, females have not been observed to have a specific acceptance response, further than passively to permit the male to mount and copulate (in this genus the two acts are simultaneous). They have, however, the following rejection signals: a kick, generally given to an approaching male before the apparent start of courtship; a buzz, usually given by sexually immature females; the depression of the tip of the abdomen so that it is inaccessible to the male; the extrusion of the vaginal plates, occasionally accompanied by the production of a drop of fluid from the anus; and decamping (Conolly & Cook, 1973). (The term "Decamping", as used throughout this thesis, is defined as rapid running or flying away from the male.) Manning (1967b) reported that in D. melanogaster females are either fully receptive, or fully unreceptive. They become

fully receptive during the first two days after emergence. They become unreceptive after mating, due to: a./ the effect of copulation itself - this wears off after 48 hours; b./ the presence of live sperm in the genital tract (where it is retained for 8-10 days). Burnet et al. (1973) report that the changes in female behaviour which occur after insemination can be mimicked by implanting a male paragonial gland in the female's abdomen. The behavioral changes are apparently effected by a secretion from this gland, coupled with information from the abdominal stretch receptors.

However, a female's response to a male's courtship is not solely dictated by her own general state of receptivity. Females discriminate between conspecific and heterospecific males, and also between conspecific males of different strains, i.e. they are choosing their mates, so Sexual selection is presumably operating among males.

Species recognition is a necessary requirement of courtship, because among the Drosophilidae there are several groups of sibling species existing sympatrically. These are often morphologically almost identical, and of sufficiently similar ecology to make interspecific encounters at breeding sites frequent events. D. melanogaster and D. simulans are two such species which have been closely studied. The two species are of very similar ecology (e.g. McKenzie & Parsons, 1972), although there is some partitioning of breeding sites between the two species (Atkinson & Shorrocks, 1977). However, the two species are often caught together in the same baited traps, so there is no doubt that they often encounter each other in

nature. Although Hardeland (1972) has shown that they are maximally sexually active at different times of day, the difference is not enough on its own to prevent interspecific courtships from taking place. Several studies of the nature of the species isolating mechanisms between this and other pairs of species have been published. These are summarised in ch. 3.

Females are also apparently able to distinguish between males of different inbred strains within a species. Petit (1958) reported the apparently paradoxical "rare-type mating advantage" effect in D. melanogaster, which has since received much study.

If males from two strains are present in a culture, females are reported sometimes to mate preferentially with males of the less numerous strain. A negative correlation between the relative abundance of males of a given strain in a culture, and their mating success rates, has been claimed. Evidence has been presented (e.g. Ehrman, 1969; Hay, 1971), that female use chemosensory cues for distinguishing between males of different cultures (see Chapter 4). I do not propose to review the published work in this field, since it is only marginally relevant to the subject of this thesis. I must mention, however, that several workers have failed to demonstrate the rare-male mating advantage effect, despite repeated attempts to do so. Some reports of it appear to be unassailable, while in others the possibility that the effect may be an artifact resulting from the technique of marking the two strains cannot be discounted. In yet other reports, the statistical analysis of the results may be criticised.

If the effect is genuine, the only plausible explanation of how rare-type mate preference could have evolved is that a certain degree of heterozygosity is advantageous, not just to a population in the long-term, but to an individual female's own offspring. Maynard-Smith (1956) gave evidence that this may be the case: inbred male D. subobscura produce less, and poorer, sperm than outbred males. Females of this species only mate once, so there must be a selection pressure for them to recognize outbred males, and to produce outbred offspring.

Outbreeding will result in greater variation among offspring than inbreeding. This may not maximise inclusive fitness in a specialist species, breeding in a relatively unchanging environment. But it may be advantageous in a generalist species, utilising a wide variety of temporary habitats. The habitat in which an individual's offspring may live and reproduce is unlikely to be identical to that of its parents. D. melanogaster is such a species, so females may possibly outbreed preferentially for this reason.

The occurrence of groups of sympatric species, morphologically and ecologically almost identical, but reproductively isolated, suggests that speciation is occurring relatively rapidly in the Drosophilidae. The ability of females to distinguish between males of different inbred strains shows that the separation of different populations into distinguishable races, reproductively isolated from each other at least to some extent, could in theory occur easily. Recent theories and evidence about speciation in Drosophilidae are summarised below.

1.3. COURTSHIP BEHAVIOUR AND SPECIATION

The classical theoretical mechanism of speciation in sexually reproducing organisms (e.g. Mayr, 1942, 1963) may be described briefly as follows: an ancestral population is divided in two by a geographical barrier; the two populations thus formed are subjected to different selection pressures, and evolve in different directions; when, or if, their geographical ranges later change so that they come into contact again, it is found that they have diverged sufficiently to preclude interbreeding. A possible instance of speciation by this mechanism in Drosophilidae is Zaprionus mascariensis (see Bennet-Clark & Leroy, 1977). This species is endemic to the Mascarene islands. It shows signs of having evolved in isolation. It is reproductively isolated from its sibling species Z. tuberculatus, which has a wide geographical range on the African mainland, and with which it co-exists on the island of Réunion (see 1.4.). Apparently, Z. tuberculatus reached Réunion considerably more recently than the ancestral Z. mascariensis population. The two species probably share an immediate common ancestor. Dobzhansky (1944) found that strains of D. sturtevanti descended from flies caught in widely separated localities in Central and South America showed incipient reproductive isolation in the laboratory. If the geographical ranges of the different races were to change, some might be found to have attained specific status.

The mechanism of speciation has also been simulated in the laboratory. Kilias et al. (1980) kept several populations of D. melanogaster under different conditions for five years, at the end of

which a degree of stable environment-dependent sexual isolation was found. Genetic differentiation was slight; the main differences between the populations were behavioral.

A variation on this mechanism of speciation was suggested by Dobzhansky (1940), in which a pre-mating reproductive barrier arises after the two populations have come into contact with each other. If hybrids are viable, but are less fit than pure-bred members of the two populations (e.g. they have reduced fertility), then there will be a selection pressure for reproductive isolation to arise. Knight et al. (1956) and Crossley (1974) were able to increase the degree of sexual isolation between two strains of D. melanogaster by allowing them to interbreed, and then artificially selecting against hybrids. Dobzhansky & Pavlovski (1971) achieved a similar effect in D. paulistorum. Ehrman (1971, 1973) mixed wild-type D. melanogaster populations with populations of the mutant Black Body. Hybrids between the wild-type and mutant strains were sterile. After two years, incipient reproductive isolation between the strains had arisen. After four more years, the degree of isolation was significantly enhanced.

There is evidence that speciation may occur by this process in nature. Ehrman (1965) reports that in the D. paulistorum species complex, strains of different races descended from individuals taken in regions of sympatry exhibit a greater degree of sexual isolation than do strains of the same races taken from regions of allopatry. Markow (1981) also reports that the degree of isolation between strains of D. arizonensis and D. mojavensis is greater when the

strains are of sympatric origin than when they are allopatric (see 3.1.).

However, some authors consider these postulated mechanisms of speciation by natural selection to be insufficient by themselves to explain the apparently rapid rate of speciation occurring in some taxa, notably the Drosophilidae. In this family, there occur groups of "sibling species", between which reproductive isolation is total, or nearly so, but morphological differences are slight. Evidently, these have acquired reproductive isolation from each other only recently. However, they are often sympatric over most, or all, of their geographical ranges, and have similar ecologies. It seems hard to believe that natural selection *per se* could have been responsible for such rapid speciation. The extreme case of rapid speciation is that of *Drosophila* radiation on the Hawaiian archipelago.

There are 300 known species of *Drosophila* (about one-quarter of the genus) endemic to these islands (Carson et al., 1970), most of them found on the five youngest islands. Speciation is apparently occurring very rapidly. It is hard to envisage populations being geographically isolated for more than a few tens of thousands of years at the very most. Genetical studies (e.g. Carson et al., 1970; Craddock & Johnson, 1979) show that the behavioral and morphological divergence of many species has been accomplished with a minimal degree of genotypic divergence. This ability of populations to diverge phenotypically very rapidly is evidently facilitating rapid speciation.

Three mechanisms have been postulated to account for this high rate

of speciation, in addition to the classical mechanism described above. The first of these is the theory of speciation by sexual selection (e.g. Spieth, 1974b; Ringo, 1977). They point out that the major differences between many sibling species are in characteristics associated with male sexual behaviour, and suggest that they have arisen as a result of sexual selection. It is suggested that this mechanism could accelerate speciation, because some of the characteristics associated with species recognition may have a very simple genetic basis, and could therefore change rapidly. e.g. Some Hawaiian species have "hammer" heads. Their Head shape is used as a cue for species recognition. Its genetic basis is known to be dependent on only four loci.

The second theory which has received considerable attention recently is the "founder-flush" theory of speciation by genetic drift. It is suggested that changes in the sexual recognition mechanisms occur as a result of genetic drift in a very small, temporarily isolated population, thus accelerating the acquisition of reproductive isolation from other populations. This idea is not entirely new (see Mayr, 1942; Müller, 1942). It has been refined by Carson (1968), who suggested that populations could show considerable genetic drift if they were originally founded by very few individuals, or even just one pregnant female. Further genetic drift might occur if the population levels then fluctuated, going through several "crashes", followed by "flushes", such as may be expected if its geographical range is limited, making it vulnerable to short-term, local environmental changes.

The feasibility of this mechanism is supported by the report of Arita & Kaneshiro (1979), that partial reproductive isolation arose between two laboratory strains of D. adiastola descended from individuals taken from the same locality on two separate occasions. They had both been kept under conditions as nearly identical as possible, so any genotypic differences between the two populations presumably arose as a result of genetic drift occurring at the time of their foundation.

An attempt to test the theory experimentally was made by Powell (1978), by creating an artificial "original" strain of D. pseudoobscura by mixing several laboratory strains. From this "original" strain several strains were derived, some of which were subjected to "founder-flush" cycles acquired a degree of reproductive isolation from one another, but strains which were not subjected to such cycles did not. The weak point of this experimental design was the creation of the "original" strain. Powell explicitly states that this "original" strain was intended to have a gene pool resembling that of a wild population. To what extent this intention was actually fulfilled, I would not care to speculate. In other respects, however, the work is interesting, and similar experiments, using a large number of wild-caught flies from one locality to found the "original" strains, should be worth performing. It is a major advantage of this theory, that, unlike the Sexual Selection hypothesis, it can be tested experimentally in such a way.

The chief criticism that can be levelled against the "Founder-flush" speciation theory is that it is superfluous. It

requires an increasing divergence in an isolated population from other populations, as a result of genetic drift repeatedly occurring. Statistically, this is extremely unlikely to occur. A selection pressure, either environmental or sexual, however, may be predicted to have this effect. The rate at which a selection pressure may change a population under natural conditions is not known in any instance, but it may be quite rapid. Personally, I would guess that genetic drift may accelerate divergence between populations on some occasions, but I consider it highly unlikely that it could ever cause speciation by itself.

The third suggested mechanism of rapid speciation is that of hybrid dysgenesis. Hybrids between some laboratory strains of D. melanogaster have reduced fertility, and this has been shown to result from members of one strain possessing extra (presumably parasitic) chromosomal elements (Bingham et al., 1982). It is suggested that such chromosomal elements could arise spontaneously in a wild population, and spread rapidly through it, thus causing at least partial sterility of hybrids with other populations. If several such chromosomal elements were present in a population, hybrids with other populations might be totally sterile. At least, any reduction in hybrid fertility will create a selection pressure for total pre-mating reproductive isolation, and thus speciation.

1.4.. ZAPRIONUS TUBERCULATUS & ZAPRIONUS SEPSOIDES

The above introduction is intended to place the study described in this thesis in context, and to show why a comparative study of the

sexual behaviour of two sibling species of drosophilids is of general interest.

The two species studied here are Zaprionus tuberculatus Malloch, 1932; and Z. sepsoides Duda, 1939. The genus Zaprionus is closely related to Drosophila, from which it diverged during the D. immigrans radiation (Throckmorton, 1975). The two species are found throughout tropical Africa. Z. tuberculatus is also found on the island of Reunion. The two species were not originally definitely known to be distinct, the two names being considered to be synonyms. Barker (1974) was the first to give evidence that Z. tuberculatus might in fact be two species. This was conclusively demonstrated by Tsacas et al. (1977) who described small morphological differences, particularly in the eggs of the two species. The two species apparently have slightly different habitat preferences - they are caught with different relative frequencies in traps baited with different fruit. However, Lachaise et al. (1982) reported that the two species breed together in rotting figs, both species favouring figs at the same stage of decomposition. The two species must therefore frequently encounter each other at breeding sites. The two species are morphologically extremely similar (there is a difference between the mean sizes of the two species, but size is variable, and there is a large size range overlap), so species recognition presumably depends on chemical and/or behavioral cues. Sex recognition is presumably affected in the same way, since sexual dimorphism in this genus is slight.

The chief aim of this project was to try to discover what these

cues for sex and species recognition are. Once they have been described, it should be possible to study their genetics. If their genetic basis proves to be simple (i.e. depending on few loci), then they may be susceptible to rapid change, either by the differential effect of sexual selection in different populations, or by genetic drift. Speciation could then occur rapidly.

CHAPTER 2

Descriptive.

Contents:
2.1. Introduction
2.2. Summary of previous published work
2.3. Experiment 1: Materials & Methods
2.4. Observations
2.5. Discussion

2.1. INTRODUCTION

2.1.1. Aims. The aims of these observations were as follows:

1/. To repeat previously published observations to check their accuracy;

2/. To compare the courtships of different strains of the two species of Zaprionus studied in this project.

3/. To familiarise myself with the courtships of Zaprionus and the methods and techniques of observation and recording.

2.1.2. Reasons for the aims. The only descriptions of the courtships of Zaprionus tuberculatus and Z. sepsoides that had been published before this study was undertaken were in Bennet-Clark et al. (1980). These are summarised in 2.2. In that study, only a small number of courtships of each species were observed, and only one strain of each species was used. The descriptions had to be

confirmed, and larger quantitites of numerical data had to be amassed to provide more reliable statistics.

In the present study, more than one strain of each species was used (see 2.3.). Differences between the courtships of the different strains might be expected for the following reasons:

1/. The different strains were descended from wild individuals originally caught in widely separated localities, and may therefore represent different subspecies;

2/. At the time of foundation, the total population of a strain may be small, or may even be one gravid female. Subsequently, the total population may be reduced to low levels on many occasions. Genetic drift may therefore cause differences to arise by the "Founder-Flush" effect (see Chapter 1).

It was therefore necessary to amass numerical data for each strain separately, and compare the data compiled for the different strains.

2.2. SUMMARY OF PREVIOUSLY PUBLISHED WORK.

The descriptions of the courtships of *Z. tuberculatus* and *Z. sepsoides* given by Bennet-Clark et al. (1980) are summarized below:

2.2.1. General features of courtship in the genus.

Normal courtship consists of: approach and "Grappling" by the male, followed after a few seconds by "song", produced by wing vibration, and an attempt to mount. If the female is receptive, copulation commences a few seconds later, and lasts for between 2 and 4 minutes in most species. After the end of copulation, the male performs a few seconds of

post-copulatory display, consisting of "Orientation" and song. More usually under experimental conditions, the female is not initially receptive, and responds to courtship song by "Whining" and "Rocking". "Whining" is the production of a pulsed or continuous high-pitched sound by wing vibration. "Rocking" is a rapid side-to-side rocking of the body. These behaviours may modify the male's behaviour in a number of ways. His song may become erratic, extended, or may cease. He may perform a few seconds of "Dance" before resuming courtship. "Dance" consists of turning around on the spot for one or two turns, with either smooth or jerky movements. It is performed at a longer range from the female than other courtship behaviours.

Mounting is not always followed immediately by copulation. Males may stay mounted for a long time, singing intermittently. The male may eventually succeed in copulating, or may dismount apparently spontaneously, or may be forced to dismount by the female's kicking or rocking.

Within 5s after the end of copulation the female becomes relatively inactive, and responds to all approaches by males with immediate vigorous Whining and/or Rocking, sustained for several seconds. She also "Points" her abdomen and "Protrudes". "Pointing" consists of turning the abdomen so that the ovipositor points directly at the head of the male. "Protrusion" is the extension of the ovipositor, occasionally accompanied by the production of a drop of liquid. (This also occurs in Drosophila species - see Spieth, 1974a). These refusal signals persist for at least 6 hours after copulation has ended.

Other behaviours associated with sexual activity not mentioned above are decribed below:

Male Behaviours:

Scissoring: A discrete flicking open and shut of the wings by the male, producing sound. This differs from song, which is continuous.

Stationary Display: The male stands in a stereotyped position, from straight ahead (0^{o}), straight behind (180^{o}), or at angles of 45^{o}, 90^{o}, or 135^{o} in relation to the direction in which the female is facing. He may also stand beside the female, with his body parallel to that of the female. Display is normally accompanied by Song.

Circling: The male may move from one display position to another by describing a smooth arc of movement with the female's body at the centre, facing the female throughout. This is also normally accompanied by Song.

Massage: During mounting or copulation, the male rubs the sides of the female's abdomen with his mesothoracic legs.

Female Behaviours:

Chasing: when a female will run towards other flies and chase them away.

Kicking: either with the middle legs towards a male orientated at 90^{o} or with both hind legs to a male at 180^{o} or attempting to mount.

Abdomen Depression: The abdomen is depressed downwards so that copulation cannot occur. This signal of unreadiness to copulate occurs in unreceptive females on being mounted by a male.

Abdomen Extension. The abdomen becomes long and thin and apparently difficult to hold; a signal of unreceptivity used after

mounting by the male.

Throughout the rest of this thesis, these behaviours will be referred to, spelt with an initial capital letter. Unless otherwise stated, the definitions of the behaviours will be as given by Bennet-Clark et al.

2.2.2. Behaviour and songs of individual species. The Songs that are described here fall into three main categories, all of which appear to be produced by the majority of species in the genus: The Male "Type 1" Song; the Male "Type 2" Song; and the Female Song. "Type 1 Song is typically monocyclic. "Type 2" Song has a longer pulse repetition period than "Type 1" Song, and the pulses are typically polycyclic. Both species produce other sounds erratically, which are of uncertain behavioral importance. These are not described or analysed.

2.2.2.1.

Z. tuberculatus. Strain used: 124.5 (see 2.3.1.).

Male sexual behaviours: as described above, with stationary displays, circling and dance. Dance is silent, and never precedes singing displays. Mounting may ensue immediately, after the first song display, or after repeated displays of all types. The male sings both Song Types before copulation and rubs the posterior ventral third of the female's abdomen with his mesothoracic legs before and at the very start of copulation. Average duration of copulation is 2.25 min.

Female behaviour is typical of the genus.

Songs: a/. Male. Two types of Song are produced, apparently indiscriminately during courtship. Characteristics of the songs are given in Table Ia. Song is produced

either by vibration of both wings opened to an angle of 30-60° from the body, or one wing opened through 15-45°.

b/. Female. Two types of song are produced, a continuous tone used after copulation or in vigorous refusal (Whining); and a pulsed song produced at low levels of excitation, mainly after mounting by a male or after copulation. Characteristics are given in Table II.

2.2.2.2.

Z. sepsoides. Strain used 123.3 (see 2.3.1.).

Courtship behaviour of this species is very similar to that of Z. tuberculatus.

Songs: a/. Male. Two types of song are produced. "Type 1" Song is often produced in repeated sequences, but trains of 1-2-1 are also common. The average pulse period within individual sequences of "Type 1" Song may not differ significantly from that of Z. tuberculatus, but, measured for the whole population, the period is significantly shorter and the sequences have about one third of the duration. The "Type 2" Song has very significantly longer pulse period than that of Z. tuberculatus. Characteristics of the songs are given in Table Ib.

b/. Female. In addition to a continuous whine similar to that of Z. tuberculatus, there are one or possibly two types of pulsed song, produced in similar contexts to

TABLE 1. Male song parameter statistics of Z. tuberculatus and Z. sepsoides as reported by Bennet-Clark et al. (1980).

A. Z. tuberculatus

Temperature	"Type 1" Song		"Type 2" Song				
	Pulse Period (ms)	Sequence Duation(ms)	Pulse Period(ms)	Pulse Duration(ms)	Carrier Frequency(Hz)	Sequence Duration(ms)	No. of Insects
18-20°C	$\bar{X}$=12.4	$\bar{X}$=730	$\bar{X}$=26.6	monocyclic	monocylic	$\bar{X}$=900	5
	±1.3 S.D.	±530 S.D.	±3.5 S.D.			±690 S.D.	
	N=183	N=10	N=240			N=10	
24-26°C	$\bar{X}$=10.7	$\bar{X}$=810	$\bar{X}$=28.9	$\bar{X}$=17.0	$\bar{X}$=170	$\bar{X}$=935	5
	±1.6 S.D.	±515 S.D.	±3.9	±8.4 S.D.	±40 S.D.	±705 S.D.	
	N=150	N=12	N=125	N=80	N=80	N=10	
B. Z. sepsoides							
18-20°C	$\bar{X}$=11.2	$\bar{X}$=325	$\bar{X}$=42.0	$\bar{X}$=14.2	$\bar{X}$=230	$\bar{X}$=1370	5
	±1.9 S.D.	±170 S.D.	±5.2 S.D.	±2.6 S.D.	±11 S.D.	±630 S.D.	
	N=356	N=15	N-284	N=74	N=74	N=15	
24-26°C	$\bar{X}$=11.1	$\bar{X}$=330	$\bar{X}$=40.4	$\bar{X}$=33.7	$\bar{X}$=185	$\bar{X}$-1100	5
	±0.9 S.D.	±200 S.D.	±7.4 S.D.	±5.4 S.D.	±6.7 S.D.	±695 S.D.	
	N=186	N=12	N=156	N=98	N=98	N=15	

TABLE II. Female song parameter statistics of Z. tuberculatus and Z. sepsoides as reported by Bennet-Clark et al. (1980).

	Pulse Period (ms)	Pulse Duration (ms)	Carrier Frequency (Hz)
Z. tuberculatus	$\bar{X}$ = 215	$\bar{X}$ = 50	$\bar{X}$ = 390
	± 63 S.D.	± 35 S.D.	± 57 S.D.
	N = 19	N = 25	N = 19
Z. sepsoides	$\bar{X}$ = 57.9	$\bar{X}$ = 18.2	$\bar{X}$ - 435
	± 12 S.D.	± 5.9 S.D.	± 85 S.D.
	N = 16	N = 19	N = 19
	or		
	$\bar{X}$ = 119		
	± 14 S.D.		
	N = 10		

Temperature = 18-20°C

the pulsed song of female Z. tuberculatus. Its characteristics are given in Table II. They are significantly different from the pulsed song of female Z. tuberculatus.

EXPERIMENT 1.

2.3. MATERIALS & METHODS

2.3.1. Strains of flies used. The courtship behaviour of the following strains of Z. tuberculatus and Z. sepsoides were examined:

Z. tuberculatus: Strain 124.5. Origin: Tarna, near Marodi, Niger, August, 1969.

Strain 164.8. Origin: St.-Denis, Island of Réunion, August, 1973.

Z. sepsoides: Strain 123.3. Origin: La Maboke, near M'Baiki, Central African Republic, April, 1969.

Strain 184.6. Origin: N'Kolbisson, near Yaoundé, Cameroun, 10th October, 1975.

Strain 209.1. Origin: N'Kolbisson, Cameroun, 24-25th August, 1978.

Z. tuberculatus 164.8 is descended from one wild-caught gravid female. All the other strains are originally descended from many individuals. Vials containing a few dozen larvae and pupae, and one or two adults, of each strain, were sent to me from the C.N.R.S. Laboratoire de

Génétique et Biologie Évolutives, Gif-sur-Yvette, France, arriving at Oxford on 27th October, 1978.

Breeding populations of these strains were kept in bottles containing standard Drosophila medium made of cornflour, molasses, agar, and yeast. The cultures were kept at 20-26°C, with a 12hr-12hr light-dark cycle.

The populations were kept at low overall levels for reasons of practical convenience. Characteristically the total population of each strain was kept in two 1/3-pint milk bottles, each with approx. 50cm^3 of standard Drosophila medium in the bottom. Fresh colonies were set up at approximately monthly intervals (these species have a generation time of approx. 3-4 weeks at 20°C), by placing some 20-50 individuals into each of two fresh bottles. The populations were thus almost certainly considerably more inbred than are populations in the wild. However, D. Roberts (pers. comm.) assures me that populations kept at these levels will retain a high level of heterozygosity, and are not therefore "inbred" in the technical sense of the term as used by geneticists.

Observations were conducted during the first four hours of "light", at temperatures of 20-22.5°C.

The observations of Z. sepsoides 123.3 described in this chapter were carried out in November, 1978. The other strains were observed in July-September, 1979.

The flies observed were all virgins, which had been kept in single-sex populations in vials since the day of eclosion. The vials contained Drosophila medium, with live yeast on the surface, and also

a small piece of tissue paper. The volume of space in which the flies lived in these vials was approx. 15-20 cm^3. Males aged 6-12 days, and females aged 8-12 days, were observed. (Males aged less than 8 days were found to court little, and have never been observed to copulate. No consistent age-related differences between the sexual behaviours of individuals of either sex have been observed within the 8-21 day age group. Flies aged > 21 days show a decline in general activity levels.)

2.3.2. Experimental apparatus. All visual observations were carried out with a X10 binocular microscope, with its attached spotlight switched on at low power, and shining through Kodak Wratten-25 red filter and an infra-red heat filter. The tape recording apparatus used was: a Grampian GR2 ribbon microphone (see Bennet-Clark 1972), connected via a X10 preamplifier to a Sony TC-280 twin-track tape recorder. The sounds recorded were monitored with headphones during recording. Recordings were photographed with a Nihon-Kohden PC-2A camera attached to a Tektronix Type 502A dual-beam oscilloscope. The speed constancy of the tape recorder was better than 1%, so time markers were placed on the second oscilloscope trace during filming.

The observation chambers in which the flies were placed for these recordings were rectangular, and made of 0.5mm wire mesh, with a floor of fine gauze to increase their acoustical transparency. The roof was of glass. These chambers had valves made of thin latex sheet at one or both ends of the chamber, through which the flies were introduced by being allowed to walk down a funnel from the vial. When a chamber was used for recording, it was placed on top of the ribbon

diaphragm of the microphone, from which half the protective screen had been removed. The bottom of the chamber was within 5mm of the ribbon diaphragm. The chambers had the following approximate internal dimensions:

Length: 22mm

Width: 8mm

Depth: 5mm

The chambers could be divided in two by a partition of thin celluloid entering through a thin slot in the glass roof. This partition was in place when a male fly was introduced into the chamber at one end, and a female at the other end. It was then removed when recording began.

These chambers were designed with the following criteria in mind: a clear view inside for visual observations; maximum acoustical transparency for sound recording; and an easy method of introducing flies and removing them. The major drawback of the design was that the chamber was liable to acquire an odour: the latex valves were stuck on with rubber solution, and the rest of the chamber was held together with "Durofix" glue. These adhesives were given 24 hours to dry at a temperature of approx. 30°C, so the odour from their solvents was probably minimal. However, they probably would have absorbed any odours emitted by the flies, and given them off. It was impossible to remove such odours by washing with organic solvents, since this would have dissolved the adhesives. The chambers may have acquired human odours as well, since they were handled with bare hands at temperatures up to 26°C. It was decided, therefore, not to try to

keep the chambers chemically clean, but to treat them all in exactly the same way, to ensure that the "chamber background odour" would as nearly as possible be standardised for all observations. In between sets of observations of the different strains, the chambers were "washed" by leaving them in distilled water for 24 hours, and then leaving them to dry at approx. 30°C for 24 hours.

2.3.3. Methods of Observation and Analysis. Pairs of flies were observed for periods of up to half an hour, but the observation periods were usually terminated soon after the end of copulation. Observations were taken during the first four hours of the artificial 12-hour "day", at temperatures of 20-22.5°C. The sounds produced by the flies were recorded.

Oscilloscope traces of all the male songs recorded were filmed. From these not more than two sequences of ≥20 pulses of "Type 1" and "Type 2" Songs were chosen at random from the recordings of each male for analysis. Analysis was of the following parameters: "Type 1" and "Type 2" pulse periods; and "Type 2" carrier frequency. All song sequences recorded were used in an analysis of "Type 1" and "Type 2" sequence durations. "Type 2" pulse durations were not analysed. Because of phase shifts caused by the recorder or the relative positions of the fly and the microphone, the number of cycles in the pulse is subject to uncertainty. I therefore decided that pulse duration statistics would be too artificial to be worth compiling.

All the female pulsed songs were included in the analysis. The parameters analysed were: pulse period, pulse duration, and carrier

frequency.

Cowling & Burnet (1981) have pointed out that in analysing pulse periods, the mode is a more meaningful statistic than the mean. A maximum pulse period that does not constitute the end of sequence has to be set arbitrarily, and the size of this maximum will affect the value of the mean pulse period. In 2.4. pulse period statistics are all accompanied by the arbitrarily-set maximum pulse period for the type of song concerned. A sequence was defined as any succession of two or more pulses separated by a period < the arbitrary maximum.

2.4. OBSERVATIONS.

2.4.1. General observations. The general features of courtship observed corresponded closely to Bennet-Clark et al.'s descriptions. All the behaviours described in 2.2.1. were noted, with the possible exception of female chasing. Grappling by the male was apparently preceded by a less violent, more exploratory tapping of the female's abdomen with the fore-legs. Males always produced "Type 2" Song when attempting to copulate. Female "Kicking" and "Leg Extension" are evidently two different behaviours, the former being apparently a method of repelling other flies, and the latter a more exploratory movement. However, it was not invariably clear which category a particular movement fell into. On the whole, it appeared that "Kicking" was used to repel male Mounting, and sometimes to fend off males when a female was in a corner of the observation chamber. Otherwise, leg movements appeared to fall into the category of "Leg Extension".

Post-copulatory display and song by males was not always observed, and not all courtships, successful or otherwise, elicited female song.

2.4.2. Z. tuberculatus. Courtships of this species were generally fairly short, lasting only a few seconds, and with a high success rate. "Type 2" Song was always produced by males before Mounting and on attempting to copulate. "Type 1" Song was generally produced in smaller quantities, and was entirely lacking from some courtships. It was typically produced by males after a bout of courtship which had not culminated in Mounting. A sequence of "Type 1" Song was also sometimes produced immediately after the end of copulation.

2.4.2.1. Male Songs. See Tables IIIa, V-IX, and Figs. 1, 2, 3, 7, 8, 12, 13. The song parameters are as defined by Bennet-Clark et al., except for Sequence Duration (see 2.3.3.). None of the song parameters had Normal distribution (see 2.4.4.1.). The Pulse Period distributions were leptokurtic, and the "Type 2" Song Carrier Frequency distributions were platykurtic. The "Type 2" Song Carrier Frequency and Pulse Period distributions were also significantly asymmetric.

The song parameter distributions of the two strains were compared using Mann-Whitney U-tests (see 2.4.4.2.). The only significant difference was in the "Type 2" Song carrier frequencies. Strain 164.8 had more broadly distributed Pulse Periods in both song types than did Strain 124.5. In fact, in Strain 164.8 the distributions of the Pulse Periods of the two song types overlapped, and it was hard to ascribe some sequences of monocyclic song with Pulse Periods of 20-25ms to one or other song type. The shape of the cycle can be of use in

distinguishing long period "Type 1" from short period "Type 2" Song.

Comparison with Bennet-Clark et al.'s statistics. In the absence of Bennet-Clark et al.'s raw data, non-parametric significance tests cannot be applied to compare their statistics with those given here for strain 124.5. In addition, the Sequence duration statistics cannot be compared, because Bennet-Clark et al.'s definition of a sequence was different from mine. The mean pulse periods of both song types are markedly longer than those given by Bennet-Clark et al., and the "Type 2" Song Carrier Frequency is lower. This is unlikely to be an effect of the different temperatures used here, since these observations were taken at a temperature intermediate between those used by Bennet-Clark et al.

2.4.2.2. Female songs. See Table IV. Both a continuous whine and a pulsed song were recorded. The two were not always distinct, so an arbitrary maximum pulse duration for pulsed song was set. The whine is a rejection response, used in conjunction with Rocking and Pointing. The context of the pulsed song is not readily apparent. It is usually produced while the male is producing "Type 2" Song, but is not always present in courtships. Of the 44 females of both strains observed, only 25 definitely produced pulsed song. All the pulsed song recorded were analysed.

Comparison with Bennet-Clark et al.'s statistics. Bennet-Clark et al. used a longer arbitrary maximum pulse period than that used here, with the result that their mean pulse period is greater than my maximum pulse period. They give a considerably longer mean pulse duration, and a higher carrier frequency.

2.4.3. Zaprionus sepsoides. This species has a much higher courtship failure rate than Z. tuberculatus. Of the 58 pairs of Z. sepsoides 184.6 and 209.1 observed, only 30 copulated during the 30-minute observation period. Most females were either receptive, copulating readily after a short courtship, or totally unreceptive, responding to all courtship by Whining, Rocking and Pointing. All were virgins aged $\geq$ 8 days. Relatively few females were initially unreceptive females but later allowed copulation. It is suspected that the unreceptive females were gravid. Females develop and lay eggs, regardless of whether or not they have mated. The surface of the medium in a vial containing females often becomes carpeted with eggs. Females from such vials are often unreceptive. They often have characteristically distended abdomens. In the course of later experiments, it was found that if a female from an old vial was found to be unreceptive, its receptivity could be restored by placing it in a new vial. It would lay at least one egg within an hour of being placed in the new vial, after which it would become receptive. On a very few occasions, an unreceptive female was observed to lay an egg in the observation chamber (a totally unsuitable substrate), after which it would permit copulation. Crossley (pers. comm.) reports observing an unreceptive female of a Drosophila species to do this on one occasion.

Male Z. sepsoides were as ready to court as Z. tuberculatus were. Rejection signals by the female did not appear to deter them from courting. Most males produced both types of song, but "Type 1" song was not obligatory for successful courtship.

2.4.3.1. Male songs. See Table IIIb, V-IX, and Figs. 1, 4, 5, 6, 9,

10, 11, 14, 15, 16.

None of the male song parameters measured had Normal distribution (see 2.4.4.1.). The "Type 2" Song carrier frequency distribution was platykurtic in strain 209.1. All the other distributions were leptokurtic in all three strains. Strains 123.3 and 184.6 differed significantly in the distributions of all song parameters except "Type 2" Song sequence durations. Strain 123.3 had considerably shorter pulse periods in both song types, a higher "Type 2" Song carrier frequency, a shorter "Type 1" Song and a longer "Type 2" Song mean sequence duration. Strain 123.3 was not observed to produce monocyclic "Type 2" Song. The other two strains produced both monocyclic and polycyclic "Type 2" Song. Strains 184.6 and 209.1 also differed slightly, but significantly, in their "Type 2" song carrier frequencies.

Comparison with Bennet-Clark et al.'s statistics: Bennet-Clark et al. recorded flies of strain 123.3. Their statistics resemble those given here for strain 123.3 more closely than those for the other strains. However, they given a shorter mean pulse period for both song types. They give a similar "Type 2" Song carrier frequency for songs recorded at a temperature of 24-26^{o}C, but a higher carrier frequency for songs recorded at 18-20^{o}C.

2.4.3.2. Female songs. See Table IV, Figs. 1, 17, 18.

The songs of female Z. sepsoides 123.3 were not analysed, because the quality of the recordings was not high enough.

In addition to a continuous whine, females also produced pulsed songs. The statistics given for the parameters of pulse period, pulse

Fig. 1. Oscilloscope traces of male songs of *Z. tuberculatus* and *Z. sepsoides*

A. *Z. sepsoides* polycyclic "Type 2" Song. x = pulse period. Two pulses of female song are marked with arrows.

B. *Z. sepsoides* monocyclic "Type 2" Song.

C. *Z. sepsoides* "Type 1" Song. y = sequence duration.

D. *Z. tuberculatus* "Type 2" Song sequence containing mono- and dicyclic pulses.

E. 50Hz. time marker.

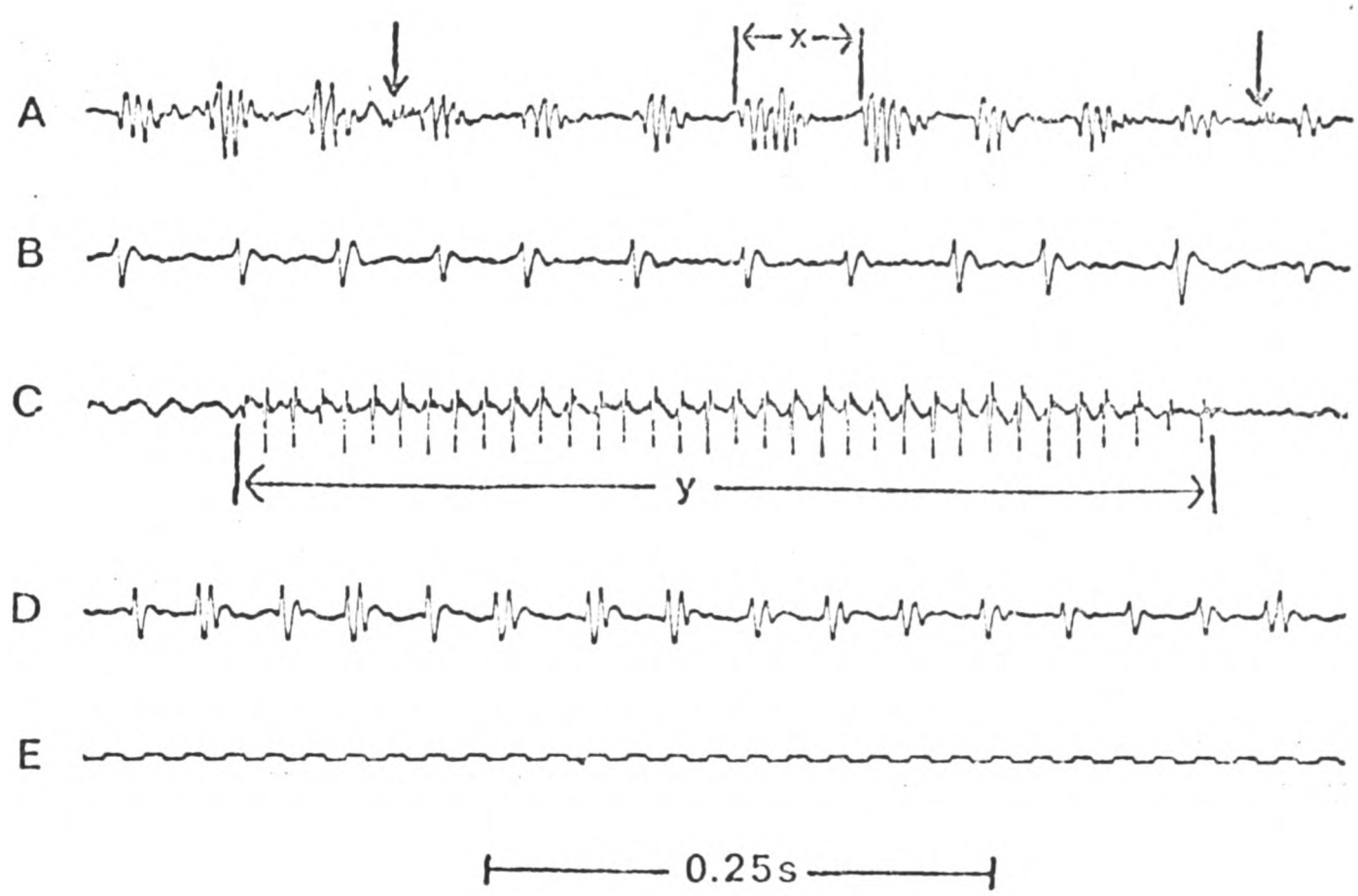

A
x
B
C
y
D
E
0.25s

TABLE III. Male song parameter statistics recorded in Experiment 1. A. Z. tuberculatus

Strain	Types of Song Observed	"Type 1" Song (monocyclic) Pulse Period(ms)	Sequence Duration(ms)	No. of Flies	"Type 2" Song (mono-and polycyclic) Pulse Period(ms)	Carrier Frequency(Hz)	Sequence Duration(ms)	No. of Flies
124.5	1,monocyclic 2,mono- and polycyclic	$\bar{X}$=13.8 +2.4 S.D. $\underline{N}$=927 mode=15 max*=20	$\bar{X}$=219 +2.86 S.D. $\underline{N}$=308	21	$\bar{X}$=33.8 +5.4 S.D. $\underline{N}$=988 mode=32, 35 max*=50	$\bar{X}$=172 +21 X.D. $\underline{N}$=619	$\bar{X}$=314 +485 S.D. $\underline{N}$=346	19
164.8	1,monocyclic 2,mono- and polycyclic	$\bar{X}$=16.6 +2.8 S.D. $\underline{N}$=949 mode=17 max*=26	$\bar{X}$=210 +236 S.D. $\underline{N}$=191	20	$\bar{X}$=33.2 +5.3 S.D. $\underline{N}$=1348 mode=31 max*=50	$\bar{X}$=158 +24 S.D. $\underline{N}$=883	$\bar{X}$=662 +874 S.D. $\underline{N}$=365	20

*max = arbitrary maximum pulse period within a sequence

TABLE III. ctd. B. Z. sepsoides

Strain	Types of Song Observed	"Type 1" Song (monocyclic) Pulse Period(ms)	Sequence Duration(ms)	No. of Flies	"Type 2" Song (mono-and polycyclic) Pulse Period(ms)	Carrier Frequency(Hz)	Sequence Duration(ms)	No. of Flies
123.3	1,monocyclic 2,polycyclic	$\bar{X}$=12.3 +1.48 S.D. $\underline{N}$=870 mode=12 max*=20	$\bar{X}$=217 +203 S.D. $\underline{N}$=657	19	$\bar{X}$=47.8 +5.51 S.D. $\underline{N}$=1243 mode=45 max*=70	$\bar{X}$=196 +12.1 S.D. $\underline{N}$=1243	$\bar{X}$=608 +721 S.D. $\underline{N}$=322	23
184.6	1,monocyclic 2,mono- and polycyclic	$\bar{X}$=13.5 +1.67 S.D. $\underline{N}$=971 mode=14 max*=20	$\bar{X}$=286 +338 S.D. $\underline{N}$=659	18	$\bar{X}$=53.9 +7.15 S.D. $\underline{N}$=1514 mode=50 Max*=80	$\bar{X}$=162 +19.6 S.D. $\underline{N}$=673	$\bar{X}$=543 +799 S.D. $\underline{N}$=446	20
209.1	1,monocyclic 2,mono- and polycyclic	$\bar{X}$=14.2 +1.80 S.D. $\underline{N}$=1255 mode=15 max*=20	$\bar{X}$=357 +457 S.D. $\underline{N}$=671	25	$\bar{X}$=56.0 +7.4 S.D. $\underline{N}$=1236 mode=54 max*=80	$\bar{X}$=167 +22.4 S.D. $\underline{N}$=938	$\bar{X}$=533 +739 S.D. $\underline{N}$=596	27

max* = arbitrary maximum pulse period within a sequence

TABLE IV Female pulsed song parameter statistics recorded in Experiment 1.

Species & Strain	Pulse Period(ms)	Pulse Duration(ms)	Carrier Frequency(Hz)	No.of Flies
Z.tuberculatus 124.5	$\bar{X}$=111.1 ±53.6 S.D. N=68 max*=200	$\bar{X}$=30.4 ±26.3 S.D. N=111 maxt=100	$\bar{X}$=353 ±56.4 S.D. N=111	10
Z.tuberculatus 164.8	$\bar{X}$=90 ±32.8 S.D. N=60 max*=200	$\bar{X}$=33.7 ±23.2 S.D. N=87 maxt=100	$\bar{X}$=309 ±53.9 S.D. N=87	15
Z.sepsoides 184.6	$\bar{X}$=96.2 ±44.4 S.D. N=79 max*=200	$\bar{X}$=25.2 ±10.4 S.D. N=170 maxt=50	$\bar{X}$=308 ±215 S.D. N=170	17
Z.sepsoides 209.1	$\bar{X}$=96.8 ±40.7 S.D. N=80 max*=200	$\bar{X}$=25.8 ±10.3 S.D. N=172 maxt=50	$\bar{X}$=302 ±50 S.D. N=172	22

max* = arbitrary maximum pulse period within a sequence

maxt = arbitrary maximum pulse duration that constitutes pulsed song

TABLE V. Modal "Type 1" Song pulse period values for each male observed in Experiment 1. Pulse periods are given in ms.

Species:	Z. tuberculatus		Z. sepsoides		
Strain:	124.5	164.8	123.3	184.6	209.1
	6	11	11	10.5	12
	13	13.5	11	11	13
	14	14	11	13	14
	14	14	12	13	14
	14	15	12	14	14
	14	15	12	14	14
	14	15	12	14	14
	14	16	12	14	14
	15	17	12.5	14	14
	15	18	13	14	14.5
	15	19	13	14	15
	15		13	14	15
	15		13	14	15
	16		13	15	15
			13	15	15
			14		15
			14		15
					15
					16

TABLE VI. Modal "Type 2" Song pulse period values for each male observed in Experiment 1. Pulse periods are given in ms.

Species:	Z. tuberculatus		Z. sepsoides		
Strain:	124.5	164.8	123.3	184.6	209.1
	25.7	26	40.7	48	46
	28	27	44	49	47
	28	27	44	50	52
	30	28.5	45	50	53
	30	29	45	50	53
	31	29	46	51	53
	32	30	46	51	54
	33	31	46	52	54
	35	31	46	53	54
	35	31	47	54	56
	36	33	47.5	54	56
	36	33	48	55	58
	37	33	49.3	56	58
	37	33	50	58	59
	38	33	50	60	59
	38	33	50	60	59
	38	34	50	70	60
		34	50		60.5
		35	51		62.5
		36	52		67.5
		36.5	52		
		40.5	53		

TABLE VII. Modal "Type 2" Song carrier period values for each male observed in Experiment 1. Pulse periods are given in ms.

Species:	Z. tuberculatus		Z. sepsoides		
Strain:	124.5	164.8	123.3	184.6	209.1
	137	129	175	155	149
	144	134	179	136	154
	161	145	188	145	155
	165	147	189	149	155
	165	148	192	154	155
	168	148	193	158	164
	168	151	193	159	164
	170	153	194	159	164
	171	154	195	159	164
	181	156	195	169	166
	181	157	196	162	167
	183	159	199	165	168
	186	161	200	170	170
	187	162	200	171	170
	188	163	200	172	177
	189	165	202	175	180
	191	165	202		184
	196		203		186
			204		186
			205		

TABLE VIII. Mean "Type 1" Song sequence durations for each male observed in Experiment 1.

Species:	Z. tuberculatus		Z. sepsoides		
Strain:	124.5	164.8	123.3	184.6	209.1
	57	80	60	79	40
	97	80	60	132	40
	101	82	99	133	78
	115	90	102	192	85
	116	98	125	197	116
	119	107	142	209	136
	147	112	168	255	145
	183	165	171	277	160
	202	177	196	297	198
	237	201	196	302	210
	244	243	208	305	215
	247	270	214	371	235
	249	276	230	379	235
	257	289	232	382	277
	258	294	240	420	280
	283	344	249	820	293
	321	810	251		320
	334		251		337
	492		322		378
					439
					442
					448
					458
					566
					582
					886

TABLE IX. Mean "Type 2" Song sequence durations for each male observed in Experiment 1. Durations are given in ms.

Species:	Z. tuberculatus		Z. sepsoides		
Strain:	124.5	164.8	123.3	184.6	209.1
	69	126	118	132	100
	89	169	379	166	120
	162	239	397	167	180
	218	254	419	245	181
	219	339	466	342	301
	224	442	505	412	314
	232	462	513	412	430
	250	472	535	482	392
	293	473	557	536	405
	296	474	559	547	427
	322	503	562	567	490
	394	523	659	647	526
	462	547	674	686	547
	477	650	680	750	586
	545	714	715	778	617
	659	768	814	827	690
	753	786	844	836	690
	993	1030	966	870	730
	1037	1123	979	1034	742
	1056	1129	2327	1150	806
		1187			909
					915
					930
					1140
					1175
					1445
					1540
					2407
					2780
					3770

Figs. 2-6. "Type 1" Song Pulse Period Populations Histograms.

Fig. 2. *Z. tuberculatus* 124.5

Fig. 3. *Z. tuberculatus* 164.8

Fig. 4. *Z. sepsoides* 123.3

Fig. 5. *Z. sepsoides* 184.6

Fig. 6. *Z. sepsoides* 209.1

Fig. 2.

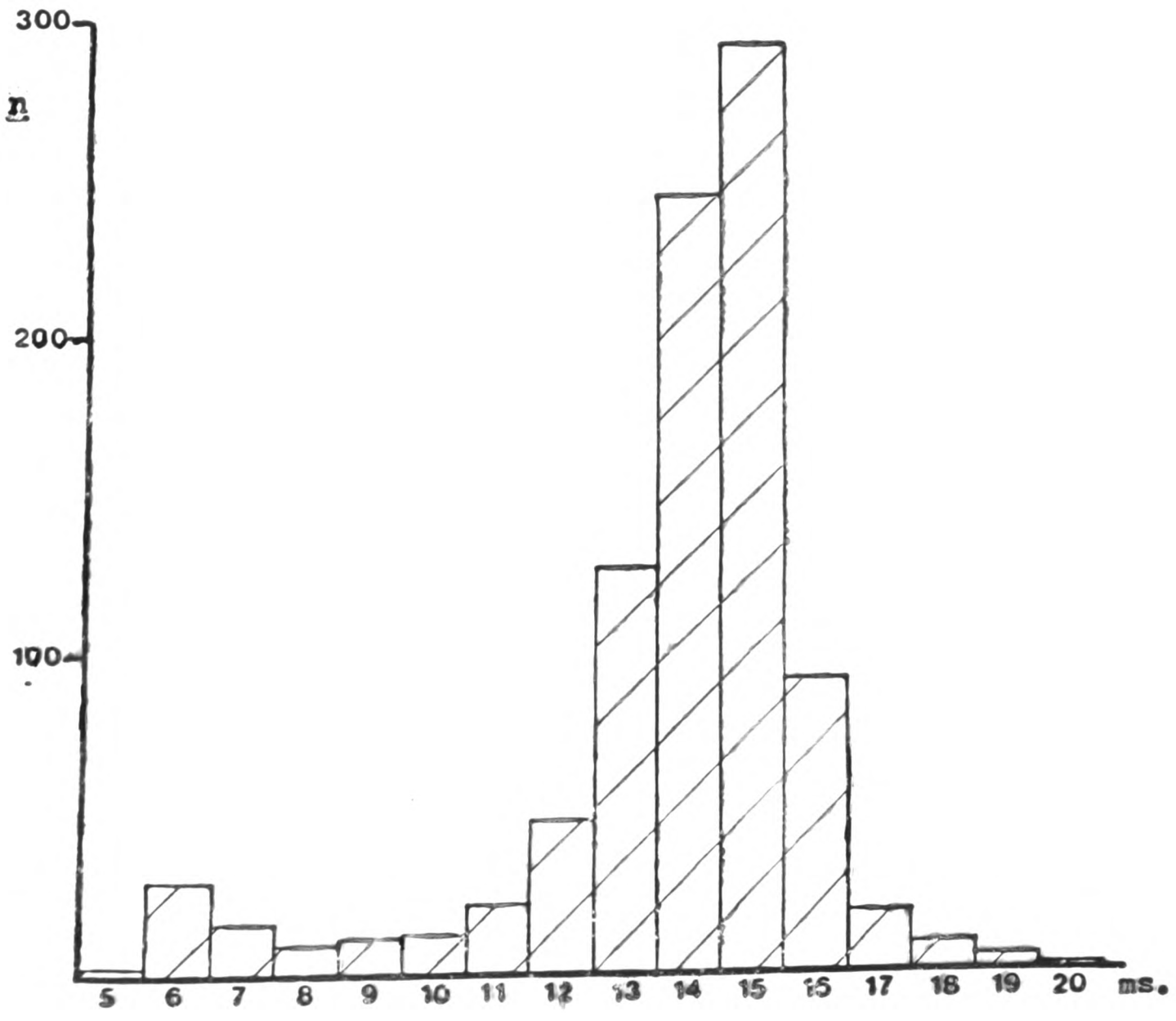


Fig. 3.

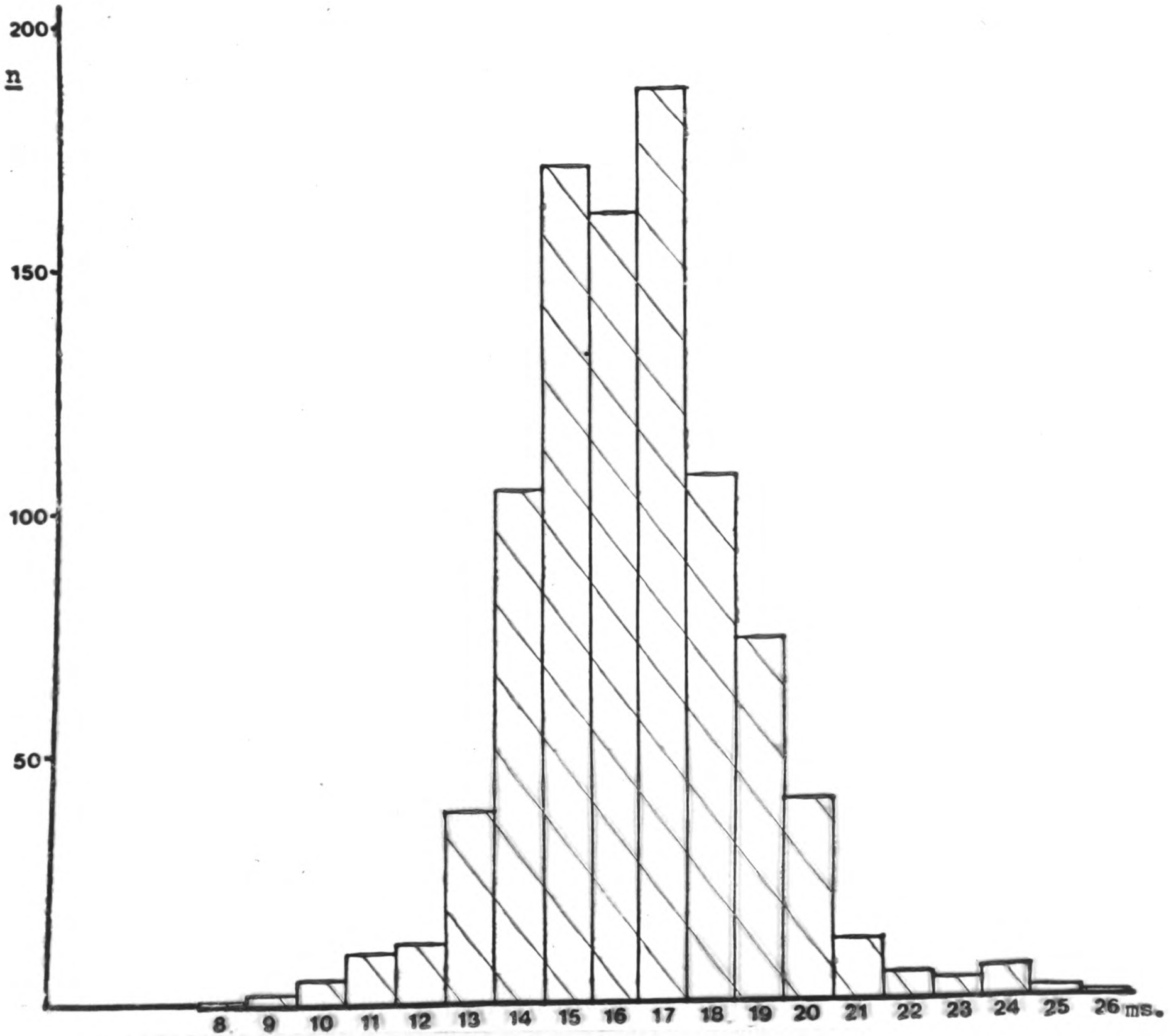

200
n
150
100
50
8
9
10
11
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14
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16
17
18
19
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21
22
23
24
25
26
ms.

Fig. 4.

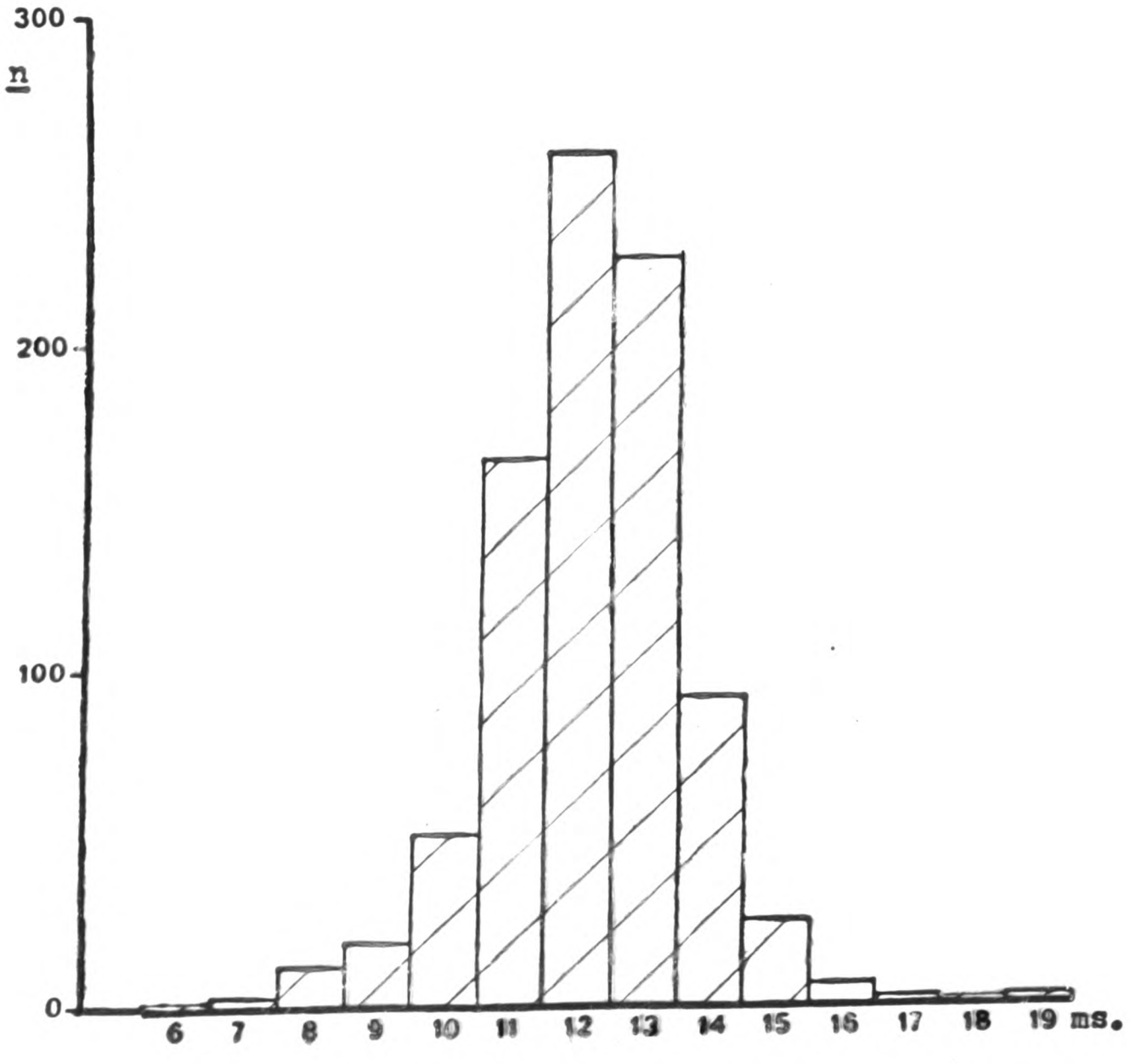
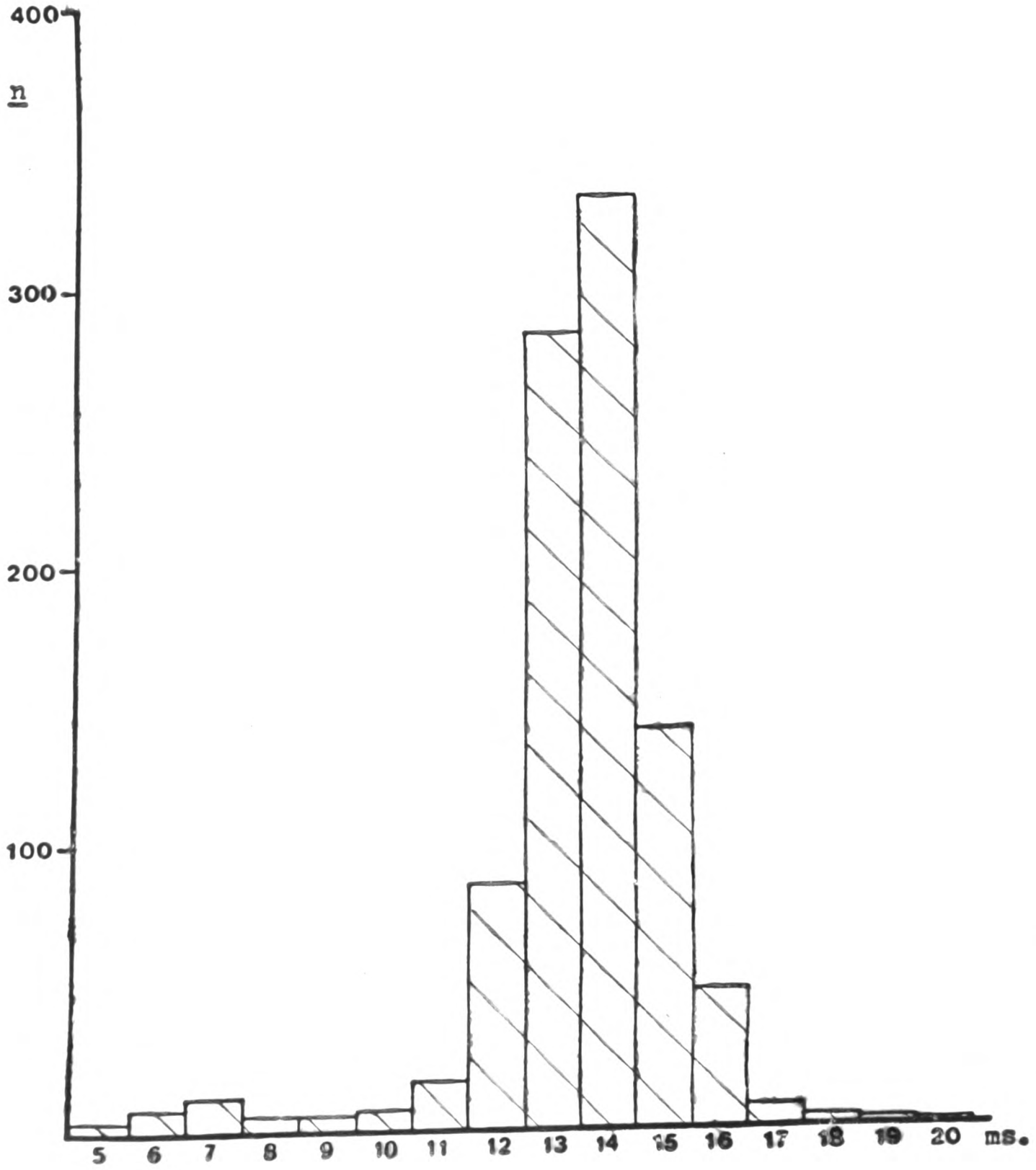


Fig. 5.



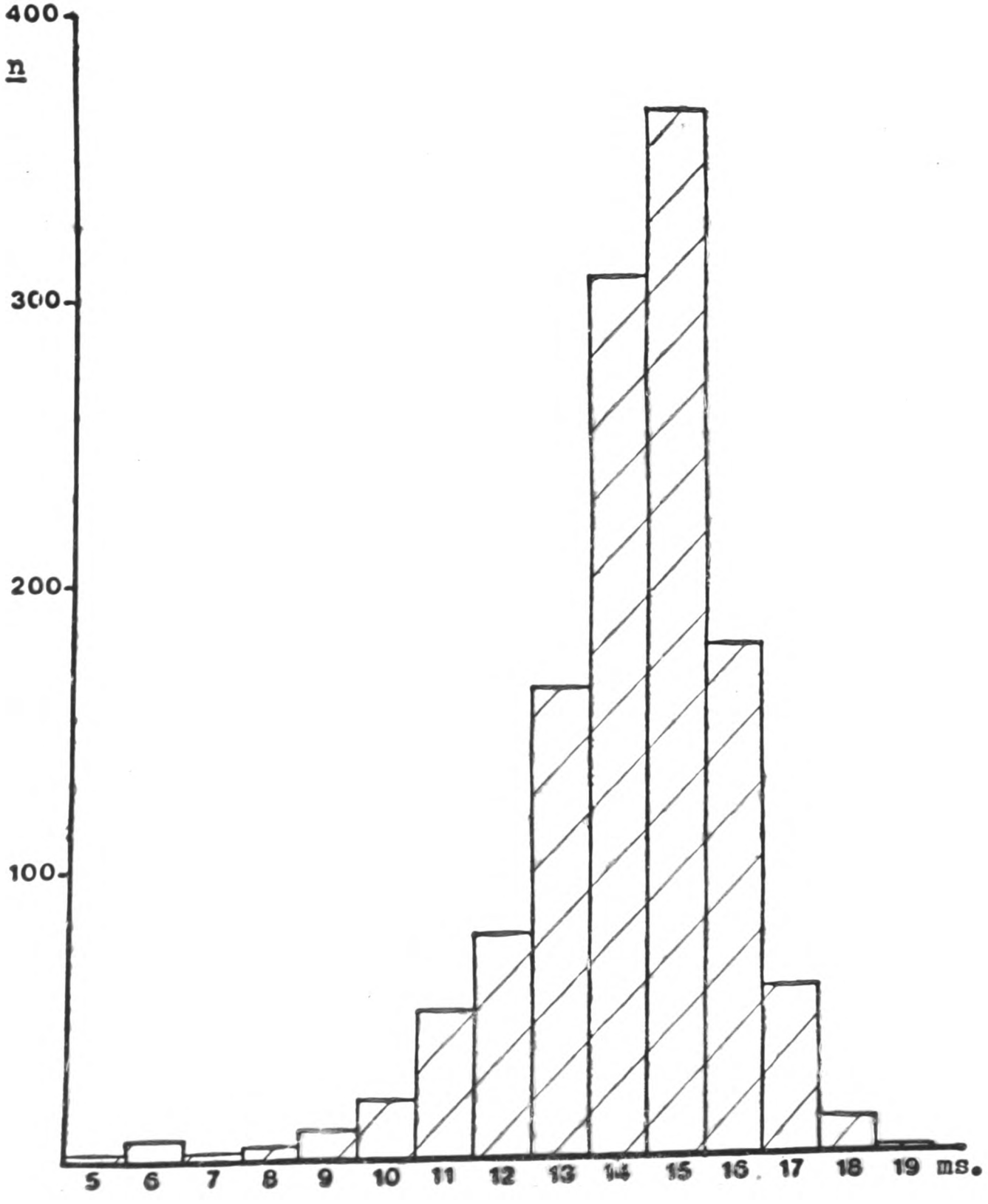


Fig. 6.

Figs. 7-11. "Type 2" Song Pulse Period Populations Histograms.

Fig. 7. Z. tuberculatus 124.5

Fig. 8. Z. tuberculatus 164.8

Fig. 9. Z. sepsoides 123.3

Fig. 10. Z. sepsoides 184.6

Fig. 11. Z. sepsoides 209.1

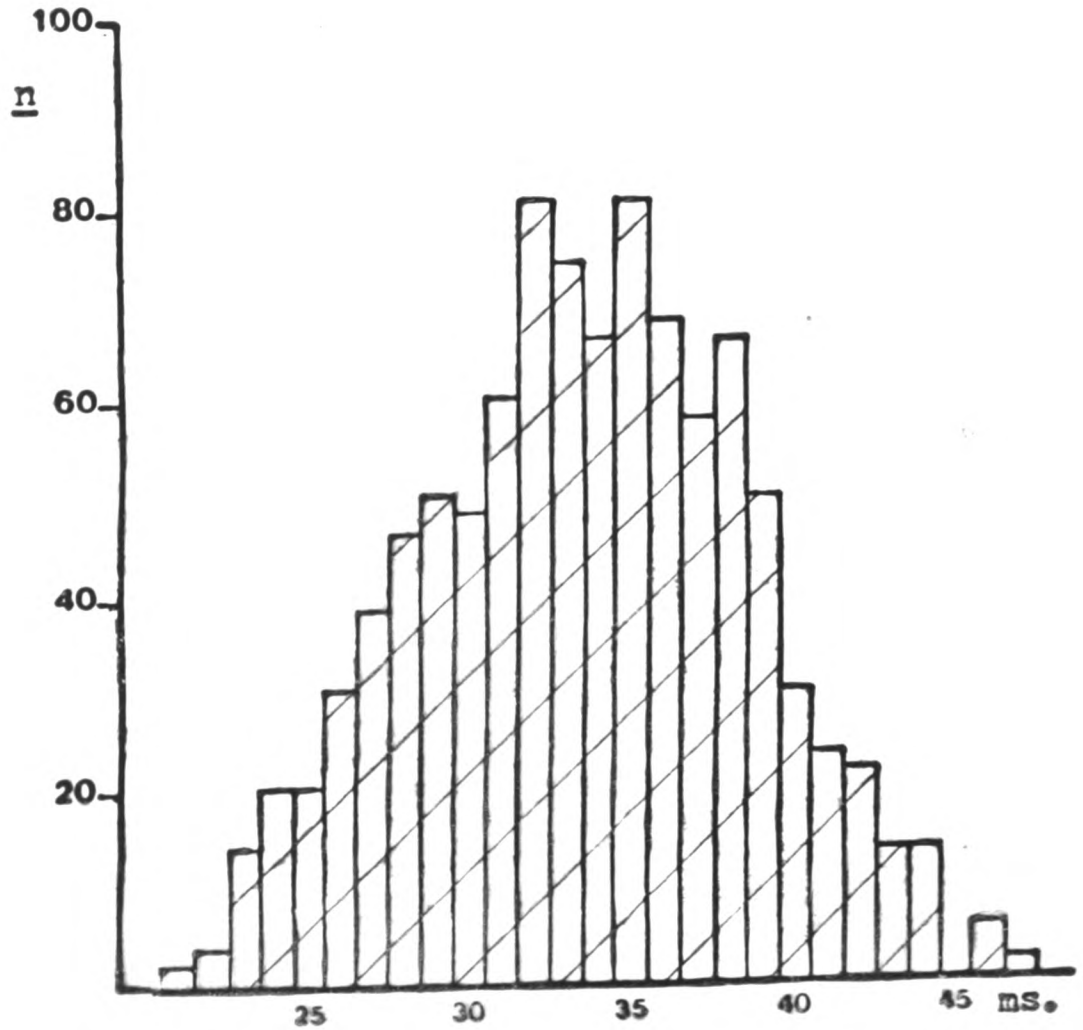


Fig. 7.

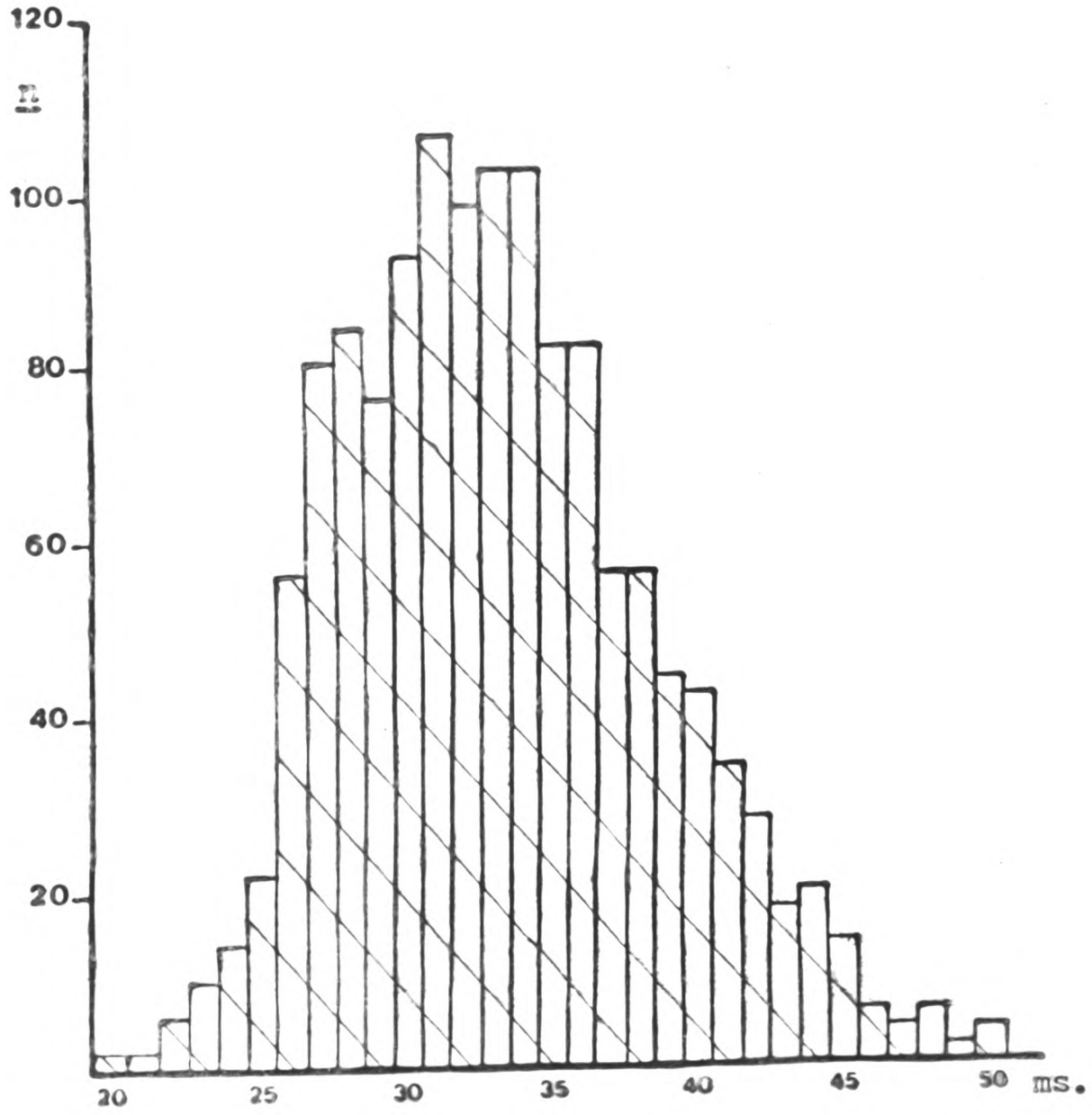


Fig. 8.

Fig. 9.

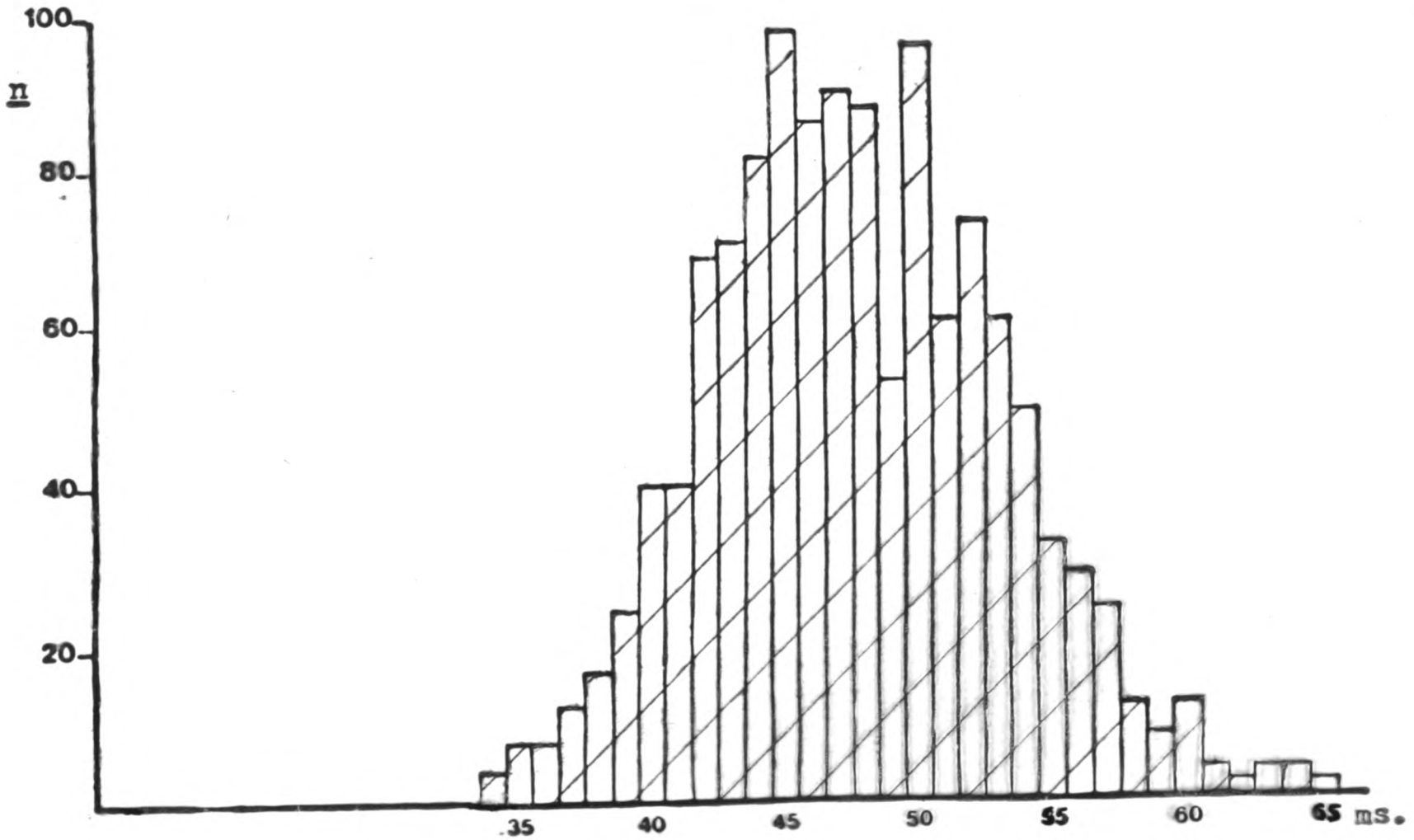


Fig. 10.

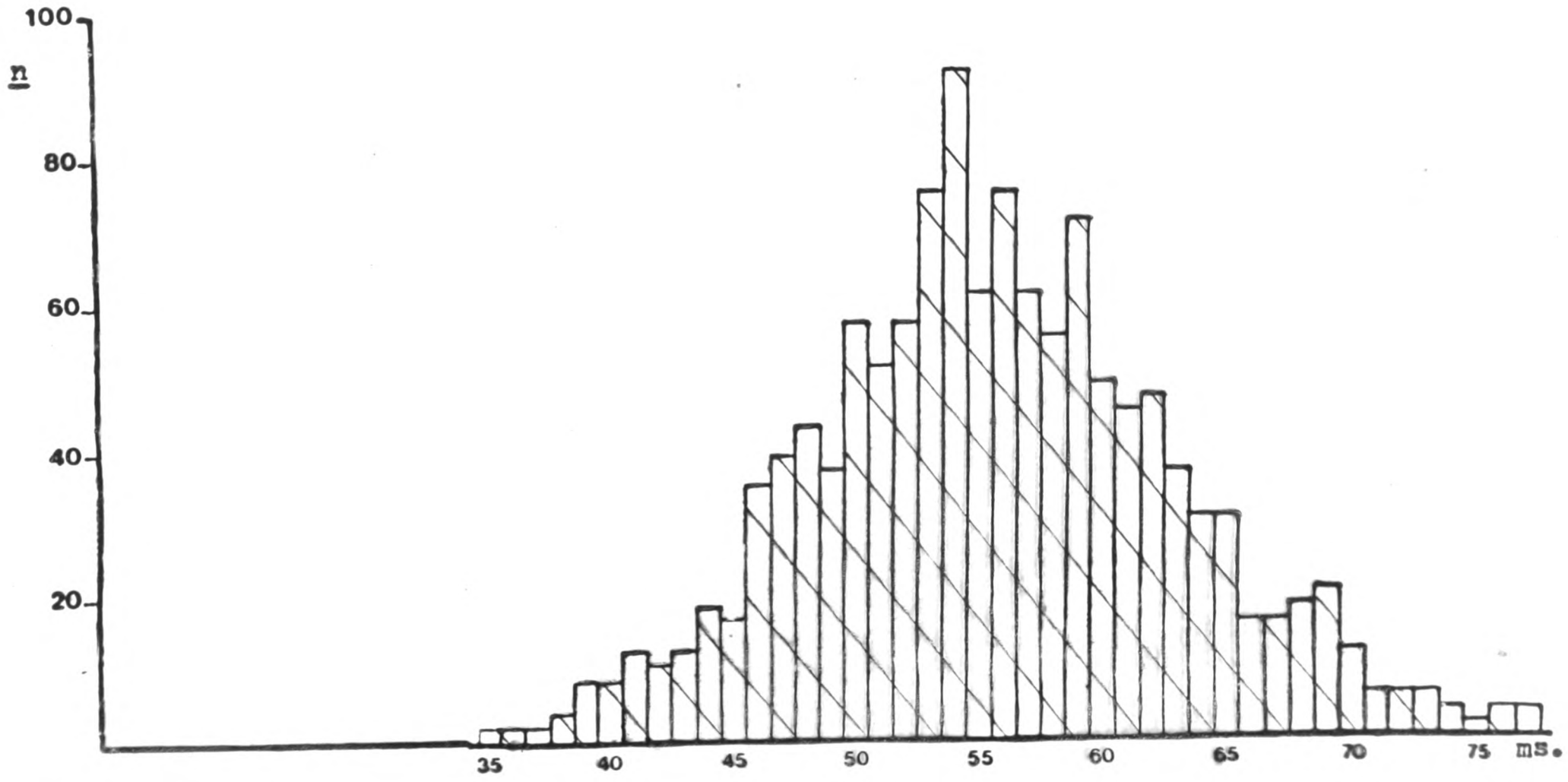
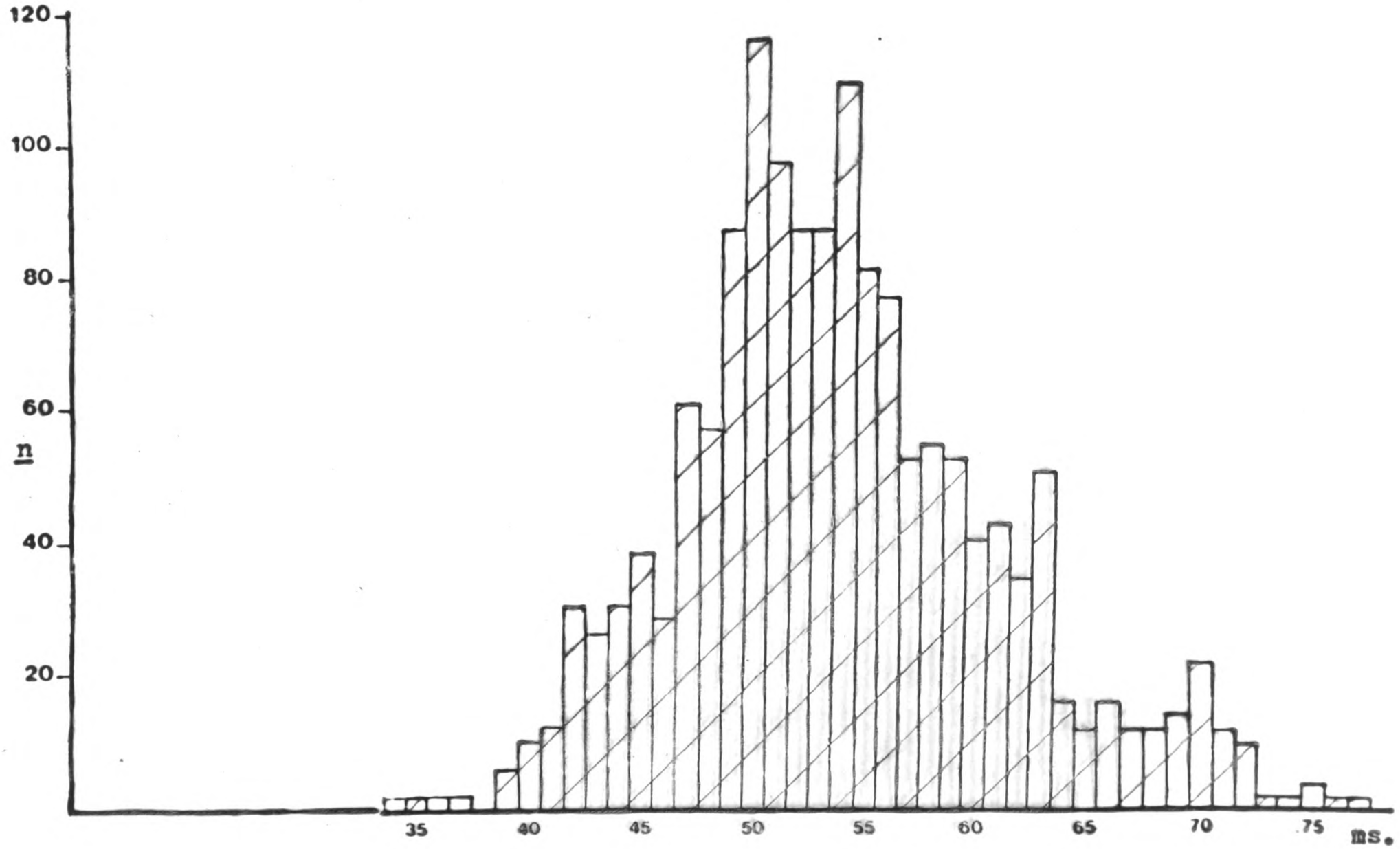


Fig. 11.



Figs. 12-16. "Type 2" Song Carrier Frequency Populations Histograms.

Fig. 12. Z. tuberculatus 124.5

Fig. 13. Z. tuberculatus 164.8

Fig. 14. Z. sepsoides 123.3

Fig. 15. Z. sepsoides 184.6

Fig. 16. Z. sepsoides 209.1

Fig. 12.

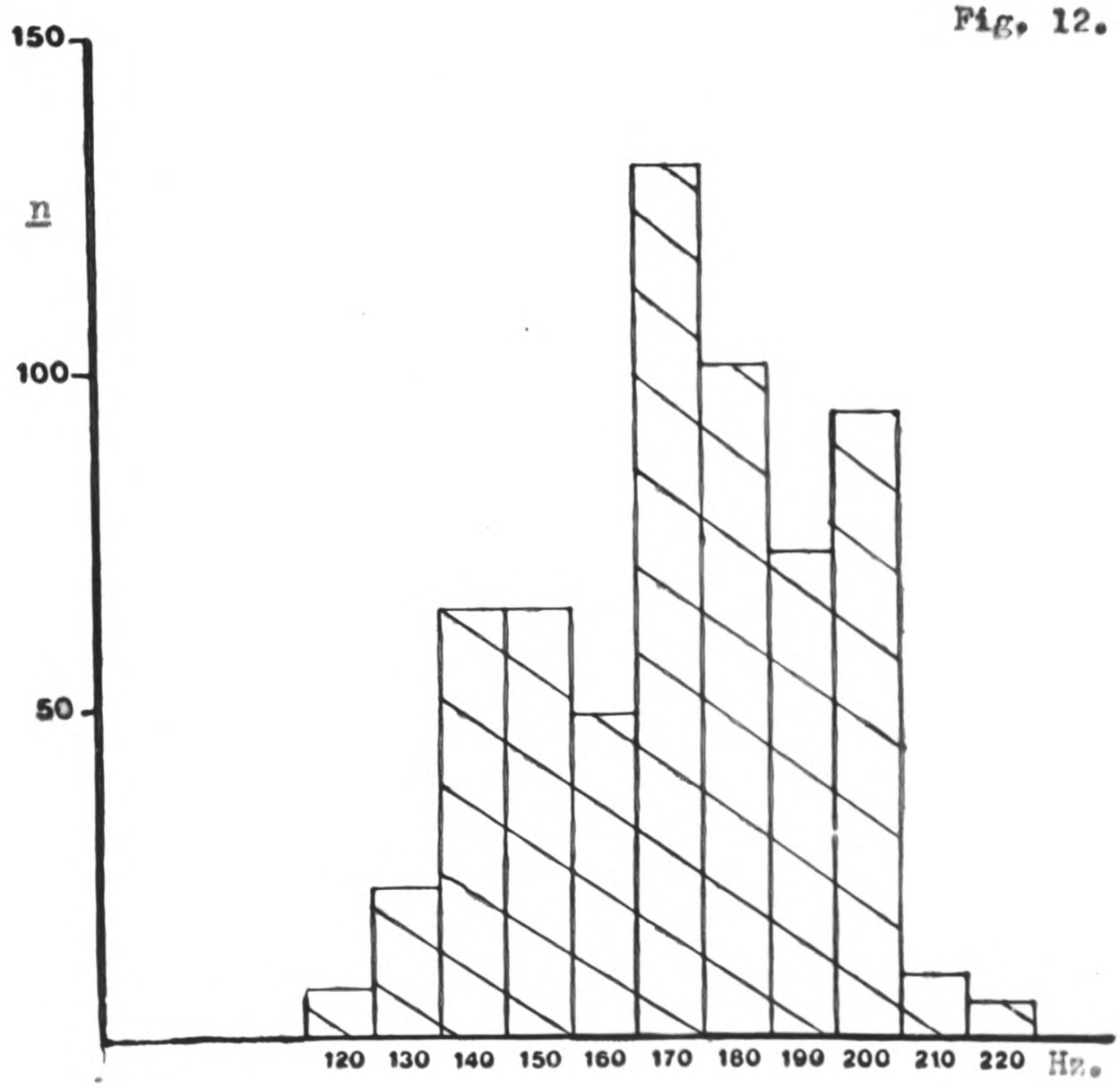


Fig. 13.

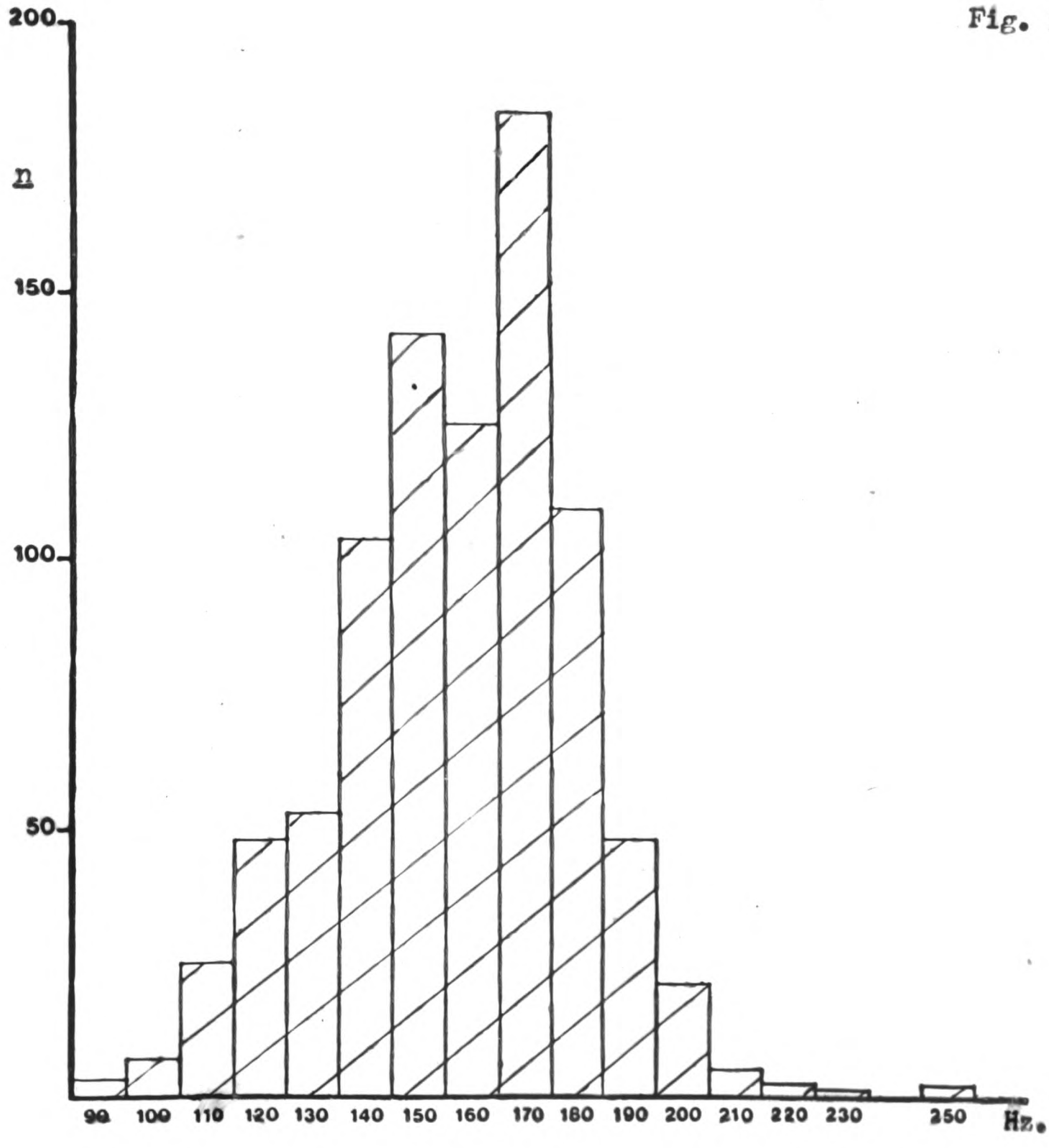

200
n
150
100
50
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
250
Hz.

Fig. 14.

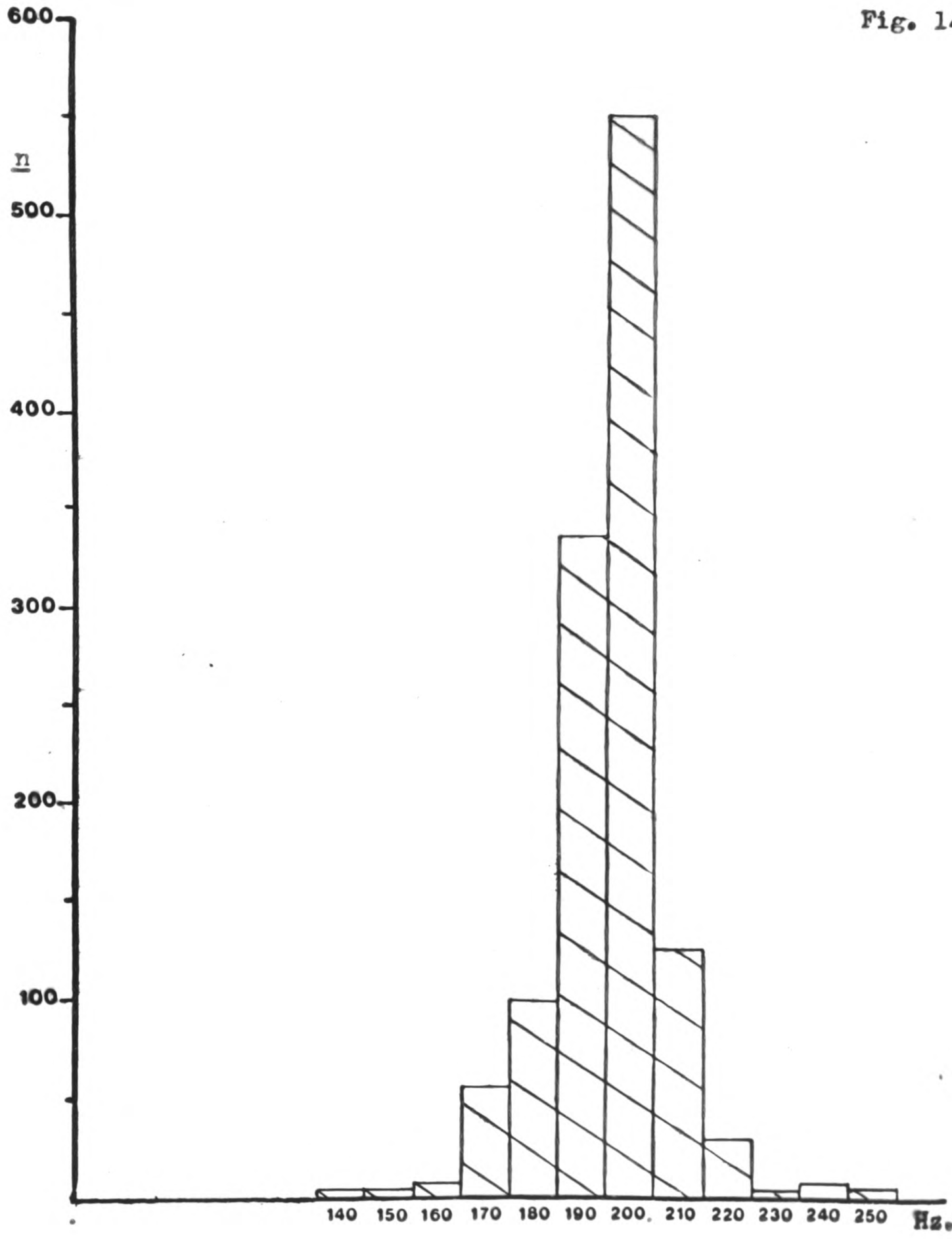

600
n
500
400
300
200
100
140 150 160 170 180 190 200 210 220 230 240 250 Hz.

Fig. 15.

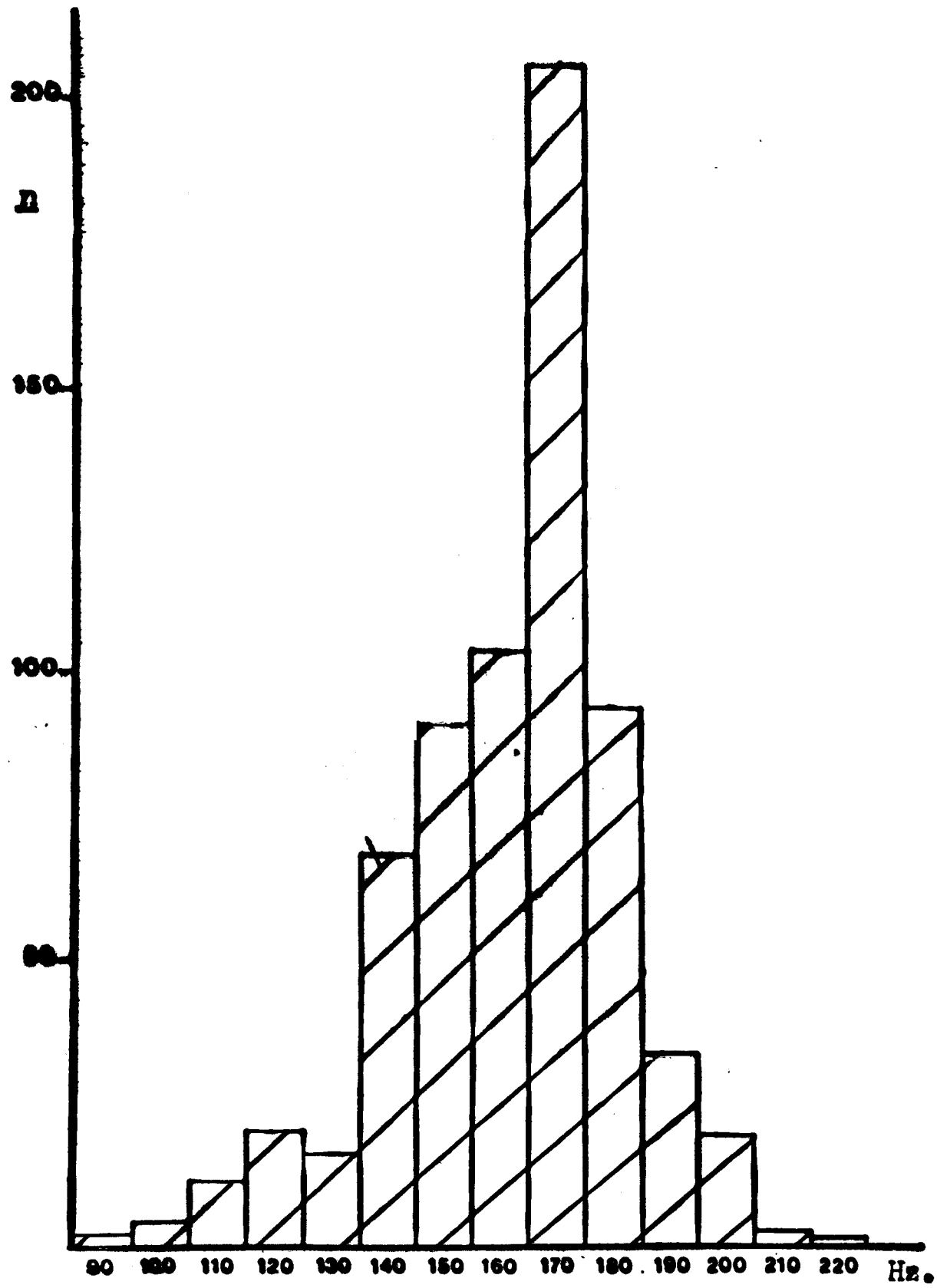

200
n
150
100
50
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
Hz.

Fig. 16.

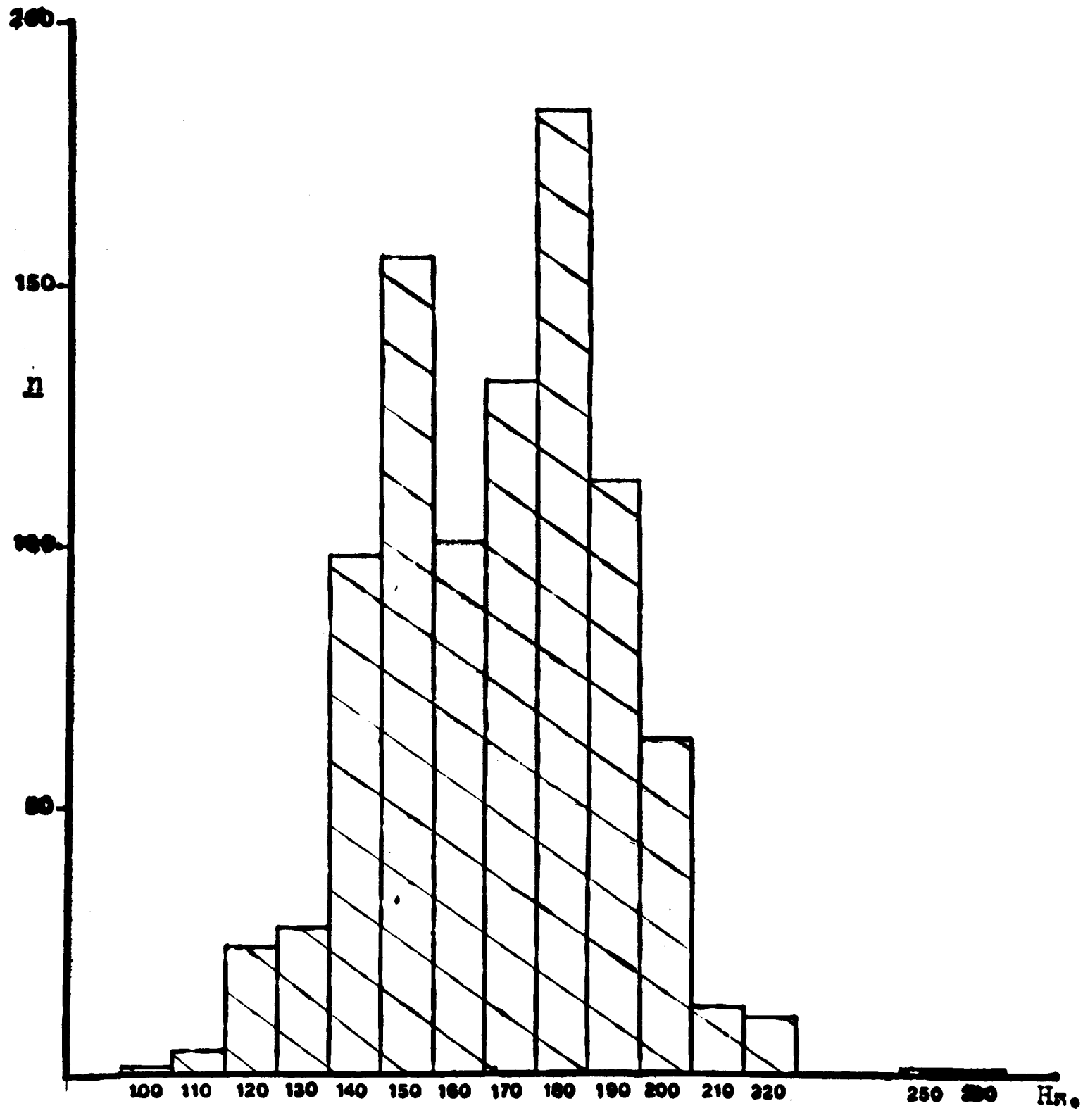
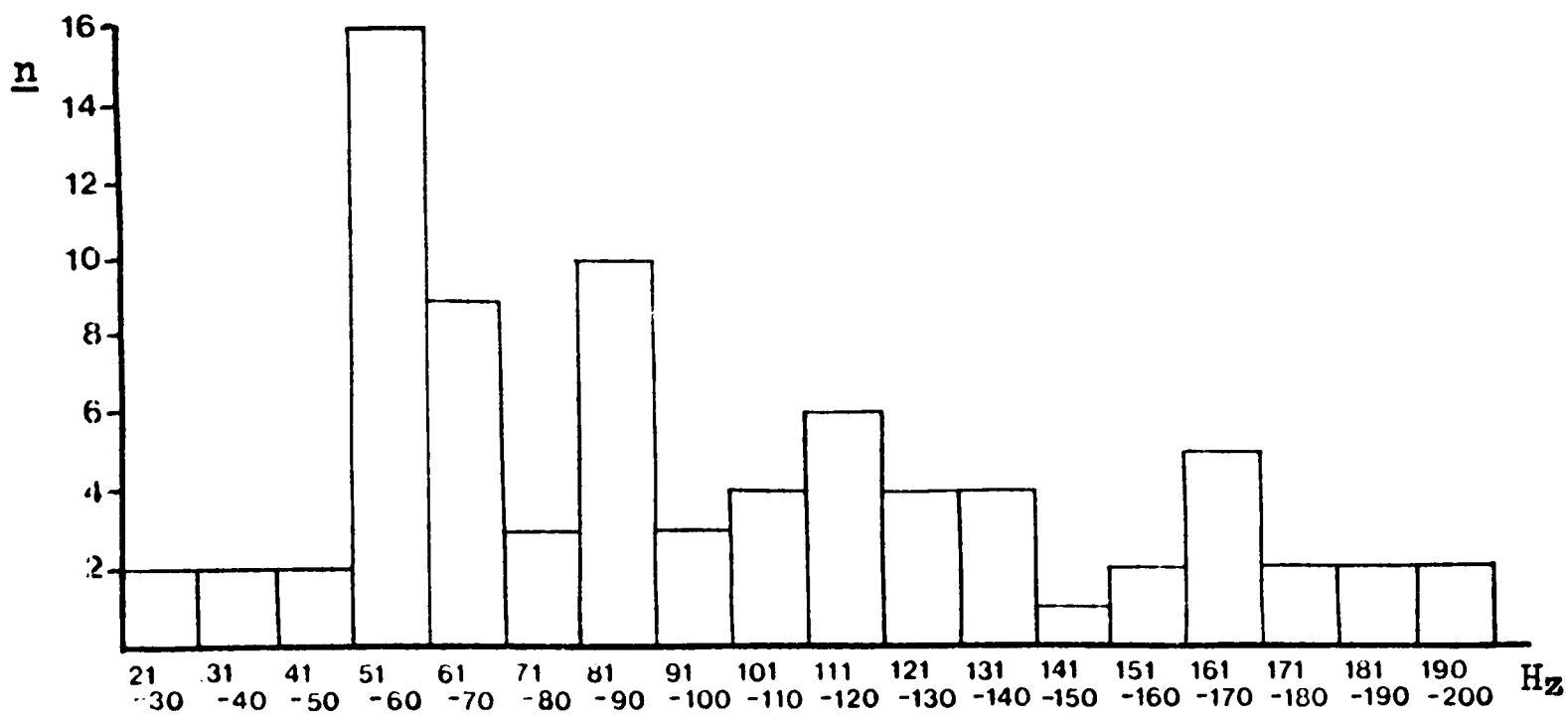

n
150
100
50
100 110 120 130 140 150 160 170 180 190 200 210 220
250
HR.

Fig. 17 *Z. sepsoides* 184.6 female pulsed song histograms.

A. Pulse Periods

B. Mean Pulse Periods Within a Sequence

A


n
16
14
12
10
8
6
4
2
21 -30
31 -40
41 -50
51 -60
61 -70
71 -80
81 -90
91 -100
101 -110
111 -120
121 -130
131 -140
141 -150
151 -160
161 -170
171 -180
181 -190
190 -200
Hz

B

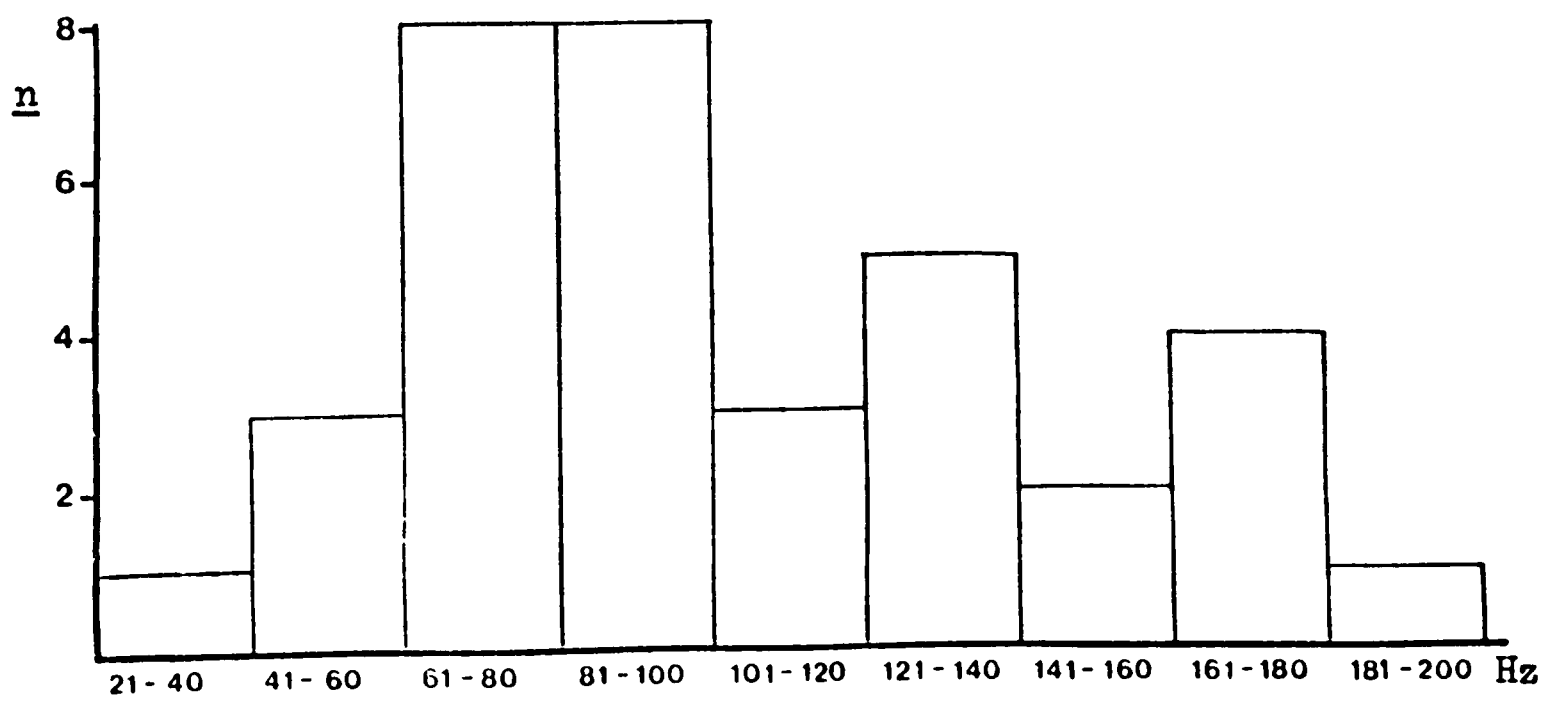
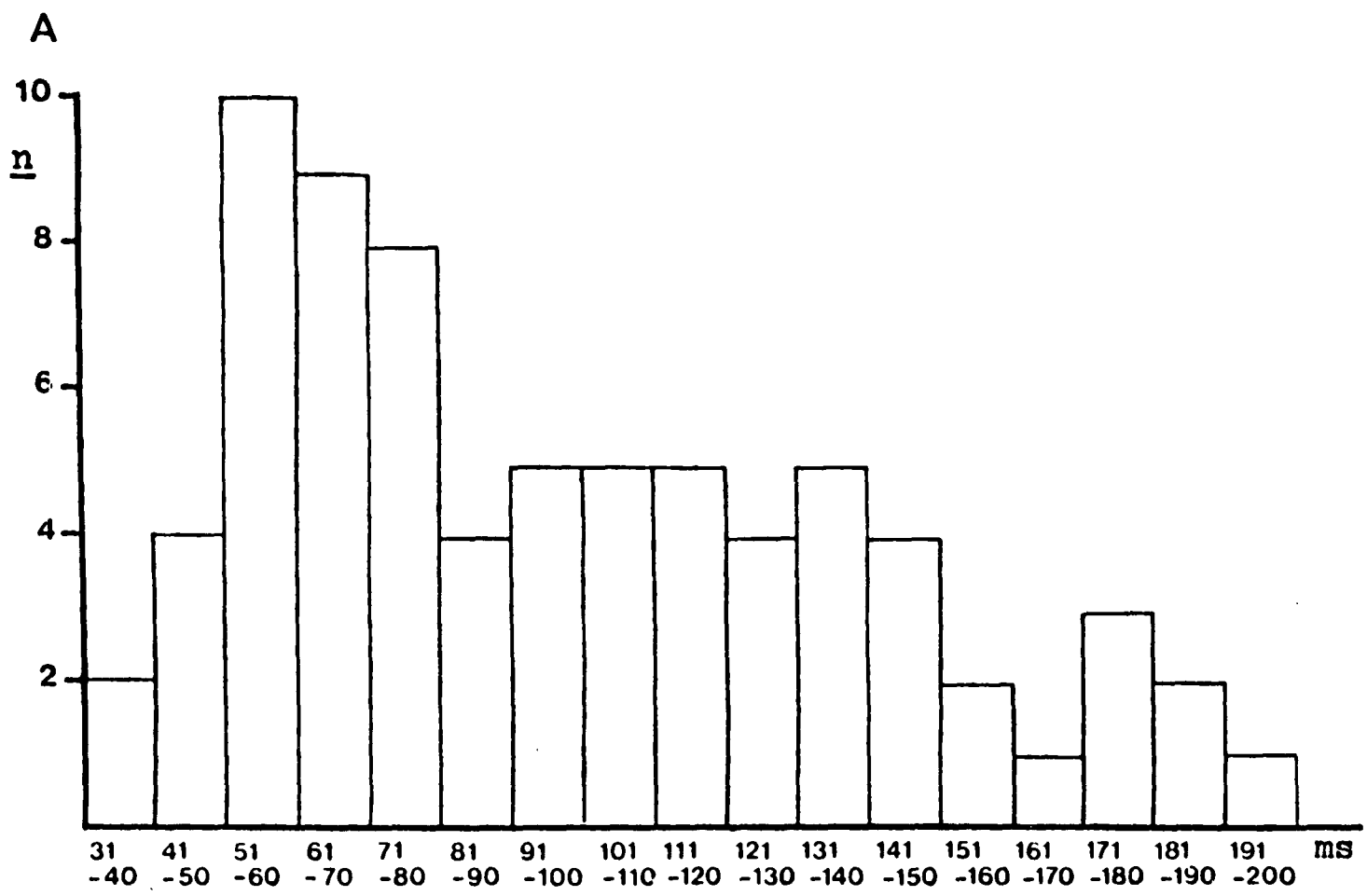
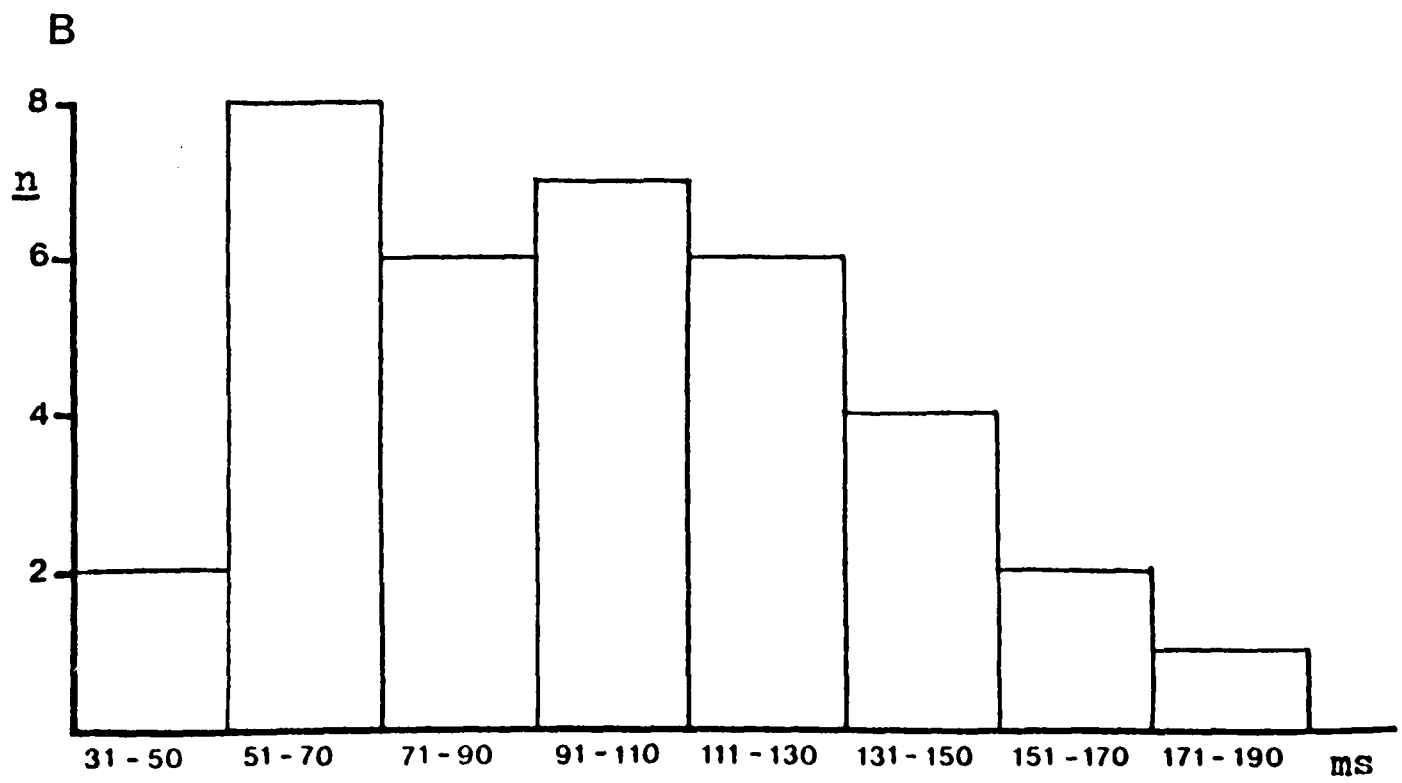

n
8
6
4
2
21 - 40
41 - 60
61 - 80
81 - 100
101 - 120
121 - 140
141 - 160
161 - 180
181 - 200
Hz

Fig. 18. *Z. sepsoides* 209.1 female pulse song histograms.

A. Pulse Periods

B. Mean Pulse Periods Within a Sequence


A
10
n
8
6
4
2
31 -40
41 -50
51 -60
61 -70
71 -80
81 -90
91 -100
101 -110
111 -120
121 -130
131 -140
141 -150
151 -160
161 -170
171 -180
181 -190
191 -200
ms


B
8
n
6
4
2
31 - 50
51 - 70
71 - 90
91 - 110
111 - 130
131 - 150
151 - 170
171 - 190
ms

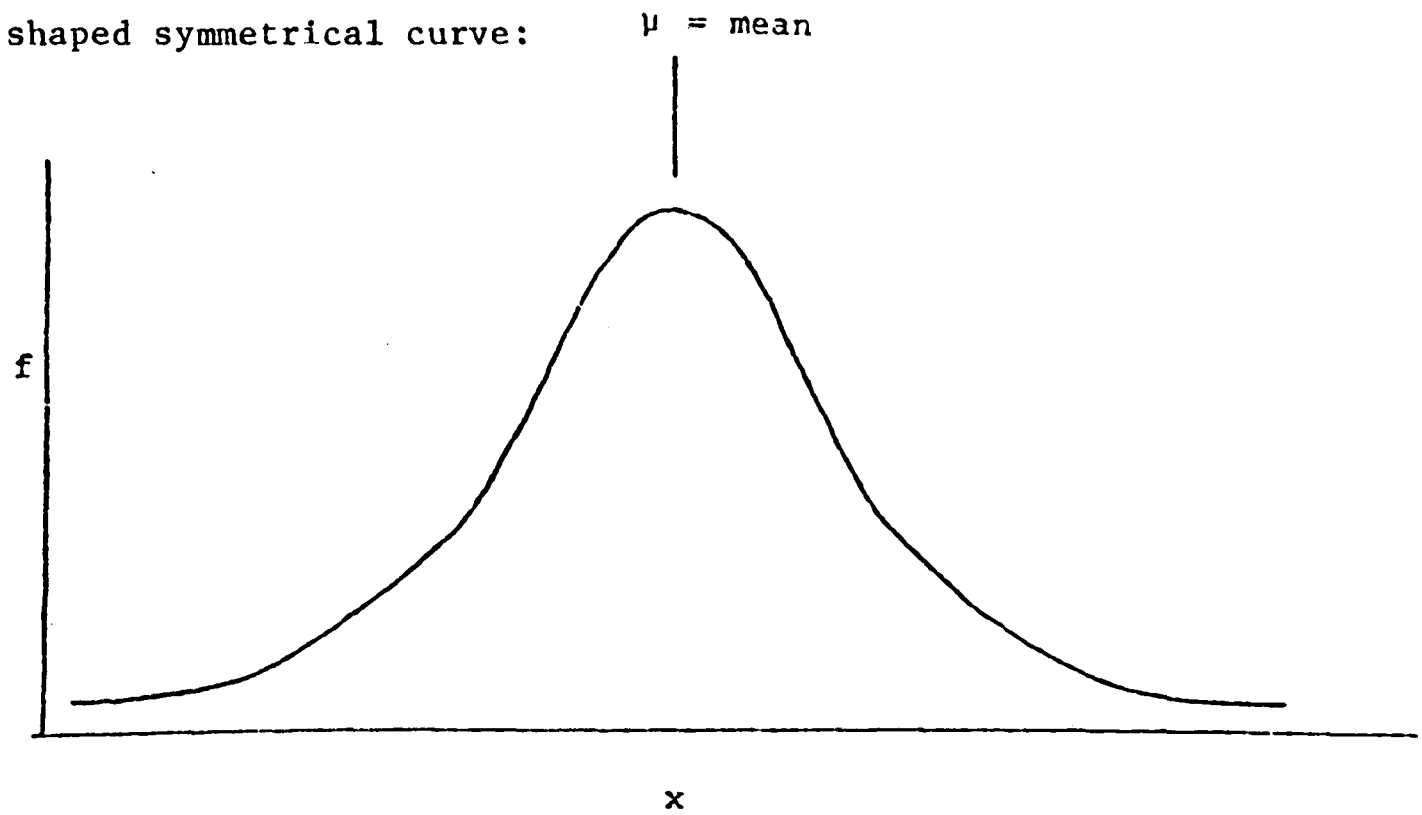
duration, and carrier frequency were almost identical for strains 184.6 and 209.1. They are also similar to those given for the songs of female Z. tuberculatus, except that pulse durations are apparently rather shorter.

Comparison with Bennet-Clark et al.'s statistics: Bennet-Clark et al. give a shorter mean pulse duration than those given here, and a higher mean carrier frequency. They claim to have observed two types of pulsed song, differing in their mean pulse periods. No indication that females produce two types of pulsed song may be found in the data presented here. The mean pulse periods (96.2 and 96.8ms) are similar to those given by Bennet-Clark et al. for "long period" song (119ms), but the modal class, as shown in Figs. 17 and 18, is 51-60ms, which corresponds to the mean given by Bennet-Clark et al. for "short period" song (57.9ms). There is no indication in Figs. 17 and 18 that the pulse periods may actually belong to two distinct populations.

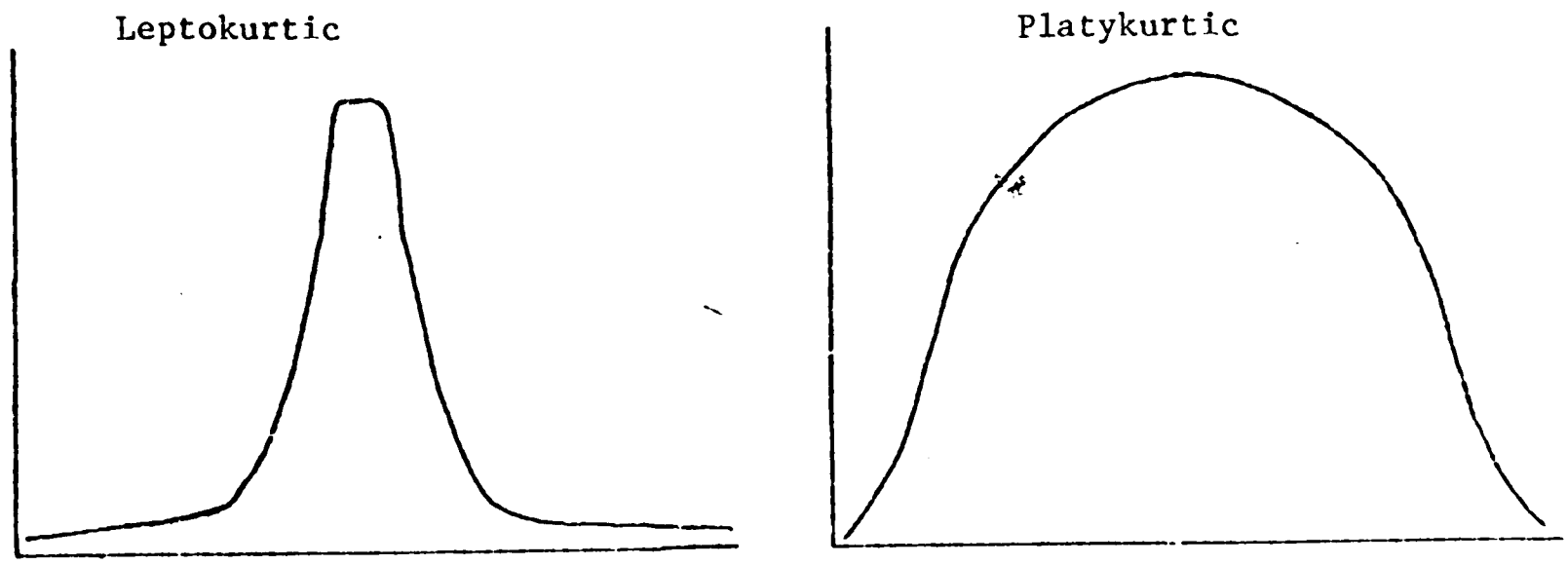
2.4.4. STATISTICS

2.4.4.1. The test for Normal distribution, and the effects of kurtosis.

The Normal distribution, when plotted as a histogram, gives a bell-shaped symmetrical curve:



Not all symmetric distributions, however, are Normal. They may exhibit two types of kurtosis:



There are various reasons why the distributions of song parameter populations may differ significantly from Normal (see 2.5.1.). It was considered worthwhile, therefore, to test the distributions of male "Type 1" and "Type 2" song pulse periods, and "Type 2" Song carrier frequencies, as given in Figs. 2-19, for Normal distribution. The test used here is given by Chambers ("Statistical Calculation", ch. 4, pp.27-29), and depends on deriving two constants γ_1 and γ_2, from the first four moments abut the mean, μ1-3. The working is as follows:

Where $fx = \bar{X} - x$,

$$\nu'_1 = \Sigma(fx)/\underline{N}$$

$$\nu'_2 = \Sigma(fx^2)/\underline{N}$$

$$\nu'_3 = \Sigma(fx^3)/\underline{N}$$

$$\nu'_4 = \Sigma(fx^4)/\underline{N}$$

$$\mu_1 = 0$$

$$\mu_2 = \nu'_2 - \nu'^2_1$$

$$\mu_3 = \nu'_3 - 3\nu'_1\nu'_2 + 2\nu'^3_1$$

$$\mu_4 = \nu'_4 - 4\nu'_1\nu'_3 + 6\nu'^2_1\nu'_2 - 3\nu'^4_1$$

$$\gamma_1 = \pm\sqrt{\beta_1}$$

$$\gamma_2 = \beta_2 - 3$$

where $\beta_1 = \mu^2_3/\mu^3_2$ and $\beta_2 = \mu_4/\mu^2_2$

The distribution is significantly asymmetric if $\gamma_1 > 2(\sqrt{(6/n)})$ and its kurtosis differs significantly from Normal if $\gamma_2 > 2(\sqrt{(24/N)})$. All the three male song parameters tested were found to differ significantly from the Normal distribution in every case (see 2.4.2., 2.4.3.).

2.4.4.2.. Significance tests comparing the song parameter populations of the different strains of Z. tuberculatus and Z. sepsoides.

The populations of all the song pulses measured, as shown in Figs. 2-21, and analysed in 2.4.5.1., give the best available picture of the shapes of the distributions of the song parameter populations of each strain. However, they cannot be used for comparing the song parameter populations of the different strains. This is because each data point in the populations represents a single song pulse, but since each song pulse is not produced by a different male, the data points are not truly independent. When comparing the song parameters of the different strains, individual figures representing the songs of each male have to be generated. In the cases of "Type 1" and "Type 2" Song pulse periods, the figure used was the modal pulse period of each male. (When the distributions had > 1 mode, the mean of the modal values was used.) In the cases of "Type 2" Song carrier frequencies,

and sequence durations of both song types, the mean value for each male was used. These parameter populations of the different strains were compared using Mann-Whitney U-tests. The data are given in Tables V-IX.

1/. Comparing Z. tuberculatus 124.5 and 164.8.

"Type 1" Song Pulse Periods.

$n_1 = 16$ $n_2 = 13$ $n_1n_2 = 208$

$R_1 = 203$ $R_2 = 214$

$U = 85$ $p > 0.05$

"Type 2" Song Pulse Periods.

$n_1 = 18$ $n_2 = 21$ $n_1n_2 = 378$

$R_1 = 382$ $R_2 = 434$

$U = 167$ $P > 0.05$

"Type 2" Song Carrier Frequencies.

$n_1 = 18$ $n_2 = 21$ $n_1n_2 = 378$

$R_1 = 477.5$ $R_2 = 303.5$

$U = 71.5$ $P < 0.05$

"Type 1" Song Sequence Durations.

$n_1 = 20$ $n_2 = 18$ $n_1n_2 = 360$

$R_1 = 414$ $R_2 = 327$

$U = 156$ $P > 0.05$

"Type 2" Song Sequence Durations.

$n_1 = 20$ $n_2 = 21$ $n_1n_2 = 420$

$R_1 = 349.5$ $R_2 = 511.5$

$U = 139.5$ $P > 0.05$

2/. Comparing *Z. sepsoides* 123.3 and 184.6.

"Type 1" Song Pulse Periods.

$n_1 = 19$ $n_2 = 16$ $n_1n_2 = 304$

$R_1 = 246.5$ $R_2 = 383.5$

$U = 56.5$ $P < 0.05$

"Type 2" Song Pulse Periods.

$n_1 = 20$ $n_2 = 16$ $n_1n_2 = 320$

$R_1 = 277$ $R_2 = 389$

$U = 46$ $P < 0.05$

"Type 2" Song Carrier Frequencies.

$n_1 = 20$ $n_2 = 16$ $n_1n_2 = 320$

$R_1 = 528.5$ $R_2 = 136.5$

$U = 0.5$ $P << 0.05$

"Type 1" Song Sequence Durations.

$n_1 = 20$ $n_2 = 17$ $n_1n_2 = 340$

$R_1 = 300$ $R_2 = 403$

$U = 90$ $P < 0.05$

"Type 2" Song Sequence Durations.

$n_1 = 20$ $n_2 = 20$ $n_1n_2 = 400$

$R_1 = 425$ $R_2 = 395$

$U = 185$ $P > 0.05$

3/. Comparing *Z. sepsoides* 184.6 and 209.1.

"Type 1" Song Pulse Periods.

$n_1 = 16$ $n_2 = 20$ $n_1n_2 = 320$

$R_1 = 229$ $R_2 = 437$

$U = 93$ $P < 0.05$

"Type 2" Song Pulse Periods

$\underline{n}_1 = 16$ $\underline{n}_2 = 19$ $\underline{n}_1\underline{n}_2 = 304$

$\underline{R}_1 = 237.5$ $\underline{R}_2 = 392.5$

$\underline{U} = 101.5$ $\underline{P} > 0.05$

"Type 2" Song Carrier Frequencies.

$\underline{n}_1 = 16$ $\underline{n}_2 = 19$ $\underline{n}_1\underline{n}_2 = 304$

$\underline{R}_1 = 232$ $\underline{R}_2 = 398$

$\underline{U} = 96$ $\underline{P} > 0.05$

"Type 1" Song Sequence Durations.

$\underline{n}_1 = 17$ $\underline{n}_2 = 27$ $\underline{n}_1\underline{n}_2 = 459$

$\underline{R}_1 = 389.5$ $\underline{R}_2 = 600.5$

$\underline{U} = 222.5$ $\underline{P} > 0.05$

$$\underline{z} = \frac{\underline{U} - 1/2\underline{n}_1\underline{n}_2}{\sqrt{(1/12\ \underline{n}_1\underline{n}_2(n_1 + n_2 + 1))}} = 0.169$$

$\underline{z} < 1.960$ therefore $\underline{P} > 0.05$

"Type 2" Song Sequence Durations.

$\underline{n}_1 = 20$ $\underline{n}_2 = 30$ $\underline{n}_1\underline{n}_2 = 600$

$\underline{R}_1 = 467.5$ $\underline{R}_2 = 807.5$

$\underline{U} = 257.5$

$\underline{z} = 0.84$ $\underline{z} < 1.960$ therefore $\underline{P} > 0.05$

4/. Comparing all Z. tuberculatus with all Z. sepsoides.

"Type 1" Song Sequence Durations.

$\underline{n}_1 = 38$ $\underline{n}_2 = 63$ $\underline{n}_1\underline{n}_2 = 2394$

$\underline{R}_1 = 1795$ $\underline{R}_2 = 3363$

$\underline{U} = 1047$

$\underline{z} = 1.004$ $\underline{z} < 1.960$ $\underline{P} > 0.05$

"Type 2" Song Sequence Durations.

$\underline{n}_1 = 41$ $\underline{n}_2 = 70$ $\underline{n}_1\underline{n}_2 = 2870$

$\underline{R}_1 = 1941.5$ $\underline{R}_2 = 4274.5$

$\underline{U} = 1080.5$

$\underline{z} = 2.166$ $\underline{z} > 1.960$ $\underline{P} < 0.05$

2.5. DISCUSSION

2.5.1. Comparison of song parameter statistics.. Differences between the distributions of song parameter populations of different strains and species can have the following possible causes:

1/. Genetically-determined interspecific and inter-strain differences;

2/. Random differences between samples;

3/. Differences between observation conditions used;

4/. Observer error, where statistics are compiled by more than one observer;

5/. Recording errors.

Possibility no. 2 can be eliminated by a statistical significance test (see 2.4.4.2.). Observation conditions can be standardised, as here, so possiblity no. 3 may safely be disregarded, except in the case of comparisons of statistics given here with those given by Bennet-Clark et al. There is also no feasible way of eliminating possibility no. 4 in the case of differences between statistics given here and those given by Bennet-Clark et al., but any differences arising from this cause are likely to be slight. Possiblity no. 5

arises from the possibilities of the recording tape stretching, or the tape recorder or the oscilloscope camera not running at exactly constant speeds (see 2.3.2.). It can be eliminated by recording a time calibration trace on one track of a two-track tape recording, while the sound is recorded on the other track simultaneously. This was done here.

Interspecific genetically-determined differences may be expected. Genetically-determined differences within a species may occur between different strains which are descended from individuals taken from different regions. They can also occur between populations of the same strain recorded at different times, as a result of genetic drift occuring within the strain during the intervening period.

The only obvious interspecific difference between the songs of Z. tuberculatus and Z. sepsoides that is shown by these observations is in the pulse periods of the male "Type 2" Songs. The modal pulse periods for the three strains of Z. sepsoides observed here are 54, 50, and 45ms, while those for the two strains of Z. tuberculatus are 35 and 32, and 31ms. Taken overall, the two species also may differ slightly in "Type 1" song pulse periods, their modal values being 12, 14, and 15ms for the three Z. sepsoides strains, and 15 and 17ms for the two Z. tuberculatus strains. However, the distributions of pulse periods are almost identical in Z. sepsoides 209.1 and Z. tuberculatus 124.5.

Sequence durations of male "Type 1" and "Type 2" songs are immensely variable, with a very large degree of overlap between the populations of the different strains. However, there are some

significant differences between the populations of the different strains, although not between the two species. The strain with the longest mean "Type 2" Song sequence duration was Z. tuberculatus 164.8, and that with the shortest was Z. tuberculatus 124.5. Z. sepsoides 123.3 has a mean "Type 1" Song sequence duration similar to those of the Z. tuberculatus strains, and significantly shorter than Z. sepsoides 184.6 and 209.1. This contrasts with Bennet-Clark et al.'s statistics, which suggest that "Type 1" Song sequences may be significantly longer in Z. tuberculatus 124.5 than in Z. sepsoides 123.3. However, their definition of a sequence is different from mine. Also, their samples are small, n = 15 being the largest.

These differences might indicate that sexual selection has favoured different courtship durations in the different strains. However, an investigation of this possibility would require observations of courtships under more natural conditions than those used here. "Type 1" Song is characteristic of unsuccessful courtships; the relatively large quantities of it produced by male Z. sepsoides 184.6 and 209.1 may reflect a relative unwillingness of females of these strains to mate under observation conditions.

Female songs are also very variable in the parameters of pulse period, pulse duration, and carrier frequency, but appear to be similar for all strains of both species. This is contrary to the report of Bennet-Clark et al., who found differences between the species, and also apparently two distinct pulsed songs produced by female Z. sepsoides. Their samples, however, were again very much

smaller than those analysed here.

Intraspecific differences in male song parameters between strains appear to be the rule rather than the exceptions. Z. sepsoides 123.3. differs markedly in male song from the other two Z. sepsoides strains, both "Type 1" and "Type 2" Song pulse periods being significantly shorter, and the "Type 2" Song carrier frequency being significantly higher. Also, it has not been known to produce monocyclic "Type 2" Song at 20-22.5°C, unlike the other two strains. The two Z. tuberculatus strains differ in "Type 2" song carrier frequencies.

These differences are between strains descended from individuals captured in different regions. The Z. sepsoides strains 184.6 and 209.1 however, are descended from several individuals each, captured at the same locality in 1975 and 1979 respectively. They were observed by me in summer 1979, and at that time they differed in "Type 1" Song Pulse Periods, the difference being small but statistically significant. This difference may be the result of genetic drift. Alternatively, they could be a pleiotropic effect of changes due to different selection pressures acting between the years 1975 and 1979, when one strain was being reared under artificial conditions, while the ancestors of the other strain were still wild.

If this inference is correct, then this is an interesting finding, because genetic drift is generally thought of as only producing discernible effects in highly inbred populations. The laboratory populations used here, although smaller than wild populations, are nonetheless sufficiently large for a considerable

degree of heterozygosity to be maintained (see 2.3.1.).

The distributions of the populations of the song parameters of Pulse Period and Carrier Frequency (in "Type 2" Song) require comment. In all cases of both song types, the distributions of the Pulse Peirods are leptokurtic, and in all cases of "Type 2" song the Carrier Frequency distributions are platykurtic. Firstly, it must be noted that the overall distribution of each population results from a combination of two factors: 1/. variations between individuals in populations containing considerable genetic heterozygosity (see 2.3.1.); 2/. variations within the song repertoire of each individual. The samples of the songs of each individual analysed were too small for inter-individual differences to be definitely apparent, but the overall impression was that, with few exceptions, intra-individual variation was greater than inter-individual differences. This impression is substantiated by a comparison of the data given in Figs. 2-19 with those given in Tables V, VI, and VII.

However, the inferences that can be drawn from the shapes of the distributions are the same, whether the variation is largely intra- or inter-individual. A leptokurtic distribution (as shown by the Pulse Periods) is one in which the range of possible values for each data-point is relatively wide, but the range of values which are actually found at all frequently is much narrower: i.e. in addition to the constraints of physical possibility, further constraints are acting, which tend to narrow the range of the distribution observed. It is tempting to infer, in the case of Pulse Periods that these further constraints may be imposed by natural selection: the

functional significance of these songs is presumably one of inter-individual communication, (see below 2.5.2.) and the pulse periods of the songs may be parameters that convey information to other individuals. A narrow distribution of pulse periods may thus be an adaption to ensure that the information conveyed by the songs is not misleading or ambiguous.

A leptokurtic distribution, however, is one in which there is a relatively even distribution over a range, with relatively sharp "cut-off points" at each end of it. These cut-off points may be imposed by the limits of physical possibility, although this is not necessarily the case. This would imply that any other constraints acting to restrict the distribution further are relatively weak. Possibly in the case of "Type 2" Songs, flies are not discriminating between carrier frequencies (since "Type 2" Song pulses are often monocyclic, this would not be surprising); or, if they are discriminating, their perception is relatively imprecise, e.g. they will recognise the difference between a 90Hz carrier frequency and one of 220Hz, but not between carrier frequencies of 140Hz and 190Hz.

2.5.2. The rôles of male and female songs. The rôle of male "Type 1" Song is unclear. It announces a male's sex, but apparently not his species. It is not obligatory, although most courtships feature at least one short sequence of it. It is particularly characteristic of long and unsuccessful courtships. It is also produced in inter-male interractions (see Chapter 4). Its context may thus loosely be described as a reaction by a male on encountering another individual that does not identify itself as a sexually receptive female. It

features in sexual interactions, and has been assumed both here and by Bennet-Clark et al. to be a part of the male's sexual behaviour, but the context described above does not eliminate the possibility of its being an aggressive signal.

A further possibility is that "Type 1" song is a general sex-recognition signal. Sexual dimorphism is slight in this genus, and the observations recorded here and the following two chapters do not eliminate the possibility that "Type 1" song is used by a male in situations where it is necessary for its sex to be made unequivocally apparent to other individuals, while not actually attempting to mount and copulate. This hypothesis would be hard to test experimentally, but a possible method is suggested by the results of Experiment 3, and some observations that were noted by chance during Experiment 5 (see Chapter 4). In Experiment 3, pairs of males were observed together. They produced "Type 1" Song, often in considerable quantities. In Experiment 5, males with their wings amputated were confined together in small chambers. These males were observed to court each other vigorously, and often mounted each other. A possible explanation of these observations is that one of the functions of "Type 1" Song is to deter courtship by other males.

The hypothesis could be tested by the following experiment. Two males, one with its wings amputated, and the other intact, would be observed together, under two sets of conditions, Experimental and Control. Under Experimental conditions, artificially-simulated "Type 1" Song would be provided (using the apparatus used in Experiment 8; see Chapter 5) every time the winged male was observed to court or

otherwise interract with the wingless male. Under Control conditions, no simulated song would be provided. The hypothesis predicts that under Experimental conditions, the winged male would be deterred from courting the wingless male, and would not be able to mount the wingless male as easily as under Control conditions.

Another possibility might be to make a female appear "male" to other females by use of artificially-simulated "Type 1" Song. A recently-mated female would be observed with a virgin female, under two sets of conditions, Experimental and Control. Under Experimental conditions, artificially-simulated "Type 1" Song would be sounded every time the two females approached each other; under Control conditions, no simulated song would be provided. If "Type 1" Song is used by males for disclosing their sex to females, I would predict that the inseminated females would respond to an approach by a virgin female by Rocking more frequently under Experimental conditions than under Control conditions.

The male "Type 2" Song would appear to be sexual. It invariably features in successful courtship, and is always produced by the male when attempting intromission, as well as earlier in courtship. It identifies both the male's sex and species. Evidence will be presented in chapter 7 to show that it is obligatory, or nearly so, for successful courtship.

It is interesting to note that, according to Bennet-Clark et al. (1980) the closely-related species Z. mascariensis does not have two distinct song types, but has one, highly variable and irregular male song. Its courtship is described as being apparently less

organised and stereotyped than those of Z. tuberculatus and Z. sepsoides.

The female whine, as noted by Bennet-Clark et al., is apparently a rejection response, produced by unreceptive females. It is invariably accompanied by Rocking, and usually by Pointing and Protrusion (see 2.2.1.).

The rôle of female pulsed song is obscure, because relatively little of it was recorded. Sometimes pulses where produced immediately before or after a Whine, and merged with it, apparently indicating that pulsed song indicates an unreceptive state. However, female pulsed song was also recorded in successful courtships.

CHAPTER 3

Comparisons of Intraspecific and Interspecific Courtships of *Z. tuberculatus* and *Z. sepsoides*.

Contents:

3.1. Introduction
3.2. Materials & Methods
3.3. Results & Statistics
3.4. Discussion & Conclusions
3.5. Appendix: Data

3.1. INTRODUCTION

3.1.1. Aims. It was decided to devote the major part of this project to a study of the species isolating mechanism operating between the two species, since this would be relevant to current theories about the process of speciation (see Chapter 1). Hybrids between these two species have not been reported (and none occurred during this project). This chapter reports a series of observations which it was hoped would show at which points before and/or after copulation the isolating mechanism operates.

3.1.2. Summary of published accounts of interspecific courtships in Drosophilidae. Two populations are said to be separate biological species if they do not interbreed in nature (e.g. Mayr, 1942, 1963).

If they are allopatric, they may be introduced to each other under artificial conditions, to see if there is a reproductive barrier. However, the presence or absence of a reproductive barrier under artificial conditions does not automatically imply the presence or absence of such a barrier in nature. Many sympatric species which do not interbreed in nature will do so in captivity, sometimes producing fertile offspring - e.g. Drosophila pseudoobscura and D. persimilis (Koopman, 1950); D. heteroneura and D. silvestris (Ahearn et al., 1974).

Reproductive barriers may be categorised as either pre-mating or post-mating (Mayr, 1942). Pre-mating barriers may be: ecological, ethological, or mechanical; post-mating barriers may be hybrid inviability or sterility. All these different types of barrier have been found between different species of Drosophilidae. The two South American species Drosophila pavani and D. gaucha interbreed readily in the laboratory, producing sterile hybrids (Koref-Santibanez, 1964). These two species, however, are largely allopatric, with only a small geographical area where their ranges overlap. The strains of the two species which were studied were allopatric in origin. Wasserman & Koepfer (1977) and Markow (1981) have shown that strains of the sibling species D. mojavensis and D. arizonensis descended from individuals caught in a region of sympatry are more strongly isolated (by a pre-mating barrier) than are strains of allopatric origin.

Two pairs of sibling species whose reproductive barriers have been most extensively studied are Drosophila pseudoobscura and

D. persimilis, and D. melanogaster and D. simulans.

D. pseudoobscura and D. persimilis do not hybridise in nature, apparently due to an ecological reproductive barrier, i.e. although they are sympatric, their ecologies are sufficiently different to ensure that they do not encounter each other. In the laboratory, however, although there is pre-mating barrier, hybridisation still occurs, and female hybrids are fertile (Mayr, 1946; Koopman, 1950). Spieth (1958) and Parsons (1973) report that the pre-mating barrier may contain a "learned" element.

The degree of ecological overlap between Drosophila melanogaster and D. simulans in nature is considerable (McKenzie & Parsons, 1972; Parsons, 1975). Hybrids do not occur in nature, and only infrequently in the laboratory. The premating reproductive barrier seems to contain an element of asymmetry. Most interspecific matings are between male D. melanogaster and female D. simulans (Barker, 1962, 1967; Wood & Ringo, 1980).

Not all of the work on reproductive barriers between Drosophilid species has entailed direct observation. Manning (1959a) performed a series of comparative observations on intra- and interspecific courtships in D. melanogaster and D. simulans. Spieth (1958) observed D. pseudoobscura and D. persimilis. But many workers have only performed "mass-mating" experiments, where numbers of males and females of two different species are left together for a period of time (e.g. Ahearn et al., 1974). If sterile hybrids are produced, or females are inseminated, the reproductive barrier is said to be post-mating. If, however, the females are not inseminated, then the

barrier is said to be pre-mating. This kind of experiment gives no indication of whether, in the case of pre-mating barriers, the male or the female of a heterospecific pair is responsible for species recognition. It is often assumed that recognition is performed by the female, because males have been observed to court heterospecific females. This assumption is made by Ahearn et al. (1974). Zouros & D'Entremont (1980) also make this assumption in the case of the partially sympatric species D. arizonensis and D. mojavensis, but Wasserman & Koepfer (1977) and Markow (1981) report that males of these species are also capable of species discrimination. Bateman (1948) and Trivers (1972) have pointed out that one of the effects of anisogamy will be that females will tend to choose their mates more carefully than males, because each mating represents a higher proportion of their total reproductive investment. However, von Schilcher & Dow (1977) point out that, although intraspecific sexual selection will mostly act on males for this reasons, interspecific sexual isolation should tend to be performed by males, since it is plainly counter-adaptive for a male to court a heterospecific female, leaving the female to recognise the male's species and terminate courtship. Their observations which were intended to support this hypothesis, however, are misinterpreted, because they assume that in an unsuccessful courtship (either intra- or interspecific), the individual which is observed to terminate the courtship is the one that has decided not to copulate. In fact, the decision visibly to terminate a courtship may be made in response to a rejection signal given by the other individual, which had made the original decision

not to copulate.

It would thus seem obvious that if a pre-mating barrier is found to exist between two species, no assumptions can safely be made about which sex is responsible for maintaining the isolation; this can only be inferred from direct observations.

3.2. EXPERIMENT 2: Rationale

Conspecific and heterospecific pairs of *Z. tuberculatus* and *Z. sepsoides* were observed, and some of their sexual behaviours were recorded. Since the recording techniques available were rather crude, it was decided to limit the analysis of data chiefly to comparisons of the rates at which conspecific and heterospecific pairs performed the different sexual behaviours. Thus it was hoped that any differences between the tendencies of males of both species to court conspecific and heterospecific females would become apparent, and also any differences between the tendencies of females to accept or reject conspecific and heterospecific males' courtships. From these indications, it was hoped that a hypothesis could be derived as to the mechanism of species isolation, which could then be tested by more refined techniques in later experiments.

MATERIALS & METHODS

See also Chapter 2 .

3.2.1. Strains of flies used. *Z. tuberculatus* 124.5

Z. sepsoides 184.6

All the flies used were aged between 8 and 17 days, and had been kept in single-sex populations in vials for the day of emergence.

Males had been kept at population densities of 2 per vial. This was because in the course of pilot observations for Experiment 4 (see 4.2.) it was noted that levels of male sexual activity were apparently affected by the population densities at which they had been kept prior to observation. Males kept at low population densities exhibited more sexual activity than males kept under more crowded conditions. A similar effect in Drosophila has been described by Ellis & Kessler (1973).

Females were kept at population densities of up to 15 per vial.

3.2.2. Observation and recording techniques. These were used in Experiment 1 (see 3.2.). Observation periods lasted for 20 minutes. The second track of the tape recordings was used for recording the output of an electronic event recorder of the Dawkins (1971) design. This was operated manually by the observer while watching the flies with the binocular microscope to give a commentary on non-acoustical behaviours. The following behaviours were recorded during observation with the electronic event recorder:

a. Male: Orientation, Grappling; Mounting; Start of Copulation; End of Copulation.

b. Female: Leg Extension; Rocking; Pointing and Extrusion.

These terms were used as defined by Bennet-Clark et al. (1980) (see 2.2.1.). "Circling" was treated as a type of Orientation. Orientation was considered to be terminated by the start of Grappling.

The commentary thus recorded was transcribed onto paper by computer. The transcription gave the time of commencement of each behaviour recorded, and the time of its termination if it lasted > 0.5s. For the purposes of analysis, all behaviours with a duration of < 0.5s were treated as having a mean duration of 0.25s. The times of commencement and termination of bursts of male "Type 1" and "Type 2" Songs, and female Whining were then added to the commentary thus produced.

3.2.3. Observations taken. Conspecific and heterospecific pairs were observed. Numbers of observation periods were:

♂ *Z. sepsoides*	X	♀ *Z. sepsoides*	11
♂ *Z. sepsoides*	X	♀ *Z. tuberculatus*	9
♂ *Z. tuberculatus*	X	♀ *Z. tuberculatus*	9
♂ *Z. tuberculatus*	X	♀ *Z. tuberculatus*	13

All heterospecific pairs which were observed to copulate were placed together immediately afterwards in a bottle containing Drosophila medium, to see if any hybrid offspring were produced.

3.3. RESULTS

3.3.1. General observations. In addition to those reported in 2.4., the following observations were noted:

1/. Female Leg Extension was characteristically given in response to an approach by the male that was not visibly courting. (Females have also been observed Leg Extending at each other).

2/. Female Rocking, Whining, and Pointing are closely associated.

TABLE X Sexual Behaviours of conspecific and Heterospecific Pairs of Z.tuberculatus and Z.sepsoides Recorded in in Experiment 2.

Species		N	No. Orients/t	No. Grapples/t	Duration Type 1/t	Duration Type 2/t	Lag Periods	No. Leg Extensions/t	Duration Rocking/t
♂ Z.sepsoides X ♀ Z.sepsoides	Copulated	7	$\bar{X}$=0.1737 SD=0.1736	$\bar{X}$=0.1912 SD=0.1700	$\bar{X}$=0.0268 SD=0.0339	$\bar{X}$=0.2788 SD=0.3103	$\bar{X}$=249.8s SD=291.9s	$\bar{X}$=0.0546 SD=0.1108	$\bar{X}$=0.0226 SD=0.0408
	No Copulation	4	$\bar{X}$=0.0306 SD=0.0207	$\bar{X}$=0.0110 SD=0.0043	$\bar{X}$=0.0452 SD=0.0529	$\bar{X}$=0.0137 SD=0.0915	$\bar{X}$=174.1s SD=164.1s	$\bar{X}$=0.0107 SD=0.0134	$\bar{X}$=0.0265 SD=0.0182
♂ Z.sepsoides X ♀ Z.tuberculatus	Copulated	0							
	No Copulation	9	$\bar{X}$=0.0106 SD=0.0189	$\bar{X}$=0.0117 SD=0.0220	$\bar{X}$=0.0020 SD=0.0030	$\bar{X}$=0.0044 SD=0.0091	$\bar{X}$=229.6s SD=164.6s	$\bar{X}$=0.0235 SD=0.0157	$\bar{X}$=0.0005 SD=0.0010
♂ Z.tuberculatus X ♀ Z.tuberculatus	Copulated	6	$\bar{X}$=0.1691 SD=0.1460	$\bar{X}$=0.1243 SD=0.0761	$\bar{X}$=0.0442 SD=0.0396	$\bar{X}$=0.1045 SD=0.1252	$\bar{X}$=32.9s SD=20.1s	$\bar{X}$=0.0288 SD=0.0257	$\bar{X}$=0.0147 SD=0.0146
	No Copulation	3	$\bar{X}$=0.0488 SD=0.0612	$\bar{X}$=0.0230 SD=0.0207	$\bar{X}$=0.0009 SD=0.0008	$\bar{X}$=0.0003 SD=0.0003	$\bar{X}$=71.9s SD=33.8s	$\bar{X}$=0.0167 SD=0.0162	$\bar{X}$=0.0020 SD=0.0022
♂ Z.tuberculatus X ♀ Z.sepsoides	Copulated	4	$\bar{X}$=0.0612 SD=0.0147	$\bar{X}$=0.1718 SD=0.1410	$\bar{X}$=0.0352 SD=0.0238	$\bar{X}$=0.1885 SD=0.1715	$\bar{X}$=75.1s SD=53.7s	$\bar{X}$=0.1787 SD=0.2525	$\bar{X}$=0.0217 SD=0.0377
	No Copulation	6	$\bar{X}$=0.0358 SD=0.0350	$\bar{X}$=0.0229 SD=0.0192	$\bar{X}$=0.0010 SD=0.0105	$\bar{X}$=0.0077 SD=0.0071	$\bar{X}$=150.0s SD=140.0s	$\bar{X}$=0.0170 SD=0.0121	$\bar{X}$=0.0016 SD=0.0018
	♀ Unreceptive No Copulation*	3	$\bar{X}$=0.1490 SD=0.0415	$\bar{X}$=0.1792 SD=0.1808	$\bar{X}$=0.0189 SD=0.0217	$\bar{X}$=0.0569 SD=0.0699	$\bar{X}$=25.8x SD=19.5s	$\bar{X}$=0.0167 SD=0.168	$\bar{X}$=0.1177 SD=0.0765

*Three female Z.sepsoides were unreceptive to conspecific courtship, and then were then observed with male Z.tuberculatus. See 5.4.2.

The chief rejection signal appears to be Rocking. This may be accompanied by Whining and Pointing. An unreceptive female generally does not commence Whining unless the male produces song. However, if the male persists in courting, the threshold stimulus required to elicit these rejection responses is apparently lowered, until all three behaviours are elicited by the male's proximity, even if he he does not court at all.

3/. If a female responded to a male courtship with these rejection responses, the male often stopped courting temporarily, and often produced a few bursts of "Type 1" Song. However, rejection responses by the female were not observed to have any lasting deterrent effect on male courtship.

4/. As has already been noted (2.4.3.), a high proportion of female Z. sepsoides were unreceptive to male courtship. Females of this species which were found to be unreceptive to conspecific male courtship were then observed immediately afterwards with male Z. tuberculatus. Observations of these females were classed in separate categories from observations of apparently receptive female Z. sepsoides for analysis.

5/. None of the interspecific copulations observed resulted in any offspring being produced. The females laid eggs, but these did not eclose.

3.3.2. Analyses of Data.

The data used in the analyses are given in the Appendix and summarised in Table X. Included in the analyses are: the total durations of male "Type 1" and "Type 2" Songs; the number of times

the male Orientated towards the female; the number of times the male Grappled with the female; the times at which the male Mounted the female and Dismounted; the number of times the female tapped the male (Leg Extension); and the total duration of female Rocking. These behaviours were defined as by Bennet-Clark et al. (1980). Male Stationary Display and Circling were not recorded; they were too often simultaneous with Grappling, and Stationary Display can only be separated from the Orientation which initiates it by arbitrary criteria. Female Whining, Pointing and Extruding were also excluded from the analyses, because the data taken on these behaviours was judged to be too inaccurate; they almost always occurred simultaneously with Rocking, and often simultaneously with male song, usually "Type 2". This made it virtually impossible to record them every time they were observed (see 3.4.1.). In the case of courtships that resulted in copulation, only the period before the start of copulation is included in the analyses. This was because the period after copulation was considered not to be directly comparable to the observation periods in which copulation was never achieved, due to the change in the female's behaviour (see Bennet-Clark et al., 1980), which may affect the male's behaviour.

The observations of intraspecific and interspecific courtships of unreceptive female Z. sepsoides (see 3.3.1.) were excluded from the analyses. It was considered that they were not directly comparable with courtships in which the female was receptive to intraspecific courtships.

The following analyses were performed on the data:

1/. The courtship success/failure rates of conspecific and heterospecific pairs were compared, using a Fisher Exact test. The courtship success/failure rates of male Z. sepsoides and male Z. tuberculatus with conspecific and heterospecific females were then compared directly, with two further Fisher Exact tests.

2/. The lag periods of all the different categories of intraspecific and interspecific, successful and unsuccessful courtships were compared, using a one-way Analysis of Variance (Anovar).

3/. Figures representing the rates of performance of the different sexual behaviours during courtship were generated by the following method: the quantities of the different behaviours given in the Appendix were divided by t′ defined as the observation period, t, minus the lag period, measured in seconds. Behaviours recorded during the lag period were excluded from the analyses. A square-root transformation was then performed on these rate figures. The rates of the different sexual behaviours in the different categories of intraspecific and interspecific courtships were then compared, using one-way Anovars.

Successful and unsuccessful courtships were compared separately, for the following reason: in a successful courtship, the time at which courtship ends is determined by the flies, when they commence copulation; in an unsuccessful courtship, however, the time at which the courtship ends is determined by the observer. Unsuccessful courtships were defined as ending with the end of the observation period. During an observation period, several bouts of sexual activity might be observed, separated by periods in which no sexual

TABLE XI. Numbers of successful and unsuccessful courtships of conspecific and heterospecific pairs observed in Experiment 2, excluding observations of "unrecptive" female Z. sepsoides.

			Copulated	No Copulation
Conspecific	♂ Z. sepsoides	X ♀ Z. sepsoides	7	1
	♂ Z. tuberculatus	X ♀ Z. tuberculatus	6	3
Heterospecific	♂ Z. sepsoides	X ♀ Z. tuberculatus	0	9
	♂ Z. tuberculatus	X ♀ Z. sepsoides	4	6

activity was observed. The bouts of sexual activity could have been defined retrospectively as separate "courtships", by deciding on an arbitrary maximum period that could elapse between two observed sexual behaviours within on "courtship". It was decided, however, that any such measures of rates of sexual behaviours thus generated would be too artificial to be worth compiling. In particular, it would be difficult, if not impossible, to apply this method to the frequent instances where a male Orientated, usually towards a moving female, but did not follow this up with any other sexual behaviour. In these instances, an arbitrary courtship duration could be ascribed (e.g. 0.5s), but it is debatable whether summating such "durations", in the manner of Manning (1959a) and Connolly et al. (1969) would produce a more realistic measure of male sexual activity than recording the frequency with which such events occurred, as was done here.

Therefore, no attempt was made to compare the rates of sexual behaviours in successful courtships with the rates in unsuccessful ones.

3.3.2.1. Comparisons of courtship success rates, using Fisher Exact Tests.

For data, see Table XI.

Comparing intraspecific courtships with interspecific courtships.

$$P = \frac{17! \times 19! \times 17! \times 19!}{13! \times 4! \times 4! \times 15! \times 72!}$$

= 0.0011 i.e. highly significant.

Comparing male Z. sepsoides courtships with conspecific and hetero-

specific females.

$$P = \frac{8! \times 9! \times 7! \times 10!}{7! \times 0! \times 1! \times 9! \times 17!}$$

= 0.0004 i.e. highly significant.

Comparing male Z. tuberculatus courtships with conspecific and heterospecific females.

$$P = \frac{13! \times 10! \times 10! \times 13!}{16! \times 7! \times 4! \times 6! \times 23!}$$

= 0.31 i.e. not significant

3.3.2.2. Comparison of Lag Periods, using a One-Way Anovar. For data, see Appendix.

Comparing all categories of intraspecific and interspecific, successful and unsuccessful courtships. The categories included were:

♂ Z. sepsoides X ♀ Z. sepsoides (copulated) n = 7

♂ Z. sepsoides X ♀ Z. tuberculatus (no copulation) n = 9

♂ Z. tuberculatus X ♀ Z. tuberculatus (copulated) n = 6

♂ Z. tuberculatus X ♀ Z. tuberculatus (no copulation) n = 3

♂ Z. tuberculatus X ♀ Z. sepsoides (copulated) n = 4

♂ Z. tuberculatus X ♀ Z. sepsoides (no copulation) n = 6

The category Z. sepsoides X Z. sepsoides (no copulation) had to be excluded, because it only contained one member.

F = 1.417, with (5, 29) degress of freedom; P = 0.247, i.e. not significant.

3.3.2.3. Comparisons of rates of performance of different sexual behaviours during courtships.

In each case, the data may be obtained from the Appendix by

dividing the quantity of the behaviour recorded (e.g. No. Orientations, Duration Type-2 Song, etc.) by $\underline{t}'$. A square-root transform is then performed on the figure thus derived:

$$f(\underline{x}) = \sqrt{(\underline{x} + 0.38)}$$

This will tend to make negatively-skewed distributions symmetric. It is necessary to add 0.38 to each value of $\underline{x}$, because $\sqrt{0} = 0$, while the square roots of all other values $\underline{x}$ are greater than $\underline{x}$. A set of anovars was performed on the rates of the different sexual behaviours in the different categories of intraspecific and interspecific successful courtships, and another set of anovars on the sexual behaviour rates in the different categories of unsuccessful courtships.

$\underline{P} > 0.05$ in every case.

3.4. DISCUSSION & CONCLUSIONS.

3.4.1. General Comments. The method of recording different behaviours used here was fairly crude, in that it only permitted one behaviour to be recorded at any given moment, although two individuals were being observed, each of which was capable of performing more than one category of behaviour simultaneously. The sound track of the recording made it possible to superimpose the male and female acoustical behaviours separately onto the commentary of the non-acoustical behaviours made with the electronic event recorder, but recordings of simultaneous non-acoustical behaviours could not be superimposed.

There were also simple observational difficulties of categorising

the behaviours as they were observed. E.g. if a male Orientates towards a female, but the female moves, the male will track the female's movements so that he remains Orientated. If the female is moving quickly, the male may not be able to remain Orientated continuously. Under such circumstances, the observer has to decide whether to record one Orientation or several.

For these reasons, it was decided that the recording technique used here was not sufficiently precise to enable detailed comparative analysis of the courtships of the two species to be undertaken, like that of the courtships of D. melanogaster and D. simulans by Manning (1959a). Therefore no attempt was made to do so, and the analysis was restricted to comparisons of intra- and interspecific courtships of these two species.

As has been mentioned above, (3.3.2.), finding an appropriate method of analysing the data presented problems. The chief difficulty was that any definition of the point at which an unsuccessful courtship ends is bound to be arbitrary, and is likely to contain a subjective element. Such an arbitrary definition was used in Experiment 8 (described in Chapter 5), based on the distance of the male from the female. However, in that experiment, cube-shaped observation chambers were used, which gave the females greater opportunity to terminate courtships by decamping than in the flattened rectangular chambers used in this experiment. Also, in a flattened rectangular chamber, it is easier for two flies to pass close to one another, while not visibly interacting sexually; e.g. when one fly is on the ceiling of the chamber, and the other on the floor. In this

experiment, the shape and size of the observation chambers was largely influenced by the design and sensitivity of the microphone on which they were placed.

For these reasons, no such definitions were made, and the rates of sexual behaviours in unsuccessful courtships were analysed separately from those in successful courtships.

However, there is one parameter of sexual activity which can be used for comparing successful and unsuccessful courtships directly. This is the "lag period" before the first observed male Orientation. This was first referred to by Manning (1959b), and it has since been accepted as a distinct phenomenon, different from subsequent periods of no observable courtship (e.g. Nissani, 1977). However, in the instance of pairs where the female elicits little sexual behaviour from the male, at least, this may not be entirely true.

3.4.2. Specific Discussion of Results. The only statistically significant differences between the different categories of intraspecific and interspecific courtships was in the success rates. There is evidently an effective pre-mating reproductive barrier between male Z. sepsoides and female Z. tuberculatus. However, these observations give no evidence for a pre-mating reproductive barrier between male Z. tuberculatus and female Z. sepsoides.

The differences between the rates of different courtship behaviours were not statistically significant in any instance, probably for the following two reasons: 1/. Insufficient replicates of the observations were performed; 2/. For the reasons given above, successful and unsuccessful courtships could not be compared directly.

It will in fact be seen from the data that in all but one of the nine observed "courtships" of male Z. sepsoides X female Z. tuberculatus, very little male sexual activity was recorded. The extent to which male Z. tuberculatus courted female Z. sepsoides was more variable.

The courtships of unreceptive female Z. sepsoides by males of both species were too few in number for statistical analysis, but they appeared to fall into separate categories of their own. The female rejection signals did not appear to deter males of either species from courtship. However, it must be remembered that the confined conditions under which the pairs were observed are probably unnatural, in that the male repeatedly encounters the same female, which is unable to decamp.

There is no evidence from these observations that the "lag periods" before the first observed male Orientation give any reliable indication of the subsequent amounts of male sexual activity, or of the probable outcome of the courtship. The subjective impression was that the "lag period" was largely affected by the duration before the first random encounter between the male and the female. If this is in fact the case, then any sex- or species-specific pheromones used by the flies would appear only to be effective at extremely short range.

3.4.3. Conclusions. These observations provide the basis for the following hypothesis: that male Z. sepsoides and female Z. tuberculatus are prevented from mating with each other by a behavioral barrier, in which the ability of male Z. sepsoides to recognise the female's species may be the most important factor. Recognition could be effected by olfactory cues.

If male Z. tuberculatus also possess such a discriminatory ability, it is plainly much less reliable, at least under observation conditions. If there is any pre-mating barrier between male Z. tuberculatus and female Z. sepsoides, therefore, the female may also be playing an active discriminatory role. Recognition cues could be olfactory and/or acoustical. The use of visual cues is considered highly unlikely, in view of the very high morphological similarity of these species. The recording technique used was too crude to show any subtle behavioral differences, but no obvious ones were noted. Experiments are described in the next two chapters, which were designed to test these possibilities. It was considered safe to discount the possibility that visual cues are being used in species recognition.

Work on other drosophilid species suggests that courtship songs and pheromones are widely used as species recognition cues. Waldron (1964) was the first to report that D. pseudoobscura and D. persimilis had species-specific male courtship songs. Mayr (1946, 1950) had already shown that the degree of sexual isolation between these two species was reduced by the removal of the female's antennae, and Petit (1959) had shown that in D. melanogaster the arista and Johnston's organ of the antennae were involved in the perception of courtship stimuli. Manning (1967) then showed that the arista and Johnston's organ worked together as the auditory receptor which is used for perceiving male songs (the arista had previously been assumed to be an olfactory organ). Taken together, these findings suggest that D. pseudoobscura and D. persimilis use species-specific

male courtship songs for species recognition.

The evidence that male songs are used for species recognition by D. melanogaster and D. simulans is more direct. Bennet-Clark & Ewing (1967, 1969) showed that the readiness of female D. melanogaster to mate with wingless males is increased if artificially-simulated courtship song is provided, and that the pulse period of the artificial "song" is critical. Ewing (1978) showed that the sound receptors in the antennae respond differentially to artificial "songs" of different pulse periods. Von Schilcher & Manning (1975) had shown that hybrid males, which have courtship songs identical to D. simulans, have a higher courtship success rate with female D. simulans than with female D. melanogaster.

The fact that sibling species almost invariably differ in their courtship songs, even when they are apparently almost identical in other respects, is circumstantial evidence for the widespread use of male songs for species recognition (Ewing & Bennet-Clark, 1968; Ewing, 1979).

By contrast, the evidence for species recognition by means of pheromones is slight. Manning (1959b) reports that male D. melanogaster and D. simulans are capable of species recognition, but that this capability is destroyed if their fore-tarsi, with which they tap the female, are removed. This suggests that they recognise species-specific contact pheromones. Markow (1981) reports that interspecific courtships between Drosophila mojavensis and D. arizonensis individuals of strains of sympatric origin are generally terminated by the male, before he has produced any courtship song.

(This generally happens in courtships between male Z. sepsoides and female Z. tuberculatus). It is inferred from this that males are capable of recognising a female's species by olfactory means.

There is also evidence that male D. melanogaster can distinguish between females of different inbred strains by olfactory means (Hay, 1971; Averhoff & Richardson, 1976). There is therefore no reason why some drosophilid species should not use olfactory cues for species recognition.

3.5 APPENDIX. DATA. Each courtship is by a different pair of flies. Courtships of ♂ Z. sepsoides X ♀ Z. sepsoides (copulated).

Courtship 1	t = 107.4s,	
	t′ = 9.5s	3.3s mounted
Lag Period		97.9s
No. Orientations		3
No. Grapples		1
Total Duration Type-1 Song during courtship		0s
during lag period		0s
Total Duration Type-2 Song		9.15s
No. leg Extensions during courtship		2
during lag period		0
Total Duration Rocking		0s

Courtship 2	t = 307.3s	
	t′ = 298.0s	2.9s mounted
Lag Period		9.3s
No. Orientations		34
No. Grapples		10
Total Duration Type-1 Song during courtship		6.13s
during lag period		2.03s

	Total Duration Type-2 Song	15.61s
	No. Leg Extensions	
	during courtship	0
	during lag period	0
	Total Duration Rocking	0s

Courtship 3 — t = 201.9s, t′ = 184.7s (1s mounted)

Lag Period	17.2s
No. Orientations	27
No. Grapples	26
Total Duration Type-1 Song	
during courtship	19.18s
during lag period	0s
Total Duration Type-2 Song	21.47s
No. Leg Extensions	
during courtship	0
during lag period	0
Total Duration Rocking	2s

Courtship 4 — t = 771.1s, t′ = 25.1s (3.6s mounted)

Lag Period	546.0s
No. Orientations	4
No. Grapples	9

Total Duration Type-1 Song during courtship	0s
during lag period	0s
Total Duration Type-2 Song	12.00s
No. Leg Extensions during courtship	0
during lag period	0
Total Duration Rocking	0s

Courtship 5

t = 327.8s

t′ = 253.8s 2.5s mounted

Lag Period	74.0s
No. Orientations	7
No. Grapples	15
Total Duration Type-1 Song during courtship	1.14s
during lag period	0s
Total Duration Type-2 Song	11.47s
No. Leg Extensions during courtship	3
during lag period	0
Total Duration Rocking	0s

Courtship 6

t = 269.0s,

t′ = 95.0s 10.5s mounted

Lag Period	174.0s
No. Orientations	6

No. Grapples	3
Total Duration Type-1 Song	
during courtship	2.09s
during lag period	0.63s
Total Duration Type-2 Song	13.04s
No. Leg Extensions	
during courtship	4
during lag period	0
Total Duration Rocking	2.64s

Courtship 7 $t = 866.8s$, $t' = 36.3s$ (20.2s mounted)

Lag Period	830.5s
No. Orientations	3
No. Grapples	7
Total Duration Type-1 Song	
during courtship	1.35s
during lag period	0s
Total Duration Type-2 Song	5.79s
No. Leg Extensions	
during courtship	0
during lag period	5
Total Duration Rocking	4.35s

Courtships of ♂*Z. sepsoides* X ♀*Z. sepsoides* (no copulation)

Courtship 1 t = 1202.5s

t′ = 769.35s 0s mounted

Lag Period	433.25s
No. Orientations	4
No. Grapples	3
Total Duration Type-1 Song	
during courtship	2.12s
during lag period	0s
Total Duration Type-2 Song	4.68s
No. Leg Extensions	
during courtship	26
during lag period	4
Total Duration Rocking	3.63s

Courtships of ♂Z. sepsoides X ♀Z. sepsoides (♀ unreceptive, no copulation)

Courtship 1

t = 1254.4s

t′ = 1215.6s 2.03s mounted

Lag Period	42.8s
No. Orientations	76
No. Grapples	20
Total Duration Type-1 Song during courtship	159.08s
during lag period	
Total Duration Type-2 Song	26.19s
No. Leg Extensions during courtship	0
during lag period	0
Total Duration Rocking	41.02s

Courtship 2

t = 1205.8s

t′ = 1008.8s 0s mounted

Lag Period	197.0s
No. Orientations	32
No. Grapples	12
Total Duration Type-1 Song during courtship	47.52s
during lag period	0s

Total Duration Type-2 Song	3.22s
No. Leg Extensions	
during courtship	5
during lag period	0
Total Duration Rocking	54.18s

Courtship 3 t = 1227.1s

t′ = 1203.8s 0s mounted

Lag Period	23.3s
No. Orientations	28
No. Grapples	15
Total Duration Type-1 Song	
during courtship	0s
during lag period	0s
Total Duration Type-2 Song	28.91s
No. Leg Extensions	
during courtship	5
during lag period	0
Total Duration Rocking	18.89s

Courtships of ♂*Z. tuberculatus* X ♀*Z. tuberculatus* (copulated).

Courtship 1 — t = 225.7s, t′ = 216.3s (6.7s mounted)

Lag Period	9.4s
No. Orientations	25
No. Grapples	10
Total Duration Type-1 Song during courtship	4.07s
during lag period	0.56s
Total Duration Type-2 Song	0.60s
No. Leg Extensions during courtship	10
during lag period	0
Total Duration Rocking	3.52s

Courtship 2 — t = 150.2s, t′ = 59.3s (6.7s mounted)

Lag Period	90.9s
No. Orientations	7
No. Grapples	8
Total Duration Type-1 Song during courtship	6.07s
during lag period	0s
Total Duration Type-2 Song	5.59s

No. Leg Extensions		
during courtship		4
during lag period		0
Total Duration Rocking		0.25s

Courtship 3 — t = 103.3s; t′ = 81.9s; 3.5 mounted

Lag Period	21.4s
No. Orientations	2
No. Grapples	6
Total Duration Type-1 Song	
during courtship	0.5s
during lag period	0s
Total Duration Type-2 Song	6.17s
No. Leg Extensions	
during courtship	1
during lag period	0
Total Duration Rocking	0s

Courtship 4 — t = 252.0s, t′ = 224.8s; 17.1s mounted

Lag Period	27.2s
No. Orientations	41
No. Grapples	41
Total Duration Type-1 Song	
during courtship	3.79s
during lag period	0s

Total Duration Type-2 Song	11.05s
No. Leg Extensions	
during courtship	5
during lag period	0
Total Duration Rocking	7.52s

Courtship 5

t = 224.2s

t′ = 184.3s 15.7s mounted

Lag Period	39.9s
No. Orientations	11
No. Grapples	6
Total Duration Type-1 Song	
during courtship	16.34s
during lag period	0s
Total Duration Type-2 Song	3.61s
No. Leg Extensions	
during courtship	2
during lag period	0
Total Duration Rocking	5.28s

Courtship 6

t = 16.4s

t′ = 7.9s 3.6s mounted

Lag Period	8.5s
No. Orientations	2
No. Grapples	1

Total Duration Type-1 Song	
during courtship	0s
during lag period	0s
Total Duration Type-2 Song	3.70s
No. Leg Extensions	
during courtship	0
during lag period	0
Total Duration Rocking	0s

Courtships of ♂ *Z. tuberculatus* X ♀ *Z. tuberculatus* (no copulation)

Courtship 1

t = 1193.9s

t′ = 1112.3s 0s mounted

Lag Period	81.6s
No. Orientations	2
No. Grapples	2
Total Duration Type-1 Song	
during courtship	0s
during lag period	0s
Total Duration Type-2 Song	0s
No. Leg Extensions	
during courtship	4
during lag period	0
Total Duration Rocking	0s

Courtship 2

t = 1251.5s

t′ = 124.9s 12.45s mounted

Lag Period	26.6s
No. Orientations	164
No. Grapples	59
Total Duration Type-1 Song	
during courtship	2.49s
during lag period	0s
Total Duration Type-2 Song	1.72s

No. Leg Extensions	
during courtship	6
during lag period	0
Total Duration Rocking	6.25s

Courtship 3

t = 1171.9s

t′ = 1064.3s 4.2s mounted

Lag Period	107.6s
No. Orientations	19
No. Grapples	17
Total Duration Type-1 Song	
during courtship	0.79s
during lag period	0s
Total Duration Type-2 Song	0.25s
No. Leg Extensions	
during courtship	6
during lag period	1
Total Duration Rocking	1s

Courtships of ♂*Z. sepsoides* X ♀*Z. tuberculatus* (no copulation)

Courtship 1

t = 1154.9s,

t′ = 979.9s 0s mounted

Lag Period	175.0s
No. Orientations	9
No. Grapples	8
Total Duration Type-1 Song during courtship	2.51s
during lag period	0s
Total Duration Type-2 Song	0s
No. Leg Extensions during courtship	22
during lag period	0
Total Duration Rocking	0.25s

Courtship 2

t = 1219.9s,

t′ = 1018.0s 0s mounted

Lag Period	201.9s
No. Orientations	6
No. Grapples	3
Total Duration Type-1 Song during courtship	2.02s
during lag period	0s
Total Duration Type-2 Song	2.09s

No. Leg Extensions		
during courtship		6
during lag period		1
Total Duration Rocking		0s

Courtship 3	t = 1205.9s,	
	t′ = 1044.4s	0s mounted
Lag Period		161.5s
No. Orientations		2
No. Grapples		0
Total Duration Type-1 Song		
during courtship		0s
during lag period		0s
Total Duration Type-2 Song		0s
No. Leg Extensions		
during courtship		29
during lag period		8
Total Duration Rocking		0s

Courtship 4	t = 1203.7s,	
	t′ = 827.6s	0s mounted
Lag Period		376.1s
No. Orientations		3
No. Grapples		6
Total Duration Type-1 Song		
during courtship		0s
during lag period		0s

Total Duration Type-2 Song	0s
No. Leg Extensions	
during courtship	18
during lag period	4
Total Duration Rocking	0.86s

Courtship 5 — t = 1221.9s, t′ = 747.7s — 0s mounted

Lag Period	474.2s
No. Orientations	1
No. Grapples	1
Total Duration Type-1 Song	
during courtship	0s
during lag period	0s
Total Duration Type-2 Song	0s
No. Leg Extensions	
during courtship	10
during lag period	1
Total Duration Rocking	0s

Courtship 6 — t = 1258.8s, t′ = 773.9s — 0s mounted

Lag Period	484.9s
No. Orientations	5
No. Grapples	4

Total Duration Type-1 Song during courtship	0s
during lag period	0s
Total Duration Type-2 Song	4.22s
No. Leg Extensions during courtship	10
during lag period	3
Total Duration Rocking	0s

Courtship 7 — t = 1297.5s, t′ = 1149.5s, 7.4s mounted

Lag Period	58.0s
No. Orientations	73
No. Grapples	83
Total Duration Type-1 Song during courtship	11.39s
during lag period	0s
Total Duration Type-2 Song	34.87s
No. Leg Extensions during courtship	32
during lag period	0
Total Duration Rocking	3.74s

Courtship 8 — t = 1215.1s, t′ = 1087.9s, 0s mounted

Lag Period	127.2s

No. Orientations	4
No. Grapples	7
Total Duration Type-1 Song	
during courtship	3.01s
during lag period	0.25s
Total Duration Type-2 Song	0.78s
No. Leg Extensions	
during courtship	69
during lag period	4
Total Duration Rocking	0s

Courtship 9 t = 1186.6s, 0s mounted
t′ = 1181.9s

Lag Period	7.7s
No. Orientations	1
No. Grapples	1
Total Duration Type-1 Song	
during courtship	0s
during lag period	0s
Total Duration Type-2 Song	0s
No. Leg Extensions	
during courtship	19
during lag period	0
Total Duration Rocking	0s

Courtships of ♂*Z. tuberculatus* X ♀*Z. sepsoides* (copulated)

Courtship 1

t = 186.6s,

t′ = 97.8s 1.06s mounted

Lag Period	88.9s
No. Orientations	8
No. Grapples	4
Total Duration Type-1 Song	
during courtship	3.87s
during lag period	0.25s
Total Duration Type-2 Song	1.57s
No. Leg Extensions	
during courtship	5
during lag period	0
Total Duration Rocking	0s

Courtship 2

t = 282.2s,

t′ = 192.0s 12.93s mounted

Lag Period	90.2s
No. Orientations	11
No. Grapples	22
Total Duration Type-1 Song	
during courtship	1.50s
during lag period	0s
Total Duration Type-2 Song	34.67s

No. Leg Extensions		
during courtship	0	
during lag period	2	
Total Duration Rocking	16.70s	

Courtship 3 — t = 178.8s, t′ = 24.4s — 19.53s mounted

Lag Period	1
No. Orientations	2
No. Grapples	4
Total Duration Type-1 Song	
during courtship	0.5s
during lag period	0s
Total Duration Type-2 Song	11.42s
No. Leg Extensions	
during courtship	3
during lag period	2
Total Duration Rocking	0s

Courtship 4 — t = 109.8s, t′ = 61.9s — 29.69s mounted

Lag Period	47.9s
No. Orientations	4
No. Grapples	7
Total Duration Type-1 Song	
during courtship	5.72s
during lag period	0s

Total Duration Type-2 Song	5.54s
No. Leg Extensions	
during courtship	3
during lag period	0
Total Duration Rocking	0s

Courtships of ♂*Z. tuberculatus* X ♀*Z. sepsoides* (no copulation)

Courtship 1	t = 1170.2s	
	t′ = 1115.7s	1.02s mounted
Lag Period		54.5s
No. Orientations		79
No. Grapples		62
Total Duration Type-1 Song during courtship		16.53s
during lag period		0s
Total Duration Type-2 Song		6.95s
No. Leg Extensions during courtship		23
during lag period		1
Total Duration Rocking		4.14s

Courtship 2	t = 1231.0s	
	t′ = 817.3s	0s mounted
Lag Period		413.7s
No. Orientations		13
No. Grapples		11
Total Duration Type-1 Song during courtship		13.02s
during lag period		6.12s
Total Duration Type-2 Song		15.74s

	No. Leg Extensions	
	during courtship	27
	during lag period	21
	Total Duration Rocking	3.60s

Courtship 3	t = 1213.4s	
	t′ = 1180.6s	0s mounted
	Lag Period	32.8s
	No. Orientations	25
	No. Grapples	0
	Total Duration Type-1 Song	
	during courtship	33.3s
	during lag period	0s
	Total Duration Type-2 Song	8.69s
	No. Leg Extensions	
	during courtship	11
	during lag period	0
	Total Duration Rocking	0s

Courtship 4	t = 1218.5s	
	t′ = 1162.6s	0s mounted
	Lag Period	65.9s
	No. Orientations	111
	No. Grapples	43
	Total Duration Type-1 Song	
	during courtship	0.25s
	during lag period	0s

Total Duration Type-2 Song		14.25s
No. Leg Extensions		
during courtship		0
during lag period		0
Total Duration Rocking		1s

Courtship 5 — t = 1149.7s; t′ = 1080.5s — 0s mounted

Lag Period	69.2s
No. Orientations	8
No. Grapples	10
Total Duration Type-1 Song	
during courtship	0s
during lag period	0s
Total Duration Type-2 Song	0s
No. Leg Extensions	
during courtship	9
during lag period	0
Total Duration Rocking	0s

Courtship 6 — t = 1164.8s; t′ = 907.0s — 0s mounted

Lag Period	257.8s
No. Orientations	3
No. Grapples	3

Total Duration Type-1 Song	
during courtship	0.75s
during lag period	0s
Total Duration Type-2 Song	0s
No. Leg Extensions	
during courtship	28
during lag period	1
Total Duration Rocking	0s

Courtships of ♂ *Z. tuberculatus* X ♀ *Z. sepsoides* (♀ unreceptive, no copulation)

Courtship 1

t = 1178.7s

t′ = 1172.6s 0s mounted

Lag Period	6.1s
No. Orientations	160
No. Grapples	50
Total Duration Type-1 Song	
during courtship	3.92s
during lag period	0.79s
Total Duration Type-2 Song	1.70s
No. Leg Extensions	
during courtship	1
during lag period	0
Total Duration Rocking	11.65s

Courtship 2

t = 1207.2s

t′ = 1152.8s 1.97s mounted

Lag Period	52.4s
No. Orientations	59
No. Grapples	12
Total Duration Type-1 Song	
during courtship	3.31s
during lag period	0s

Total Duration Type-2 Song	4.23s
No. Leg Extensions	
during courtship	11
during lag period	0
Total Duration Rocking	20.80s

Courtship 3 t = 1185.8s

t′ = 1185.8s 482.48s mounted

Lag Period	19.1s
No. Orientations	51
No. Grapples	47
Total Duration Type-1 Song	
during courtship	58.79s
during lag period	0s
Total Duration Type-2 Song	184.62s
No. Leg Extensions	
during courtship	28
during lag period	1
Total Duration Rocking	197.21s

CHAPTER 4

Experiments to Investigate the Possible Use of Pheromones by *Z. tuberculatus* and *Z. sepsoides* for Sex and Species Recognition.

Contents:

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4.1. INTRODUCTION

4.1.1. Evidence for the use of pheromones in Drosophilidae. As mentioned above (3.4.), the evidence for the use of pheromones for species recognition among Drosophilidae is largely circumstantial. However, the evidence for the use of sex-specific pheromones is conclusive, and such pheromones have been isolated.

The original report of the use of pheromones in Drosophilidae was by Sturtevant (1915) who claimed that mating speed in *D. melanogaster* was increased if pairs were placed in vials which had previously contained other pairs, and were thus "scented". However, Ewing & Manning (1963) failed to reproduce this result.

It is reported that specific female behaviour is not necessary to

stimulate male courtship, since males will court immobilised females (Spieth, 1966; Hall, 1978; Siegel & Hall, 1979). Indeed, males of some species, including D. melanogaster, will court in the dark (Hardeland, 1972; Grossfield, 1966), or when blind (Siegel & Hall, 1979).

Shorey & Bartell (1970) and Averhoff & Richardson (1974) claimed to have shown that female odour increased male sexual activity in D. melanogaster. Male odour also had the same effect, but to a very much lesser extent. In their experiments, air from a chamber containing females was blown into the chamber containing males under observation. The pheromome could apparently be detected by males over a distance of 5 cm.

More direct evidence is produced by Tomkins et al. (1980). They failed to reproduce the effects reported by Shorey & Bartell (1970), or Averhoff & Richardson (1974), but they found that an extract from virgin female D. melanogaster stimulated inter-male courtship, and they used gas chromatography to show differences between volatile compounds extracted from males and females. Jallon et al. (in press) identified sex-specific long-chain hydrocarbons in cuticular extracts from D. melanogaster, and Jallon & Hotta (1979) and Antony & Jallon (in press) showed that the male-specific hydrocarbons are not present in very young males (which attract male courtship). Venard & Jallon (1980) isolated a pheromone by washing vials which had contained females with cyclohexane (a highly volatile solvent). This pheromone was found to stimulate male wing vibration.

The production of sex-specific pheromones appears to be mediated

by tissues in the abdomen. Jallon & Hotta (1979) found that isolated female abdomens elicit male courtship. Sex mosaic gynandromorphs elicit courtship if female tissue is present in the abdomen (Nissani, 1975, 1977; Hotta & Benzer, 1976; Hall, 1977; Jallon & Hotta, 1979). Hall (1978) reports that the stimulus which causes fru mutant male D. melanogaster to be courted by other males appears to be a tissue in the abdomen.

4.1.2. Evidence for the use of pheromones in Zaprionus. Bennet-Clark et al. (1980) stated: "Tactile and possibly olfactory stimuli ... ... appear to be common but rather variable signals (in Drosophilidae) ... It seems likely that olfactory and chemical stimuli that appear to be important in Drosophila ... ... are reduced and/or replaced by visual and acoustical signals in Zaprionus ...". My own observations described in Chapters 2 and 3, however, appear to suggest that males, at least, may recognise the sex and species of other individuals by chemical means. Visual cues are unlikely to be implicated, since the two species are morphologically almost identical, and sexual dimorphism is slight. No behavioral differences between females of the two species have been noted, except for the higher incidence of unreceptivity in female Z. sepsoides. The possibility of the use of visual and/or behavioral cues by males for sex and species recognition has therefore been discounted in this project.

During this project, one chance observation was made which seemed to suggest that in Zaprionus, males produce a sex-specific pheromone

which inhibits male courtship, and its production is mediated by abdominal tissue. A sick and dying male Z. tuberculatus in a vial containing an all-male population was persistently courted and mounted by other males. This individual had an abnormally small abdomen. Its corpse was still being courted by other males 48 hours after its death (dead males have not been observed to elicit courtship on any other occasion).

There is as yet no evidence that females use chemical cues for sex or species recognition. The observations of female behaviour described in Chapters 2 and 3 are adequately explained by the hypothesis that a female only recognises a male's sex when he initiates courtship, and can only distinguish between males of the two species by their "Type 2" Songs.

The aims of the experiments described in this chapter were to present more conclusive evidence for or against the use of pheromones as cues for recognition of the sex and species of other individuals by male and female Zaprionus.

4.2. EXPERIMENT 3.

4.2.1. Method & Materials

4.2.1.1. Aims and Rationale.

To investigate whether the sexual behaviour of male Z. tuberculatus is affected differentially by sex-specific chemical residues left on the substrate on which flies have walked and/or excreted.

Pairs of mature virgin males were recorded together twice, once in an observation chamber which had previously contained females, and once in an observation chamber which had previously contained other males. It was hoped that if the two sexes emit sex-specific pheromones, residues of these would be left in the observation chambers, and these would differentially affect male sexual activity levels. The tendency of males to court each other was considered to be a useful method of assessing levels of male sexual activity for the folllowing reasons: 1/. Unlike in heterosexual courtships, male behaviour is not affected by the response of a female, whose behaviour is not the subject of study; 2/. Since both males remain virgins, they can be observed twice consecutively, under the different conditions, and their behaviours in the two seprate observation periods are directly comparable; 3/. The duration of the observation period can be standardised by the observer. In heterosexual courtships, the duration is determined by the flies themselves, since the courtship ends with the start of copulation.

The observation conditions are arguably no more "unnatural" than those of heterosexual courtships. In nature, males presumably encounter each other, as well as encountering females, at oviposition sites. It should therefore be no less "natural" to confine two males in an observation chamber for a given period of time than it would be to confine a male and a female together.

It may be noted that other authors have also used inter-male courtship as a bioassay of male sexual activity levels in Drosophilidae (e.g. Nissani, 1975; Tomkins et al., 1980).

The parameters of male sexual activity measured were the quantities of "Type 1" and "Type 2" Song produced during an observation period of standard duration (vide Venard & Jallon, 1980).

4.2.1.2. Materials.

Strain of flies used: Z. tuberculatus 164.8 (see 2.2.).

Males and females were kept in single-sex populations in vials from the day of emergence. Males were kept at population densities of 2 per vial (see 3.2.1.) until observation. They were observed when aged between 8 and 21 days. Females were kept at population densities of up to 12 per vial, and were used when aged between 8 and 21 days.

4.2.1.3. Techniques & methods of observation. (See also chapter 2.)

Pairs of males were placed together in an observation chamber of similar design to those used in Experiment 1, and their songs were recorded.

The internal dimensions of the chambers were approximately:

Length: 10 mm

Breadth: 9 mm

Depth: 5 mm

All observation periods lasted for 1200s and were taken during the first four hours of the artificial 12-hour "day".

Oscilloscope traces of the sound recordings were filmed, and the total numbers of pulses of each song type were summated.

Two observation chambers were used, hereinafter referred to as the "Male Odour" and "Female Odour" chambers respectively. Immediately before observations were taken, the "Male Odour" chamber had two males placed in it for a period of 45 min- 2 hr, and the "Female Odour"

chamber had two females placed in it for a similar period. It was judged that this was sufficient time for two flies effectively to "saturate" a chamber of approx. 0.5 cm^3 volume with any odours they might produce. These were then removed before the pair of males to be recorded were introduced into the chamber.

Each pair of males was observed twice, at an interval of 24 hours, once in the "Male Odour" chamber and once in the "Female Odour" chamber. The order in which they were observed in the two chambers was randomised by use of random number tables. Care was taken to ensure that both observations were carried out as nearly as possible at the same time of day.

Twenty pairs of males were observed. The total numbers of pulses of "Type 1" and "Type 2" Songs produced by each pair in the two chambers were compared, and the statistical significance level of the differences was assessed by Wilcoxon matched-pairs T-tests.

4.2.2. Results & Statistics. Tables XII and XIII give the total numbers of pulses of "Type 1" and "Type 2" Songs produced by each pair of males in each case. The mean numbers of pulses produced of both song types by males when in the "Female Odour" chamber were greater than those produced in the "Male Odour" chamber. The analyses are given below:

T = the sum of the ranks of the negative values of (B - A).

n = 20 pairs

P = < 0.05 if $T \leq 52$

"Type 1" Song: $T = 82$

$P > 0.05$

TABLE XII Total numbers of pulses of "Type 1" Song produces by pairs of males in "Male Odour" and "Female Odour" chambers in Experiment 3.

Pair no.	No. of pulses "Male Odour"(A)	No. of pulses "Female Odour"(B)	(B - A)	Rank of |B - A|
1	1 259	4 800	3 541	17
2	1 287	2 231	944	5
3	4 211	3 048	837	4
4	3 742	1 082	-2 260	16
5	2 913	3 241	328	2
6	7 640	6 325	-1 315	8
7	2 777	4 082	2 305	15
8	3 283	11 778	8 495	19
9	5 324	3 178	-2 156	13
10	7 073	16 924	9 851	20
11	251	1 231	980	6
12	5 816	3 635	-2 181	14
13	255	986	731	3
14	4 347	4 463	116	1
15	12 573	6 922	-5 653	18
16	4 649	6 553	1 904	12
17	870	2 403	1 533	10
18	1 117	2 760	1 643	11
19	2 177	829	-1 348	9
20	7 079	6 090	-1 011	7

TABLE XIII Total numbers of pulses of "Type 2" Song produces by pairs of males in "Male Odour" and "Female Odour" chambers in Experiment 3.

Pair no.	No. of pulses "Male Odour"(A)	"Female Odour"(B)	(B - A)	Rank of |B - A|
1	470	2 585	2 115	17
2	253	2 106	1 853	14
3	666	3 655	2 989	20
4	103	8	-95	4
5	170	401	231	6
6	584	494	-90	3
7	1 504	50	-1 454	11
8	23	1 964	1 941	16
9	710	743	33	1
10	1 122	3 721	2 599	19
11	0	789	789	9
12	1 947	474	-1 473	12
13	0	472	472	7
14	1 384	2 598	1 214	10
15	2 240	4 111	1 871	15
16	1 576	2 060	484	8
17	2 701	493	-2 208	18
18	45	1 552	1 507	13
19	198	271	73	2
20	346	484	138	5

"Type 2" Song: $\underline{T} = 48$

$\underline{P} < 0.05$ i.e. significant.

4.2.3. Discussion. In this experiment, the same males were observed in two chambers, designed to differ only in the sex of the flies which had previously inhabited them. This one factor apparently caused a difference in the quantities of "Type 2" Song, and possibly "Type 1" Song also, produced by the males in the two chambers. From this it can be inferred that flies leave sex-specific residues in their environment, and that female and male residues affect the behaviour of males differentially, i.e. they act as sex pheromones. Either male residues inhibit male sexual behaviour, or female residues promote it, or both these effects occur.

Since the observation chambers are designed to allow the free passage of air (the sides are made of wire mesh, and the floor of gauze), it would seem that the pheromone must be left on the substrate, rather than as a vapour in the air. It would therefore seem more likely that it is perceived as a chemotactile stimulus, via the flies' tarsi and/or proboscis. However, it may also contain a volatile component, acting as an olfactory stimulus. The possible existence of volatile sex-specific pheromones was investigated in the following experiment.

4.3. EXPERIMENT 4.

4.3.1. Method & materials

4.3.1.1. Aims and Rationale.

To investigate whether the sexual behaviour of male

Z. tuberculatus is affected differentially be sex-specific elements (pheromones) in the odour produced by other conspecific flies.

Heterosexual pairs of Z. tuberculatus were observed in a chamber through which a stream of air was being passed. The air reached the chamber via another chamber, containing other flies, as a source of putative volatile pheromones. In half of the replicates performed, these flies were male, and in half they were female. The flies used as a source of putative pheromones had previously had their wings amputated to ensure that they could not produce sex-specific sounds that might be heard by the pair under observation. All the flies used in this experiment were sexually mature virgins.

It was decided to observe heterosexual pairs, rather than pairs of males as in the previous experiment, because the easiest sexual behaviour in inter-male interractions to quantify is song; this could not be recorded in this experiment, because the experimental design precluded the use of observation chambers suitable for taking sound recordings.

The parameter of sexual behaviour recorded was the "lag period" before the first observed males Orientation (see Manning, 1959b; Nissani, 1977; etc.). This parameter was chosen because it is quantitative, and is less influenced by the females' behaviour than is the rest of a male's courtship.

4.3.1.2. Materials.

Strain of flies used: Z. tuberculatus 164.8 (see 2.2.).

All individuals were virgins aged 8-14 days, which had been kept in single-sex populations in vials since the day of emergence. The

Fig. 19.

A. Exploded diagram of the apparatus used in Experiments 4 and 5, seen in side view.

a, b, & c: Tubes a, b, and c.

d: Chamber 1.

e: Chamber 2.

f: gauze covering to end of Tube a.

g: glass cover-slips.

h: sealing-wax.

j: direction of air flow through apparatus.

B. Experiment 4; the method of introducing the female fly into Chamber 2. See 4.3.1.3.

a: the female fly.

b: the male fly.

c: Chamber 2.

d: end of Perspex funnel.

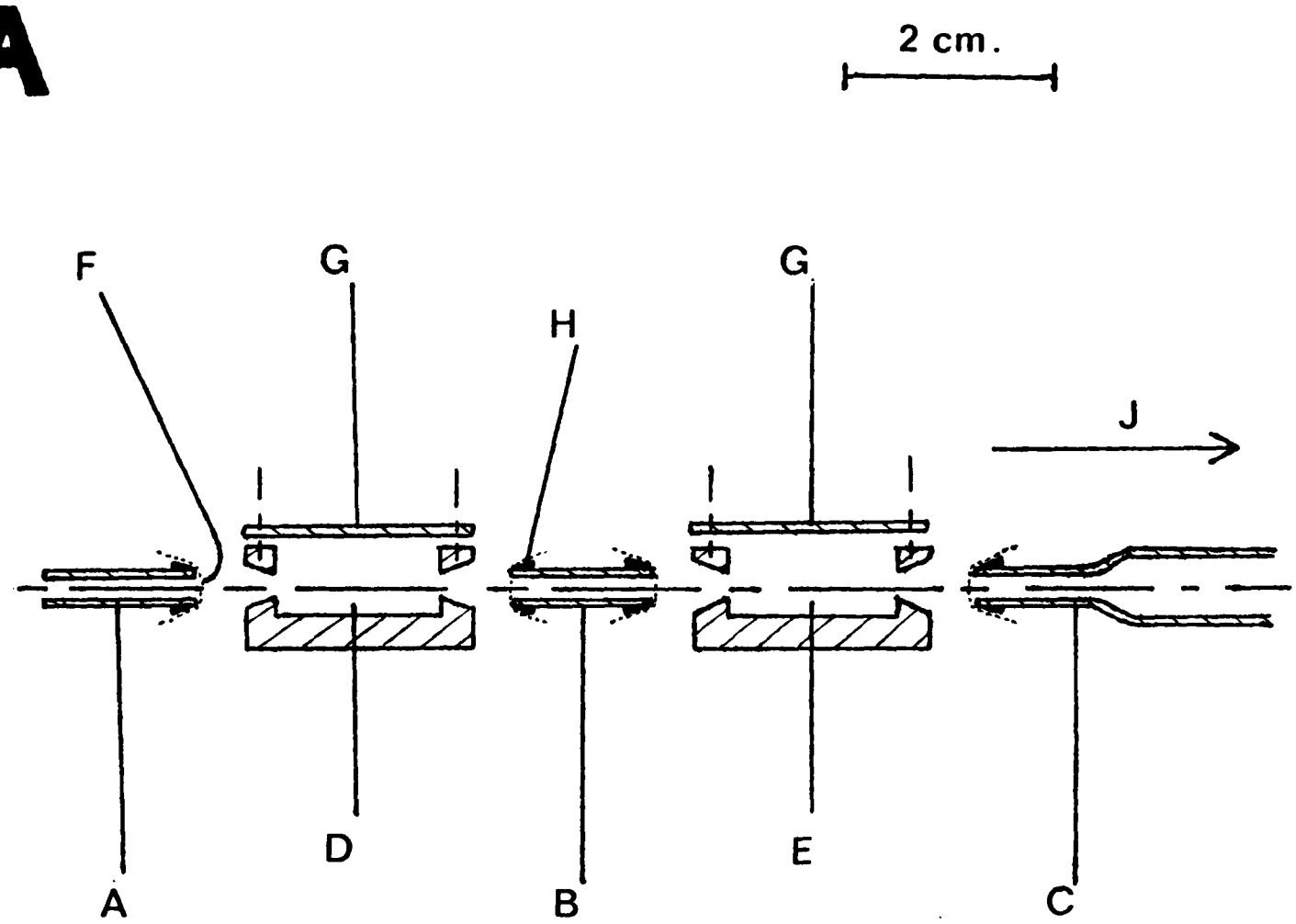
e: opague glass cover-slip.

f: piston tipped with cotton-wool.

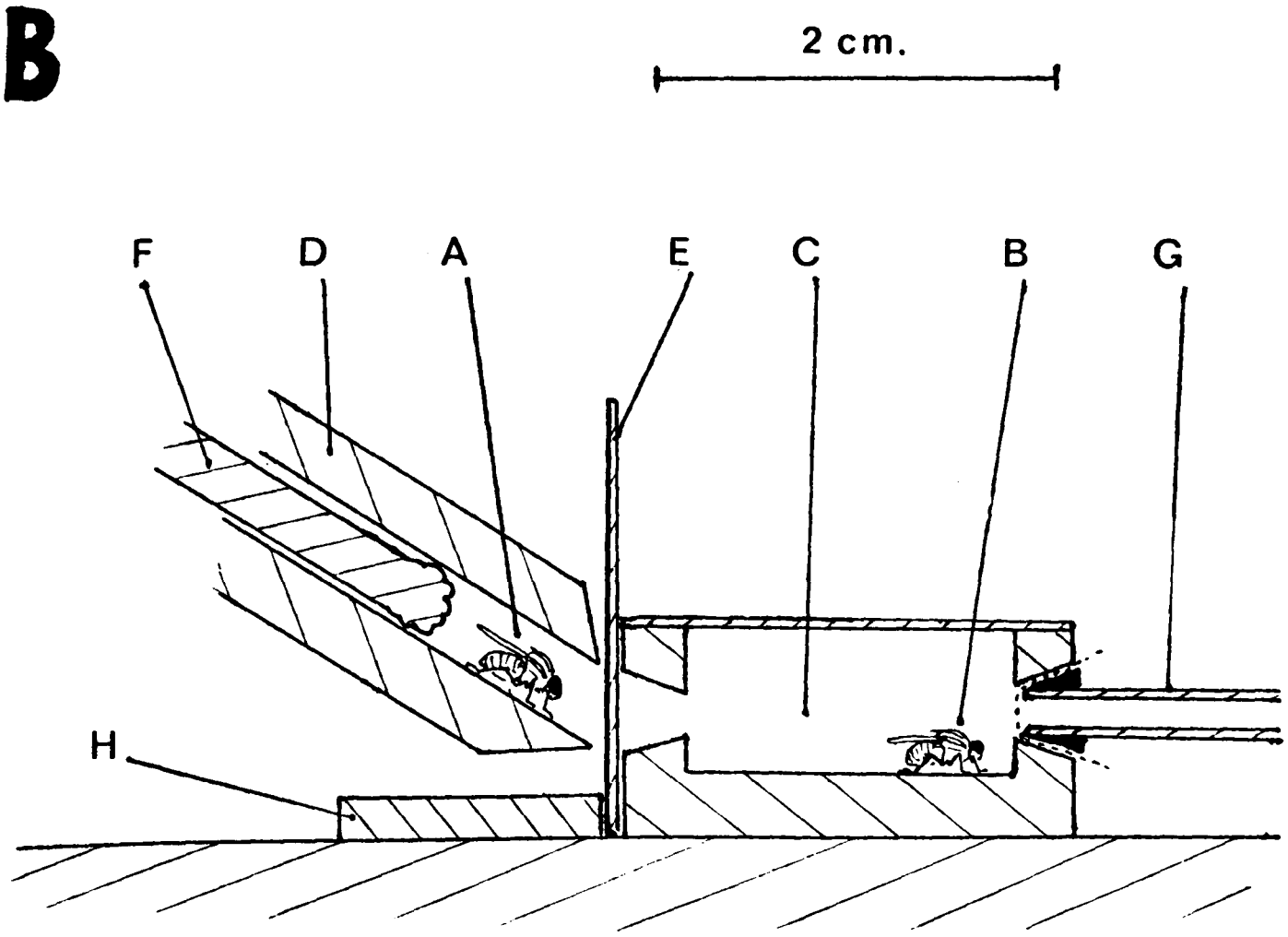
g: Tube c.

h: foam plastic support for (e).

A



B



males had been kept at population densities of 2 per vial (see 3.2.1.), while the females were kept at densities of up to 16 per vial. In order to standardise further the conditions under which the flies had been kept before observation, all the flies were transferred to fresh vials 13-20 hours before the observations were undertaken. All observations were performed during the first four hours of the 12-hour artificial "day", at temperatures of 20-22.5^{o}C.

4.3.1.3. Techniques & methods of observations.

The apparatus is shown in Fig. 19a. The observation chambers were flattened cylinders made of Perspex, with a removable glass cover-slip for a roof. Each chamber had two tapered holes drilled through the side, opposite each other, through which the flies were introduced by being allowed to walk down a Perspex funnel. It was also via these holes that air entered and left the chambers through the glass tubes a, b, and c. The ends of these tubes were covered with gauze, attached with sealing-wax.

Chamber 1 (the "up-wind" chamber) contained two flies of the same sex, hereinafter referred to as the "Pheromone-Source", or "P-S" flies. These flies had had their wings amputated on the day before they were used in the experiment. They were rendered torpid by cooling for this operation. The same P-S flies were never used for more than two replicates of the observations.

Chamber 2 contained the heterosexual pairs of flies under observation.

A new Chamber 2 was used for each replicate during a day's run of observations. Two Chamber 1's and two Tube b's were used: one for

replicates in which the P-S flies were male, and another for replicates in which the P-S flies were female. At the end of each day's run of replicates, all the Chamber 2's and both the Chamber 1's were washed with distilled water and scrubbed dry with disposable tissues, before being used again on the following day.

Air was sucked slowly through the apparatus by a venturi-pump attached to a water-tap. Before each day's run of replicates, the rate of suction was adjusted by attaching tube c to a dummy apparatus, identical to that used for observations. A piece of smouldering tissue paper was held close to the open end of Tube a, so that the smoke could be seen passing through the dummy apparatus, and the rate of air passage thus guaged. The rate of suction was adjusted to be as slow as possible, while remaining definitely observable. Smoke was never passed through the apparatus used for observations.

The procedure for conducting each replicate of the observations was as follows: 1./ Two P-S flies of the same sex were introduced into Chamber 1, and the entrance holes were stoppered with Tubes a and b.

2/. A male fly was intoduced into Chamber 2. Chamber 2 was then attached to Tube c. The other entrance hole was covered over with a glass cover-slip with a piece of black tape stuck on it to make it opaque. (This cover-slip was supported in position by a small piece of foam plastic.) See Fig. 19b.

3/. The method of introducing the female fly into Chamber 2 was as shown in Fig. 19b. The female, having been allowed to walk from her vial into a Perspex funnel, was then confined in the end of the funnel

by introducing a piston, tipped with cotton-wool. The opening at the end of the funnel was placed against the cover-slip directly over the entry hole of Chamber 2. At a moment when the male fly was on the opposite side of the chamber from the entry hole, the cover slip was removed, so that the female could walk into the chamber. Chamber 2 was then immediately attached to chamber 1 via Tube b, and a stop-watch was started. This procedure marked the start of the observation period. It took < 3 seconds to perform.

4/. The length of time which elapsed from the start of the observation period to the first observed male Orientation (the lag period) was recorded with the stop-watch.

Forty replicates were performed: in twenty of these, the P-S flies were male ("Male Odour Conditions"); and in twenty they were female ("Female Odour Conditions"). The lag periods of the pairs observed under the different "odour conditions" are given in Table XIV.

4.3.2. Results.

4.3.2.1. General observations.

In general, lag periods were very short. The male typically courted the female on encountering her for the second or third time, and sometimes on the first encounter. The big majority of courtships resulted in Mounting and copulation within a few seconds. No consistent difference between the lag periods under the two different "odour conditions" was immediately apparent.

In eight of the forty replicates, the lag period was very much

TABLE XIV. Lag periods of male Z.tuberculatus observed in Experiment 4 under "Male" and "Female Odour" conditions.

Replicate No.	"Odour Conditions"	Lag Period(s)	arc-sine	Replicate No.	"Odour Conditions"	Lag Period(s)	arc-sine
1	Male	37	21.71	2	Female	2	1.14
3	"	7	4.01	4	"	3	1.72
5	"	12	6.89	6	"	16	9.21
7	"	38	22.33	8	"	54	32.68
9	"	23	13.30	10	"	14	8.05
11	"	57	34.75	12	"	54	32.68
13	"	39	22.95	14	"	54	32.68
15	"	22	12.71	16	"	35	20.49
17	"	41	24.20	18	"	21	12.12
19	"	33	19.27	20	"	42	24.83
21	"	24	13.89	22	"	5	2.87
23	"	8	4.59	24	"	6	3.44
25	"	33	19.27	26	"	75	48.59
27	"	77	50.35	28	"	9	5.16
29	"	52	31.33	30	"	42	24.80
31	"	3	1.72	32	"	6	3.44

longer (between 140 and 800s) than in the other thirty-two (< 80s). It would seem that these eight males were gnerally less sexually active than the rest. They were deemed to be "sexually inactive males", and were excluded from the statistical analysis. Four of these males were observed under "Male Odour Conditions", and four were under "Female Odour Conditions".

4.3.2.2. Statistical analysis.

The lag periods of males under "Female Odour Conditions" were compared with those of males under "Male Odour Conditions". The distributions of the lag period populations are one-tailed. An arc-sine transformation was therefore performed on the data, and the two populations were then compared, using a one-way Anovar (see Table XIV). The result of this test is given below.

$n_1 = 16 \quad n_2 = 16$

Variance Ratio = 0.129

No. degrees of freedom = 1, 30

$P \gg 0.05$ i.e. no significant difference

4.3.3. Discussion. Unlike Experiment 3, the results of this experiment give not the smallest indication that male Z. tuberculatus may use chemosensory cues for discriminating between male and female conspecifics. Possible reasons for this are discussed below.

1/. This species may, in fact, not use pheromones for sex recognition, and the effect observed in Experiment 3 may be spurious. I consider this possibility highly unlikely, because males do, in fact appear to distinguish between the sexes readily, despite the fact that the degree of sexual dimorphism is slight. Also, other drosophilids

are known to use pheromones for sex recognition (see 4.1.).

2/. The species may use tactile sex-specific pheromones, but not volatile air-borne ones. In Experiment 3, males would have been perceiving sex-specific residues in the observation chambers by means of their chemotactile organs, rather than a volatile component given off by these residues. While the existence of such tactile pheromones is not unlikely (again, other drosophilids are known to use them), it would seem unlikely that this species relies on them solely for sex recognition. Males can definitely recognise another fly's sex without touching it, and it seems unlikely that a male has to walk over ground that another fly has itself just stood on for it to recognize that other fly's sex.

3/. Volatile pheromones are used for sex recognition, but they are only perceived at very short range (a few millimetres). Thus the odour of the female in the observation chamber "swamps" that of the P-S flies when that female is close to the male. This is, in my view, by far the most likely possibility, and is entirely consistent with all male behaviour observed in this and the other experiments described in this thesis. A male's behaviour has not been noted to be changed by the proximity of a female until the female is < 5 mm. away from the male.

In order further to investigate this possibility, this experiment should be repeated, with the following modification: the Chamber 1 in the apparatus should contain more than two P-S flies - perhaps six of them. In this way, it might be possible for Chamber 2 to be sufficiently filled with sex-specific volatile pheromone from Chamber

1 to "swamp" the odour of the female in Chamber 2. Also the quantities of male locomotory activity and "grooming" behaviours during the lag period could be recorded as well.

4.4. EXPERIMENT 5.

4.4.1. Method & Materials.

4.4.1.1. Aims and Rationale.

To investigate whether the behaviours of female Z. sepsoides and Z. tuberculatus are affected differentially by sex-specific and species-specific elements (pheromones) in the odours produced by other flies of these two species.

The experiment was performed in two parts: in the first, the possible ability of female Z. tuberculatus to distinguish air-borne sex-specific pheromones was investigated; in the second, the ability of females of both species to distinguish between air-borne species-specific pheromones was investigated.

Single females were observed. The parameter of behaviour measured was the quantity of locomotory activity over a given time period (see von Schilcher, 1976a) under conditions when they were exposed to air that had been passed through a chamber containing other flies. In Part 1, these other flies were conspecific, and their sex was varied between replicates. In Part 2, they were males, and their species was varied between replicates. All the flies used in this experiment were sexually mature virgins.

4.4.1.2. Materials.

Strains of flies used: Z. sepsoides 123.3. and Z. tuberculatus 124.5 (see 2.2.).

All individuals were virgins aged 8-20 days, which had been kept in single-sex populations since the day of emergence, at population densities of up to 16 per vial. In order to standardise further the conditions under which the flies had been kept before observation, the females observed had been transferred to fresh vials 13-20 hours before observation, at population densities of 6 per vial. All observations were carried out during the first four hours of the 12-hour artificial "day", at temperatures of 23-26^{o}C.

4.4.1.3. Techniques & methods of observation.

The apparatus was of the same design as that used in Experiment 4 (see 4.3.1.3., and Fig. 19a). Six "P-S" flies, with their wings amputated, were placed in Chamber 1. The same P-S flies were never used for more than three replicates of observation. In Part 1, two Chamber 1's were used: one for replicates in which the P-S flies were male, and another for replicates in which the P-S flies were female. In Part 2, different Chamber 1's were used for replicates in which the P-S flies were Z. sepsoides, and replicates in which they were Z. tuberculatus. Each female observed in a day's run of replicates was observed in a fresh Chamber 2. At the end of the day's observations, all the Chamber 2's were washed with distilled water, and scrubbed dry with disposable tissues, before being used on the following day.

Part 1. Method: Species of flies used: Z. tuberculatus (see above).

The procedure was as follows:

1/. The female to be observed (the Subject) was introduced into Chamber 2. Chamber 2 was connected to Tube c (see Fig. 19a), but not to Chamber 1, so that a stream of "clean" air was sucked through it. After a "settling-down" period of 15s, the Subject was observed for 300s. The amount of time spent in locomotory activity was summated with a stop-watch.

2/. Chamber 1, containing six P-S flies of the same sex, with their wings amputated, was connected to Chamber 2 by Tube b. After another 15s "settling-down" period, the Subject was then observed for a further 300s, and the amount of time spent in locomotory activity was summated.

3/. Chamber 1 was disconnected again, and the Subject was observed for a third 300s period.

4/. A second Chamber 1, containing six P-S flies of the opposite sex to those in the first, was connected to Chamber 2, and the Subject was again observed for 300s.

5/. Chamber 1 was again disconnected, and the Subject was observed for a final 300s period.

Since locomotory activity in a fly can easily be stimulated by visual cues, especially movement, the observer took care to remain as motionless as possible during all observation periods.

Fifteen Subjects were observed. Eight of these were observed first under "Male Odour Conditions" and then under "Female Odour

Conditions"; the other seven were observed first under "Female Odour Conditons" and then under "Male Odour Conditions".

The amounts of time spent in locomotory activity by each Subject observed under "Male Odour Conditions" and "Female Odour Conditions" were compared, using a Wilcoxon matched-pairs T-test.

The amounts of time spent in locomotory activity by each Subject observed in both Parts 1 and 2 (see below) during the three periods when Chamber 2 was not connected to Chamber 1 were subjected to a linear regression analysis, to see if a consistent change in levels of locomotory activity with time occurred. In theory, however, such a change should not give rise to spurious experimental results, since half the Subjects were observed first under "Male Odour Conditions" and then under "Female Odour Conditions", and in half the Subjects this order was reversed.

Part 2. Method: In two separate sets of replicates, 15 female *Z. tuberculatus* and 15 female *Z. sepsoides* were observed under "*Z. sepsoides* Odour Conditions" and "*Z. tuberculatus* Odour Conditions". To create "*Z. sepsoides* Odour Conditions" Chamber 1 contained six wingless male *Z. sepsoides*; and to create "*Z. tuberculatus* Odour Conditions" Chamber 1 contained six wingless male *Z. tuberculatus*. The procedure was the same as in Part 1. The amounts of time spent in locomotory activity by each female observed under the two different "odour conditions" were compared using Wilcoxon matched-pair T-tests.

All the flies used were virgins aged 8-18 days.

TABLE XV Experiment 5, Part 1. Total amounts of time, measured in seconds, spent by each female Z.tuberculatus Subject in locomotory activity under "Male Odour" and "Female Odour" conditions.

Subject No.	Total duration of locomotory activity "Male Odour"(A)	"Female Odour"(B)	(B - A)	Rank of |B - A|
1	3	13	-10	5
2	134	39	95	14
3	88	95	-7	3
4	23	4	19	7
5	0	4	-4	2
6	2	2	0	
7	31	13	18	6
8	1	95	-94	13
9	12	43	-31	8
10	18	103	-85	12
11	36	2	34	9
12	79	0	79	11
13	7	51	-44	10
14	2	1	1	1
15	11	3	8	4

TABLE XVI Experiment 5, Part 2. Total amounts of time, measured in seconds, spent by each female Z.tuberculatus Subject in locomotory activity under "Z.spesoides Odour" and "Z.tuberculatus Odour" conditions.

Subject No.	Total duration of locomotory activity "Z.tuberculatus Odour"(A)	"Z.sepsoides Odour"(B)	(B - A)	Rank of |B - A|
1	67	21	46	6
2	10	106	-96	12
3	117	6	111	13
4	2	40	-38	5
5	81	21	60	8.5
6	33	93	-60	8.5
7	16	90	-74	11
8	77	7	70	10
9	1	1	0	
10	1	5	-4	2.5
11	14	0	14	4
12	11	11	0	
13	60	1	59	7
14	1	0	1	1
15	5	9	-4	2.5

TABLE XVII Experiment 5, Part 2. Total amounts of time, measured in seconds, spent by each female Z.sepsoides Subject in locomotory activity under "Z.sepsoides Odour" and "Z.tuberculatus Odour" conditions.

Subject	Total duration of locomotory activity			Rank of
No.	"Z.tuberculatus Odour"(A)	"Z.sepsoides Odour"(B)	(B - A)	|B - A|
1	21	32	-11	7
2	1	0	1	1
3	2	9	-7	5
4	22	0	22	11
5	4	0	4	2.5
6	13	0	13	8
7	85	67	18	10
8	37	33	4	2.5
9	84	54	30	12.5
10	0	0	0	
11	47	17	30	12.5
12	1	16	-15	9
13	84	51	33	14
14	65	60	5	4
15	45	37	8	6

4.4.2. Results & Statistics

Part 1. The amounts of time, measured in seconds, spent in locomotory activity by each Subject under "Male Odour Conditions" and "Female odour Conditions" are given in Table XV. The result of the Wilcoxon matched-pair T-test is given below:

T = the sum of the ranks of the negative values of (B - A)

N = 14 pairs (the pair in which (B - A) = 0 is discounted)

$P < 0.05$ if $T \leq 21$

T = 53 $P > 0.05$

Part 2. a. *Z. sepsoides*. The amounts of time spent in locomotory activity by each female *Z. sepsoides* under "*Z. sepsoides* Odour Conditions" and "*Z. tuberculatus* Odour Conditions" are given in Table XVI. The result of the Wilcoxon matched-pairs T-test is given below:

T = the sum of ranks of the negative values of (B - A)

N = 14 pairs (the apir in which (B - A) = 0 is discounted)

$P < 0.05$ if $T \leq 21$

T = 21 $P < 0.05$, i.e. statistically significant

b. *Z. tuberculatus*. The amounts of time spent in locomotory activity by each female *Z. tuberculatus* under "*Z. sepsoides* Odour Conditions" and "*Z. tuberculatus* Odour Conditions" are given in Table XVII. The result of the Wilcoxon matched-pairs T-test is given below:

T = the sum of tied ranks of the negative values of (A - B)

N = 13 pairs (the two pairs in which (A - B) = 0 are discounted)

$P < 0.05$ if $T \leq 17$

T = 41.5 $P > 0.05$

The amounts of time spent by females of the two species in

Fig. 20. Experiment 5, Parts 1 and 2. Total durations of locomotory activity of female *Z. tuberculatus* Subjects during the three 300s periods when Chamber 2 was not connected to Chamber 1 (see 4.4.1.3.). The scale of the y-axis is logarithmic. The three recorded instances in which no locomotory activity was recorded are therefore not included.

Gradient of regression line = -0.023

Observed *t* = 0.2444

With *n* - 2 = 85 d.f., *P* > 0.8

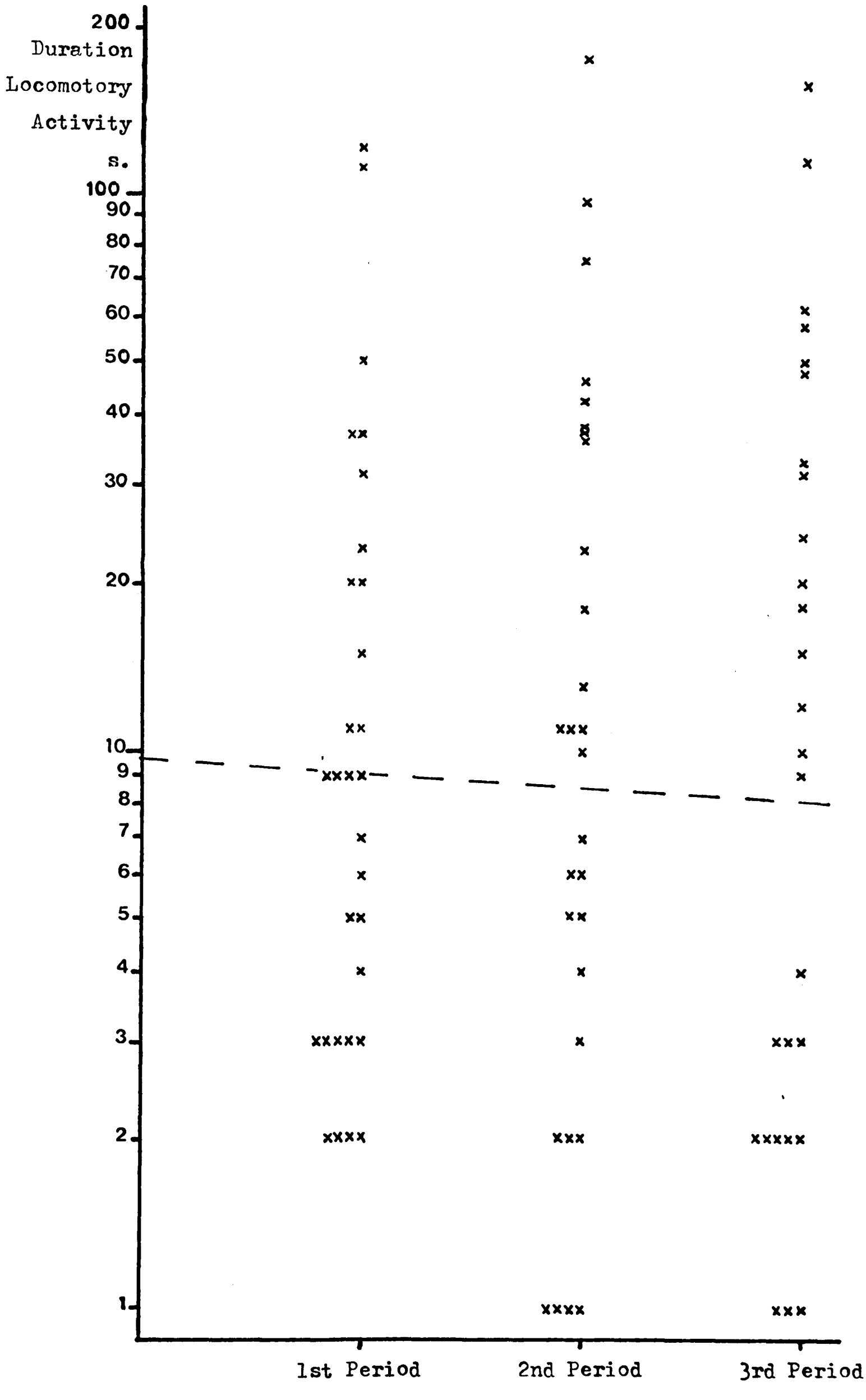
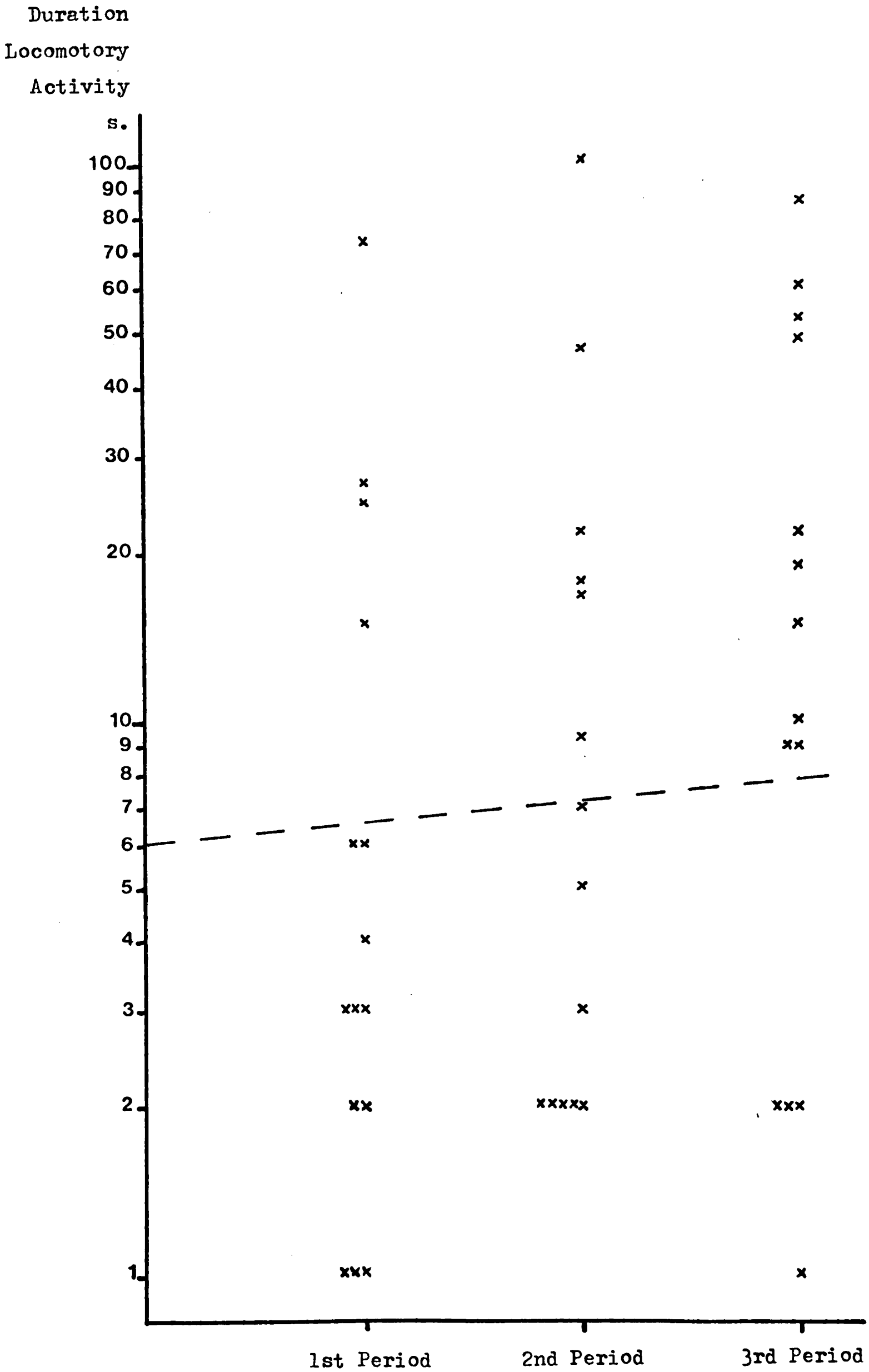

200
Duration
Locomotory
Activity
s.
100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
1st Period
2nd Period
3rd Period

Fig. 21. Experiment 3, Part 2. Total durations of locomotory activity of female Z. sepsoides Subjects during the three 300s periods when Chamber 2 was not connected to Chamber 1 (see 4.4.1.3.). The scale of the y-axis is logarithmic. The two recorded instances in which no locomotory activity was observed are therefore not included.

Gradient of regression line = 0.037

Observed t = 1.7576

With n - 2 = 26 d.f., $P > 0.05$


Duration
Locomotory
Activity
s.
100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
1st Period
2nd Period
3rd Period

locomotory activity during the three 300s periods when Chamber 2 was not connected to Chamber 1 are given in Figs. 20 and 21. Since the distributions were strongly negatively skewed, a logarithmic transformation was performed on them. A linear regression analysis was then performed as shown. The object of this was to see if there was any tendency for the Subjects' levels of locomotory activity to change with time during the course of observations. The gradients of the regression lines do not differ significantly from zero, i.e. no such change with time is descernible in these data.

4.4.3. Discussion. The results of this experiment suggest that female Z. sepsoides may be able to distinguish between males of the two species by olfactory means. However, it gives no evidence that female Z. tuberculatus can distinguish between conspecific and heterospecific males, or between conspecific males and females, by this means. This, of course, does not definitely mean that they are unable to do so.

If female Z. tuberculatus can distinguish other flies' sex and/or species by olfactory means, there could be various reasons why this experiment failed to demonstrate this ability. Firstly, they may have a less sensitive olfactory sense than Z. sepsoides, and the concentrations of sex-specific and species-specific pheromones to which they were exposed in this experiment may have been too low. Secondly, the ability to discriminate between different sex-and species-specific pheromones need not manifest itself in differential levels of locomotory activity in the presence of the different

pheromones. Another observable behaviour performed by females in the absence of other flies is "Grooming" with the tarsi. Levels of this behaviour in drosophilids is believed to be affected by odours of other nearby flies (see Hay, 1971). The amount of time spent by females in 'grooming', rather than in locomotory activity, could have been recorded, or both could have been recorded simultaneously. Thirdly, pheromones may only discernibly affect females' behaviour towards other flies, in which case this experiment would not demonstrate the effect. The following experiment was designed with this possibility in mind.

4.5. EXPERIMENT 6.

4.5.1. Methods & Materials.

4.5.1.1. Aims & Rationale.

To assess the ability of female *Z. sepsoides* and *Z. tuberculatus* to identify the sex of conspecific flies, and the species of male flies, by olfactory means.

Female experimental subjects of the two species were presented with conspecific males, conspecific females, and heterospecific males; all of which had had their wings and legs amputated to minimise their behavioral repertoire. The subjects' reactions were recorded. As a control, the subjects were also presented with small rectangular pieces of paper of similar size to the flies. The purpose of this was to check that the observed behaviours of the subjects on being presented with other flies were indeed specific responses to encounters with flies, rather than general responses to encounters

with any object.

4.5.1.2. Materials.

Strains of flies used: Z. tuberculatus 124.5

Z. sepsoides 123.3 (see 2.2.).

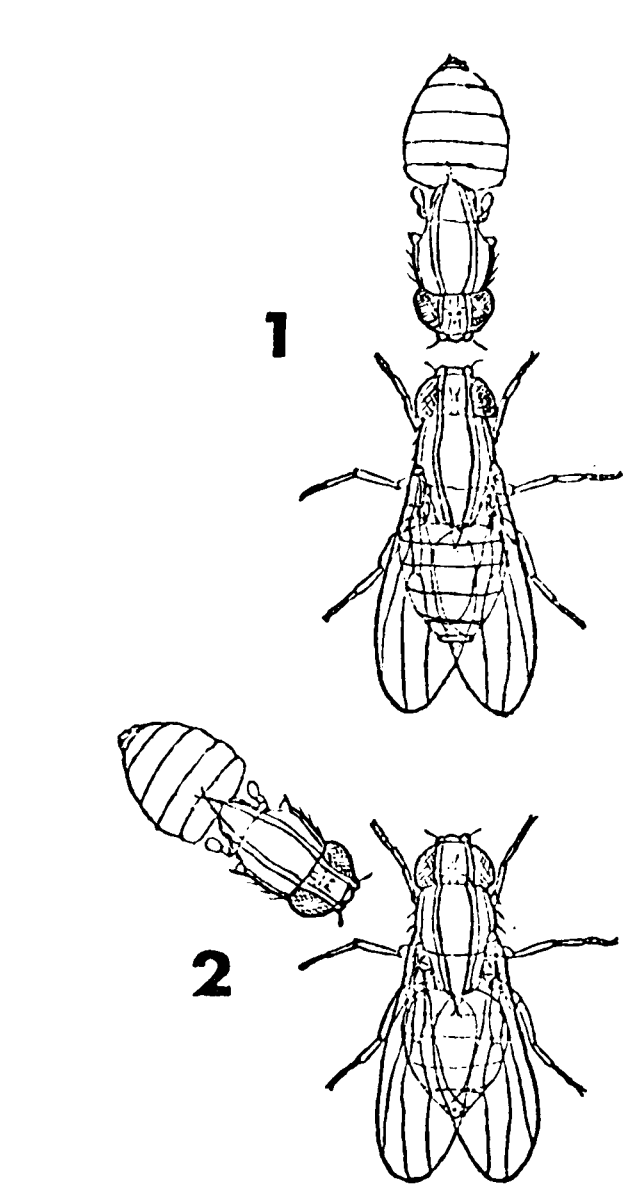
All flies were kept in single-sex popuations in vials from the day of emergence, at population densities of 2-12 per vial. In order further to standardise the conditions under which flies were kept before observation, the female "Subjects" were transferred to fresh vials 13-20 hours before observation, at population densities of 2 per vial. Flies used were aged 8-20 days. All observations were carried out during the first four hours of the artificial 12-hour "day", at temperatures 23-26^{o}C.

4.5.1.3. Techniques and methods of observation.

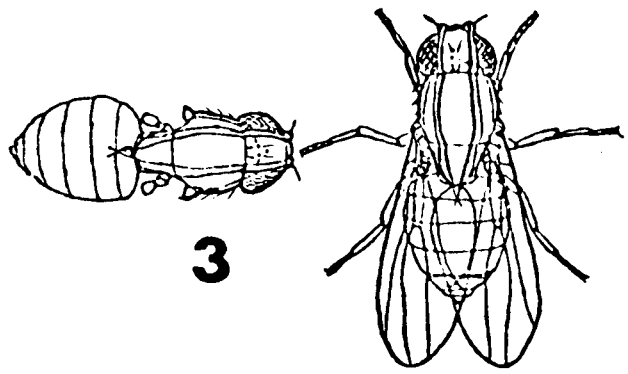
The female Subjects were rendered torpid by cooling, and were attached to a piece of flexible copper wire 0.04 mm in diameter by means of a small blob of wax on the pronotum. The piece of wire was approx. 10 cm long, and the other end of it was attached to a X8 binocular microsocpe, so that the fly was hanging in the focal plane (approx. 9 cm below the objective). The fly was allowed to perch on a small piece of expanded polystyrene (approx. 1 cm squre), attached to the microscope stage, on which it had a limited freedom to walk. It could also fly off this perch. This method of restraint was used rather than an observation chamber, so that the Subject was accessible to the observer.

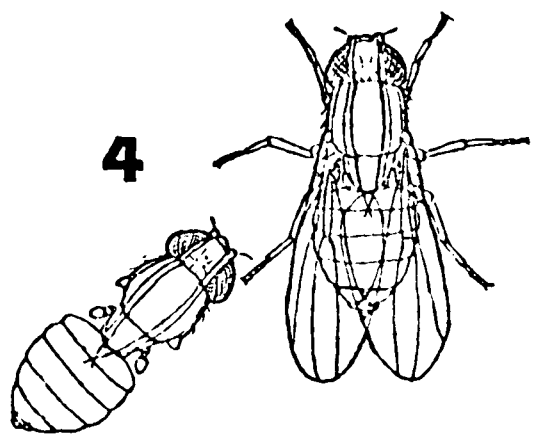
Each Subject was "Presented" (see below) with the following "Subjects": a conspecific male; a conspecific female; a

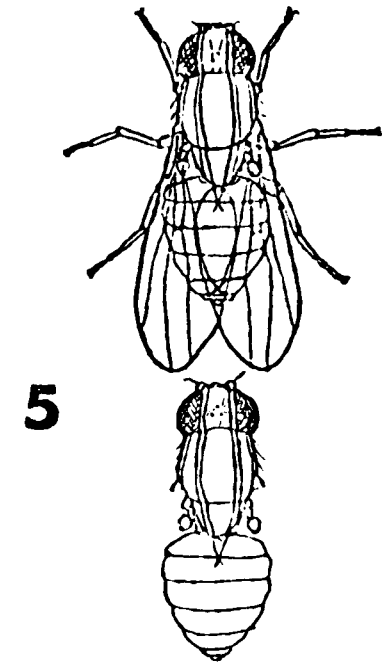
Fig. 22. Experiment 6: The eight directions of Presentations of Objects. The Subject is shown with wings and legs. The Object is shown as a fly without wings or legs.

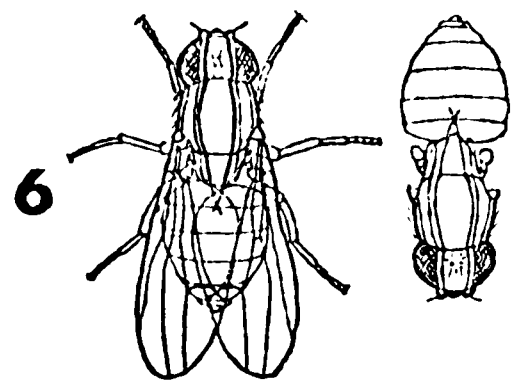

1

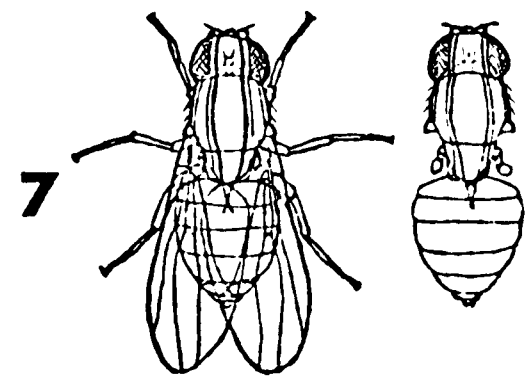
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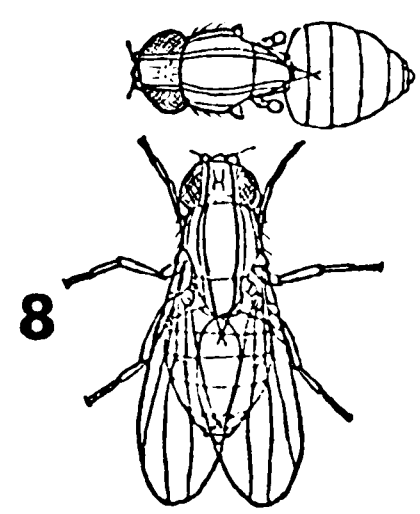

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heterospecific male; and an "Inert Object", consisting of a small slip of white paper approx. 3 mm long X 2 mm wide, with a V-shaped longitudinal fold. The order in which they were presented was varied systematically between Subjects.

The Object flies had previously had their wings and legs amputated. They were rendered torpid by cooling for this operation. They were mounted on the end of a stiff piece of wire 0.08 mm in diameter, attached with wax to the underside of the thorax, with which they were manipulated by the observer. The Inert Objects were also mounted on the ends of similar pieces of wire.

The Objects were presented to the Subjects in the following manner: they were made to approach the Subject until nearly touching, three times at intervals of less than 1s, followed by an interval of approx. 5s before repeating this approach procedure from a different direction. Such a set of three approaches is hereinafter termed a "Presentation". All Presentations were in the horizontal plane. The directions from which the Presentations were made are illustrated in Fig. 22. These directions were supposed to mimic "Orientation" by a courting male. Directions 1 to 5 are typical of courting males of these species. Directions 6 to 8 are more common in fortuitous encounters, but during courtship a male may often momentarily be Orientated in these directions. Each Presentation was repeated six times with each Object.

The Subjects were scored for the number of Presentations from each direction to which they responed with "Leg Extension" (see 2.4.1.). It was recorded as an all-or-nothing response. "Rocking" was not

recorded, since the attachment of the Subject to a length of wire inevitably restricted its ability to Rock. Decamping was also not recorded, because any realistic definition of what movements by the Subject constituted a "decamp" was likely to be highly arbitrary, and contain a large subjective element.

4.5.2. Results & Statistics. Fifteen Subjects of each species were observed. They frequently responded with Leg Extension to Presentations of live flies, less frequently to Presentations of Inert Objects. They also frequently responded to Presentations of all Objects with movements that could be construed as attempts to Decamp. Rocking, however, was only definitely observed on a very few occasions. Tables XVIII and XIX give the number of Presentations of each Object to which each Subject responded by Leg Extension, expressed as fractions of the total number of Presentations.

The statistical analysis was as follows: Wilcoxon matched-Pairs T-tests were used to compare:

1/. the overall rates of Leg Extension per Presentation of live Object flies with the rates of leg Extension per Presentation of Inert Objects;

2/. the rates of leg Extension per Presentation of Conspecific Males with the rates of Leg Extension per Presentation of Conspecific Females;

3/. the rates of Leg Extension per Presentation of Conspecific Males with the rates of Leg Extension per Presentation of Heterospecific Males.

TABLE XVIII Experiment 6, a/. Z.tuberculatus. Numbers of Presentations of different Objects to which each Subject responded by Leg Extension, expressed as fractions of the total numbers of Presentation.

Subject	Objects						
	Conspecific ♂	Conspecific ♀	Heterospecific ♂	Inert			
No.	(A)	(B)	(C)	Object (D)	(B - A)	(C - A)	(1/3(A+B+C)-D)
1	0.42	0.40	0.33	0.26	-0.02	-0.09	0.12
2	0.40	0.35	0.40	0.21	-0.05	0	0.17
3	0.58	0.48	0.48	0.26	-0.10	-0.10	0.25
4	0.42	0.52	0.40	0.24	0.10	-0.02	0.21
5	0.50	0.60	0.50	0.29	0.10	0	0.24
6	0.60	0.67	0.42	0.21	0.07	-0.18	0.35
7	0.67	0.48	0.56	0.31	-0.19	-0.11	0.26
8	0.73	0.67	0.58	0.26	-0.06	-0.15	0.40
9	0.48	0.65	0.65	0.33	0.17	0.17	0.26
10	0.44	0.46	0.42	0.17	0.02	-0.02	0.27
11	0.38	0.33	0.46	0.14	-0.05	0.08	0.25
12	0.52	0.48	0.40	0.31	-0.04	-0.12	0.16
13	0.44	0.29	0.48	0.14	-0.15	0.04	0.26
14	0.52	0.40	0.52	0.19	-0.12	0	0.29
15	0.44	0.50	0.48	0.26	0.06	0.04	0.21

TABLE XIX Experiment 6, b/. Z.sepsoides. Numbers of Presentations of different Objects to which each Subject responded by Leg Extension, expressed as fractions of the total numbers of Presentation.

Subject No.	Objects: Conspecific ♂ (A)	Conspecific ♀ (B)	Heterospecific ♂ (C)	Inert Object (D)	(B - A)	(C - A)	(1/3(A+B+C)-D)
1	0.48	0.33	0.56	0.26	-0.15	-0.22	0.20
2	0.50	0.54	0.54	0.38	0.04	-0.12	0.15
3	0.40	0.22	0.50	0.21	-0.18	0.10	0.16
4	0.42	0.25	0.37	0.17	-0.17	-0.05	0.18
5	0.42	0.42	0.54	0.24	0	0.12	0.22
6	0.37	0.33	0.31	0.24	-0.04	-0.06	0.10
7	0.35	0.62	0.50	0.17	0.27	0.15	0.32
8	0.48	0.62	0.69	0.29	0.14	0.21	0.31
9	0.48	0.40	0.33	0.21	-0.08	-0.15	0.19
10	0.67	0.58	0.52	0.31	-0.09	-0.15	0.28
11	0.42	0.31	0.31	0.09	-0.11	-0.11	0.26
12	0.67	0.60	0.62	0.31	-0.07	-0.05	0.32
13	0.50	0.44	0.54	0.36	-0.06	0.04	0.13
14	0.69	0.60	0.67	0.43	-0.09	-0.02	0.22
15	0.46	0.42	0.42	0.24	-0.04	-0.04	0.19

Results are given below:

a/. *Z. tuberculatus*. Data is given in Table XVIII.

1/. Comparing responses to Presentations of live flies (1/3(A+B+C)) with responses to Presentations of Inert Objects (D):

N = 15 Subjects

T = 0 P << 0.01, i.e. highly significant.

2/. Comparing responses to Presentations of Conspecific Males (A) with responses to Presentations of Conspecific Females (B):

N = 15 Subjects

T = 50 T > 25, therefore P > 0.05

3/. Comparing responses to Presentations of Conspecific Males (A) with responses to presentations of Heterospecific Males (C):

N = 12 Subjects (excluding the three Subjects for which (C - A = 0)

T = 23 T > 14, therefore P > 0.05

b/. *Z. sepsoides*. Data are given in Table XIX.

1/. Comparing responses to Presentations of live flies (1/3(A+B+C)) with responses to Presentations of Inert Objects (D):

N = 15 Subjects

T = 0 P << 0.01, i.e. highly significant.

2/. Comparing responses to Presentations of Conspecific Males (A) with responses to Presentations of Conspecific Females (B):

N = 14 Subjects (excluding the Subject for which B - A = 0)

T = 26 T > 21, therefore P > 0.05

3/. Comparing responses to Presentations of Conspecific Males

(A) with responses to Presentations of Heterospecific Males (C):

N = 15 Subjects

T = 45 T > 25, therefore P > 0.05

4.5.3. Discussion. This experiment confirms that female Zaprionus can discriminate between flies and other objects of similar size by visual and/or olfactory means, but gives no evidence to suggest that they can distinguish between sexes or species within the genus. Thus it does not corroborate the result of the previous experiment, which suggested that female Z. sepsoides could distinguish a male's species by olfactory means.

It is possible that females indeed cannot distinguish between sexes by olfactory means, but this would be hard to demonstrate conclusively. All this experiment has demonstrated is that if females do possess such a discriminatory ability, it does not manifest iself in their tendencies to Leg Extend to flies of different sexes and/or species. Such an ability could also manifest itself in differential levels of overall locomotory activity (as investigated in the previous experiment), or tendencies to Decamp or other female-specific behaviours (see 2.2.1.), which have not yet been investigated.

4.6. EXPERIMENT 7.

4.6.1. Method & Materials

4.6.1.1. Rationale

To investigate the possible involvement of pheromones in the discrimination by male Z. tuberculatus and Z. sepsoides between

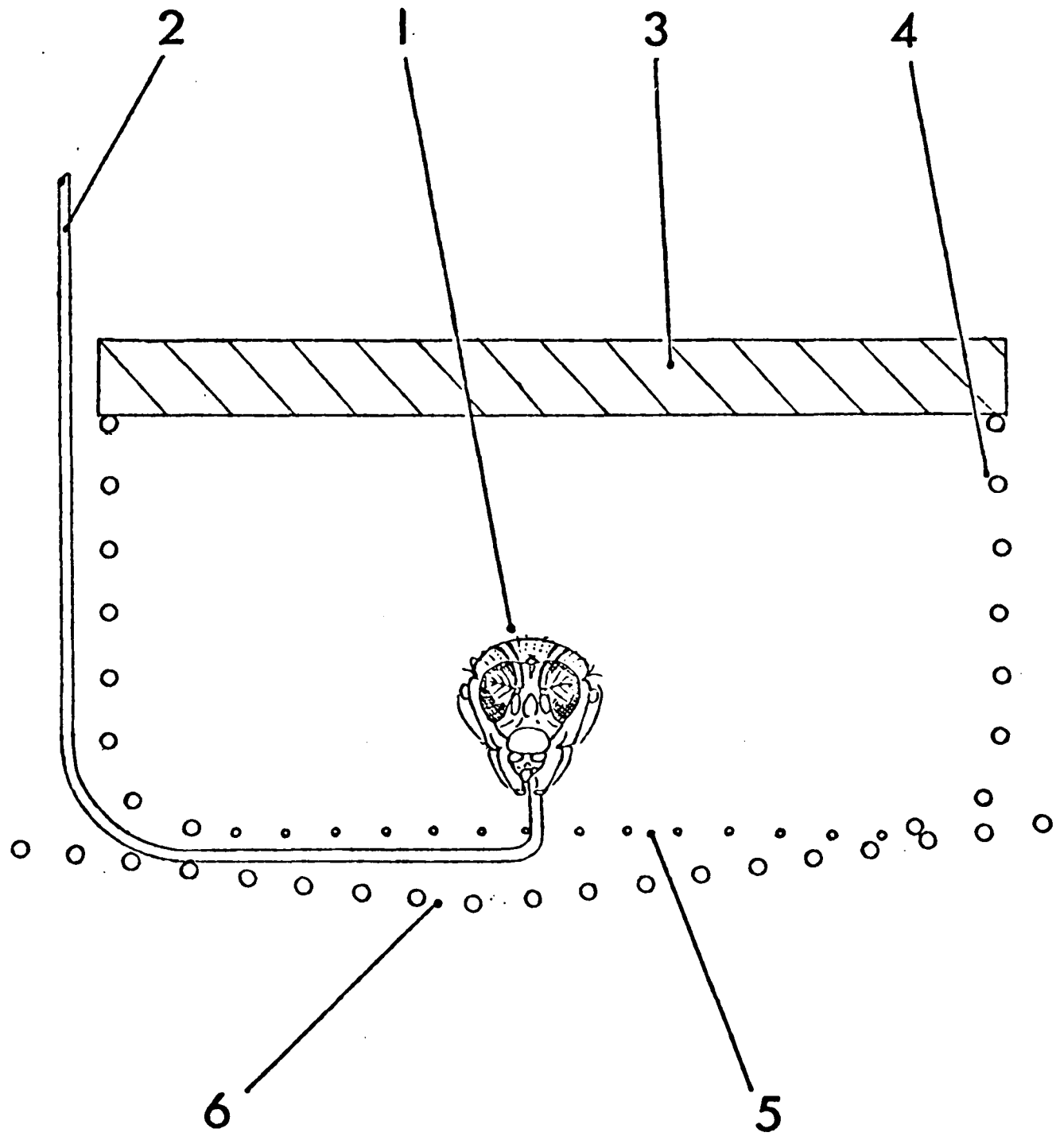
Fig. 23. Experiment 7: The method of positioning a female fly without wings and legs in an observation chamber.

1: the fly, viewed head-on;

2: 0.6 mm. dia. wire;

3,4,5: glass roof, wire mesh sides and gauze floor of the observation chamber;

6: wire mesh covering of the microphone ribbon.


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1
3
4
6
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conspecific and heterospecific females. The tendencies of males of the two species to court conspecific and heterospecific females that had had their wings and legs amputated were compared. The object of amputating the females' wings and legs was to minimize their behavioral repertoire. Observations were of pairs of flies placed together in an observation chamber.

4.6.1.2. Materials

Strain of flies used: Z. tuberculatus 124.5

Z. sepsoides 184.6 (see 2.2.).

Males and females were kept in single-sex populations in vials from the day of emergence. Males were kept at population densities of 2 per vial (see chapter 4) until observation. they were observed when aged between 9 and 18 days. Females were kept at population densities of up to 12 per vial, and were used when aged between 9 and 18 days.

4.6.1.3. Techniques & methods of observation.

The wings and legs of females were amputated. In order to perform the amputations, the females were rendered torpid by cooling. While still torpid, they were mounted on the end of a piece of wire of 0.06 mm diameter, attached to the underside of the thorax with a blob of wax. This piece of wire served as a mounting by which the female was attached to the floor of the observation chamber, with the tip of the abdomen clear of the floor and accessible to the male (see Fig. 23). The exact attitude of the fly could be adjusted to a limited extent by moving the end of the wire.

Two separate observation chambers were used; one for pairs in which the female was Z. tuberculatus, and the other for pairs in which

the female was Z. sepsoides. The male fly was introduced through a valve of thin latex sheet at one end of the chamber. The other end was made of thin celluloid sheet, to which the glass roof was attached by means of a hinge of latex sheet. The roof could thus be lifted off, to allow the installation of the female. The internal dimensions of the chambers were approximately:

Length: 10 mm

Breadth: 9 mm

Depth: 5 mm

At the beginning of a day's observations, the chamber which was to be used had one or more intact virgin females of the requisite species placed in it for a period of at least half an hour. They were then removed before the female without wings and legs was installed. Thus the observation chamber was intended to contain residual odours only of females of the species used in the observations.

Each observation period lasted for 1200s.

Recordings were made with the systems described in chapter 2.

Numbers of conspecific and heterospecific pairs observed were:

♂ Z. sepsoides X ♀ Z. sepsoides: N = 13

♂ Z. sepsoides X ♀ Z. tuberculatus: N = 10

♂ Z. tuberculatus X ♀ Z. tuberculatus: N = 14

♂ Z. tuberculatus X ♀ Z. sepsoides: N = 10

4.6.2. Results & Statistics. The following measures of male sexual activity were recorded: the total number of pulses of "Type 1" Song; the total number of pulses of "Type 2" Song; and the total duration of non-acoustical male sexual behaviours. Non-acoustical sexual

TABLE XX Comparisons of Sexual Behaviours of Male Z.sepsoides and Z.tuberculatus towards Conspecific and Heterospecific Females as Recorded in Experiment 7.

	♂ Z.sepsoides X ♀ Z.sepsoides	♂ Z.sepsoides X ♀ Z.tuberculatus	♂ Z.tuberculatus X ♀ Z.tuberculatus	♂ Z.tuberculatus X ♀ Z.sepsoides
n	13	10	14	10
Total durations of non-acoustical sexual behaviours	$\bar{X}$=81.7s SD=186.0s	$\bar{X}$=0.94s SD=2.82s	$\bar{X}$=103.1s SD=185.1s	$\bar{X}$=36.4s SD=70.4s
No. of copulations And attmepted copulations	2	0	2	0
Total Type 1 song durations	$\bar{X}$=13.3s SD=40.7s	$\bar{X}$=0s SD=0s	$\bar{X}$=5.7s SD=12.4s	$\bar{X}$=7.5s SD=19.0s
Total Type 2 song durations	$\bar{X}$=4.4s SD=10.7s	$\bar{X}$=0s SD=0s	$\bar{X}$=7.5s SD=18.1s	$\bar{X}$=1.0s SD=2.1s

behaviours were defined as consisting of the following: Orientation (including Grappling, which is performed during Orientation, and Circling and Stationary Display, in which the male remains Orientated towards the female); and time spent Mounted or copulating. The measure of non-acoustical male sexual activity generated by summating the durations of these behaviours shows a bias towards those males which copulated or repeatedly attempted to do so, since these behaviours take longer to perform than do other sexual behaviours. This bias is considered to be realistic; males which copulate, or attempt to do so, are showing higher levels of sexual activity than those which do not.

Results are given in Table XX and below. The differences in these total durations between males paired with conspecific females and those paired with heterospecific females where tested for significance with the following Mann-Whitney U-tests:

a/. ♂*Z. tuberculatus* X ♀*Z. tuberculatus*:

Total durations, s: 0, 0, 0, 0, 0, 0, 0. 13.01, 34.51, 71.96, 360.89, 363.66, 579.76

$R_1 = 179$

♂*Z. tuberculatus* X ♀*Z. sepsoides*:

Total durations, s: 0, 0, 0, 0, 0, 2.83, 6.83, 7.42, 137.05, 209.56

$R_2 = 121$

$N_1 = 14$; $n_2 = 10$; $n_1 n_2 = 140$

$P < 0.05$ if $U \leq 36$

$U_1 = 66$; $U_2 = 73$; $P > 0.05$

b/. ♂*Z. sepsoides* X ♀*Z. sepsoides*:

Total durations, s: 0, 0, 0, 1.98, 1.98, 2.02, 2.91, 7.05, 7.52, 10.63, 201.16, 385.52, 622.18

$R_1 = 196$

♂*Z. sepsoides* X ♀*Z. tuberculatus*:

Total durations, s: 0, 0, 0, 0, 0, 0, 0, 0, 0, 9.41

$R_2 = 74$

$n_1 = 13$; $n_2 = 10$; $n_1 n_2 = 130$

$P < 0.05$ if $U \leq 33$

$U_1 = 25$; $U_2 = 111$; $P < 0.05$

Thus male *Z. sepsoides* courted conspecific females significantly more than heterospecific females, but the differences between levels of sexual behaviours of male *Z. tuberculatus* twoards conspecific and heterospecific females were not significant.

4.6.3. Discussion. The results of this experiment appear clearly to demonstrate the ability of male *Z. sepsoides* to discriminate between conspecific and heterospecific females by recognising differences that are not behavioral, and presumably not morphological, since the two species are morphologically almost identical. it is therefore reasonable to assume that the discrimination is based on chemosensory cues. However, this experiment does not show whether these are olfactory cues, or whether they are chemotactile, perceived through the tarsi on contact with the female or with pheromone residues on the walls of the observation chamber.

There is some indication that male *Z. tuberculatus* also court conspecific females more than female *Z. sepsoides*, but the difference is not statistically significant. Their faculty for species recognition

by chemosensory cues, if it exists, is plainly unreliable, at least under experimental conditions.

The results of this experiment are closely consistent with those of Experiment 2. However, males generally showed less tendency to court wingless and legless females in this experiment than the intact females in Experiment 2. From this it would appear that males may use movement as a cue for recognition of females, or at least of flies generally. This is not improbable. Connolly et al. (1969) and Burnet & Connolly (1973) have shown that mutant Drosophila which are unable to perceive movement visually have reduced mating success rates.

4.7. CONCLUSIONS.

On the whole, the results of the experiments described in this chapter are inconclusive. Experiment 7 appeared to demonstrate fairly clearly that male Z. sepsoides can distinguish between females of the two species by chemosensory means. The method of quantifying male sexual behaviour was relatively crude, the number of replicates performed was small, and the statistical analysis applied to the results was therefore of necessity a conservative one. Nevertheless, the effect noted was statistically significant ($P < 0.05$). Male Z. tuberculatus, however, were not demonstrated to possess this ability, although the results suggest that this could possibly have been because insufficient replicates were performed.

The other experiments appeared to give conflicting results. Experiment 3 appeared to demonstrate that male Z. tuberculatus can distinguish the sex of conspecifics by chemosensory means, but Exper-

iment 4 failed to corroborate this. Again, the results of Experiment 5 appeared to indicate that female Z. sepsoides can distinguish a male fly's species by oflactory means, but the results of Experiment 6 failed to give any such indication.

Taken as a whole, such results as were obtained are broadly consistent with the observations taken in Experiments 1 and 2. It is reasonable to believe that males can distinguish a conspecific fly's sex by chemosensory means, especially since this has been demonstrated in other Drosophilidae. The results of Experiment 2 seemed to indicate that Z. sepsoides can distinguish between the two species more reliably than can Z. tuberculatus. The results of Experiments 5 and 7 appear to support this conclusion. However, the results of Experiment 5 remain the only evidence that females of either species may use chemosensory cues for identifying other flies.

CHAPTER 5

Experiments to Investigate the Possible Use of Male Courtship Songs by Female *Z. tuberculatus* and *Z. sepsoides* for Species Recognition.

Contents:

5.1. Introduction

5.2. Experiment 7: Materials & Methods

5.3. Results & Statistics

5.4. Discussion

5.1. INTRODUCTION

5.1.1. Aims. It was hypothesised in chapters 2 and 3 that in *Z. tuberculatus* and *Z. sepsoides* females will not allow a male to copulate unless he produces "Type 2" Song, and female *Z. sepsoides*, at least, can distinguish between "Type 2" Songs of the two species, and will only copulate with a male if he produces conspecific song. The observations described in this chapter were made with the following aims:

1/. To investigate whether or not females would allow copulation if the male did not produce any "Type 2" Song; and, if not, whether they would accept artificially-simulated "Type 2" Song as a substitute.

2/. To investigate whether females of either species could discriminate between artificially-simulated conspecific and heterospecific "Type 2" Songs.

5.1.2. Summary of previously published work. Courtship songs are produced by males by wing vibration, and perceived via the aristae of their antennae. Experiments designed to investigate the functional roles of courtship songs have mostly entailed the amputation of the male's wings (thus silencing the male), or amputation of the female's antennae or aristae (thus deafening the female).

Removal of the female's antennae was first shown to reduce her receptivity by Mayr (1950), in a study on Drosophila pseudoobscura and D. persimilis. Manning (1959a) reported the same in D. melanogaster, and Petit (1959) identified the aristae and Johnston's Organ as being the part of the antennae which were needed to perceive courtship stimuli. However, Ikeda et al. (1981) claimed that in D. mercatorum removal of the aristae affected receptivity less than removal of the entire antennae. Manning (1959a) also showed that removal of the male's wings also reduced mating success. Manning (1967a) then showed conclusively that females only respond to male wing vibration if they have the aristae on their antennae. These reports clearly indicate that male songs generally increase female receptivity.

Previously published evidence that male courtship songs are used as cues for species recognition has been summarized in 3.4.3.

The most direct method of investigating the functional roles of male song is the use of artificially simulated "song". Bennet-Clark & Ewing (1967) showed that in D. melanogaster courtship success rate was greatly reduced by amputating the male's wings but was to a

considerable extent restored by the provision of artificially simulated song. Bennet-Clark & Ewing (1969) then showed that the pulse period artificially simulated song was critical for achieving this effect. If the pulse period was one-half, or twice, that of natural conspecific male song, courtship success rate was only very slightly greater than if no simulated song was provided. The courtship success rate was also significantly reduced if simulated D. simulans male song was provided. However, the effect of simulated D. melanogaster male song on the courtship success rate of wingless male D. simulans has not yet been reported.

Bennet-Clark et al. (1973) used artificially-simulated male song to show that female D. melanogaster summate the auditory stimuli that they receive during courtship. Their receptivity was enchanced if they were "pre-stimulated" with five minutes of simulated song before they were introduced to males. Von Schilcher (1976a) described a second male song in D. melanogaster, called "sine song". Von Schilcher (1976b) then reported that while sine song increases female receptivity generally, pulsed song chiefly increases receptivity if the female is actually being courted by a male at the time.

Von Schilcher (1976a) also reported that simulated pulse song affects male behaviour, increasing their locomotory activity (it decreases female locomotory activity). The sound intensity has to be increased greatly in order to produce this effect in males which have had their aristae amputated.

EXPERIMENT 8

5.2. MATERIALS & METHODS

5.2.1. Strains of flies used: Z. tuberculatus 124.5

Z. sepsoides 184.6 (see 2.2.).

All the flies used were aged between 8 and 21 days, and had been kept in single-sex populations in vials from the day of emergence. Males had been kept at population densities of 2 per vial (see Chapter 3); females were kept at population densities of up to 15 per vial.

5.2.2. Observation Techniques. The effect of electronically-simulated conspecific "Type 2" Song on the intraspecific courtship success rate of males of both species which had had their wings amputated was assessed. It was then compared with the effect of simulated heterospecific "Type 2" Song on their intraspecific courtship success rates.

An electronic insect sound simulator of the design described by Robinson & Ewing (1978) was used. It was connected to a loudspeaker, positioned 20 cm directly beneath an observation chamber, and pointing directly upwards. The observation chambers were made of glass, with a roof and floor of fine gauze, the latter consisting of two slightly overlapping pieces forming a valve through the flies were introduced via a funnel. They were approximately cubic, with an internal side length of 1 cm.

The output of the sound simulator was monitored and calibrated

TABLE XXI Characteristics of the electronically-simulated "Type 2" Songs used in Experiment 8.

	Sound Particle Velocity Level	Pulse Period	No. Cycles per Pulse	Carrier Frequency
Z.tuberculatus	78dB approx.	32ms	1	---
Z.sepsoides	78dB approx.	54ms	3	170Hz approx.

Fig. 24. Experiment 8: Oscilloscope traces of the simulated "Type 2" male songs.

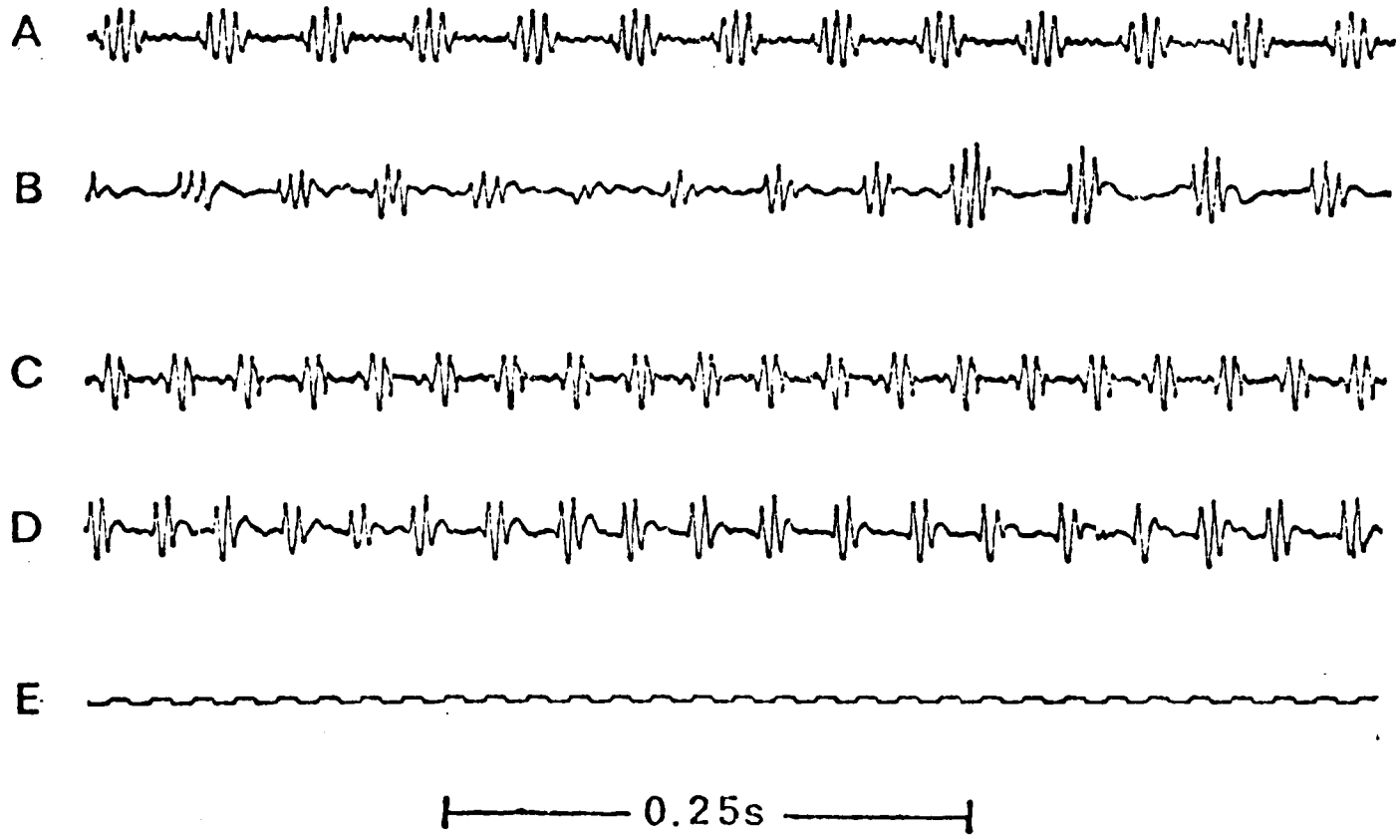
A. Simulated Z. sepsoides song.

B. Genuine Z. sepsoides song, for comparison.

C. Simulated Z. tuberculatus song.

D. Genuine Z. tuberculatus song, for comparison.

E. 50Hz. time marker.


A
B
C
D
E
0.25s

with a Grampian GR2 ribbon microphone positioned approx. 5 cm above the observation chamber, connected to an oscilloscope via a X10 pre-amplifier. This microphone was also used to measure the sound particle velocity level by the following method: it was calibrated using an A.C. voltmeter in the far-field, where sound pressure and particle velocity obey the inverse square law, against the output of a calibrated pressure-sensitive microphone (B&K type 4117). Using tis calibration, the sound particle velocity at the experimental chamber was obtained from the ouptut on the same voltmeter. This calibration is subject to the following errors: in the near field, the rate of change of particle velocity with distance from the sound source obeys the inverse 6th power law; and particle velocity is sensitive to the effects of such obstructions in the sound field as the microphone itself.

The characteristics of the simulated "Type 2" Songs of the two species are given in Table XXI. Fig. 24 shows oscilloscope traces of the simulated songs, with traces of recordings of the genuine songs produced by the two species for comparison. In a series of pilot observations these simulated songs were found to increase the courtship success rates of wingless males of both species. Mono- and dicyclic simulated Z. sepsoides songs of the same pulse period did not apparently enable wingless males to copulate. This is interesting, because although it was noted in Experiment 1 that Z. sepsoides produced proportinately more polycyclic "Type 2" Song than did Z. tuberculatus (see 2.5.1.), the ratio of mono-: polycyclic pulses did not apparently affect the courtship success rate in either

species. Many Z. sepsoides courtships contained almost entirely monocyclic "Type 2" Song, and a high proportion of the polycyclic pulses produced were dicyclic.

Two observation chambers were used, one for observations of each of the two species. They were periodicially washed by leaving them in distilled water overnight, and the gauze roofs and floors were replaced.

Methods of observation were as follows: each male had its wings amputated on the day previous to observation, and was observed for a period of up to 30 minutes with a conspecific female. If copulation occurred, observation was terminated at the start of copulation. The durations of courtships by the male were summated. The following behaviours were defined as "courtship": Orientation, Grappling and Mounting. A successful courtship ended with the start of copulation. In observations where song simulation was provided, the song simulator, producing continuous artificial "song", was switched on whenever the male started courtship, and switched off again whenever he stopped courting. The total duration of artificial "song" provided was, therefore, the same as the total courtship duration.

The following observations were excluded from the analyses: 1/ those in which no courtship was observed; 2/ those in which the female was found to be "unreceptive". All females which were courted but did not copulate during the observation period were observed again on the same day with a winged male, with no song simulation provided. A female was deemed to be "unreceptive" if she did not copulate with the winged male.

Two sets of observations were taken:

1/ The courtship success rates of wingless males with and without simulated conspecific "Type 2" Song were compared. Each male was observed with two females consecutively. In one observation period, simulated conspecific song was provided, and in the other, no simulated song was provided. The order in which the observation periods with and without simulated song were carried out was varied.
2/ The courtship success rates of wingless males with simulated conspecific and heterospecific "Type 2" Songs were compared. The procedure was similar to that of the first set of observations. In each case, the courtship success rates were compared by means of 2X2 χ^2 tests.

5.3. RESULTS & STATISTICS

5.3.1. The Intraspecific Courtships of Wingless Males With and Without Simulated Conspecific "Type 2" Songs. See Table XXII.

a. *Z. tuberculatus*. Copulation always occurred when song simulation was provided, but only occurred once when no song simulation was provided. The contingency table is thus:

	With simulated song	Without simulated song
Copulated	O = 7	O = 1
	E = 4	E = 4
No copulation	O = 0	O = 6
	E = 3	E = 3

Where O = observed score, E = score predicted by the null hypothesis,

$\chi^2 = 10.5$

with 1 d.f., $P < 0.01$

Of the six males that failed to copulate without song simulation, four succeeded in Mounting at least one. None of the females were unreceptive.

b. Z. sepsoides. Seven out of the ten males observed copulated when song simulation was provided, but only one copulated when no song simulation was provided. The contingency table is thus:

	With simulated song	Without simulated song
Copulated	O = 7	O = 1
	E = 4	E = 4
No copulation	O = 3	O = 9
	E = 6	E = 6

$\chi^2 = 10.5$

with 1 d.f., $P < 0.01$

Of the nine males that failed to copulate without song simulation, four succeeded in Mounting at least once. Three of the females observed were unreceptive.

5.3.2. The Intraspecific Courtships of Wingless Males With Simulated Conspecific and Heterospecific "Type 2" Songs. See Table XXIII.

a. Z. tuberculatus. Of the nine males observed, six copulated both when conspecific and heterospecific simulated songs were provided. The

only male which failed to copulate when conspecific song was provided did copulate when heterospecific song was provided. The contingency table is thus:

	Conspecific Song	Heterospecific Song
Copulated	$\underline{O} = 8$	$\underline{O} = 7$
	$\underline{E} = 7.5$	$\underline{E} = 7.5$
No copulation	$\underline{O} = 1$	$\underline{O} = 2$
	$\underline{E} = 1.5$	$\underline{E} = 1.5$

$\chi^2 = 0.4$

with 1 d.f., $\underline{P} > 0.1$

The mean duration of successful courtship is shorter with conspecific than with heterospecific simulated song, but when tested with a Mann-Whitney U-test this difference did no reach significance ($\underline{P} < 0.1$). Three of the females observed were unreceptive.

b. Z. sepsoides. All seven males observed copulated when simulated conspecific song was provided, but only one also copulated when simulated heterospecific song was provided. The contingency table is thus:

	Conspecific Song	Heterospecific Song
Copulated	$\underline{O} = 7$	$\underline{O} = 1$
	$\underline{E} = 4$	$\underline{E} = 4$
No copulation	$\underline{O} = 0$	$\underline{O} = 6$
	$\underline{E} = 3$	$\underline{E} = 3$

$\chi^2 = 10.5$, with 1 d.f., $\underline{P} < 0.01$

TABLE XXII. The intraspecific courtships of males With and Without simulated conspecific Type-2 song

Total courtship durations, s			With simulated song	Without simulated song
Z. tuberculatus	winged, copulated	N		8
		$\bar{X}$	----------	111s
		SD		88s
	wingless, copulated	N	7	1
		$\bar{X}$	29s	170s
		SD	17s	0
	wingless, copulated	N	0	6
		$\bar{X}$		1226s
		SD		501s
Z. sepsoides	winged, copulated	N		19
		$\bar{X}$	----------	53s
		SD		54s
	wingless, copulated	N	7	1
		$\bar{X}$	49s	102s
		SD	45s	0
	wingless, no copulation	N	3	9
		$\bar{X}$	806	585s
		SD	503s	404s

TABLE XXIII. The intraspecific courtships of wingless males with Conspecific and Heterospecific simulated "Type 2" Songs provided.

Total courtship durations		Conspecific Song	Heterospecific Song
	N	8	7
copulated	$\bar{X}$	43	113s
	SD	25	82s
Z. tuberculatus			
	N	1	2
no copulation	$\bar{X}$	571s	336s
	SD	0	304s
	N	7	1
copulated	$\bar{X}$	66s	84s
	SD	98s	0s
Z. sepsoides			
	N	0	6
no copulation	$\bar{X}$		919s
	SD		629s

Of the six males that failed to copulate when simulated heterospecific song was provided, four succeeded in Mounting at least once. Eight of the females observed were unreceptive.

5.4. DISCUSSION

The results of this experiment indicate that "Type 2" Song is obligatory, or nearly so, for successful courtship by a male, and that the simulated "Type 2" Songs used here provide an adequate substitute for it in the courtships of males which have had their wings amputated.

Female *Z. tuberculatus* can probably discriminate between conspecific and heterospecific "Type 2" Song, and copulate after a shorter courtship if the accompanying song is conspecific. However, they will still copulate with males when *Z. sepsoides* song is provided, under experimental conditions. It must be remembered that male *Z. sepsoides* do not readily court female *Z. tuberculatus*, so under natural conditions females probably only encounter *Z. sepsoides* song rarely, and there is little selection pressure for them to be able to distinguish between it and conspecific "Type 2" Song.

Females of this species mate much more quickly with wingless males when simulated conspecific song is provided than with winged control males. The simulated song may be a supernormal stimulus in any of the following possible ways:

1. Simulated song was provided continuously throughout courtship, unlike genuine song produced by winged males.

2. Simulated song was more regular than genuine song.

3. Simulated song may be louder than genuine song. It is virtually impossible to compare the amplitudes of simulated and genuine songs perceived by the female, because simulated song is produced from a large source at a considerable distance from the female (in the far field), while genuine song is produced by a small source (the male's wings) very near the female (in the near field).

It is possible, therefore, that female Z. tuberculatus may be stimulated sufficiently to allow copulation by simulated Z. sepsoides song, but not by genuine Z. sepsoides song. However, there is evidently no total barrier preventing female Z. tuberculatus from mating with a male when only Z. sepsoides-like "Type 2" Song is provided during courtship.

Female Z. sepsoides, however, usually refuse to copulate with a male that does not produce conspecific song. It must be remembered that male Z. tuberculatus will court female Z. sepsoides fairly readily, so there is presumably a strong selection pressure for female Z. sepsoides to distinguish between conspecific and Z. tuberculatus "Type 2" Song.

One other interpretation of these results is possible. It has been reported by Von Schilcher (1976a) that male song also affects male behaviour - see 5.1.2. Failure of wingless male Zaprionus to copulate when no simulated conspecific song is provided could be because they court less vigorously than when it is provided. If this is the case, then male Z. sepsoides are more discriminating between conspecific and heterospecific songs than are male

Z. tuberculatus.

However, the courtship duration statistics show that the males which failed to copulate did generally court vigorously. Even in the absence of conspecific song, they often mounted the female, and attempted to mount even more frequently. Males which did mount were observed to attempt copulation repeatedly. Thus the almost total failure of wingless males to copulate when no song simulation was provided, and of male Z. sepsoides to copulate when heterospecific song was provided, is not easily accounted for by this hypothesis alone.

This possibility will, however, have to be investigated by repeating this experiment with males that have had both their wings and the aristae of their antennae amputated, thus deafening as well as silencing them. Lack of time prevented this from being tried. If this treatment does not completely restore their ability to copulate in the absence of simulated conspecific song, then it can be concluded that females can discriminate between the songs, and mate preferentially with males whose courtship is accompanied by conspecific song. But until this experiment is performed, this interpretation of the results given here must remain tentative.

CHAPTER 6

Conclusions

Contents:
6.1. The Courtships of Z. tuberculatus and Z. sepsoides
6.2. The Species Isolating Mechanism Operating between Z. tuberculatus and Z. sepsoides.

6.1. THE COURTSHIPS OF Z. TUBERCULATUS AND Z. SEPSOIDES.

The observations taken in Experiment 1 (chapter 2) of flies aged > 8 days confirmed the basic descriptions given by Bennet-Clark et al. (1980). Non-acoustical male courtship behaviours consists primarily of Orientation, Grappling, Mounting, and the start of copulation, performed in that order. These are accompanied by "Type 2" Song, produced in sequences of highly variable duration, throughout courtship, and always when the male is attempting intromission. Courtship can be brief, lasting only a few seconds from the initital Orientation to the start of copulation, or it can be more protracted, especially if the female is initially unreceptive. Longer courtships often include the optional components of Stationary Display, Dance, Circling, "Type 1" Song, and Scissoring.

Females are described as being either receptive or unreceptive. Some initially unreceptive females copulate after persistent courtship. Receptive females are sometimes observed to spread their wings slightly when courted, but this often occurs simultaneously with male Mounting, and so is not obvious. No other definite acceptance

response hs been observed. Unreceptive females respond to courtship by Rocking, Whining, Pointing, and Extruding, as described by Bennet-Clark et al. (1980), and also by decamping. Males are unable to mate with unreceptive females, but nonetheless often court them persistently. Females become unreceptive within a few seconds of the end of copulation. Virgin female Z. sepsoides are also often initially unreceptive. There are some indications that such females will become receptive after laying an (unfertilized) egg, and that under natural conditions they might respond to courtship by doing so, and then mating. However, this possibility has not yet been investigated with a formal experiment. Virgin female Z. tuberculatus are rarely unreceptive.

Sexual dimorphism in these species is slight. The results of Experiments 3, 5, and 6 (chapter 4) seem to indicate that sexual recognition in Z. tuberculatus is effected by olfactory cues percived by the male (although this was not corroborated by Experiment 4). Z. sepsoides probably employs a similar mechanism. This is similar to the mechanism of sex recognition used by Drosophila melanogaster (e.g. Tomkins et al., 1980; Venard & Jallon, 1980).

No consistent qualitative differences between the non-acoustical behaviours of the two species has been observed. The only major consistent interspecific difference is in the "Type 2" Song pulse periods. These are longer in Z. sepsoides than in Z. tuberculatus, with little overlap between the pulse period populations of the two species.

6.2. THE ISOLATING MECHANISM OPERATING BETWEEN Z. TUBERCULATUS AND Z. SEPSOIDES.

The observations taken in Experiment 2 (chapter 3) suggested the following: that the "Type 2" male song was obligatory for successful courtship; that there was an effective mechanism preventing male Z. sepsoides from copulating with female Z. tuberculatus, in which the male's recognition of the female's species appeared to be the most important factor; that male Z. tuberculatus, however, courted female Z. sepsoides quite readily, and under observation conditions sometimes copulated with them; so if there is an effective pre-mating reproductive barrier between male Z. tuberculatus and female Z. sepsoides, it must depend to a large extent on the female Z. sepsoides' ability to recognise the male's species; and that the courtships of males of the two species apparently only differed in the pulse periods of the "Type 2" Songs of the two species.

These observations were used as a basis for the following hypothesis: that the species isolating mechanism is asymmetric between these two species, the onus of species recognition lying largely on Z. sepsoides. Males of this species appear to recognise a female's species by olfactory or possibly chemotactile means. Females recognize a male's species by auditory and/or olfactory means. This is assuming, as is most likely, that the flies cannot distinguish between the two species by visual means. The results of Experiments 5, 7, and 8 support this hypothesis. The results of Experiment 6 did not give any evidence that females of either species use olfactory cues for sex or species recognition, but

the results of Experiment 5 suggested that female Z. sepsoides could recognise a male's species by olfactory means. The results of Experiment 7 appeared to demonstrate that male Z. sepsoides can recognise a female's species by chemical means. Results suggested that male Z. tuberculatus may also possess this ability to a limited extent, but these were not statistically significant. In Experiment 8, female Z. sepsoides were able to distinguish between conspecific and Z. tuberculatus "Type 2" Songs with a high degree of reliability, and refused to copulate with males unless their courtships were accompanied by conspecific song. Female Z. tuberculatus, however copulated with males if either species' "Type 2" Song was provided, although they apparently copulated after a shorter courtship if it was accompanied by conspecific song than if it was accompanied by Z. sepsoides song.

An asymmetry of this nature has not yet been described comprehensively in the isolating mechanisms between any other drosophilid species whose courtships have been studied, although there appears to be some asymmetry in the mechanism isolting Drosophilia melanogaster from D. simulans. Female D. melanogaster discriminate between the pulsed songs with a pulse period typical of their species, and those with other pulse periods (Bennet-Clark & Ewing, 1969). Male D. simulans discriminate between females of the two species more reliably than do male D. melanogaster (Wood & Ringo, 1980). However, no investigation of the ability or otherwise of female D. simulans to discriminate between the male songs of the two species has yet been published.

There is nothing inherently unlikely about such an asymmetry. A possible means by which it might evolve is suggested by Arita & Kaneshiro (1979), who report a partial asymmetric isolation which arose between two laboratory cultures of Drosophila adiastola (see 1.3.). These cultures were both descended from single gravid females taken from the same locality on different occasions. It is suggested that the sexual isolation resulted from the effect of genetic drift at the time of foundation of the two cultures (see Powell, 1978).

This could also occur in nature, causing incipient speciation by the "founder-flush" effect (see 1.3.). A new colony may be founded by a few individuals, or a single female, and may undergo population "crashes". Under such conditions, genetic drift may occur, and partial asymmetric reproductive isolation may arise as a result of this. If two populations acquire partial reproductive isolation, there may be a selection pressure for the isolation to become total. This will occur if the two populations have become adapted to different ecological niches, and hybrids are less viable than their parents in either niche. Partial asymmetric isolation between two populations, A and B, occurs if one sex (sex a) of population A recognises, and mates preferentially with, members of its own population (n.b. this is unlikely to occur if the "rare-type mating advantage" effect reported by some authors operates - see 1.2.). Isolation may then become total in either of the following ways: sex a of population b may also acquire a mating preference for its own population; or the other sex (sex b) of population A may acquire a mating preference for its own population. In the former case, the isolating

mechanism is symmetric, relying on population recognition by sex a. In the latter case, the mechanism is asymmetric, the onus of recognition lying on both sexes of population A.

If it is assumed that Z. tuberculatus and Z. sepsoides share an immediate common ancestor, then the asymmetric isolating mechanism between the two species may have evolved via a state of partial asymmetric isolation as described above. The "reduced viability" of hydrids may have been in fact the infertility of hybrid eggs, or this may have arisen later, after the two populations had acquired specific status.

Another possible means by which asymmetric isolation might evolve is suggested by Zouros & D'Entremont (1980). They found that females of a population of Drosophila mojavensis which was sympatric with the sibling species D. arizonensis were highly discriminating in their choice of mates, and were sexually isolated from males from regions where D. arizonensis did not occur. (In this species, mate choice is apparently performed entirely by the female.) They suggested that the presence of D. arizonensis in one region was causing the local population of D. mojavensis to evolve towards specific status. This mechanism is, however, unlikely to have been the cause of the ancestral Z. sepsoides population acquiring reproductive isolation from other populations of Z. tuberculatus, because there is today no other known sibling species that is sympatric with Z. sepsoides. (The only other known species in the tuberculatus species group is Z. mascariensis, which appears to have evolved in isolation on the Mascarene Islands, where

Z. sepsoides is not found. See Bennet-Clark & Leroy, 1977).

Nonetheless, it is still tempting to suggest that inferences about the phylogenetic relationship between the two species may be drawn from the nature of the asymmetry in the reproductive isolating mechanism operating between them. Kaneshiro (1976) reports that in the planitibia species complex of Hawaiian Drosophila, ethological isolating mechanisms operating between allopatric species under laboratory conditions are partial and asymmetric, and that "females of the ancestral species are more discriminating against males of the derived species than conversely". Watanabe & Kawanishi (1979) proposed a different model of speciation, which predicted an asymmetry in the opposite direction. Wasserman & Koepfer (1980) proposed a third model, and gave results of experiments on the sibling species Drosophila arizonensis and D. mojavensis intended to test their hypothesis.

However, in the instance of these two species of Zaprionus it is debatable whether the words "ancestral" and "derived", as describing the two species, can have any real meaning. The term "derived" is presumably meant to describe a species that was originally descended from a small isolated population, while the "ancestral" population remained large and wide-ranging; the "derived" population, it is supposed, underwent faster evolutionary change than the "ancestral" population. But since there is no way of checking any of these preassumptions in the case of these two species, which are sympatric throughout tropical Africa, I suggest that it is meaningless to call one species "ancestral", and the other "derived". The two

species are presumed to share an immediate common ancestor, from which both are "derived".

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