THE COURTSHIP BEHAVIOUR AND SONGS OF THE DROSOPHILA VIRILIS SPECIES GROUP

by

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I hereby declare that this thesis was composed by myself, and that the work it contains is my own.

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NEWBURY, S. F., DONEGAN, J. and STEWART, J. A. (in press) *Drosophila virilis* and its cosmopolitan relatives in urban islands.

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ABSTRACT

Courtship in species of the Drosophila virilis group consists of a series of bouts, or sequences, of courtship behaviour punctuated by periods of non-courtship behaviour. Within each sequence, male behaviours tend to occur in a set order and are superimposed upon one another. This hierarchical patterning is interpreted in terms of a simple motivational model involving separate thresholds for the release of these behaviours under common internal sexual excitation.

Courtship sequence length (the time available to the male to perform his courtship repertoire) is largely under the control of the female since courtship can only occur when the male has contact with the female. Evidence is presented that the female summates the sexual stimulation provided by the courtship of the male, and that the most important component is the courtship song which results from wing vibration.

Female pulse song, which results from wing fluttering, is neither obviously attractive to the courting male, nor is it species-specific or sex-specific. It is proposed that fluttering behaviour in general is a spacing behaviour, which is probably most effective in maintaining the "individual distance" between females at feeding/mating and oviposition sites.

In general, the similarity of the courtship songs of the virilis phylad, and the dissimilarity of the songs of the montana phylad, reflect the close, and more distant, genetic relatedness, respectively, of the species of these two phylads. However, passive change in song characteristics as a by-product of other adaptive processes may not be a sufficient explanation for present-day song patterning since a more detailed examination reveals that certain species of the montana phylad possess similar songs. Evidence from sexual isolation studies and the present-day distribution of the virilis group species is used to examine the possibility that, as a conservative character, large changes in song patterning only occur where there is selection pressure for effective premating isolation mechanisms, i.e. in zones of species overlap.

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 ACOUSTIC COMMUNICATION IN INSECTS

1.1.1 INTRODUCTION

The purpose of this chapter is to provide a short, general review of acoustic communication in insects. There is, of course, a wealth of material available on this topic, so clearly, in a brief review of this kind, it is only possible to point out the main areas of interest and provide some examples. Hopefully, though, this should be sufficient to allow the reader: (i) to place the particular subject of this thesis, the courtship behaviour and songs of the Drosophila virilis species group, in the general context of insect acoustic communication; (ii) to identify the general principles which are involved in the examination of any acoustic communication system, namely: the identification of the mechanisms of sound production and sound reception, and the investigation of the function and evolution of the acoustic signal(s). A short introduction to the present research itself is presented in Section 1.2.

At this point it might be wise to state exactly what is meant by the term "communication". The following definition comes from BURGHARDT (1970): "Communication is the phenomenon of one organism producing a signal that, when responded to by another organism, confers some advantage (or the statistical probability of it) on the signaller". In other words intent, viewed in the context of the emitter's adaptive behaviour based upon previous phylogenetic (and ontogenetic) events, is a necessary aspect of signalling. Similarly OTTE (1974) distinguishes between the function and effect of a signal: ""function" designates the special action of any part of a living organism that evolved because such action fostered survival or reproduction. An "effect", on the other hand, is a mere by-product of a characteristic". For instance, the function of a male orthopteran calling song may be to attract sexually receptive females, but an effect may be that it attracts predators to the signaller. Also, as BURGHARDT (1970) points out, communication must involve information transmission; for example, telling a man to jump off a bridge is an example of communication whereas physically pushing him off is not! This problem will be encountered in Chapter 4 where the role of female song and male aggressive song as spacing signals is considered: these songs are produced as a result of wing fluttering, which may itself have a direct spacing effect in that it may physically delineate a "personal space" around the fly producing the song.

1.1.2 MECHANISMS OF SOUND PRODUCTION

(General references: DUMORTIER (1963a); SOTAVALTA (1963); CHAPMAN (1969a); RICHARDS and DAVIES (1977a); LEWIS and GOWER (1980); EWING (1984)).

Sounds of different kinds and intensities are produced by a number of species in all the major orders of insects. Sound may be produced in one of four main ways: (i) stridulation; (ii) vibration; (iii) percussion; (iv) emission of air. (i) Stridulation , which involves the rubbing of two parts of the body against one another, has evolved many times in different groups of insects, with perhaps the best-known being the Orthoptera. In the grasshoppers (Acrididae), the hind leg is rubbed against the forewing (tegmen); the plectrum, which may be on the tegmen (Acridinae) or the femur

(Oedipodinae), is drawn across a series of teeth (the file or comb) on the opposing structure. In the crickets (Gryllidae) and the bush crickets (Tettigoniidae), the plectrum and file are situated on the two tegmina. In many cases, parts of the tegmen act as a resonator and radiate sound energy. Sound may also be produced by the rubbing together of the two antennae, e.g. in some species of the Phyllidae (Phasmida) where a plectrum and file are situated on the third segment of both antennae. In the waterboatman (Corixidae:Heteroptera) the file is present on the inner surfaces of the prothoracic femora whilst the plectrum consists of the thickened flanges of maxillary plate. Sound production by stridulation is also found in the Coleoptera and Lepidoptera.

(ii) Vibration may involve the wings, parts of the body or specialised structures called tymbals. The wing vibration of certain Hymenoptera and Diptera is usually employed for close-range acoustic signalling. In the Hymenoptera, certain species of braconid wasp produce a characteristic sound with their wings during courtship and syrphid males court females by producing a high pitched hum as they fly over a female on a flower. This behaviour has also been observed in certain bees (Megachile, Anthopora, Anthidium). Examples from the Diptera include the courtship songs of Drosophila species which involve the vibration of one or both wings in the vicinity (within 1 cm) of the female, and the flight tone of sexually receptive Aedes aegypti females which males of this species find attractive (ROTH (1948)). Vibration of the body is found in several groups: examples include the gall midges (Lipara), the tettigoniid, Copiphora rhinoceros, and the acridoid, Zonocerus variegatus. The vibrations are transmitted via the substrate.

Finally, the paired tymbal organs, each of which consists of a "drum" which is vibrated by the action of powerful muscles, are situated in the abdomen of many species of the Cicadidae (Homoptera), and the metathorax of some Lepidoptera.

(iii) Percussion involving the striking together of body parts is not very common. The best example comes from the genus Hecatesia (Lepidoptera) where modified costae on the forewings of these moths are struck together like castanets, producing a series of sound pulses. Percussion involving the tapping or drumming of part of the body against the substrate is more frequently encountered; for example, the abdomen is used in some stonefly (Plecoptera) species and in the psocopteran Trogium pulsatorium, and some wood-boring beetles ,e.g. Anobium, produce sound by striking their head against the wood.

(iv) Sound production by the emission of air is not common. The "cry" of the death's head hawk moth ,Acherontia atropos, results from the emission of air from the pharynx near the base of the proboscis, and the "hiss" produced by cockroaches of the genus Gromphadorhina is produced as air is forced out of partly closed spiracles.

1.1.3 MECHANISMS OF SOUND RECEPTION

(General references: AUTRUM (1963); CHAPMAN (1969b); RICHARDS and DAVIES (1977b); LEWIS and GOWER (1980).

Insects possess two basic types of sound receptor: moveable hairs, supplied with sensory cells and articulating on the body surface, and scolopophorus organs (also called chordotonal organs). The latter are the most important in the context of acoustic communication and can be divided into four basic types:

(i) Simple chordotonal organs, situated between the tibia and the tarsus (Diptera, Coleoptera, Hemiptera).

(ii) Subgenual organs (absent in the Coleoptera and Diptera), located in the legs at the proximal end of the tibia (Homoptera, Heteroptera, Neuroptera, Lepidoptera; the organ may also be in two parts: Odonata, Orthoptera); both (i) and (ii) respond to substrate-borne vibration.

(iii) Paired Johnston's organs, located between the second and third antennal segments (the organ is relatively simple in some Orthoptera, but is highly developed in the Culicidae and Chironimidae).

(iv) Paired tympanal organs (with tympanic membranes) (simple in Lepidoptera and Corixidae (Heteroptera), but highly specialised in the Cicadidae (Homoptera) and Orthoptera)). These organs are sometimes present in the wing-bases (some Lepidoptera, e.g. Satyridae) but are more commonly found in the thorax (Noctuidae (Lepidoptera), Corixidae), or the abdomen (Acridoidea, Cicadidae, some Lepidoptera), or the base of the fore-tibia (Tettigonioidea, Grylloidea).

1.1.4 FUNCTIONS OF ACOUSTIC COMMUNICATION

(General references: DUMORTIER (1963b); OTTE (1977); EWING (1984)). This section is concerned solely with sexual and agonistic signals, but it should be remembered that acoustic signals can also serve a warning function, e.g. the "hiss" produced by cockroaches of the genus Gromphadorhina (although this sound is also produced during courtship and aggressive interactions).

Acoustic signals are often classified according to the particular context in which the sound is emitted and its relation to other behaviours, e.g. sexual, agonistic, and the response of the receiver, or receivers, e.g. phonotaxis, acceptance posture. The loud "calling songs" performed by males of the Cicadidae, Tettigoniidae, Gryllidae and Acrididae serve to attract conspecific females from a distance. They may also result in the aggregation of males, e.g. many species of Gryllidae, Tettigoniidae and Cicadidae, or the spacing of males, e.g. katydid (Tettigoniidae) males. In addition, most Orthoptera produce "aggressive songs" which promote the spacing of males, and the calling song ("spontaneous call") of corixid species males may function in this way (AIKEN (1982)). In the Acrididae, females may produce an "agreement song" perhaps indicating sexual maturity and willingness to mate.

Males of many species of the Gryllidae and Acrididae produce "courtship song" whilst in the vicinity of the female and, in species of the Acrididae, males may produce a "jumping song" which is produced just prior to mounting. In courtship involving Palmacorixa nana (Corixidae) the "courtship call" is produced by the male alone whilst "mounting" and "copulation sounds" result from the action of the peg rows on the male mesothoracic femora on the hemelytra of the female (AIKEN (1982)). All these songs which are associated with courtship may serve in the identification of species and sex, sexually stimulate the female and/or ensure that she remains stationary. As with calling songs, courtship songs may also be responded to by other males, e.g. in Drosophila melanogaster courtship song results in an increase in the locomotor activity of nearby males (SCHILCHER (1976a)).

However, care must always be exercised when ascribing a particular function to a particular acoustic signal on the basis of the behavioural context alone. For example, BOAKE and CAPRANICA (1982) have shown that the "courtship song" of the gregarious cricket, Amphiacusta maya, is identical to that produced during male fighting and it appears that it may function in both contexts as an aggressive signal to other males.

The recording and analysis of acoustic signals allows their patterning to be investigated in detail. These techniques have shown that there are assemblies of closely-related species with distinct male calling songs within the Hemiptera, Diptera, Coleoptera, Orthoptera and Plecoptera. The courtship songs of many species of Gryllidae and Acrididae are also species-specific, as are those of many Drosophila species (e.g. EWING and BENNET-CLARK (1968); CHANG and MILLER (1978); EWING (1979); COWLING and BURNET (1981)). The species-specificity of these acoustic signals is good circumstantial evidence that they may be involved in sexual isolation.

Various experimental techniques have been employed in order to investigate the possible role of acoustic signals in sexual isolation. Y-maze or open arena experiments, in which the female is given a choice between the recorded calling songs of conspecific and heterospecific males, have been performed for several orthopteran genera, e.g. Allonemobius, Teleogryllus, Gryllotalpa; the female approaches the source of the preferred song. Alternatively, attempts have been made to elicit female agreement songs using conspecific and heterospecific songs; this method has been used successfully for pentatomid bug species (COKL, GOGALA and BLAZEVIC

(1978)) and corixid species (JANSSON (1973)).

The use of simulated song allows the song parameters which are important in providing sexual stimulation and/or species information to be determined. For example, in Teleogryllus oceanicus the male calling song consists of chirps (syllables packaged in discrete groups) and trills (syllable repetition rate is stable and continues for an extended period of time, sometimes several hours); however, females appear to prefer a song consisting of 100 percent chirps to the normal mixed song (POLLACK and HOY (1981)). In Drosophila melanogaster, the courtship song component may be artificially removed by male wing amputation and this results in a greatly extended courtship. If simulated conspecific male courtship song is then supplied to the courting pair, mating speed is returned to normal (e.g. BENNET-CLARK and EWING (1967); see also Chapter 6).

1.1.5 EVOLUTION OF ACOUSTIC COMMUNICATION

(General references: OTTE (1974,1977); LEWIS and GOWER (1980)).

Unlike vertebrate displays and signals which are probably derived from three principle sources (intention movements, displacement activities, autonomic responses), the displays and signals of insects may be ultimately traceable to movements serving other functions (flying, walking, etc.). The wing vibration involved in the production of song in many Drosophila species may have evolved from flight, requiring only the superimposition of further neural patterning upon the basic flight system. The same is probably true for members of the Gryllidae, Tettigoniidae and Acrididae. For example, in the Gryllidae, the wing beat frequency of Gryllus campestris, G.bimaculatus and Acheta domesticus during flight is

20-30 Hz which is almost exactly the same as the frequency of tegminal movements during stridulation. In the Acrididae, the basic stridulatory rate of *Chorthippus mollis*, *Ch.biguttulus* and *Gomphocerippus rufus* , for example, is 50 Hz which is exactly the flight frequency (the muscles which move the wings also move the legs). In other species, the individual movements of stridulation occur in steps which follow each other at full, half or double the flight frequency.

The first stage in the evolution of an acoustic signal probably involves the production of a sound which has no communicatory function but is incidentally perceived by other organisms. If there is some survival or reproductive advantage to the producer of the sound resulting from its perception by other organisms, selection will favour its production. Once an acoustic signal comes into being, diversification will occur, the rate of change and the amount of diversification being dependent on the function of the signal. For instance, in the Acrididae, the present diversity of the calling songs and courtship songs indicates that they have undergone extensive changes, presumably because of their importance in sexual isolation, whereas changes in agonistic signals have been more conservative.

OTTE (1974) provides a list of the various selective forces which may act to shape signalling systems, including: (i) identification and discrimination; (ii) sex-specific selection; (iii) predation; (iv) intrasexual competition; (v) mate choice.

(i) Identification and discrimination: evidence of various sorts exists (see Section 1.1.4) that species-specific acoustic signals function in preventing interspecific mating. Therefore, the clearest cases of song divergence might be expected to occur in areas of sympatry where selection would favour premating isolation mechanisms which prevent energetically wasteful hybridisation (see Chapter 5; Section 5.4.2). This would involve selection pressure on males to produce species-specific songs and on females to discriminate between songs. However, despite extensive studies of the acoustic Orthoptera of North America, unequivocal examples have not been found. Evidence from _Drosophila_ species is also fairly scanty, partly because of the difficulty of establishing whether species are truly sympatric or not, and partly because of the difficulty of estimating the contribution of change in song characteristics as a by-product of other adaptive processes (see Chapter 5). However, in the _Drosophila_ _affinis_ subgroup, MILLER, GOLDSTEIN and PATTY (1975) and CHANG and MILLER (1978) have found that the courtship songs of two, closely-related sympatric races, _D.athabasca_ ("eastern A") and ("eastern B") are distinctly different, whereas the songs of more distantly-related allopatric species, e.g. _D.athabasca_ ("eastern B") and _D.azteca_, are more similar to each other.

(ii) Sex-specific selection: one sex (usually the male) has a more active role to play in signalling and this can be related to differences in reproductive investment (see TRIVERS (1972)).

(iii) Predation: male calling songs are often loud, perhaps increasing the risk of predation, but this cost must be balanced (in an evolutionary sense) against the benefits of attracting females. Courtship songs, which are produced in the vicinity of the female, are generally soft and so are less likely to attract predators and rivals.

(iv) Intrasexual competition: many ground-dwelling Acrididae have similar aggressive songs and this may be due to the convergence of song patterning for interspecific effects, although it may simply result from common descent. In the genus Syrbula, the calling of one male elicits the calling of other males nearby. Perhaps the song of the second male reduces the probability that the first male will attract sexually receptive females, and so ensures that receptive females remain available. In the New Guinea longhorned grasshopper, Hexacentrus mundus (Tettigoniidae), a competing male may insert short sounds into the rhythmic calling song of another male, which presumably reduces the the song's attractiveness to females (LLOYD (1979)).

(v) Mate choice: if males differ in genotypic fitness (contribute inferior or superior genes to offspring) and this is reflected in observable phenotypic differences, then natural selection should favour those females that choose the phenotypes representing the most fit genotypes. This is sexual selection theory, but in practice it is often difficult to separate female mate choice from other factors: for example, louder calling songs could attract more females simply because they are perceived over greater distances, rather that because females prefer (or have preferred in the past) those males which produce louder songs. Similarly, the faster mating speed of large D.melanogaster males, which produce more courtship song than smaller males, may be due to the fact that these males provide more sexual stimulation (courtship song), or that females actively choose males that produce more song (PARTRIDGE and EWING (unpublished)).

In all probability, the evolution of insect acoustic communication systems has involved various combinations of these, and other, selective forces.

1.2 THE PRESENT RESEARCH

Since each chapter of this thesis is provided with its own detailed introduction, I shall restrict myself here to a discussion of some general points regarding the purposes of the research.

The general methods employed in this investigation of the courtship behaviour and songs of the Drosophila virilis species group are presented in Chapter 2. For all behavioural observations and sound recordings, the flies were placed in a small, wire-mesh trough cell (14mm x 27mm x 5mm). Since the flies are only about 3mm in body length, the dimensions of the cell allowed for a good deal of movement, although a courted female could never completely escape the attentions of an ardent male suitor.

As stated in Section 1.1.4, an understanding of the function of the songs employed by any species requires a detailed examination of the behavioural context in which the songs are produced. For this reason, a detailed description and analysis of courtship is performed (Chapter 3). The patterning of male courtship behaviour (Section 3.4.2.2) and the stimulatory function of male courtship song are investigated. The latter involves an investigation of both the temporal occurence of male wing vibration (Section 3.4.2.3) and the processing, by the female, of the sexual stimulation provided by male courtship as a whole but particularly the song component (Section 3.4.2.4). Females of the virilis group are particularly useful for the investigation of female courtship processing since they adopt a clearly recognisable acceptance posture when they are ready to accept the male; this allows the observer to pinpoint the exact moment in which the courtship of the male has proven satisfactory to the female. The general concept of female courtship summation is then examined in the light of the findings of the present research and the recent criticisms of ROBERTSON (1982) (Section 3.5).

Chapter 4 is concerned with the phenomenon of female song. The work is illustrative of the sort of problems which are encountered when investigating the function of a particular acoustic signal (see Section 1.1.4). Since the production, by the female, of a patterned song during courtship has not been reported for any other Drosophila species (although it has been investigated in species of the closely-related genus Zaprionus), its function is of particular interest. The first stage in the investigation involves an examination of the contexts in which females (D.a.americana) produce the song: is it only produced by sexually mature females or during courtship? A detailed analysis of the song produced in the various contexts is then performed (Section 4.2). Next, the songs produced by females of several species of the virilis group are compared both with each other and also with the aggressive songs produced by the males of these species, in order to determine whether female song is species-specific (Section 4.3) or sex-specific (Section 4.4), respectively. Finally, the effect on courtship of artificially removing the female song component is investigated; the method involves the observation and analysis of courtships involving either "silenced" females (wings amputated) or "deafened" males (aristae amputated) (Section 4.5).

The findings of these investigations are contrasted with those obtained for species of the genus Zaprionus (Section 4.6.2), and are also used in a re-examination of the circumstantial evidence supporting the view that female song might function as an attractive signal or sexual isolation mechanism. (Section 4.6.3). Female song is then examined ,not in isolation, but as a component of fluttering behaviour (Section 4.6.4). Finally, the role of the so-called "rejection responses" is reviewed (Section 4.6.5).

In Chapter 5, attention is turned to the male courtship songs of the virilis group species. The songs of several species are compared with each other, and similarities and dissimilarities are compared with what is known of the species' phylogenetic relationships, degree of sexual isolation and geographical distribution. The purpose of this work is to evaluate the relative importance of evolutionary change in song as a by-product of other adaptive processes, and as a direct result of selection for an effective sexual isolation mechanism in zones of overlap (see Section 1.1.4 and 1.1.5).

Chapter 6 returns to the subject of the stimulatory function of male courtship song first discussed in Chapter 3. The male courtship song component is artificially removed by "silencing" the males (wing amputation) and "deafening" the females (arista amputation); courtships involving intact and operated individuals are compared, particularly with regard to mating speed and courtship duration (if no mating occurs) (Section 6.2). In Section 6.3, the role of the arista in the reception of song is examined neurophysiologically by recording from the antennal nerve of intact and aristaeless females whilst recorded or simulated courtship song is played back to them. Finally, in Section 6.4, attempts are made to elicit the acceptance posture from females using simulated song alone.

CHAPTER TWO

GENERAL METHODS

2.1 STOCKS

The following strains were used in this research. Abbreviations given in brackets are taken from THROCKMORTON (1982):

VIRILIS PHYLAD

D.virilis (Vi) strain 1801.1 (Texmelucan,Puebla,Mexico)

D.virilis (brewery) (Vi(b)) (Leeds,England)

D.lummei (Lu) strain 3264.1 (Moscow,Russia)

D.novamexicana (No) strain 1952.2 (Antlers,Colorado,U.S.A.)

D.americana texana (At) strain 2320.2a (Jamestown,South Carolina,U.S.A.)

D.americana americana (Aa) strain 1760.8 (Poplar,Montana,U.S.A.)

MONTANA PHYLAD

D.montana (Mo) strain 1218.8d (Cottonwood Canyon,Utah,U.S.A.)

D.flavomontana (Fl) strain 1950.1c (Chester,Idaho,U.S.A.)

D.borealis (Bo) strain 2077.4b (Itasca,Minnesota,U.S.A.)

D.littoralis (Lt) strain 1026 (Strangrias,Sweden)

D.mercatorum strain 2509.4 (New York State,U.S.A.)

2.2 CULTURING AND COLLECTING THE FLIES

The procedures for culturing and collecting the flies were standardised as far as possible. The stocks were housed in a constant temperature room at 25 ± 1 degrees Centigrade. A large container of water was placed in the room in order to increase the humidity of the air. The main light was timed to come on at 8 a.m. and go off at 8 p.m., giving a 12 hour light:12 hour dark daily cycle. All experiments were performed in this room, except for the electrophysiological work (Chapter 6) which required more specialised equipment housed in a separate room.

Each species was kept in half-pint milk bottles, with cotton wool stoppers, containing standard *Drosophila* medium to a depth of about 3 cm. Within each bottle flies were allowed to breed freely and lay eggs. Under these conditions the development period from egg to sexually mature adult was about 3 weeks. Stocks were knocked over weekly into fresh bottles to avoid overcrowding.

In order to obtain virgin flies for use in experiments, the following procedure was adopted: the newly emerged adults were etherised on the morning of their eclosion by knocking over into an etherising bottle. The flies were then sexed under the binocular microscope and placed individually in 7.75cm x 2.25cm vials, with cotton wool stoppers, containing 0.5-0.75cm of standard *Drosophila* medium; for asymmetric mass matings flies were housed 5 to a vial. The flies were kept isolated until they reached sexual maturity (see Section 4); for practical purposes this period was a minimum of 8 days. During this period of isolation, the flies were knocked over into fresh vials every 3 or 4 days.

2.3 ASYMMETRIC MASS MATINGS

The chief advantage of asymmetric mass matings, where approximately twice as many males as females are present in the mating chamber, over symmetric mass matings, where equal numbers are present, is that it is possible to regularly achieve 100 percent mating success, i.e. all the females have mated by the end of the trial. Thus the problems of truncation (see DOW (1975)) encountered in symmetric mass matings are effectively removed.

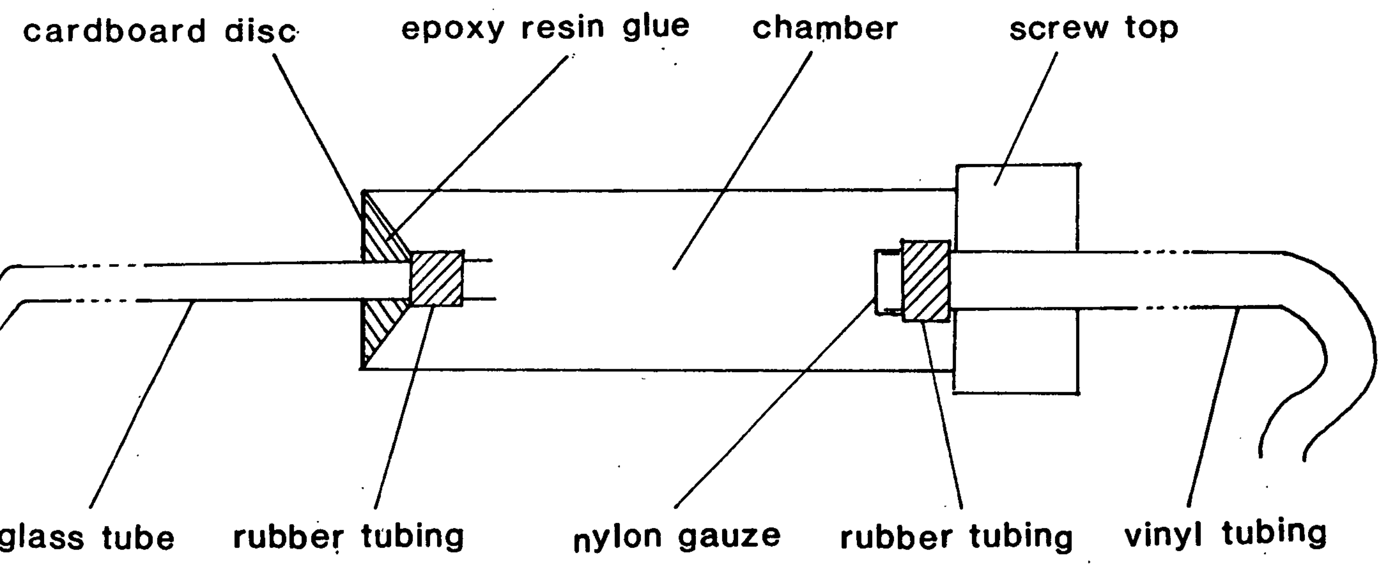
When a mass mating was to be performed, 15 females were knocked over into a 250ml flask and the flask covered with a plastic lid. 30 males were then knocked over into a half-pint milk bottle and transferred, en masse, to the 250ml flask. A small, nylon gauze cloth was then placed over the mouth of the flask and secured with an elastic band stretched around the neck. The flask was then clamped onto a retort stand and turned into a horizontal position such that its base was adjacent to the water heat-shield in front of a 100 Watt lamp. Flies were removed as they mated with the aid of a pooter whose glass tube end was pushed through a small, rubber-reinforced hole in the cloth. The pooter is illustrated in Figure 2-1(a) (actual size). The pootered flies remain in the chamber and can be removed at the end of the mass mating and counted to check on the cumulative total of the number of matings which have been recorded for each minute of the trial. The females may also be removed and placed individually in vials for egg-laying to check on fertility, if this is desired. Each asymmetric mass mating was terminated after all the females had mated, or after 30 minutes.

Figure 2-1

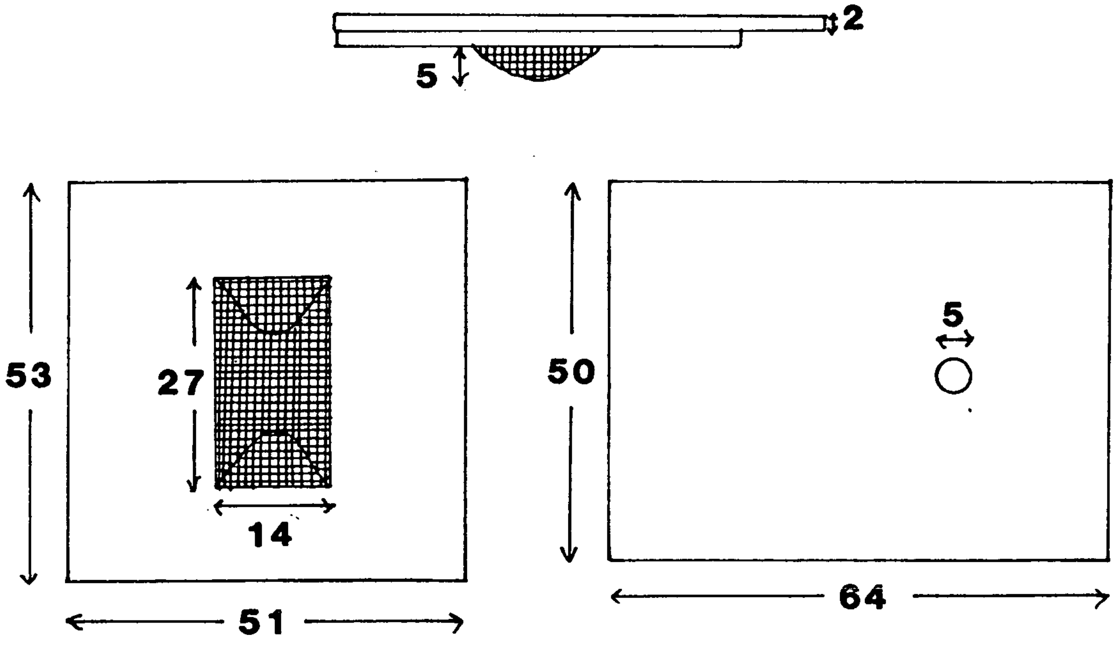
(a) Pooter for extracting flies from mass mating chamber (drawn to scale).

(b) The standard cell (drawn to scale); measurements are given in millimetres.

(a)



(b)



METAL MESH TROUGH

SET INTO PERSPEX

PERSPEX LID

2.4 SEXUAL MATURITY

STALKER (1942) and PATTERSON, McDANALD and STONE (1947) discovered that species of the virilis group do not reach sexual maturity until 5 or 6 days after eclosion. However, a preliminary series of asymmetric mass matings involving D.virilis males and females of different ages revealed that the sexes differ in their maturation periods: females are sexually mature 3 days after eclosion whereas males require 5 or 6 days. This situation is very different to that found in D.melanogaster where males court actively after only 24 hours whereas females require between 1 and 2 days to reach sexual maturity (MANNING (1967b)). The behavioural evidence concerning the late maturation of D.virilis males is supported by studies of spermatogenesis in this species (CLAYTON (1957,1962)): although the testes of males of 1 and 2 days old contain large masses of sperm bundles, the spermatozoa are not motile, and therefore not functional, until the sixth day. By contrast, the testes of the adult male D.melanogaster contain an ample supply of motile sperm within 24 hours of emergence. The advantages of late male maturation in species of the virilis group are unknown; more information concerning the natural ecology of these species is required before this question can be explored further.

Finally, a note on female sexual receptivity. MANNING (1967b) has shown that in D.melanogaster the transition from unreceptive to receptive female is not gradual but is a sudden, or "switch-on", process. Once receptive, females may differ in their courtship requirements but the process of courtship summation (see Chapter 3) is quite independent of the "switch-on" of sexual receptivity. However, results obtained with sexually receptive D.virilis females are somewhat at odds with these findings.

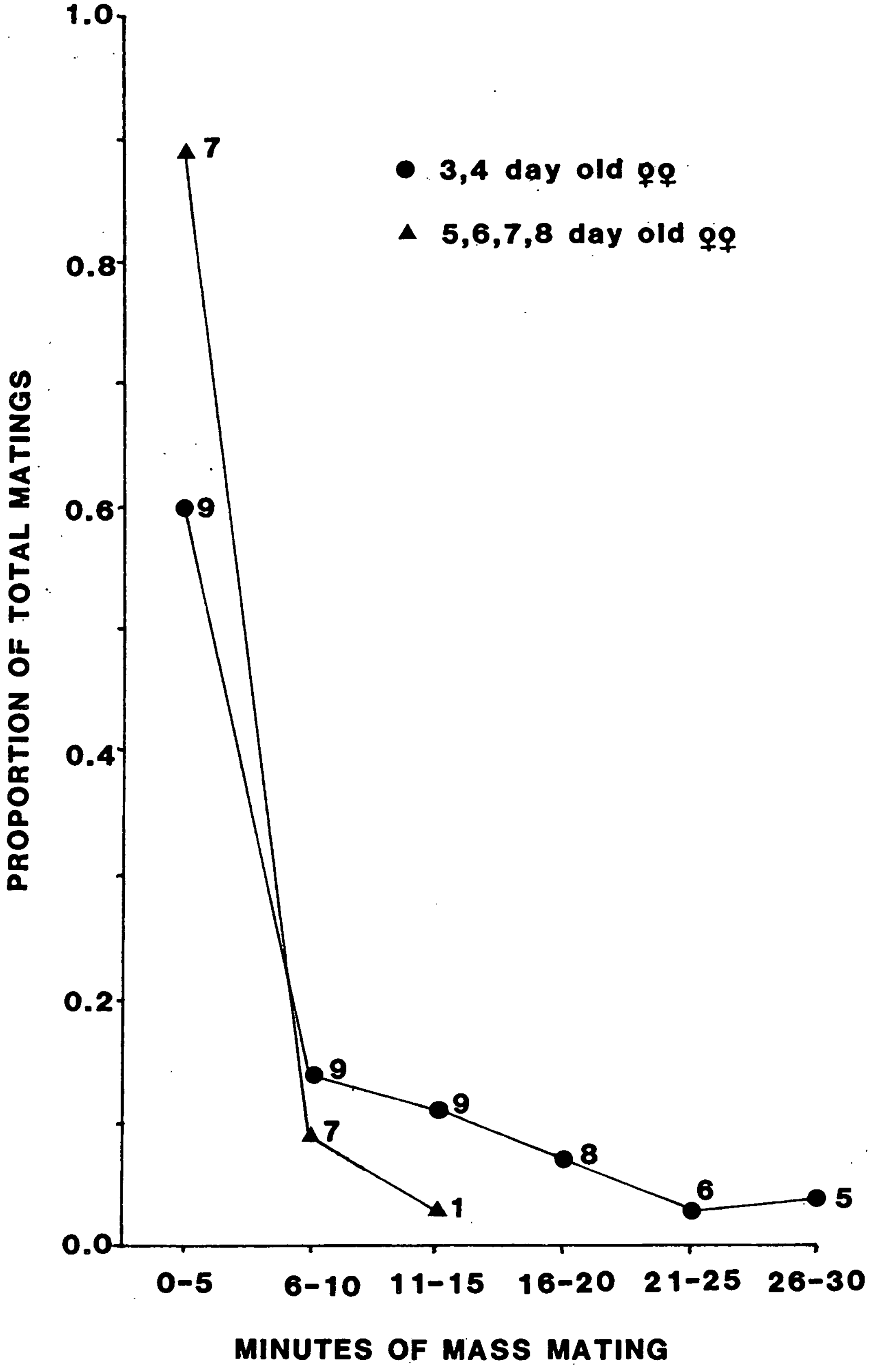
Figure 2-2 shows the proportion of total matings (combined data) which occured in each 5 minute asymmetric mass mating period for 3 or 4 day old females (9 replicates) and 5,6,7 and 8 day old females (7 replicates). The number of replicates which contained matings within the given time period is indicated at the side of the appropriate data point. It would be predicted from the findings of MANNING (1967b) that, since females have all reached sexual maturity by the third day, both age groups should show a similar mating speed distribution over the 30 minute period of the mass mating as determined by the courtship requirements of individual, sexually receptive females. This is clearly not the case. Instead, matings in the younger age group (3,4 days old) are distributed over the entire 30 minute period of the mass matings whereas most of the females of the older age group (5,6,7,8 days old) have mated within 10 minutes and all have mated by 15 minutes. From these findings it would appear that younger receptive females require more courtship stimulation from the male than older receptive females and this is strongly suggestive of a more gradual process of sexual maturation, as opposed to the more sudden, "switch-on" process which MANNING (1967b) found in D.melanogaster. These findings are in accordance with those of COOK (1973b) for D.melanogaster.

Figure 2-2

Proportion of females mating within each 5 minute period of asymmetric mass mating. Combined data: 3,4 day old females (9 replicates); 5,6,7,8 day old females (7 replicates).

(Males; IO-I2 day old)

The actual number of replicates represented in each data point is also given.


1.0
0.8
0.6
0.4
0.2
0.0
PROPORTION OF TOTAL MATINGS
● 3,4 day old ♀♀
▲ 5,6,7,8 day old ♀♀
7
9
9
7
9
8
1
6
5
0-5
6-10
11-15
16-20
21-25
26-30
MINUTES OF MASS MATING

2.5 THE STANDARD CELL

The standard cell was designed (by Arthur Ewing) to facilitate observation and sound recording and to provide adequate room for the activity of the flies. The cell is illustrated in Figure 2-1(b) (actual size). The positioning of the hole in the lid is such that it allows the cell to be completely sealed off by sliding the lid back after the flies have been introduced. The trough shape of the cell allows it to sit neatly in the well of a Grampian GR2 ribbon microphone from which the protective screen has been removed. Nine such cells were made, so that a fresh cell could be used for each experiment of a particular run. At the end of each experimental session, cells were washed in warm, soapy water and rinsed through with hot water before drying.

Flies were introduced individually, without etherisation, into the standard cell using an introducer consisting of the bottom (tap) end of a burette. The unstoppered vial was placed in the introducer and the fly was encouraged to move up the spout by pointing the spout upwards towards the bench lamp. With the fly in the spout, a finger was placed over the end to prevent the fly escaping and the tap was closed. The introducer was then gently lowered and the spout placed over the entrance hole of the cell. The tap was then opened and the fly was gently blown into the cell. The lid of the cell was then slipped back to seal it off. The same procedure was followed for the next fly (or flies).

2.6 SOUND RECORDING AND ANALYSIS

2.6.1 Basic procedure

Both males and females of the virilis group species produce sound, or "songs", during courtship and other interactions. Songs are produced as a result of wing vibration. The standard cell was used for all observations of behaviour and recording of these songs. As mentioned in the previous section the cell was designed to fit into the well of a Grampian GR2 microphone from which the protective screen has been removed. The microphone was placed on a polystyrene block which acted as a shock absorber. Observations were performed under a Kyowa binocular microscope.

The cell was illuminated from the left-hand side by a 100 Watt lamp. A large flask of water positioned between the lamp and the cell acted as a heat shield. The temperature around the cell was maintained at 26 ± 2 degrees Centigrade. A small wad of cotton wool soaked in water was placed in the cell and helped to maintain a high level of humidity in the cell for the short observational period and also allowed the flies to drink if they wished.

The output from the microphone was amplified and passed to a Tandberg Series 3000X tape recorder. All the sounds produced by the flies within the cell were continuously recorded during the observation periods. Brief comments were also made into the microphone from time to time to facilitate the analysis, e.g. the sex of the fly producing the song, whether the acceptance posture had been adopted, etc.

The tape recordings were played back through a Telequipment D53 oscilloscope, which had functioned as an amplifier during the recording stage, in order to ascertain which songs were of sufficient amplitude and clarity for analytical purposes. This selection, or editing, process involved the noting down of the tape counter number at the beginning and end of each chosen section.

The original tape recordings were made at a tape speed of 71/2 inches per second. For the purposes of filming the chosen sections were played at a quarter of this speed (17/8 inches per second) through the oscilloscope fitted with a Nihon Kohden PC-1B continuous recording oscilloscope camera. The speed of the film passing through the camera was normally set at 5cm per second, although 10cm per second was also used occasionally if a more expanded song trace was required. The copious lengths of 35mm Kodak Linagraph photographic paper which resulted from this filming process were developed and fixed and cut into sections suitable for gluing onto large (64cm x 52cm) cardboard sheets. This facilitated the measurement of the various song parameters. The basic terminology used in song description is listed below (taken from EWING and BENNET-CLARK (1968); EWING (1970,1979)):

PULSE: a discrete unit of sound consisting of one or more cycles

PULSE TRAIN: a series of pulses

INTERPULSE INTERVAL (ipi): the measured distance, converted into milliseconds, from the beginning of one pulse to the beginning of the next.

TONE BURST: a symmetrical sound pulse of several cycles; it may be amplitude-modulated.

INTRAPULSE/INTRABURST FREQUENCY: the average frequency of the sound within a pulse/burst measured in cycles per second (Hertz).

2.6.2 Measurements

The measurements which can be taken depend, of course, on the patterning of the particular song. Males and females of several species of the virilis group produce song in the form of pulse trains. Here the pulse length (ms) and interpulse interval (ms) were measured directly from the song trace with a pair of dividers. The number of cycles per pulse was also measured from the trace, and a rough estimate of intrapulse frequency was obtained by dividing the number of cycles per pulse by the pulse length, and multiplying the resultant figure by a thousand, giving a value in cycles per second, or Hertz. The courtship song produced by males of the species and subspecies of the virilis phylad takes the form of an amplitude-modulated tone burst. Here it was possible to measure the number of pulses per burst, burst length (ms), interburst interval (ms) and intraburst frequency (Hertz). However, the number of cycles per pulse was often difficult to determine since, in the main body of the burst, the beginnings and ends of individual pulses were not clearly distinguishable. The courtship songs of the montana phylad are more varied in form; details of the measurements which were taken are given in the text.

In some cases the intrapulse and intraburst frequency were determined directly from sonagrams produced from a Kay Digital Sonagraph 7800 with a Kay Sonagraph printer 7900.

2.6.3 Possible sources of error and variation

Some of the possible sources of error and variation in the measurement of the various song parameters are reviewed below.

(i) Song traces can only be realistically measured to an accuracy of

0.25mm (1.25ms). They are given to the nearest 0.5mm (2.5ms) (the film speed = 5cm per second, tape speed = 0.25 recording speed). For certain purposes a film speed of 10cm per second was used. This gives greater accuracy (0.125mm (0.625ms)) but also uses up twice as much film, so it was only used occasionally.

(ii) The thickness of the trace line itself is approximately 1.0 mm.

(iii) Slight variations in film and tape speed. This was checked periodically by recording and filming a signal of known frequency (50 Hertz).

(iv) It is not always exactly clear where a particular pulse begins and ends. This is largely because of noise within the system and also to varying amplitude within each pulse, particularly at the beginning and end. Only song of sufficient amplitude can be measured accurately; if it is too low it will get lost in the noise. A faster film speed (10cm per second) and the use of filters helps to solve these problems where greater accuracy is required, but fortunately these additional techniques are not usually necessary since the songs of these species are, in general, very loud.

(v) The method employed for measuring the number of cycles per pulse involves the counting of the number of cycle peaks above an imaginary base line running through the centre of the pulse. This means that half cycles above the line will be counted as whole cycles, and those below the line will not be counted at all. However, since the same procedure is used in measuring all pulses, this problem should even itself out.

(vi) Cycle amplitude may affect pulse length and intrapulse frequency. To produce a louder song the wing must be moved through a greater distance and/or at a higher velocity. Pulse length will increase, and intrapulse frequency will decrease, if the velocity of the wing is not increased sufficiently to cover the greater distance in the same time period as that required to produce a softer song. The problem is compounded by the fact that variations in cycle amplitude may also be due to song production at differing distances from the microphone ribbon. It is not possible to distinguish between these two sources of variation in cycle amplitude on the basis of the song trace alone.

CHAPTER THREE

DESCRIPTION AND ANALYSIS OF COURTSHIP

3.1 INTRODUCTION

Much of the work in the field of Drosophila courtship has employed D.melanogaster as the experimental animal. The courtship which has been observed in the virilis group, however, differs in several respects from that of D.melanogaster, which has been described elsewhere (e.g. BASTOCK and MANNING (1955); BURNET and CONNOLLY (1974)). It is important, therefore, to begin by presenting a detailed account of courtship in the virilis group. Although there are some differences between species, and these will be referred to where necessary, the patterning of courtship is fairly uniform within the group, and so it is acceptable to present a description of the courtship of D.a.americana as a model for the group as a whole (Section 3.2).

Following on from this general courtship description, the important behaviours are described and photographs of several of these behaviours are provided (Section 3.3).A detailed sequence analysis of courtship is then performed (Section 3.4).The purposes of this analysis are: to demonstrate the variability both within and between courtships (3.4.2.1); to examine the patterning of male behaviour (3.4.2.2); to investigate male wing vibration (3.4.2.3); to examine female courtship processing (3.4.2.4).

The investigation of female courtship processing leads on to a consideration of the concept of female courtship summation (3.5). A short history of this concept is presented (3.5.1), along with a discussion of some of the problems of this model (3.5.2).

3.2 GENERAL COURTSHIP DESCRIPTION

The following is a description of courtship in D.a.americana as observed in the laboratory (26 ± 2 degrees Centigrade). A mature virgin female of known age is introduced into the standard cell followed, about thirty seconds to a minute later, by a mature virgin male of known age. Both have been isolated on the morning of eclosion and kept separately and individually in vials.

The precourtship period, or latency, i.e. the time elapsing between the introduction of the male and the commencement of any sexual behaviour, varies greatly. During this period the flies may walk around, remain stationary, or clean themselves. Normally within a minute or so the flies come within close proximity and the male taps the female with his fore-tarsi, usually on the side of her body. The female response to this initial tap, and to subsequent courtship by the male, can take several forms: she may fend off the male with a single mesothoracic leg; flick out a single wing on the same side as the male is standing; bend her abdomen away from the male; flutter her wings; walk, run or fly away. She may also perform a combination of the above, e.g. flicking out a wing and extending a mesothoracic leg.

The male, meanwhile, attempts to move to the rear of the female. Once in position at the rear he attempts to lick her genitalia with his proboscis whilst simultaneously rubbing the underside of her abdomen with his fore-tarsi. Since the commonest of the female responses to tapping is fluttering whilst walking or running away, the male often becomes involved in an active pursuit sequence where he must follow the female closely in order to maintain his position at her rear. Such pursuit, or tracking, sequences vary in length and there may be several such sequences within any one courtship.

A sequence is ended when for one reason or another the male loses contact with the female-the female may fly off, for example. During the breaks in courtship the flies may clean themselves or remain motionless. Courtship begins again when the male taps the female once more and moves into position at her rear. Occasionally the male may circle around the female, sometimes scissoring or fluttering his wings as he does so, or flicking out a single wing.

During any one pursuit sequence the female may remain stationary long enough for the male to establish a position immediately behind her from where he is able to lick her genitalia and rub the underside of her abdomen for the period which seems necessary before he is able to extend a single wing and vibrate it. The single wing is moved up and out, diagonal to the body, and vibration is performed only on the outstroke. The wing is then returned to the body ready for the next vibration. Several such vibrations are normally performed in quick succession and either wing may be used. Each time the wing is vibrated the labellum of the male proboscis temporarily loses contact with the genitalia of the female but it is replaced immediately. The male continues throughout to rub the underside of her abdomen. While the male is vibrating the female will often flutter her wings and she may also kick back at the male with her hind-legs.

If the female is to accept the male she adopts an acceptance posture. This involves depressing the abdomen tip down towards the substratum whilst raising the wings into a high-V formation. The male perceives this signal and immediately mounts the female. As the male clambers onto the back of the female he curls his abdomen under the depressed abdominal tip of the female and clasps, with his genital claspers, the sclerotised sides of the female ovipositor and pulls it out before achieving copulation with the insertion of his penis. Incidentally, in contrast to some other species, e.g. D.melanogaster, the male makes no attempt to part the wings with his fore-tarsi during mounting. This is evidenced on the rare occasions when the male is slow to respond to the acceptance posture of the female,so that she has already brought her wings back into the closed position when he attempts to mount her. In this situation the female wings are forced up by the male head as he scrambles onto her back;such mounts are invariably unsuccessful.

Whilst in copula the male fore-tarsi clasp the female about the thorax under the wings whilst the hind legs remain on the substratum. Periodically he rubs her abdomen with his mesothoracic legs. The male invariably produces wing vibration during the first part of copula. Towards the end of copula (which normally lasts between 2 and 3 minutes) the female usually attempts to dislodge the male by kicking backwards with her hind legs at his lower abdomen. However copula does not end until the male loosens the grip of his genital claspers, withdraws his penis and dismounts.

After a short period of cleaning (normally genital-cleaning) by both flies the male will normally resume courtship once more. The female may respond in the ways mentioned earlier. In addition she may extrude her ovipositor. Despite persistent courtship by the male a second copulation is never achieved with the inseminated female.

3.3 DEFINITIONS OF BEHAVIOURS

MALE BEHAVIOURS

LATENCY - The time elapsing between the introduction of the male and the commencement of sexual activity.

ORIENTATION - This term can be variously defined but here it is taken to mean an observable sexual interest on the part of the male. Thus tapping, and the movement to the rear of the female are included, and all the subsequent sexual behaviour of the male is superimposed upon orientation.

TAPPING - This is where the male taps the body of the female with his fore-tarsi.

TRACKING - This is a particular case of orientation where the male follows closely behind a moving female. This often requires considerable agility and vigour since pursuit sequences may last a long time (several seconds) and the female is able to change both her direction and speed very rapidly, zig-zagging about the cell. The male must also be able to track the female visually since a male which has temporarily lost contact with the female is able to locate her with great accuracy and resume courtship once more.

LICKING/RUBBING - The male licks the female genitalia with his proboscal labellum, and rubs the underside of the female abdomen with his fore-tarsi. The two behaviours are normally produced simultaneously.

VIBRATION - The male extends a single wing and vibrates it. The wing is extended diagonally, about 30-40 degrees in the vertical plane and 45-60 degrees in the horizontal plane. It is vibrated only during the outstroke and is immediately returned to the body in preparation for the next vibration. The male is orientated to the female and performing licking and rubbing when he vibrates.

MOUNTING - The male climbs onto the back of the female. Simultaneously he curls his abdomen tip downwards and clasps with his genital claspers the sclerotised sides of the female ovipositor which he pulls outwards. He is then able to insert his penis into the vagina of the female and copulation is achieved.

The following male behaviours are not so commonly encountered in single pair courtships:

CIRCLING - The male moves around the female whilst still facing her. He may perform a 180 degree arc where he moves around to her head and back again to his position at her rear, or he may perform a full 360 degree circle around her.

SCISSORING - The male extends both wings then brings them back to resting position in a single, scissor-like movement.

FLICKING - The male extends and retracts a wing in a single,rapid movement.

FLUTTERING - The male extends both wings a short distance from the body and vibrates them in both a vertical and horizontal plane.

FENDING - The male extends a single mesothoracic leg to fend off the female. This sometimes occurs before the male has become sexually aroused,i.e. during the latency period, and it is produced in response to the female coming within close proximity of the male.

FEMALE BEHAVIOURS

(These behaviours are produced in response to male courtship)

WALKING,RUNNING,DECAMPING - The female walks, runs, or flies away from the male.

FENDING - The female extends a single mesothoracic leg to fend off the male.

FLICKING - The female rapidly extends and retracts a single wing. The wing is flicked out towards the male and this behaviour sometimes accompanies fending although fending is much the commoner of the two.

KICKING - The female kicks backwards with her hind legs at the head of the courting male.

ABDOMEN-BENDING - The female bends her abdomen to the side, away from the courting male. She may also depress or elevate her abdomen.

FLUTTERING - The female extends both wings a short distance from the body and then vibrates them in both a vertical and a horizontal plane.

SCISSORING - The female extends both wings then brings them back to the body in a single scissor-like movement.

ACCEPTANCE POSTURE - The female depresses her abdomen tip slightly whilst raising both wings some 60-80 degrees from resting position and lifting them about 60 degrees into a high-V formation. This behaviour immediately precedes mounting by the male.

EXTRUSION - The female extrudes her ovipositor, visible as a white tube at her rear end. This behaviour is normally only seen in inseminated females.

Plate 3.1

Male and female prior to initial orientation (Aa).

(Abbreviations: see Chapter 2).

Plate 3.2

Female flutter in response to male tapping (Aa).

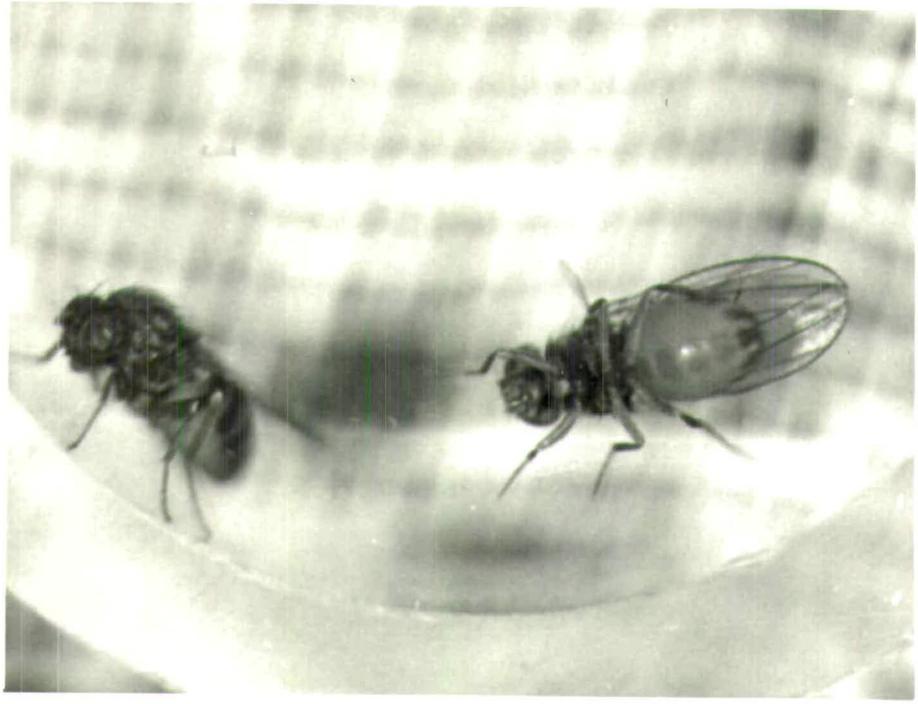


Plate 3.3(i)(ii)

Male wing vibration; female acceptance posture (Vi).

Plate 3.4

Male mounts female (Vi).

Plate 3.5

Male clasps female ovipositor (Vi).

Plate 3.6

Pair in copula (Vi).

Plate 3.7

Female extrusion (post-copula) (Vi).

3.4 A SEQUENCE ANALYSIS OF COURTSHIP

3.4.1 MATERIALS AND METHODS

The standard cell set-up was used for observing the flies in courtship. Two additional pieces of equipment were used:

(i)"Esterline Angus" event recorder

(ii) An electronic "bleeper" timing device (constructed from a design by Dr.D.A.Robinson).

Each behaviour was assigned a specific key on the event recorder keyboard. In the recorder itself a row of pens are in contact with a roll of chart paper which moves at a preset speed beneath them. The depression of a particular key causes a particular pen to flick momentarily sideways. In this way the performance of a particular behaviour can be recorded. The electronic "bleeper" device provided an audible cue every 1.5 seconds to depress the relevant keys. A switch situated above each key on the keyboard allows the pen to be permanently deflected and this was used for "acceptance posture" and "mounting" in order to indicate that copulation had occured. A switch was also used to indicate "start".

Each courtship was recorded from the introduction of the male to the separation of the flies at the termination of copulation, or for 15 minutes if no copulation occured. The "start" switch was flicked on when the male was introduced and at the end of copula the "acceptance posture", "mounting" and "start" switches were all flicked off. From each courtship a length of chart roll was produced which could then be analysed. All experiments were performed between 10 and 12 a.m. and between 2 and 5 p.m. at 26 ± 2 degees Centigrade. The flies were 8 to 12 days old.

Recorded Behaviours

MALE BEHAVIOURS: ORIENTATION; LICKING/RUBBING; CIRCLING; VIBRATION; MOUNTING

FEMALE BEHAVIOURS: FLUTTERING; ACCEPTANCE POSTURE

(N.B. In these experiments the male is said to be engaging in orientation only when he is physically touching the female).

(N.B. The data on FLUTTERING is analysed in Chapter 4).

3.4.2 ANALYSIS AND DISCUSSION

Sequence Diagrams

A sequence consists of a string of courtship behaviours which are separated from each other by a 1.5 second gap. The exception is the final courtship sequence which also contains the pair in continuous copula in a successful courtship. A sequence is separated from the preceding and the succeeding sequences by a gap of 3 seconds or more. This gap contains behaviours which are not recorded, e.g. standing still, cleaning.

One way of representing courtships is in the form of sequence diagrams which show the frequency of transition from one particular behavioural element, or elements, to the next. Clearly a particular behaviour may also be followed by itself, as opposed to a different behaviour. The number of times this occurs is shown on the sequence diagram, under the particular behaviour concerned. The letter "X" is used to denote all behaviours which are not recorded, i.e. "non-sexual behaviours". The behaviour immediately following X is the first behaviour of a sequence and that behaviour which immediately precedes X is the last behaviour of a sequence. The exception is the final sequence which ends in mounting and copulation.

In addition to the use of sequence diagrams, other forms of analysis were used in this investigation and these will be explained in the text. The analysis is split into four main sections:

Variability within and between courtships (3.4.2.1)
Male behaviour patterning (3.4.2.2)
Male wing vibration (3.4.2.3)
Female courtship processing (3.4.2.4)

3.4.2.1 Variability within and between courtships

A general courtship description provides only a superficial indication of variability. In fact, no two courtships are identical and a sequence analysis of male behaviours provides a useful way of demonstrating this variability.

First of all, the number of behaviour sequences which constituted each courtship was found to be very variable: of the 26 succesful courtships, the number of sequences making up each courtship varied from 1 to 23. Secondly, the behavioural transitions occurring within each sequence varied between sequences of the same courtship. This is illustrated in Figure 3-1, which shows 12 sequences of a 12 sequence courtship. The numbers by the arrows are the transition frequencies. The sequences differ with regard to which behaviours they contain, how much of each behaviour they contain, and the number of transitions occurring between particular behaviours. Thirdly, courtships differ from each other with regard to some of these parameters; this is illustrated in Figure 3-2 which compares the sequence diagrams representing four courtships. In this case each sequence diagram represents the sum of all the behavioural transitions occurring within each courtship. Although each courtship contains the same male behaviours, the transition frequencies and their direction vary a good deal between courtships. In addition, circling is produced in one courtship. This behaviour is fairly rare- it occured in only 4 of the 26 successful courtships.

Leaving aside sequence diagrams for the moment it is interesting to look at the variability found in the various measures of courtship length (see Table 3-1).

Figure 3-1

A courtship consisting of 12 sequences.

The numbers beneath the thick arrows are the intervals between sequences (seconds). Each of the thin arrows represents a single transition. The small numbers within the circle indicate the number of times the behaviour was followed by itself.

(Abbreviations: X = non-sexual behaviour; O = orientation;

OLR = orientation/licking/rubbing;

OLRV = orientation/licking/rubbing/vibration;

M = mounting).

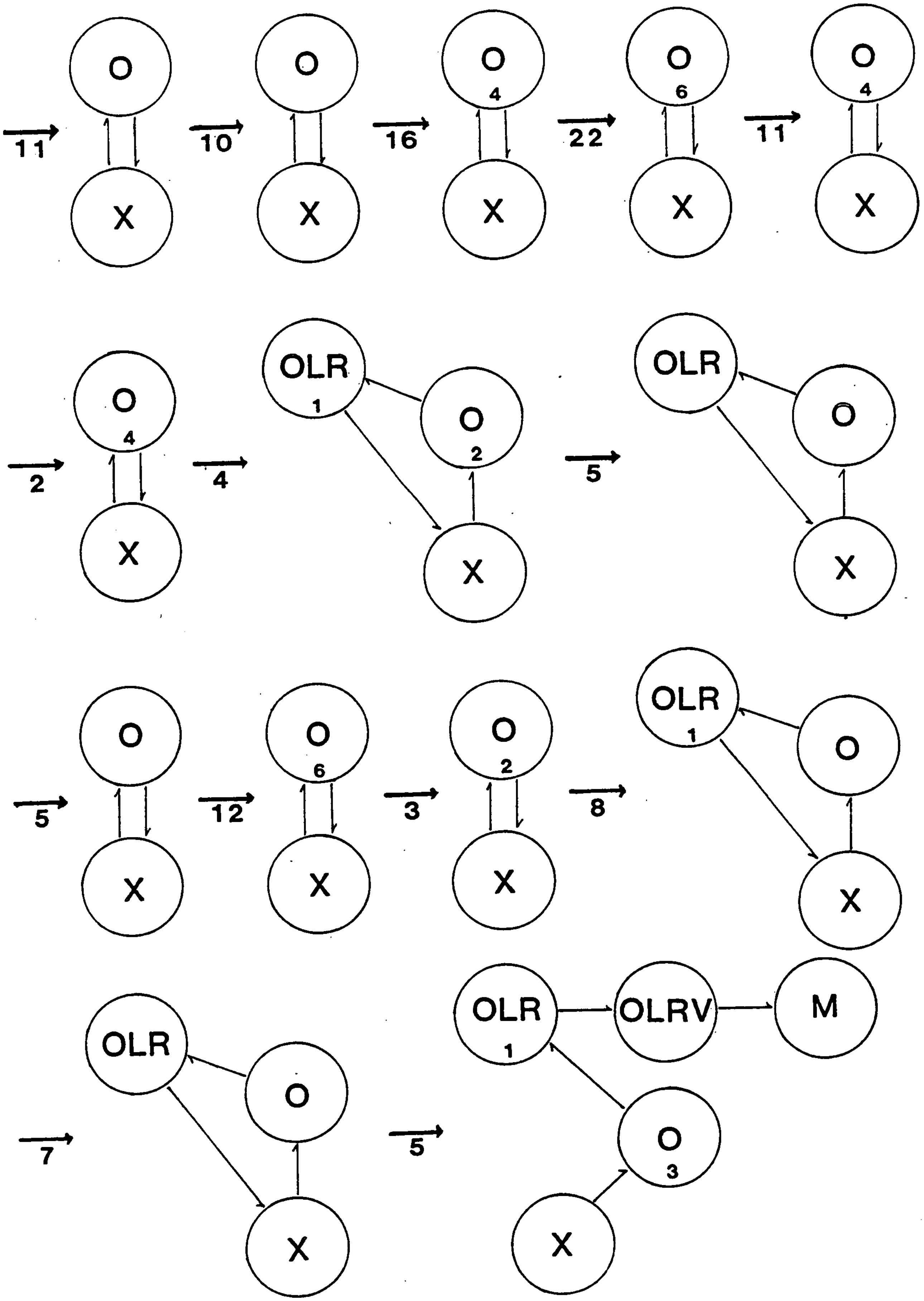
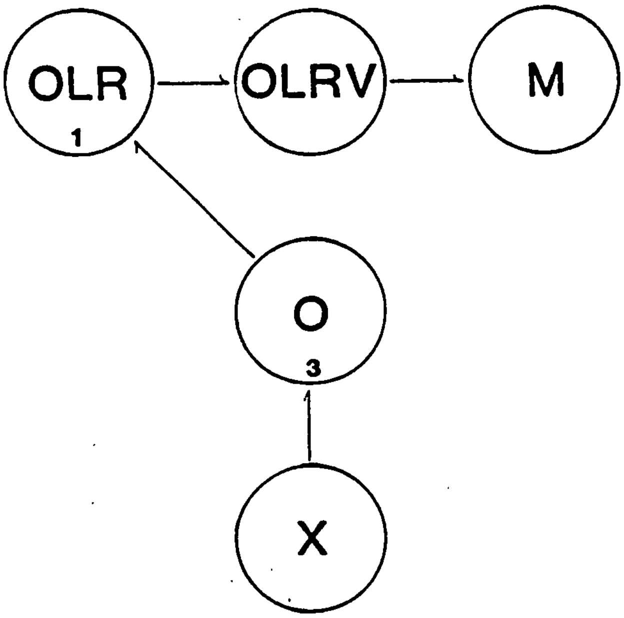
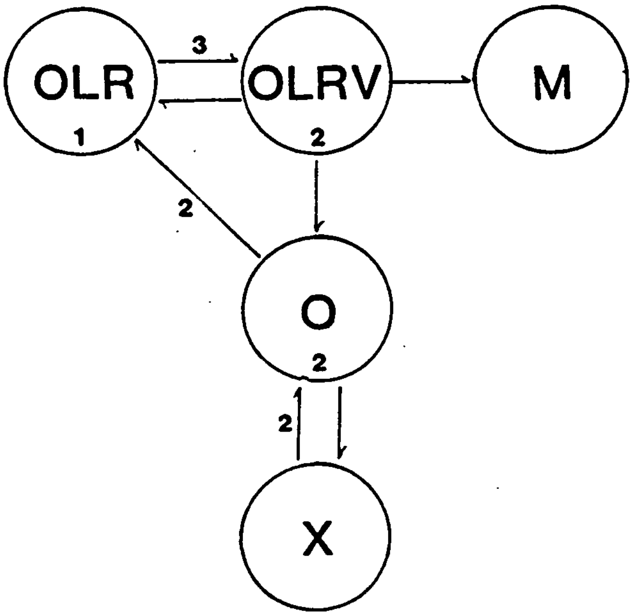

11
O
X
10
O
X
16
O
4
X
22
O
6
X
11
O
4
X
2
O
4
X
4
OLR
1
O
2
X
5
OLR
O
X
5
O
X
12
O
6
X
3
O
2
X
8
OLR
1
O
X
7
OLR
O
X
5
OLR
1
OLRV
M
O
3
X

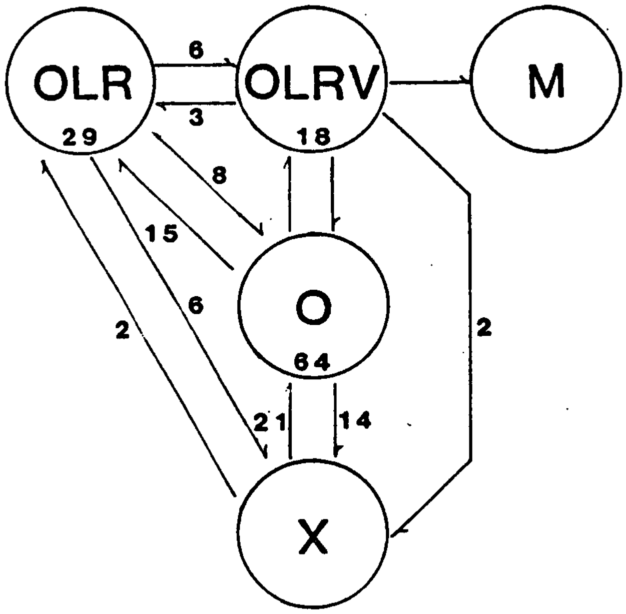
Figure 3-2

Compound sequence diagrams for 4 courtships.

(Abbreviations: as Fig.3-1; C = circling).


OLR
1
OLRV
M
O
3
X


OLR
1
3
OLRV
2
M
2
O
2
2
X


OLR
29
6
3
OLRV
18
M
8
15
6
2
O
64
2
21
14
X

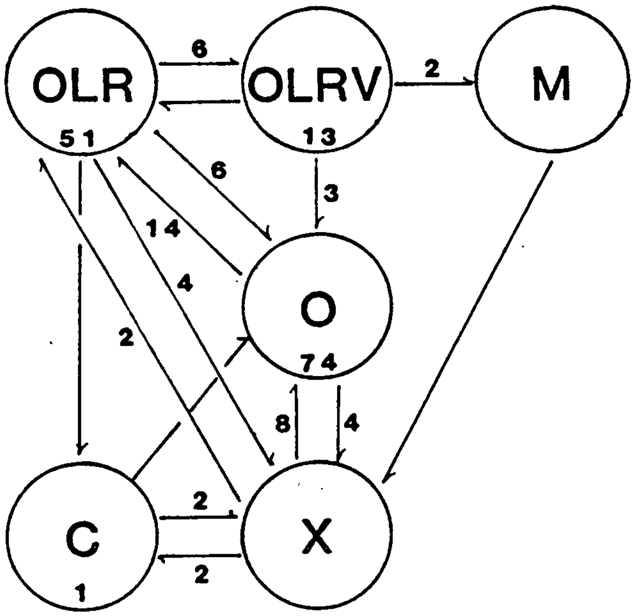

OLR
51
6
OLRV
13
2
M
6
3
14
4
2
O
74
8
4
C
1
2
2
X

Table 3-1

Basic data from the event recorder analysis

(N=26; Ranges given in brackets)

Mean total time to mount (introduction of male to mounting)

229.6 ± 37.0(188.4)s (17s-777s(12mins.53s))

Mean time to first orientation (latency)

55.1 ± 12.3(62.8)s (6s-254s(4mins.14s))

Mean time from first orientation to mounting (mating speed-1)

174.5 ± 37.0(188.9)s (5s-766s(12mins.46s))

Mean time from first vibration to mounting (mating speed-2)

88.0 ± 26.1(133.0)s (1s-465s(7mins.45s))

Mean time from first vibration to mounting excluding intervals between sequences (mating speed-3)

43.5 ± 11.8(60.1)s (1s-220s(3mins.40s))

Mean number of sequences

6.6 ± 1.2(6.3) (1-23)

Mean number of vibratory bouts

8.5 ± 1.9(9.6) (1-41)

Mean number of sequences containing vibration

2.2 ± 0.3(1.5) (1-5)

Mean copula time

121.6 ± 3.3(16.9)s (86s-150s(2mins.30s))

The time elapsing between the introduction of the male and his mounting of the female is extremely variable, being anything from 17 seconds to about 13 minutes. The latency is the time taken for the male to initiate courtship with the first orientation. It varies between 6 seconds and about 4 minutes. Even when the latency periods are subtracted from each courtship, values for mating speed are still extremely variable, being anything between 5 seconds and about 13 minutes.

Many workers now measure mating speed as the time elapsing between the first male wing vibration and mounting. This reduces the variability but the range is still great:1 second to about 8 minutes. It could be argued that the best measure of mating speed would be one which excluded all the periods when the male is not actually courting the female. This can be done by calculating the total length of all the sequences from the first male wing vibration to mounting and ignoring the intervals between the sequences. This produces a mating speed range of between 1 second and 4 minutes. The fact that this is still a wide range demonstrates the enormous variability in mating speed.

An important cause of variability

Using sequence diagrams it has been possible to demonstrate both within- and between-courtship variability in male courtship behaviour. Between-courtship variability has also been shown using the various measures of mating speed. When seeking the causes of this variability it is important to remember that courtship is an interactive process, and what the female does may have a large effect on what the male is able to do. Close observation has shown this to be the case. More specifically, it was the movement of the female which seemed to have the greatest effect on male behaviour.

All the males employed in these experiments appeared to be vigorous courters. However, a male is best able to perform all the elements of his courtship up to vibration and mounting when the female is stationary, or very nearly so. A moving female requires close tracking from behind by the male if he is to remain in contact. Often she may move too fast or turn too quickly for the male to follow; occasionally she may also fly off. In this way courtship may be interrupted for varying periods until the male has regained contact once more. Thus sequence length-the time available to the male to perform his courtship repertoire-is controlled by the female.In this way the behaviour of the female is in large part responsible for the variability found in male behaviour.

3.4.2.2 Male behaviour patterning

A compound sequence diagram of male sexual behaviours from all 26 successful courtships is presented in Figure 3-3. With reference to the last section, the vast majority of the transitions to X (non-courtship behaviour) are the result of the male losing contact with the female. What I am interested in here are the behaviours which the male performs in the periods permitted by the female. These are: orientation (O); orientation / licking / rubbing (OLR); orientation / licking / rubbing / vibration (OLRV). (Mounting is not included here because, as will be seen, this requires a specific acceptance signal from the female).

The direction and the frequency of the transitions between these behaviours indicate that the predominant order is: O - OLR -

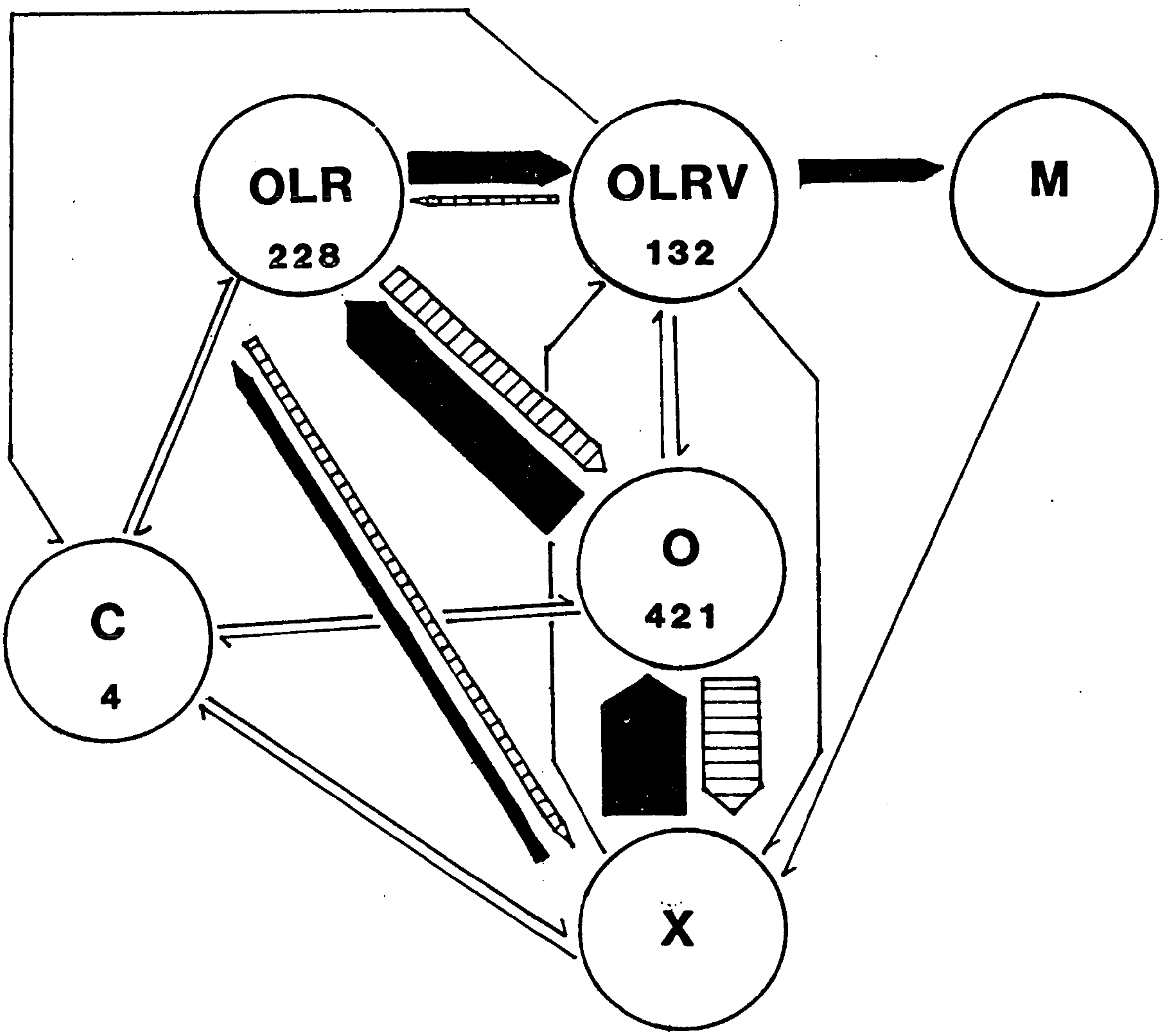
OLRV. Succeeding behaviours are superimposed upon, but do not replace, the preceding behaviour(s). In other words the male must orientate to the female before he can engage in licking and rubbing, and he must be engaged in licking and rubbing before he will produce vibration. This can be termed a hierarchy because of the superimposition of one element upon the preceeding element(s) in a set order. Differing transition frequencies are also evident within this hierarchy, with the transition O - OLR being more frequent than the transition OLR - OLRV. In addition, the transition O - O (421 occurences) is more common than OLR - OLR (288 occurences) which is more common than OLRV - OLRV (132 occurences). Finally, if mounting does not occur, there is also a tendency for the order to be maintained in reverse direction (OLRV - OLR - O), as indicated by the direction and frequency of the transitions between these elements.

Figure 3-3

A compound sequence diagram for all 26 successful courtships.

The thickness of the arrows indicates the relative frequencies of the transitions.

(Abbreviations: as Figs.3-1;3-2).


OLR
228
OLRV
132
M
O
421
C
4
X

What can be deduced about the internal state of the male based upon the hierarchical patterning of these behavioural elements? BASTOCK and MANNING (1955) investigated courtship behaviour in D.melanogaster and their analysis revealed a hierarchical patterning in the male courtship behaviours (orientation; vibration; licking). They explained this hierarchy in terms of a detailed motivational model. In this model the centres in the brain of the male which control the various behavioural elements receive common excitation but have different thresholds for firing. During courtship the female supplies the male with both inhibitory and excitatory stimuli which interact, together with internal factors, to produce the "common excitation".

This model can be used to explain the hierarchical courtship pattern found in D.a.americana. Vibration is assigned the highest threshold for firing, licking/rubbing is intermediate, and orientation has the lowest. However the thresholds for the release of orientation and licking/rubbing are probably very close since O is sometimes by-passed completely (see Fig. 3-3) giving X - OLR. Before courtship begins, i.e. during the latency period, the flies move around fairly randomly often avoiding one another. Then, often by accident, the male touches the female and courtship begins. As long as his internal sexual excitation level is above the threshold for orientation he will continue to track the female. Even after contact has been broken he will seek out the female and tap her. No such seeking behaviour occurs before the initial tap by the male and this suggests that tapping is important in sexually stimulating the male, presumably by physical and/or chemical means.

Once at the rear of the female he begins licking/rubbing. As has been mentioned previously, the thresholds for the release of orientation (tapping and moving to the rear of the female) and licking/rubbing must be fairly close since sometimes, after a break in courtship, the male will move directly up to the female rear and begin licking/rubbing without prior tapping. Once in position at her rear and licking/rubbing his sexual excitation, in time, reaches a level sufficient to trigger vibration. It is easy to see, in the light of this hierarchical model, how fluctuating sexual excitation could contribute to courtship variability in terms of which behaviours and how much of each behaviour are performed within any given sequence.

Previously I emphasised the important role of the female in limiting sequence length. It is easy enough to conceive of how the sexual excitation level of the male might decrease when a courtship sequence is terminated by temporary loss of contact with the female (as in O - X or OLR - X), but it is more difficult to understand why a vibrating male stops vibrating and engages in licking/rubbing and then possibly reverts to orientation again (as in OLRV - OLR - O). An essential component of the model of BASTOCK and MANNING (1955) is the supply of inhibitory stimuli to the male from the female. I have been concerned so far with the excitatory stimuli which the male receives from the female, but she also produces so-called "rejection responses" whilst the male is courting. For reasons which will be discussed in a later chapter I do not believe there is any good evidence that these "rejection responses" provide inhibitory stimuli to the male (see Chapter 4). How then is the sequence OLRV - OLR - O to be explained? I have no answer for this except to say that possibly the internal sexual excitation level of the male begins to drop after a certain amount of time below the thresholds for vibration and licking/rubbing. This decrease may involve some form of feedback mechanism, or an habituation of the centres which control these behaviours to the sexual excitation level.

Finally, a look at circling behaviour. It is rare in this species; it occured in only 4 out of 26 courtships and in two of these it was performed only once and in the other two it was performed two and three times respectively. It tends to occur in the later sequences of a long courtship after the male has been courting vigorously for some time. In single pair situations it probably blocks the female's path and allows her to see the male who is courting her. It may have become ritualised since in some cases of circling the male only advances up one flank of the female before returning to the rear to resume courtship once more. Fluttering, scissoring and flicking of the wings are also sometimes associated with circling and this may have additional importance in male-male interactions when both males are attempting to court the same female. More will be said of male-male interactions in a later section.

Circling may bear some relation to "wing-posturing" described by BROWN (1964) in his sequence analysis of the courtship behaviour of D.pseudoobscura. While he found no evidence for anything more than a single courtship/non-courtship threshold in this species, he suggested an alternative model for the internal control of "wing-posturing": whether a male will "wing-posture" or turn away from the female is determined by the relative balance between two tendencies- to copulate with or to avoid the female. I found no evidence for the approach-avoidance model; once sexual behaviour has been initiated the male will continue to pursue and court the female. However there are aspects of circling which are similar to "wing-posturing": both are produced after the male has been courting vigorously for some time; both involve a sudden breaking-off of the normal courtship and moving round to the front of the female, and both involve wing movements which are not normally seen in a standard courtship.

3.4.2.3 Male wing vibration

Male wing vibration produces an acoustic stimulus- the courtship song. Vibration is probably the most important component of the courtship repertoire of the male. Two pieces of evidence from the sequence analysis can be used to support this statement:

(a) There are three main components making up the courtship repertoire of the male: orientation, licking/rubbing and vibration. Orientation and licking/rubbing were insufficient on their own, within the time allowed, to provide the sexual stimulation which the female requires before allowing the male to mount and copulate. Of the 8 unsuccessful courtships recorded, 5 contained only these behaviours whereas all 26 successful courtships contained vibration as well. Thus vibration is a necessary component of a successful courtship (although it may not always be sufficient since 3 of the 8 unsuccesful courtships did contain vibration).

(b) In addition to the male behaviours, the occurrence of the female acceptance posture was also recorded for the purposes of this analysis. The analysis revealed a direct temporal correlation between male wing vibration and the female acceptance posture- when the acceptance posture occurred it was always immediately preceded by vibration. The acceptance posture acts as a signal to the male to mount the female- the analysis also revealed that mounting does not occur unless the acceptance posture has been adopted by the female. None of the 8 unsuccesful courtships contained the acceptance posture.

Having established the importance of male wing vibration, the data can now be used to look in greater detail at this particular behaviour.

The 26 successful courtships contained a total of 171 sequences of male courtship behaviour. Only 57 of these sequences actually contained vibration. Also, only a small number of courtship sequences within any one successful courtship actually contain vibration, as shown in Figure 3-4 (see also Table 3-2). In only 4 of the 26 courtships did as many as 5 sequences contain vibration; most commonly (12 courtships) only 1 sequence contains vibration. Unfortunately there is insufficient data to establish whether the total number of sequences containing vibration, or the absolute number of vibrations, is directly related to the number of sequences occuring within any one courtship. It is unlikely however that the relationship is a simple one.

Four representative courtships are shown in Figure 3-5 to illustrate some further general points (see also Table 3-2):

(i) Vibration tends to occur in the later sequences of courtship and, of course, the final sequence always contains it. This can be shown generally by dividing the courtship into two halves, on the basis of sequence number, and then comparing the two halves in terms of whether or not they contain vibration. (In courtships with an odd number of sequences the middle sequence is omitted from the analysis). Of the 14 courtships which contain 5 or more sequences, 10 have vibration occuring exclusively in the second half.

(ii) Although the vibrating male may elicit the acceptance posture from the female, the subsequent male mount may fail. Normally this is because the male is unable to clasp the sides of the female ovipositor with his genital claspers or achieve intromission because the female has withdrawn the ovipositor and/or closed the vaginal plates. Out of a total of 57 sequences which contained vibration, 26 were final sequences,i.e. also contained mounting and copulation. Of the remaining 31 sequences, 13 contained the acceptance posture. In 9 of these 13, mounting was attempted but failed.

(iii) There is great variability in the amount of vibration each courtship contains: (a) 1 vibration; (b) 2 vibrations; (c) 8 vibrations; (d) 26 vibrations. This is probably due, in the main, to differing individual requirements for vibratory stimulation on the part of the females (see Section 4), although males may also vary in terms of the quality of their courtship song, e.g.its intensity, and this may affect the number of vibrations required to satisfy the female.

(iv) Sequences which contain the acceptance posture other than the final sequence of each courtship,i.e. those in which no mount is attempted by the male or the mount fails, immediately precede the final sequence. This is true in 9 of the 10 courtships which contain such sequences (see Table 3-2). The importance of this finding will become clear in the next section but essentially it means that once the acceptance posture has been elicited it is easier to elicit it again. One can imagine that a certain quantity of vibration is required to raise the internal sexual excitation level of the female up to the threshold required for the release of the acceptance posture. Once the acceptance posture has been produced it should be easier to elicit it again, as has been shown, because the internal sexual excitation level of the female should still be high.

Figure 3-4

Frequency distribution of the number of sequences in each courtship which contained vibration.

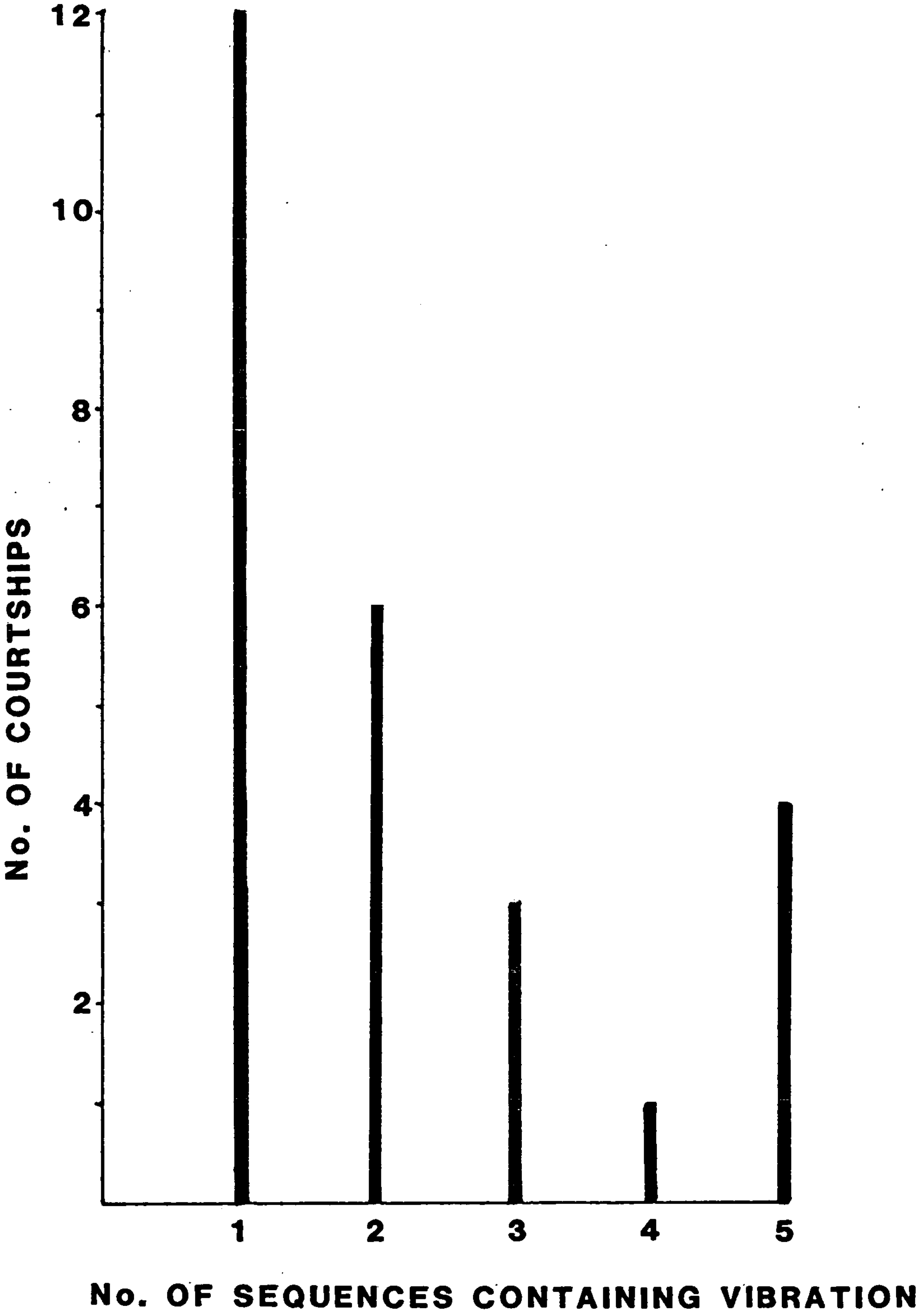

12
10
8
6
4
2
No. OF COURTSHIPS
1
2
3
4
5
No. OF SEQUENCES CONTAINING VIBRATION

Figure 3-5

The number of vibratory bouts contained in each sequence of 4 courtships: a,b,c,d.

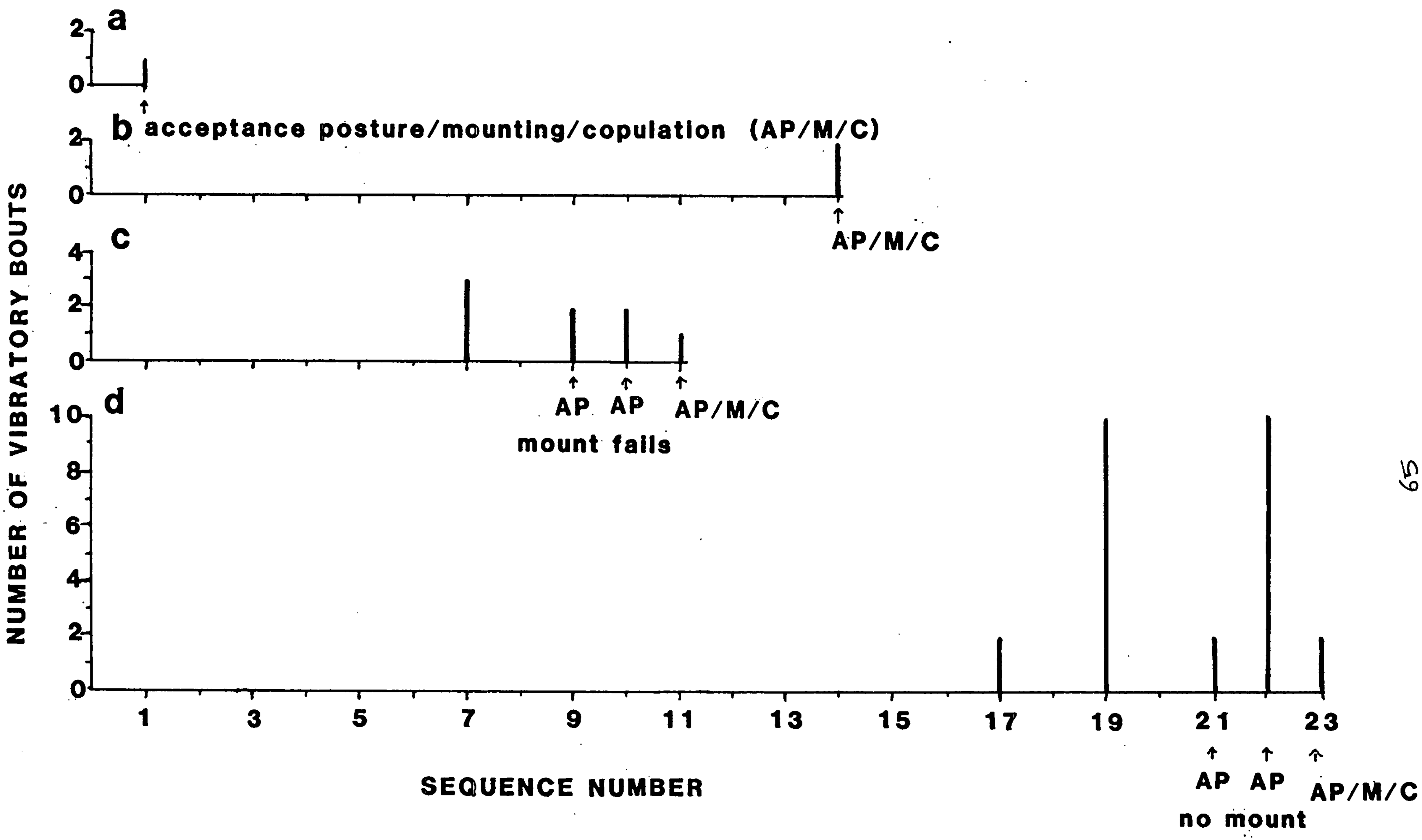

a
b acceptance posture/mounting/copulation (AP/M/C)
AP/M/C
c
AP
AP
AP/M/C
mount fails
d
AP
AP
AP/M/C
no mount
NUMBER OF VIBRATORY BOUTS
SEQUENCE NUMBER
0
2
4
6
8
10
1
3
5
7
9
11
13
15
17
19
21
23

Table 3-2

Data extracted from sequence analysis concerning vibration and acceptance posture (A.P.) (26 successful courtships); note: all final sequences contain A.P. and mounting.

VIBRATORY BOUTS (V)	SEQUENCES CONTAINING V	TOTAL SEQUENCES	ACTUAL SEQUENCES CONTAINING V	ACTUAL SEQUENCES CONTAINING A.P.
2	1	14	14	
1	1	1	1	
1	1	1	1	
7	1	2	2	
20	5	12	6,7,8,11,12	11 mount fails
26	5	23	17,19,21,22,23	21,22 no mount
1	1	8	8	
3	2	3	1,3	1 no mount
19	5	6	1,2,3,5,6	5 no mount
16	2	2	1,2	1 mount fails
2	1	1	1	
2	1	6	6	
1	1	2	2	
1	1	2	2	
8	1	15	15	
6	3	4	2,3,4	
8	2	6	2,6	
8	4	11	7,9,10,11	9,10 mount fails
2	1	5	5	
5	2	5	4,5	4 mount fails
41	5	23	9,15,20,21,23	9,21 mount fails
7	2	2	1,2	1 mount fails
6	3	6	1,4,6	4 mount fails
8	2	3	1,3	
17	3	8	2,6,8	
2	1	1	1	

3.4.2.4 Female courtship processing

In the section on variability it was stated that it is the female which normally determines the length of a sequence. She terminates a sequence by physically removing herself from the attentions of the male. This fact can be used to investigate the effect which the courtship of the male has upon the female, since one would expect the internal state of the female to affect her willingness to terminate a sequence. If the effect on the female is cumulative, i.e. if the female is summating courtship stimuli from the male, particularly the acoustic stimuli provided by male wing vibration, one might expect that she will gradually allow the male to court for longer periods as courtship proceeds (COOK (1973a) has shown in D.melanogaster that lowered female activity is correlated with the successful outcome of courtship). One would expect this to be reflected in the records in two ways:

(i) Later sequences should be longer than earlier sequences.

(ii) Sequences containing vibration should be longer than those that do not.

(i) In order that sequence length be compared, the total number of sequences occuring in each courtship is halved, and the average length of the sequences occuring in the first half is compared to the average length of the sequences occuring in the second half. This can then be expressed as a ratio for each courtship. In courtships with an odd number of sequences the middle sequence is omitted from the analysis. In the 14 courtships containing 5 or more sequences, 9 contained longer sequences in the second half and

4 contained longer sequences in the first half; in one courtship sequence lengths in both halves were identical. This analysis is rather crude (the numbers are too small) but it does show that there is a tendency for later sequences to be longer than earlier sequences.

In section 3.4.2.3(i) it was stated that in most courtships male wing vibration occurred only in the sequences of the second half. Perhaps the effect of male wing vibration on the female contributes to the overall increase in sequence length found in the later sequences of courtship. To test for this effect the length of sequences containing vibration can be compared to the length of sequences which do not contain vibration. Out of a total of 171 sequences (from the 26 succesful courtships) 56 contained vibration whilst 115 did not. The final sequences (total 26) of each courtship which invariably contain vibration must be separated from the remaining vibration-containing sequences (total 30) because the former end in mounting and copulation whilst the latter are normally terminated by the female. The data were log-transformed according to the formula log(n+1), and Student's t-Tests were performed.

Sequences without vibration: N=115; MEAN=6.69s; RANGE:1 to 60s

0.74 ± 0.03 (0.33) log s (i)

Final sequences: N=26; MEAN=13.46s; RANGE:4 to 42s

1.11 ± 0.04 (0.21) log s (ii)

Sequences containing vibration (other than final sequences):

N=30; MEAN=29.50s; RANGE:7 to 65s

1.41 ± 0.05 (0.26) log s (iii)

Comparing (i) with (ii): $t(139)=7.4$; $p<0.001$

Comparing (i) with (iii): $t(143)=11.17$; $p<0.001$

Thus, sequences which contain vibration (including final sequences) are significantly longer than those which do not contain vibration, as predicted.

These findings can be interpreted in the following way: Before the male is able to produce wing vibration it is necessary that the female remains more or less stationary long enough for the male to take up his position to her rear where he is able to lick her genitalia and rub the underside of her abdomen with his fore-tarsi. Thus the preliminary, non-vibratory,courtship of the male must have some stimulatory effect in that the female becomes stationary or moves slowly enough for the male to produce vibration. The proboscal and tarsal contact which the male has with the female, in addition to providing some sexual stimulation to the female, enables him to maintain contact with the female,even in the dark and provided she is not moving too fast. It also allows the male to be in the best position to respond to the female acceptance posture.

The increased length of sequences which contain vibration suggests that it is this element of the male courtship repertoire which functions most effectively in stimulating the female,i.e. she keeps still for longer as compared to when no vibration is present.Whilst the female is stationary with the male courting behind she is in a good position to sample vibratory acoustic stimuli from the male.

How is the courtship of the male, in particular the vibration component, processed by the female? The model I want to use, which is explained in detail in the next section, assumes that the female is able to summate the courtship stimuli provided by the male. Vibratory acoustic stimuli from the male cause the internal sexual excitation level of the female to gradually rise. At some threshold point the acceptance posture is produced. The other courtship stimuli provided by the male will also contribute to this internal sexual excitation but not to the same extent as vibration. Before the threshold point is reached the rising sexual excitation level should result in the female being less willing to terminate a sequence and more willing to let the male continue courting.This is what has been found. In addition, once the acceptance posture has been elicited it should be easier to elicit it again since the internal sexual excitation level of the female should still be high. This has also been demonstrated (see Section 3.4.2.3 (iv)).

3.5 THE FEMALE COURTSHIP SUMMATION MODEL

3.5.1 History of the concept

The idea that the female summates male courtship song (the acoustic stimuli resulting from male wing vibration) is not new. The evidence for the concept of female courtship summation has come exclusively from experiments performed using D.melanogaster.

EWING(1964) removed progressive amounts of the male wing and measured the subsequent mating speeds of these males. He found a positive, straight-line relationship between wing area and mating speed. Since, other things being equal, the intensity of the sound produced by wing vibration should be directly related to the area of the wing, it is likely that mating speed is directly related to the intensity of the acoustic stimulus. In other words, the lower the intensity of the courtship song, the more the female requires before accepting the male. In this way the female can be said to be summating male vibratory stimuli.

There is further evidence available which indicates that the amount of vibration (i.e. courtship song) which a male produces is related to his eventual courtship success. BASTOCK (1956) demonstrated that the inferior mating success (mass matings) of the "yellow" mutant of D.melanogaster was due to the fact that this mutant vibrates for shorter periods and with longer intervals between bouts of vibration than the wild type. KYRIACOU, BURNET and CONNOLLY (1978) have shown that the "ebony"/wild type heterozygote enjoys an improved mating success (competitive matings; courtship duration) because of an increase in the vibration bout length, i.e. an increase in the amount of vibration produced during courtship.

Finally, PARTRIDGE and EWING (1980 - unpublished) have shown that large males, which mate faster than small males, show an increase in both the amount and the volume of the song produced during courtship.

Following on from the work of EWING (1964) there were several attempts to demonstrate female courtship summation employing simulated male courtship song. The courtship song of D.melanogaster is actually composed of two distinct types of song: pulse song and sine song. BENNET-CLARK, EWING and MANNING (1973) exposed females to simulated pulse song for a prescribed period before males were allowed to court them. Mating speed was used as a criterion of the effective persistence of courtship song. They found that pre-stimulation resulted in an increase in mating speed and they interpreted this result as indicating that females are able to summate and store pulse song. They proposed that this ability probably depended upon some neural representation of stimulation (a central excitatory state) which persists and, since it decays with time, bears some functional resemblance to short term memory.

The important influence that female movement has upon male courtship behaviour has been stressed in the previous sections. The effect the male is having upon the female, i.e. her internal state, should be reflected in her locomotor activity. SCHILCHER(1976a) found that simulated pulse and sine song resulted in a reduction in locomotor activity in females, although pulse song had no effect on one-day old immature females, i.e. it only slows females down if they are sexually mature. Interestingly, he found that the simulated songs resulted in an increase in the locomotor activity of males. This latter result has important consequences since

BENNET-CLARK, EWING and MANNING (1973) employed an experimental set-up where both the males and the females were exposed to the simulated song, and the presence of more active males might contribute to any observed increase in mating speed. SCHILCHER (1976b) repeated their experiment but this time made sure that the males were not exposed to the pulse song. He found that pulse song had no significant effect on female receptivity (as indicated by mating speed). He concluded that pulse song is not summated by the female but instead is likely to act as a species-specific trigger stimulus since it only increases the receptivity of females if they are exposed to it whilst being courted by silent (wingless) males.

More significantly, with regard to the present discussion, he found that pre-exposure to sine song did increase the receptivity of females. He suggested that sine song acts rather like a pump, being summated by the female over a certain period of time. As the author says, "an unambiguous demonstration of a summating function of sine song would of course have to provide evidence that the amount of time of pre-stimulation is positively correlated with female receptivity". This latter experiment has not, to my knowledge, been performed.

The explanation given for the hierarchical patterning of male courtship behaviours proposed both a common internal excitatory state and individual thresholds for the release of these behaviours. In the female courtship summation model there must also be a threshold level, or setting, for the amount of sexual stimulation required before the female will accept the courting male. Other things being equal, this courtship requirement should remain fixed for an individual mature female. COOK (1973b) tested this hypothesis by measuring the number of bouts, and the total duration, of vibration required by the same group of females, tested individually, over a number of days. He also measured courtship durations. He found, as predicted by the model, that all three variables remained constant. In other words, all three variables are female-controlled and each female must have an individual setting for the amount of vibration she requires before accepting the male.

CROSSLEY and McDONALD (1979) took this investigation a stage further. Using five flies of each sex, they paired each individual twice with an individual of the opposite sex in order to discover which sex determines which courtship parameters. Using this more sensitive method, which allows the contributions of individual males and females to be determined, they confirmed the findings of COOK (1973b) that females largely control the number of bouts of vibration and their duration; in other words females have an individual vibration requirement.

3.5.2 Problems with the female courtship summation model

The evidence presented so far strongly supports the view that the female summates vibratory acoustic stimuli supplied by the male during courtship and that each female has an individual setting, or threshold level, for the amount of vibration she requires before the male is accepted. There are some problems with this model however and these are considered here.

(i) There is no direct physiological or neurophysiological evidence for the model. All the evidence is behavioral.

(ii) "Rejection responses" are produced by the female throughout courtship. However a more detailed investigation might reveal a gradual decrease in these "rejection responses" as courtship progresses and courtship stimuli from the male are summated. Alternatively, the system controlling the "rejection responses" might be entirely separate and thus unaffected by changes in the courtship processing system. All that is required is that the former system is "turned off" when the latter system thresholds. Evidence that such a "switch" system operates are the following observations: during copula the female remains completely static until just before the end when she begins kicking backwards at the point of contact between the male and female genitalia. It is as if the female suddenly becomes aware of the male presence and the system which controls kicking behaviour is immediately activated; although the female must stop producing fluttering behaviour in order to adopt the acceptance posture, she often produces fluttering both immediately prior to, and immediately following (if there is no mount, or the mount is delayed), the adoption of the acceptance posture; i.e. she is able to switch from fluttering to acceptance posture and back again very rapidly. It should be noted at this point, however, that the performance of a "rejection response" probably does not indicate a rejection of the male on the basis of his courtship (see Chapter 4). However, it is necessary that the female stops producing "rejection responses" in order that she can adopt the acceptance posture and allow the male to mount so the relationship between female courtship summation and the performance of "rejection responses" is important, irrespective of the motivational basis and the function of these responses.

(iii) It is possible to remove the male wings such that these males are no longer able to provide any vibratory acoustic stimuli. Even without this vibratory component these males can be successful in courtship ((MANNING (1967b); COOK (1973a); Chapter 6). However, they take a good deal longer than intact males to achieve copulation.

It has been noted previously (for D.a.americana) that the non-vibratory courtship of the male must have some stimulatory effect on the female. The various tactile, chemical and visual stimuli involved are less effective than vibratory acoustic stimuli but given enough time a heterogeneous summation of these stimuli would be sufficient to threshold the courtship processing system. Thus the success of wingless males presents no serious problem for the courtship summation model.

(iv) In all 26 successful courtships the D.a.americana male produced vibration whilst mounted, always during the first period of copula. Since the male has already produced sufficient vibration to elicit the acceptance posture from the female, it would seem that to produce vibration whilst mounted is superfluous to requirement. I can think of two possible reasons for vibration during copula. Firstly, it is necessary that the female remains stationary and maintains the acceptance posture whilst the male is mounted. It has been noted before that if, in response to the acceptance posture the male does not mount or his mount fails, the female quickly ceases to adopt the acceptance posture. Therefore vibration in the early stages of copula may be necessary to maintain the sexual excitation level of the female at a level high enough to ensure that she maintains the acceptance posture and does not walk around. Presumably other factors (e.g. sperm transfer) have this effect in the later period of copula when no vibration is produced.

Secondly, this vibration may not have a necessary function in stimulating the female (wingless D.virilis (brewery) males (Chapter 6) which are unable to produce any vibration at all achieve normal copulations) but may merely indicate that the male's own sexual excitation has remained at a high level so that vibration continues to be produced for a short while whilst mounted.

(v) Recently ROBERTSON (1982) has cast doubt on the validity of the female courtship summation model. He has repeated experiments of EWING (1964) and COOK (1973b) but failed to confirm their findings. In addition he has failed to find evidence that sine song is summated by the female (the so-called "pump" model of SCHILCHER (1976b)). He proposes that courtship durations are not governed by the female requirement for vibratory stimuli but depend rather upon the "agitation" of the female. This agitation is caused by the transfer of the female to the mating cell, female "reactivity" to this new environment and, to some extent, the courtship ("pestering") of the male. The time taken for the female to switch from agitation to sexual responses (the "female latency") comprises most of the courtship duration whilst male courtship song acts only as a species-identification signal to which the female responds almost immediately she has settled down. He concludes: "... the variation in courtship durations, and particularly the existence of long courtships, is considered to be artifactual."

Before examining the evidence for this alternative explanation of courtship durations, there are some statements in the text which require further explanation. Firstly, in order to explain the increase in courtship duration as a result of progressive removal of sections of the male wing (a repeat of EWING (1964)) he states:"That is, partial wing removal reduces the volume of sound generated, making it harder for the female to perceive and recognise the signal being transmitted, so that she must sample more courtship before mating." It is difficult to imagine how an increase in the amount of time spent in sampling an inferior signal would result in an improvement in the female's ability to perceive and recognise it. Further, it is difficult to understand the eventual success of wingless males since they are unable to provide any (acoustic) species-identification signal at all and so, according to this explanation,would serve only to aggravate the female with their courtship.

Secondly, according to this explanation, short male latencies lead to longer courtships because courtship by the male before the female has settled down increases her agitation:"--- the male's courtship sometimes appeared to aggravate the female's agitation so much so that she would react with the "curling" rejection response and not mate during that courtship. This seemed to be the explanation of most long courtships." However in order to explain some of the short male latency-short courtship duration data he suggests: "Here the courtship appeared to calm the female, thus reducing the female latency." He suggests that this is due largely to the slowing-down effect which pulse song has upon the female (see SCHILCHER (1976a). However, this statement is contradictory in two ways:firstly it contradicts the first statement and secondly it is not in agreement with his explanation which presumes that the female is unable to respond to the species-identification signal (pulse song) until she has settled down (switched from agitation to sexual responsiveness).

The evidence for this alternative explanation of courtship duration comes, in the main, from a plot of courtship duration (the period of time from the first vibration to mounting, excluding breaks in courtship) against male latency (the time elapsing from the introduction of the male to the first vibration). A "strongly hyperbolic relationship" is said to imply that, as male latency increases, courtship duration decreases such that short male latencies are associated with long courtships. The explanation of this relationship is said to be that if the male latency is long the female has had time to settle down and so is immediately receptive to male courtship whereas if the male latency is short the courtship of the male leads to an increase in courtship duration because it serves only to aggravate the agitation of a female who is not yet sexually responsive.

I believe this interpretation of the data is misleading. I think a fairer interpretation of the data is that the majority of the courtships are of short duration, independent of the male latency. I can test this using data from 78 courtships involving D.virilis (brewery). Unlike ROBERTSON (1982) there were no repeat matings and truncated courtships are not included. The data reveal that 33/42 (79%) of the courtships with male latencies less than 50 seconds, and 29/36 (81%) of the courtships with male latencies equal to, or greater than 50 seconds, had courtship durations of less than 30 seconds. In addition, a statistical comparison of the two groups using log-transformed courtship duration data reveals no significant difference between them ($t(76) = 1.45; p>0.1$). So, as predicted, most courtships are of short duration, independent of male latency. If, as is likely, the same is true of the ROBERTSON (1982) data, his alternative explanation of courtship duration is based on the occurence of the minority of abnormally long courtships and fails to take into account the majority of courtships which are, in fact, short.

Nevertheless, the data of ROBERTSON (1982) do reveal that short male latency courtships do contain a greater proportion of long duration courtships than long male latency courtships (although he does not attempt to explain long male latency-long duration courtships). It is perfectly feasible to consider that, in some cases, too early an approach by the male might elicit an escape response from an agitated female since, in general, flies like to keep their distance from one another (SEXTON and STALKER (1961)). However it is the contention of the female summation model that one of the functions of courtship is to reduce this escape response by sexually stimulating the female such that she gradually slows down and, finally, accepts the male. In addition, placing the emphasis on female latency (independent of male courtship) and the species-identification function of the pulse song component of male courtship song, ROBERTSON (1982) fails to take into account the functions of either sine song or the other visual and chemo-tactile components of the male's courtship.

In conclusion, the attempt by ROBERTSON (1982) to find an alternative explanation for variation in courtship duration which does not involve female courtship summation is flawed in several respects. Whilst allowing that the agitation of the female may affect the duration of courtship, it is only one of the factors involved. He has not, in my opinion, successfully excluded female courtship summation as a major contributory factor to courtship duration.

CHAPTER FOUR

FEMALE SONG

4.1 GENERAL INTRODUCTION

To begin with, it is useful to place female sound production in the context of the other behaviours exhibited by the female during courtship, and the other stimuli with which the female may supply the courting male. First of all, there are believed to be various "attractive" stimuli: the presence, movement, size and general body form of the female (SPIETH (1966)); contact or close-range pheromones (JALLON and HOTTA (1979); ANTONY and JALLON (1982)); air-borne distance pheromones (SHOREY and BARTELL (1970); AVERHOFF and RICHARDSON (1976a,b) - although there is considerable doubt as to the existence of distance pheromones, e.g. see EWING (1983)). The acceptance posture is a special case since it is an actual signal to the male to mount (see Chapter 3).

The remaining behaviours exhibited by the female during courtship are normally grouped under the general heading "rejection responses"(also termed "refusal responses" or "repelling actions"), although there are some problems with the use of these terms (see Section 4.6): fluttering; flicking; scissoring; fending; kicking; curling (abdomen bending); extrusion. Definitions of these terms can be found in Chapter 3. It is fluttering which results in the production of sound.

The female may also walk, or run, away from the male, or decamp (fly off). However, it is likely that the male finds these "escape" movements by the female attractive, except for decamping itself which results in the termination of courtship. Tracking of the moving female by the courting male is an integral part of courtship in the virilis group species (see Chapter 3), and vigorous and persistent males are favoured in this activity. This behaviour also provides ample opportunity for male-male interaction in challenging for the position at the rear of the female.

The available literature concerning female sound production in courtship, and its function, is rather sparse. In D.melanogaster the sound resulting from fluttering is in the form of irregular pulse trains. In addition, females of the D.melanogaster and D.obscura group species produce a buzz sound. Although both sorts of sound were believed to inhibit male courtship (EWING and BENNET-CLARK (1968); EWING (1977)), experimental evidence for this function is lacking.

CHANG and MILLER (1978) found that the sounds produced by females of the D.affinis subgroup species bore a striking resemblance to the two song types ("staccato" and "buzz") produced by the males of these species during courtship. Unfortunately, they did not analyze the female sounds in sufficient detail to establish whether or not they were species-specific. Nor did they observe the courtship behaviour in detail, or perform experimental studies, in order to determine the possible function of female sound in this subgroup.

BENNET-CLARK, LEROY and TSACAS (1980) have examined in detail the courtship behaviour and the sounds produced by both the males and the females in the genus Zaprionus , which is closely related to the genus Drosophila. Their investigations have revealed that the females of Zaprionus species produce regular species-specific, and in most cases sex-specific, songs. They speculate that the male may be able to use the information contained in the female song and so avoid wasting time and energy courting the wrong sex and species. This would also serve to reinforce sexual isolation. In addition, the intensity of the female song, and the ease with which it is elicited, may inform the male of the level of sexual excitation of the female and her reproductive, or receptive, state.

DONEGAN and EWING (1980) discovered that in several species of the *virilis* group fluttering behaviour by the female during courtships results in the production of a regularly structured female song. By contrast, fluttering behaviour by *D.melanogaster* females results in irregular pulse trains (EWING (1977)). Perhaps ritualisation of this generalised rejection response has occured (see ZAHAVI (1980)) in order to produce a signal in the form of a regular song containing information relating to the sex and species, and perhaps the receptive state of the female. If female song in *virilis* group species does contain such information, then direct comparisons with the female song of species of the *Zaprionus* genus (BENNET-CLARK et al (1980)) are possible.

The purpose of the following series of investigations was to discover, using both comparative and direct experimental methods, the function of female song in this group. The work is divided into four main sections:

4.2 FEMALE SONG - AN ATTRACTIVE SIGNAL?

Is the production of female song restricted to sexually mature virgin females? If not, do immature virgin females and fertilised females produce a different song?

4.3 FEMALE SONG - A SPECIES-SPECIFIC SIGNAL?

Comparisons are made between the female songs of the species of the *virilis* phylad and four species of the *montana* phylad.

4.4 FEMALE SONG - A SEX-SPECIFIC SIGNAL?

Comparisons are made between the male aggressive songs and the female songs of the species of the *virilis* phylad and four species of the *montana* phylad.

4.5 AN ALTERNATIVE APPROACH

What effect does female song have on the courting male? In order to effectively remove the female song component, courtships are performed with wingless females and aristaeless males. Asymmetric mass matings and event recorder analysis of single pair courtships are employed. Subsidiary experiments are undertaken to determine whether the surgical removal of the female wings and the male aristae has other effects on courtship. As a result, new information is obtained regarding the signal function of the female acceptance posture.

4.2 FEMALE SONG - AN ATTRACTIVE SIGNAL?

4.2.1 INTRODUCTION

Female wing fluttering occurs in virtually all *D.a.americana* courtships (23/26 and 46/48 observed courtships). Sound is produced as a result of fluttering and it has been suggested (DONEGAN and EWING(1980)) that this sound, the "female song", may be important in inducing the male to continue courting and may also have a role to play in maintaining sexual isolation.

Apart from various pieces of circumstantial evidence which will be discussed later (see Section 4.6), these claims are based largely on the fact that female song appears to have a regular structure. This patterning is revealed in the filmed oscilloscope traces produced from recordings of sexually mature virgin females producing fluttering during courtship (Figure 4-1(a)). Each bout of female song consists of a train of polycyclic pulses (each train contains 3,4,5 or 6 pulses). Each pulse is approximately 10 milliseconds in duration and contains, on average, 4 cycles. In general interpulse intervals fall within the 20-50 millisecond range, averaging around 35 milliseconds, and the intrapulse frequency is in the region of 300-400 Hertz (Sonagram: Appendix 4).

If one important function of female song is to induce the male to continue courting, one might predict that only sexually motivated females, i.e. mature virgin females, would produce it. In addition, it should only be produced by such a female if she is being courted by a male, i.e. in a sexual context; it should not be produced during female-female interactions. This leads to the following series of predictions which these experiments are designed to test:

(i) Immature virgin females do not produce "female song" when courted. They may produce a separate song.

(ii) Fertilised females do not produce "female song" when courted. They may produce a separate song.

(iii) Mature virgin females only produce "female song" when courted. In other circumstances, e.g. being tapped by another female, either the female remains silent or a separate song is produced.

4.2.2 METHODS

D.a.americana males and females were isolated on the day of eclosion in the routine manner. All observations and recordings were performed in the standard way (see Chapter 2). Experiments were carried out from 9.30 a.m. to 12.30 p.m. and from 2.00 to 5.00 p.m. at 26 ± 2 degrees Centigrade. All males used in these experiments courted vigorously. The experimental combinations were as follows:

(i) Mature virgin female + mature virgin male

The pair were left together for 10 minutes or until copulation occured, and for a further 5 minutes after copula. Time in copula was noted for each pair and each female was transferred to a fresh vial after the experiment for use in (ii) the very next day.

(ii) Fertilised female + mature virgin male

The pair were left together for 10 minutes. After this period the female was placed in a fresh vial and allowed to lay eggs. The presence of larvae after a few days confirmed that the female had been fertilised previously.

(iii) Immature (1 day old) virgin female + mature virgin male

Females were used the day after they had eclosed. The pair were left together for 10 minutes.

(iv) Four immature (2 day old) virgin females (+ mature virgin male)

In order that individual females could be recognized it was necessary to mark each one, on the thorax, with a small spot of paint. The method used for this marking was as follows: four small dabs of paint of the required colours were placed on a slide which was held on the stage of a binocular microscope. The paint took 2 or 3 minutes of exposure to air to reach the required viscosity and during this period four immature (1 day old) females were lightly anaesthetised and placed on the slide. A small dot of paint of the required colour was placed on the the dorsal thorax of each fly using a fine-pointed probe. After 1 or 2 minutes the paint had hardened and the flies, which were beginning to recover from the anaesthetic, were placed individually in vials for use the next day. The paint colours used were: "signal red", "signal green", "pageant blue" and "leyland white".

The next day these females were placed in the cell together for 10 minutes, after which time a mature virgin male was added.

(v) Four mature (6 day old) virgin females (+ mature male)

In one experiment the same four females from (iv) were used, except one which escaped and had to be replaced. In the other, a new group of marked females was prepared the day before the experiment. Again the females were kept together in the cell for 10 minutes and then a mature virgin male was added. A second male was added if the first became engaged in copula. Females which had been observed in copula, along with those which had not, were vialled and allowed to lay eggs. In this way it was possible to confirm that copulation had resulted in fertilisation.

4.2.3 RESULTS

The results are divided into two sections. The first concerns basic observational and fertility data. The second contains the results of a detailed analysis of the female song itself.

4.2.3.1 OBSERVATIONAL AND FERTILITY DATA

Summary of results (the number of courtships or trials in which song was produced is given in brackets):

(i) mature virgin females were observed to produce song both prior to (5/9) and immediately following (3/5), copula; females (4/9) which did not mate within the time period were also observed to produce song

(ii) fertilised females were observed to produce song (5/5)

(iii) immature virgin females were observed to produce song (7/8)

(iv) immature virgin females were not observed to produce song when placed together, except in the presence of a courting male (0/1)

(v) Mature virgin females were observed to produce song during physical encounters with their own sex (2/2)

Thus the production of song by the female is not dependent upon her being a mature virgin. Immature virgins and fertilised females also produce song in response to male courtship. In addition, mature virgin females produce song in intrasexual interactions.

4.2.3.2 ANALYSIS OF FEMALE SONG

It has been shown that the production of female song is not confined to sexually mature females or the courtship context. However, the song produced by immature virgins, fertilised females and mature virgin females in a non-sexual context may differ from that produced by mature virgin females whilst they are being courted. The former may produce some form of song which acts to repel the courting male. In addition, the song produced by a courted mature virgin female may change with time; perhaps a repelling song is produced by the female prior to the first male vibration but a different attractive song is produced thereafter.

To test for these possibilities it is necessary to analyse the female song in detail. Female song was analysed with respect to the following four parameters, or variables: PULSE LENGTH (milliseconds); NUMBER OF CYCLES PER PULSE; INTERPULSE INTERVAL (milliseconds); INTRAPULSE FREQUENCY (Hertz).

To begin with the data for all individuals (21) was lumped together for each song parameter so that the overall frequency distribution of values within the range could be determined. By plotting these frequency distributions out in the form of bar graphs it is possible to ascertain whether or not the range of values for each parameter are normally distributed. If two very distinct types of song are present which differ with respect to these measured parameters then this should be revealed in this analysis. The clearest demonstration that two very distinct song types are present would be a clear bimodal distribution of the frequency data.

Both pulse length (1103 pulses) and number of cycles per pulse (1103) gave relatively normal distributions. All interpulse intervals up to 1 second in duration were measured (995 ipi's) and the distribution was heavily skewed with 82 percent of all pulses falling in the 20 - 50 millisecond range. It was possible to normalise the distribution using a log-transformation.

Intrapulse frequency was estimated by dividing the number of cycles per pulse by the pulse length (milliseconds) and multiplying by a thousand (196 pulses from 11 individuals), and this method indicated that about 80 percent of all values fell within the 300 - 400 Hertz range. Since the exact beginning or end of a pulse may be lost in noise on the song trace, and half cycles may be present (see Chapter 2), this method for estimating intrapulse frequency gives only approximate values. A second method for determining frequency is to analyse the song with the aid of a sonagram. This technique is used elsewhere in this chapter (as indicated in the tables) and the results agree well with the calculated values. Some examples are given in the Appendix 4.

Thus the preliminary analysis revealed no clear bimodal distribution with respect to any of the four parameters. Therefore, two very distinct song types are not present. However, it is still possible that two fairly similar, though distinct, song types are being produced. To test for this possibility it was decided to compare the song produced by mature virgin females, fertilised females and immature virgin females (1 and 2 days old) in response to male courtship.

For each of the three reproductive states a frequency bar graph was plotted for each song parameter in order to ensure that the distributions were normal. Interpulse interval data was log-transformed. Intrapulse frequency was estimated for each data set but, because of the inaccuracy of this method, no further analysis was performed and the data can only be used for general comparisons. A One-way Analysis of Variance revealed that in all cases between-individual variation was greater than within-individual variation (F values are given in Table 4-1). This necessitated using the mean value of the data for each individual as the data point. Thus the data set for each parameter is expressed as the mean of the individual means, plus or minus the standard error and standard deviation (Table 4-1). (N.B. Since large between-individual variation is likely to be a general phenomenon, all song data is presented in this form where possible and appropriate).

The data for the three reproductive states were compared using the Student's t-Test. No statistically significant differences were found (at the five percent level) between mature virgin females, fertilised females and immature virgin females with respect to pulse length, number of cycles per pulse or interpulse interval.

Table 4-1(i)

Analysis of female song (*D.a.americana*: different physiological states) (MEAN OF MEANS ± S.E.(S.D.)).

	PULSE LENGTH (ms)	NUMBER OF CYCLES PER PULSE	INTERPULSE INTERVAL (log ms) (12.5ms-92.5ms)
MATURE	10.60 ± 0.57(2.20)	4.03 ± 0.18(0.70)	1.55 ± 0.03(0.12)
VIRGIN	N=15; n=610		N=15; n=493
FEMALES	Range:2.5-30.0	Range:1-10	
	(F=17.08;$p<0.01$)	(F=9.43;$p<0.01$)	(F=6.24;$p<0.01$)
FERTILISED	11.76 ± 0.78(1.56)	4.15 ± 0.39(0.77)	1.57 ± 0.05(0.10)
FEMALES	N=4; n=235		N=4; n=177
	Range:2.5-20.0	Range:1-7	
	(F=7.61;$p<0.01$)	(F=14.54;$p<0.01$)	(F=27.58;$p<0.01$)
IMMATURE	13.32 ± 1.22(3.24)	4.55 ± 0.34(0.90)	1.52 ± 0.03(0.07)
VIRGIN	N=7; n=154		N=7; n=132
FEMALES	Range:5.0-32.5	Range:1-11	
	(F=6.25;$p<0.01$)	(F=3.50;$p<0.01$)	(F=3.14;$p<0.01$)

INTRAPULSE FREQUENCY = 300 - 400 Hz.

Table 4-1(ii)

Statistical comparisons of songs produced by D.a.americana females in different physiological states (Student's t-Test).

PULSE LENGTH

Mature virgins v fertilised females: t(17)=1.20;p>0.05; NOT SIG.

mature virgins v immature virgins: t(20)=2.01;p>0.05; NOT SIG.

NUMBER OF CYCLES PER PULSE

Mature virgins v fertilised females: t(17)=0.28;p>0.05; NOT SIG.

Mature virgins v immature virgins: t(20)=1.38;p>0.05; NOT SIG.

INTERPULSE INTERVAL

Mature virgins v fertilised females: t(17)=0.34;p>0.05; NOT SIG.

Mature virgins v immature virgins: t(20)=0.71;p>0.05; NOT SIG.

4.2.4 DISCUSSION

DONEGAN and EWING (1980) have claimed that female song may have an attractive function in that it induces the male to continue courting. The regular structure of the song means that it could be employed as a signal for this purpose. If the female song does have an attractive function one would expect that its production would be limited to sexually mature virgin females which are being courted by suitable males. Females that are not in a position to accept the male - immature virgin females, fertilised females - should not produce this song. Neither should it be produced by females in a non-sexual context.

The results of Section 4.2.3.1 indicate that song production is not limited to sexually mature virgin females or the sexual context. Immature females, fertilised females and females placed together in the absence of males were all observed to produce song.

It is possible that the song produced by mature virgin females in the sexual context differs in some way from the song produced by immature virgins, fertilised females and females which are not being courted by a male. Perhaps these latter females produce some form of repelling song. To test for this possibility the song records were analysed in considerable detail (Section 4.2.3.2). The analysis indicates that the songs produced by mature virgins, fertilised females and immature virgins are similar with respect to pulse length, number of cycles per pulse and interpulse interval. There is insufficient data to properly compare the song produced by females in the non-sexual context (no male) with that produced by mature females during courtship but the data that are available reveal no important differences with respect to these parameters.

To conclude, it is highly unlikely that the female produces song in order to induce the male to continue courting. However, it is still possible that the male may find the female song, and the fluttering of the wings which accompanies its production, attractive in some way. This possibility is considered in Sections 4.5 and 4.6.

4.3 FEMALE SONG - A SPECIES-SPECIFIC SIGNAL?

4.3.1 INTRODUCTION

DONEGAN and EWING (1980) suggest that the female song may function as a sexual isolation mechanism. The male courtship song of D.a.americana (and other members of the virilis phylad) is a rather irregular,amplitude-modulated tone burst, whereas the female song has a more regular structure consisting of a train of polycyclic pulses. Therefore, since female song is more stereotyped, it has more of the characteristics of a sexual isolation mechanism than male courtship song. In addition, in 46/48 courtships, the female began producing song before the male, so it would be possible for the courting male to quickly discover whether or not he is courting a conspecific, and so avoid wasting time courting a foreign female. It has been suggested that male tapping may be important in this respect (SCHILCHER and DOW (1977)).

For female song to function as a sexual isolation mechanism it must be species-specific. In order to investigate this a comparative song survey was carried out involving all the species of the virilis phylad and four species of the montana phylad

4.3.2 METHODS

Stocks were cultured, collected and handled in the normal way. Courtships were performed in the standard cell at 26 ± 2 degrees Centigrade from 0900hrs to 1200 hrs and from 1400 hrs to 1700hrs. Sounds produced by the female during courtship were recorded and analysed in the routine manner (see Chapter 2). In some cases it was possible to measure frequency directly with the aid of a sonagram. To begin with, courtships were performed using a single male and a single female. After a while a second male was added to discover what effect the courtship of two males might have on the female in terms of song production. DONEGAN and EWING (1980) suggest that females may find courtship by more than one male aversive and so may produce special repelling songs.

The virilis group is divided into two phylads: the virilis phylad (3 species, 2 subspecies) and the montana phylad (7 species). The following species were used:

VIRILIS PHYLAD: D.virilis ;D.lummei ;D.novamexicana ;D.a.texana ; D.a.americana

MONTANA PHYLAD: D.montana ;D.flavomontana ;D.borealis ;D.littoralis

4.3.3 RESULTS

The songs produced by the females of the virilis phylad species are shown in Figure 4-1.

D.virilis produced no sound at all when courted by a single male. When courted by two males a buzz song was very occasionally produced.

D.lummei produced a small quantity of pulse song when courted by a single male but no song at all was produced in response to the courtship of two males.

D.novamexicana produced a small quantity of pulse song when courted by a single male. A loud and distinctive buzz song was produced when the female was courted by two males.

D.a.texana produced a great deal of pulse song in response to the courtship of a single male. This pulse song was also produced if the female was courted by two males; a buzz song was also occasionally produced in this situation.

D.a.americana produced a great deal of pulse song in response to the courtship of a single male. This pulse song was also produced if the female was courted by two males; a buzz song was also occasionally produced in this situation.

The songs were analysed and the results are presented in Table 4-2. (Sonagrams: *D.novamexicana* (buzz song); *D.a.americana* (pulse song) in Appendix 4).

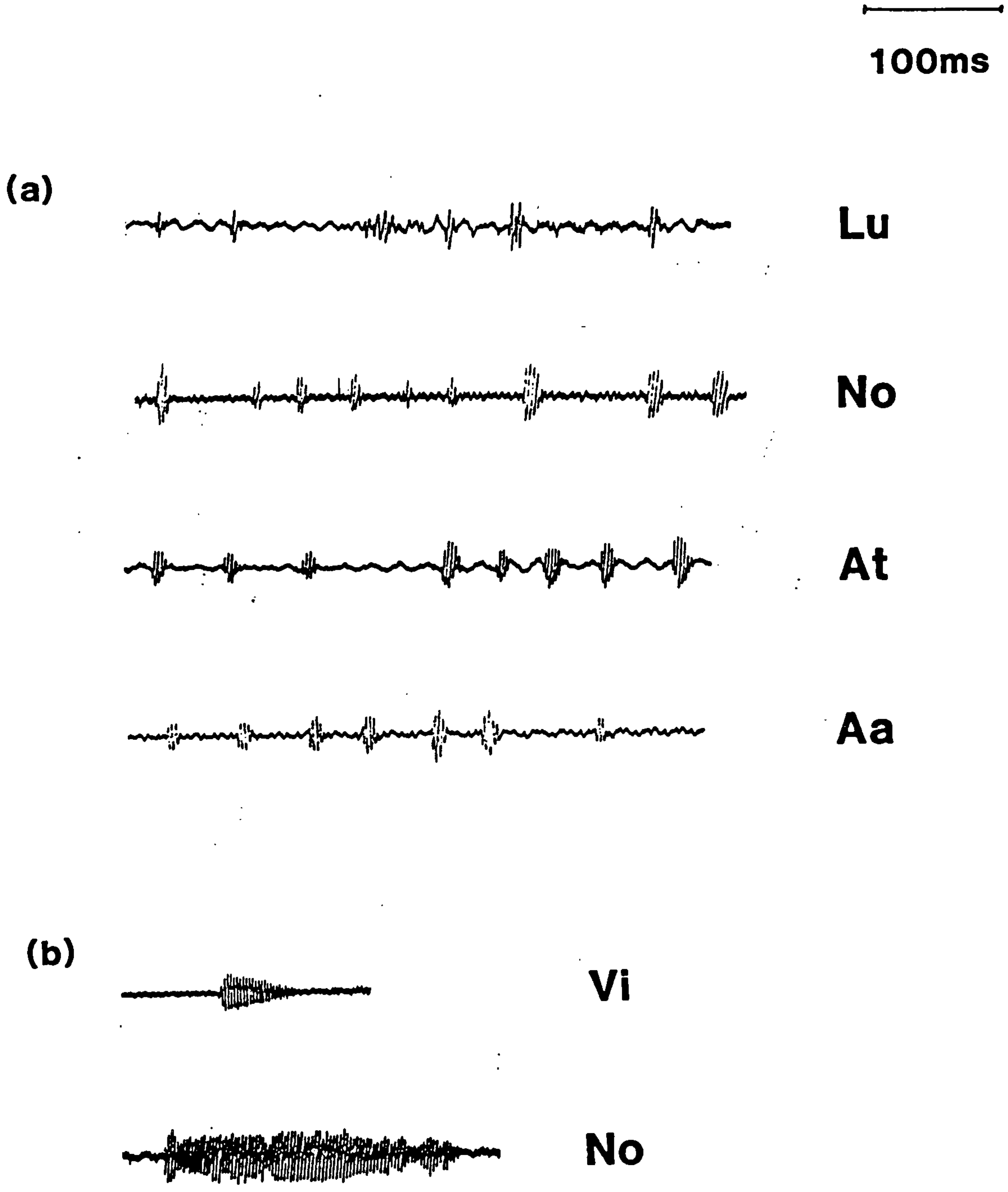

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Table 4-2

Analysis of female pulse songs (MEAN OF MEANS ± S.E.(S.D.)) and buzz songs of the *virilis* phylad.

PULSE SONGS:

D.novamexicana	*D.a.texana*	*D.a.americana*
PULSE LENGTH (ms)		
N=3; n=53	N=2; n=87	N=15; n=610
10.29 ± 1.00(1.74)	10.81 ± 0.48(0.67)	10.60 ± 0.57(2.20)
Range:5.0-15.0	Range:7.5-20.0	Range:2.5-30.0
No. CYCLES PER PULSE		
3.79 ± 0.44(0.77)	4.32 ± 0.12(0.17)	4.03 ± 0.18(0.70)
Range:2-6	Range:3-8	Range:1-10
INTERPULSE INTERVAL (ms)		
n=42	n=67	n=493
51.93 ± 8.20(14.21)	65.33 ± 5.93(8.38)	35.68 ± 2.96(11.46)
Range:25.0-92.5	Range:35.0-100.0	Range:12.5-92.5
INTRAPULSE FREQUENCY (Hz) (SONAGRAM ANALYSIS)		
300-400	300-500	300-400

D.lummei: produces very little pulse song

BUZZ SONGS: *D.novamexicana*: high pitched "twang" sound;350-400Hz (harmonics:700-800Hz); *D.virilis*; *D.a.texana*; *D.a.americana*: rare

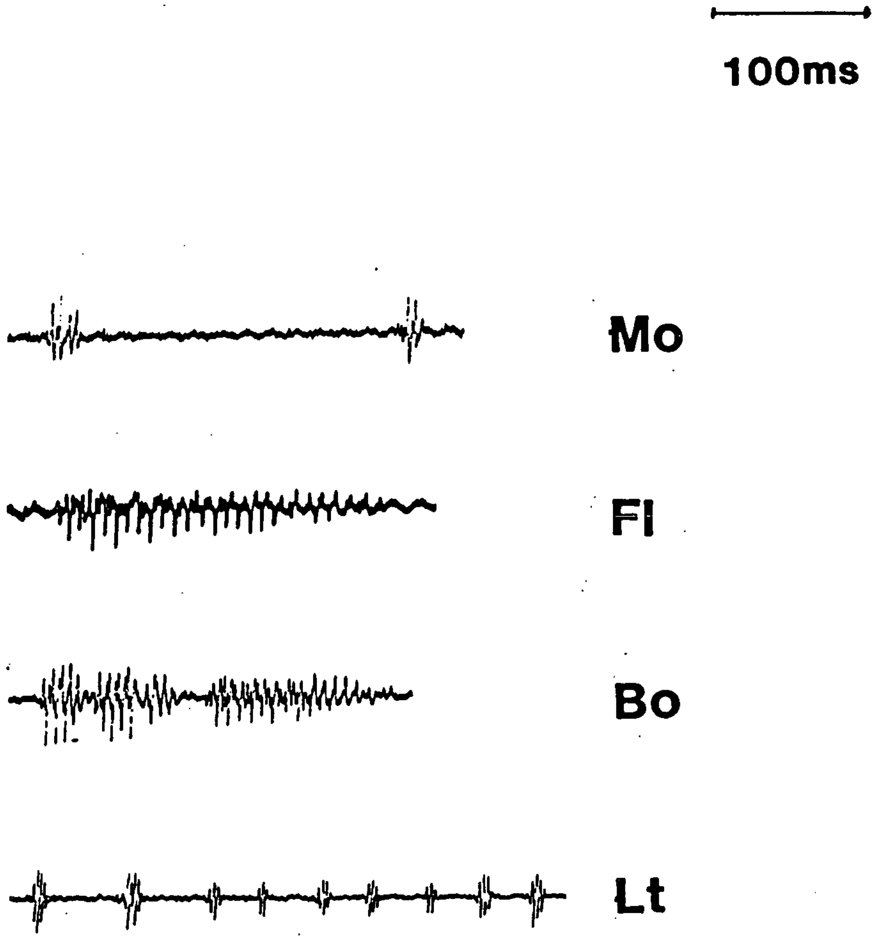
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Table 4-3

Analysis of female pulse song (*D.littoralis*) (MEAN OF MEANS ± S.E.(S.D.)) and buzz songs of 3 species of the *montana* phylad.

PULSE SONG	BUZZ SONGS
PULSE LENGTH (ms)	*D.montana*; *D.flavomontana*;
N=2; n=250	*D.borealis*: buzz song has a complex
10.40 ± 0.30(0.42)	wave form
Range:2.5-20.0	Frequency: wide energy band, but most
No. CYCLES PER PULSE	energy concentrated in 200-400Hz range
3.76 ± 0.39(0.55)	Duration:
Range:1-6	*D.montana* - short (15-20ms)
INTERPULSE INTERVAL (ms)	*D.flavomontana* - variable (20-205ms)
n=210	*D.borealis* - variable (10-355ms)
45.15 ± 6.11(8.63)	Short duration buzz of all species
Range:25.0-92.5	has similar acoustic properties to that
INTRAPULSE FREQUENCY (Hz)	of a single pulse
200-400	

4.3.4 DISCUSSION

Male courtship song in the 3 species and 2 subspecies of the *virilis* phylad consists of a rather irregular, amplitude-modulated tone burst. For this reason DONEGAN and EWING (1980) have suggested that the more regular female pulse song may function as a species-specific sexual isolation mechanism. It is unlikely that buzz song could perform such a function since it is even more irregular than male courtship song and it is only produced by females when they are courted by two males (for a discussion of female buzz song in the *virilis* phylad see Section 4.6). One might expect to find then that the females of each species of the *virilis* phylad produce a distinct, species-specific pulse song when courted by a single male. This is clearly not the case.

D.virilis produces no pulse song at all. *D.lummei* produces very little pulse song, insufficient for use as a sexual isolation mechanism (some females produced no song at all). *D.novamexicana* produces a little more pulse song than *D.lummei* but the song is similar to that produced by *D.a.texana*. However, both *D.a.texana* and *D.a.americana* produce a good deal of pulse song and their songs are distinguishable on the basis of interpulse interval.

Thus only the two subspecies of the *virilis* phylad, *D.a.texana* and *D.a.americana*, produce songs in sufficient quantity and that are sufficiently different to function, in theory at least, as a sexual isolation mechanism. These two subspecies are sympatric and such a sexual isolation mechanism would serve a useful function. However, laboratory evidence suggests there is little sexual isolation and, in any case, hybrids have been found in nature (THROCKMORTON (1982)pp.233-234).

Finally, whilst some interstrain variation in song parameters might be expected for a species-specific song, the finding (see Section 4.5) that females of D.virilis (brewery), a strain of the stock D.virilis, produce copious amounts of pulse song whilst females of the stock D.virilis are silent makes it unlikely that the female song of this species, at least, can be considered species-specific.

These findings indicate that it is highly unlikely that female song functions as a species-specific sexual isolation mechanism in the virilis phylad species.

It is of interest to compare these female songs with those produced by females of the four selected montana phylad species. The montana phylad songs are shown in Figure 4-2 and the analysis in Table 4-3. D.montana, D.flavomontana and D.borealis all produce buzz songs when courted by either one male or two males. D.montana and D.flavomontana produce very little but D.borealis regularly produces buzz song. D.littoralis, on the other hand, produces copious quantities of pulse song when courted by either one or two males which is similar to the pulse song of D.a.americana.

DONEGAN and EWING (1980) have speculated that a regular, species-specific female song may function in sexual isolation in species where the male courtship song is irregular and therefore inadequate for this purpose. This has been shown not to be the case in the virilis phylad species. However, some evidence for this speculation is found in the montana phylad species. Females of three species produce buzz song which, because of its irregularity, could not function as a sexual isolation mechanism. Male courtship song in these three species is regularly structured. On this basis one would expect the male courtship song of D.littoralis to be irregular since the female song of this species consists of regular pulse song. This is not the case however: the male courtship song has a regular structure.

It is important to bear in mind in this context that many factors are involved in determining the structure of male courtship song. The evolutionary history of the group as a whole must be considered. Whether the female produces regular pulse, or irregular buzz song may bear little or no relation to the regularity or irregularity of the male courtship song.

To conclude, on the basis of the evidence presented here, it is highly unlikely that female song functions as a sexual isolation mechanism.

4.4 FEMALE SONG - A SEX-SPECIFIC SIGNAL?

4.4.1 INTRODUCTION

It is possible that female song may function as a signal informing the male that he is courting a female. In the *virilis* phylad, pulse song is the most likely candidate for this role because of its regular structure; buzz song is restricted to situations where the female is being courted by two males and, except in the case of *D.novamexicana*, very little is produced anyway. In the *montana* phylad, *D.littoralis* females produce a pulse song which could serve this sex-identifying role, but *D.montana*, *D.flavomontana* and *D.borealis* females produce only buzz song. It is possible that buzz song may function as a sex-identifier in these latter three species but it is unlikely since buzz song is irregular and, in any case, only *D.borealis* produces large quantities of buzz song.

Both forms of female song are very different from the male courtship songs of both phylads. Even in species where the male courtship song is in the form of a pulse song (*D.montana* and *D.flavomontana*) it is easily distinguishable from female pulse song. However, in addition to courtship songs, males also produce what are known as "aggressive" songs. As the name suggests, the production of aggressive song is normally restricted to interactions between males. For instance, a male which is being courted by another male will often produce aggressive song. However, it is sometimes also produced during courtship although this is quite rare and has only been observed in three of the species tested.

The aim of this work then was to compare female song with male aggressive song to discover whether female song is sex-specific.

4.4.2 METHODS

Recordings were made of male aggressive songs at the same time as female song was being recorded for the previous experiment. The vast majority of male aggressive song was produced during male-male interactions, i.e. in the situation where two males were present with a single female.

4.4.3 RESULTS

The aggressive songs of the *virilis* phylad are shown in Figure 4-3 and the results of the analysis are given in Table 4-4. (Sonagram: *D.a.americana* male aggressive song in Appendix 4).

Males of all species of the *virilis* phylad produce aggressive song in the form of long bursts of pulse song, each burst containing a long train of pulses. As with female song, the song is produced as a result of fluttering both wings. *D.virilis* and *D.lummei* do not produce aggressive song very often. In general aggressive song is produced in response to courtship by another male and in agonistic encounters. These agonistic encounters consist of one male charging at another male, or one male attempting to barge or jostle another male out of the way. This may occur where one male is courting the female and the other male comes up and attempts to barge the other male out of position, or it may occur between two males who are away from the female. One or both males may produce aggressive song. In addition, a courted male has been observed on several occasions to respond to the first vibration of the courting male by turning rapidly to face him; both males then engage in "grappling" behaviour accompanied by the production of aggressive song. *D.a.americana* occasionally produces aggressive song during courtship when another male is not present, usually whilst circling.

In addition to pulse song, D.novamexicana and D.a.americana (and occasionally D.lummei) produce a buzz song. It is produced during an agonistic behaviour which I term "jabbing". In this, the males stand side by side, often facing in opposite directions. As they barge each other and produce pulse song, one male bends his abdomen tip into the head of the other male and at the same time jabs at him with a rapid flick, out and back, of a single wing. This wing is vibrated in the process and results in the buzz song. Both males may perform it simultaneously. (Sonagram: D.novamexicana male "jabbing" buzz song in Appendix 4).

The aggressive songs of the four species of the montana phylad are shown in Figure 4-4 and the results of the analysis are given in Table 4-5.

D.montana, D.borealis and D.littoralis all produce pulse song as a result of wing fluttering. In D.littoralis long bursts of pulse song are produced, as in the virilis phylad, but D.montana and D.borealis produce only short bursts containing relatively few pulses. All three species produce pulse song in response to male courtship or in agonistic encounters. However, in the absence of another male, D.borealis produces pulse song quite frequently when courting a female, and D.littoralis produces occasional bursts of pulse song whilst circling.

D.flavomontana does not produce pulse song but does produce buzz song. (Sonagram: D.flavomontana male buzz song in Appendix 4). D.montana and D.borealis also produce buzz song. In all three species buzz song is produced in response to courtship by another male and in agonistic encounters, although *D.flavomontana* sometimes produces buzz song while courting a female when another male is not present. Both *D.montana* and *D.flavomontana* also produce buzz song during jabbing behaviour, as described previously.

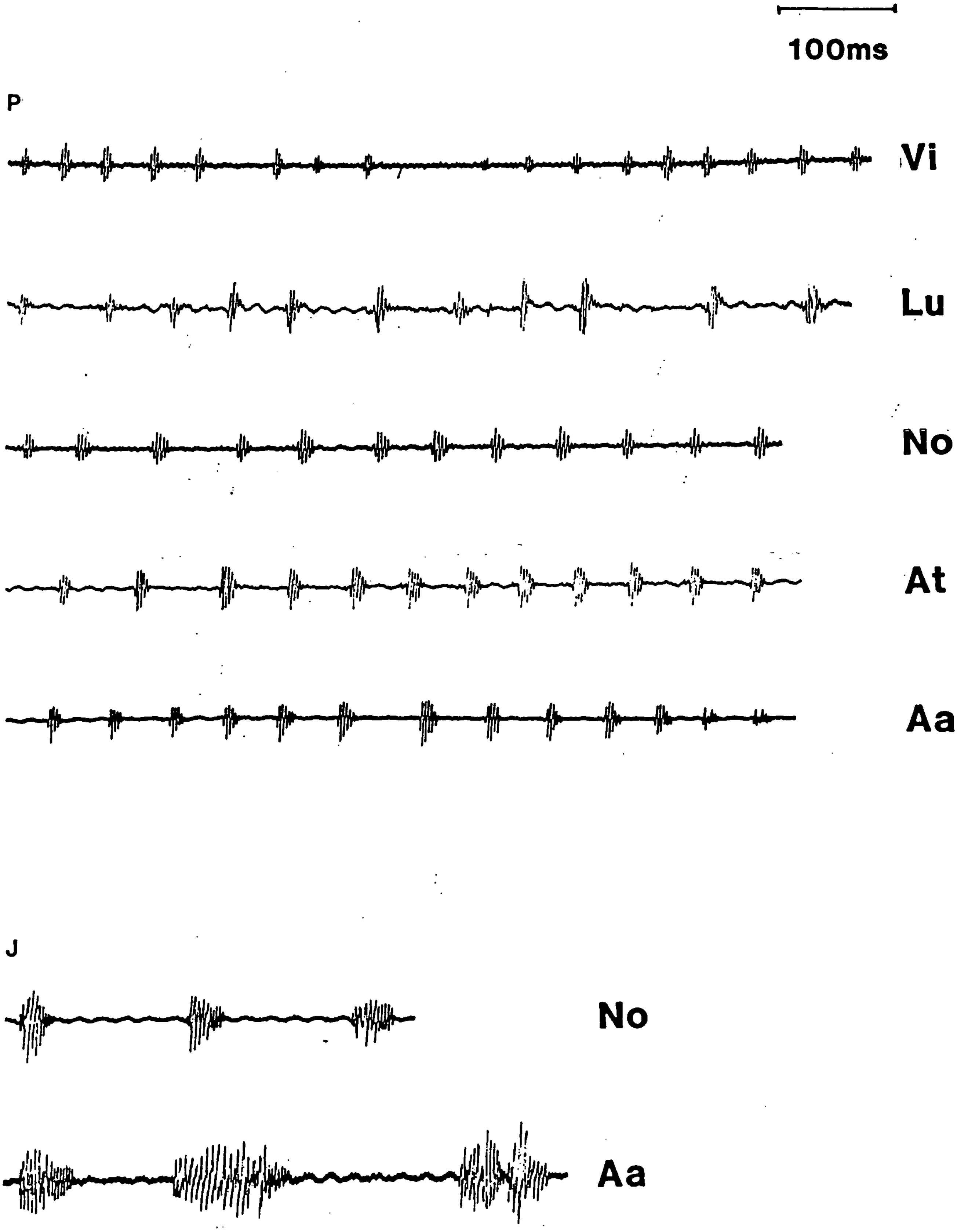

100ms
P
Vi
Lu
No
At
Aa
J
No
Aa

Table 4-4(i)

Analysis of male aggressive pulse songs (MEAN OF MEANS ± S.E.(S.D.)) and jabbing buzz songs of the *virilis* phylad.

PULSE SONGS:

D.virilis	*D.lummei*	*D.novamexicana*
PULSE LENGTH (ms)		
N=5; n=51	N=1; n=30	N=3; n=157
10.18 ± 0.44(0.99)	9.50 ± 0.63(3.44)	11.16 ± 0.56(0.97)
Range:7.5-17.5	Range:5.0-15.0	Range:5.0-15.0
No. CYCLES PER PULSE		
3.49 ± 0.18(0.40)	3.60 ± 0.21(1.13)	3.83 ± 0.13(0.23)
Range:2-5	Range:2-7	Range:2-6
INTERPULSE INTERVAL (ms)		
n=44	n=23	n=146
40.69 ± 1.01(2.25)	64.24 ± 2.74(13.15)	57.75 ± 1.86(3.22)
Range:30.0-67.5	Range:50.0-100.0	Range:35.0-100.0
INTRAPULSE FREQUENCY (Hz)		
300-400	300-400	300-400

JABBING BUZZ: *D.novamexicana*: most energy, 200-300Hz

D.lummei:rare

Table 4-4(ii)

PULSE SONGS:

D.a.texana	*D.a.americana*
PULSE LENGTH (ms)	
N=1; n=78	N=2; n=355
13.01 ± 0.26(2.33)	9.86 ± 0.20(0.28)
Range:7.5-17.5	Range:2.5-27.5
No. CYCLES PER PULSE	
4.83 ± 0.09(0.76)	3.74 ± 0.26(0.37)
Range:3-7	Range:1-9
INTERPULSE INTERVAL (ms)	
n=73	N=3; n=420
54.59 ± 0.95(8.14)	42.51 ± 2.70(4.67)
Range:42.5-85.0	Range:20.0-50.0
INTRAPULSE FREQUENCY (Hz)	
300-400	300-400

JABBING BUZZ: *D.a.americana*: most energy around 200 and 400 Hz

Figure 4-4

Aggressive songs of the montana phylad.

(Abbreviations: see Chapter 2; P = pulse song; B = buzz song; J = jabbing buzz song).

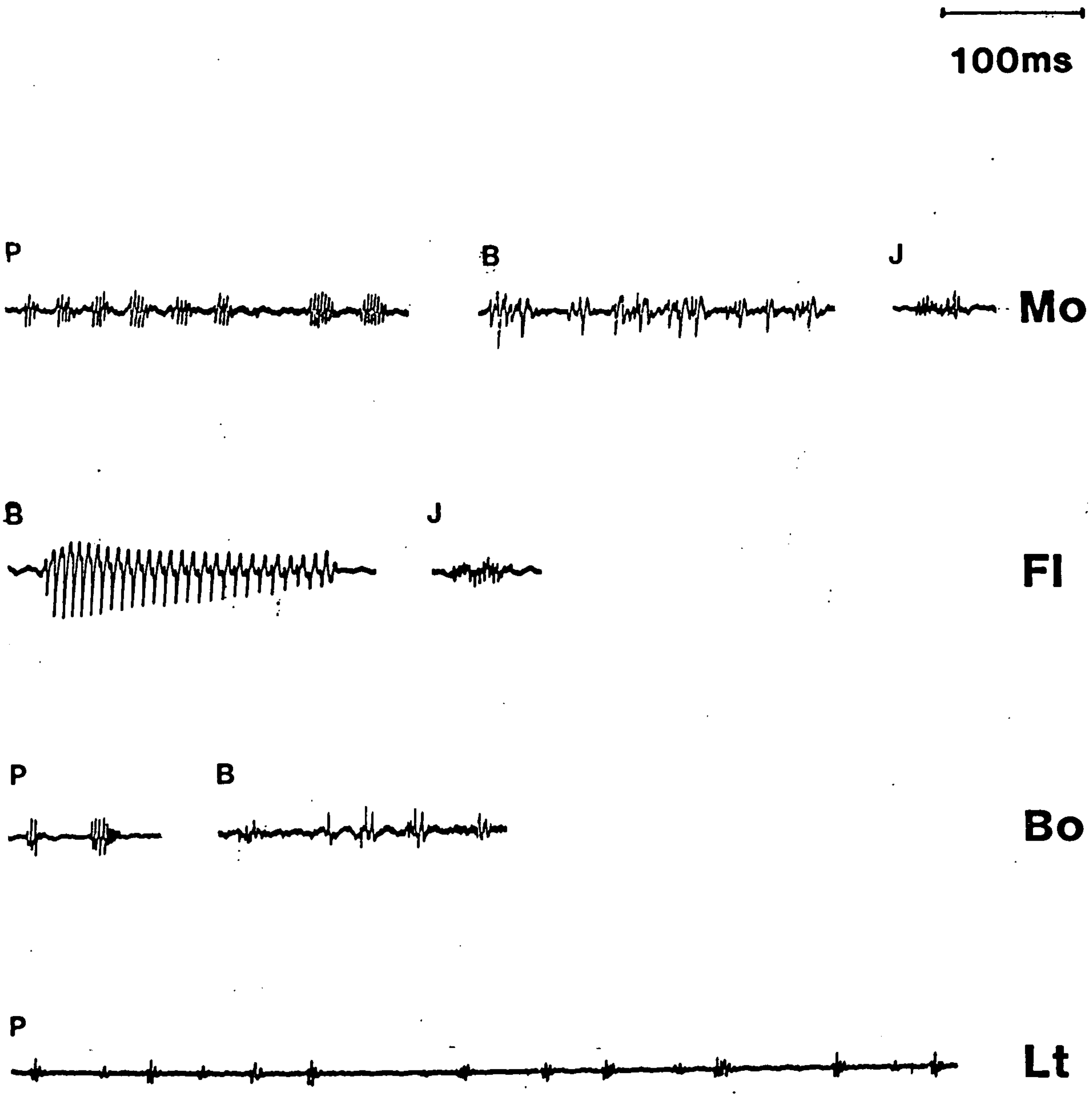

100ms
P
B
J
Mo
B
J
Fl
P
B
Bo
P
Lt

Table 4-5

Analysis of male aggressive pulse songs (MEAN OF MEANS ± S.E.(S.D.)) and buzz songs of the *montana* phylad.

PULSE SONG	BUZZ SONGS
D.littoralis	*D.montana*; *D.flavomontana*;
PULSE LENGTH (ms)	*D.borealis*
N=3; n=116	
9.27 ± 0.26(0.45)	Frequency: most concentrated 100-400 Hz
Range:5.0-15.0	Duration:
No. CYCLES PER PULSE	*D.montana*: variable (20-225ms)
3.00 ± 0.23(0.40)	*D.flavomontana*: variable (25-410ms)
Range:1-5	*D.borealis*: short (5-30ms)
INTERPULSE INTERVAL (ms)	
n=106	JABBING BUZZ
46.77 ± 4.02(6.97)	*D.montana*; *D.flavomontana*
Range:25.0-105.0	Frequency: 200-400 Hz
INTRAPULSE FREQUENCY (Hz)	
200-500	

PULSE SONGS: *D.montana*: record is poor (N=1; n=15): pulse length:10-22.5ms; No. cycles per pulse:4-10; interpulse interval:22.5-65ms; intrapulse frequency:300-400Hz.

D.borealis: only produced occasionally; insufficient for analysis

Table 4-6

Songs of the *virilis* group

(Abbreviations: A=amplitude-modulated tone burst; O=other; P=pulse song; B=buzz song; J=jabbing buzz song).

SPECIES	MALE COURTSHIP SONG		MALE AGGRESSIVE SONG			FEMALE SONG	
	A	O	P	B	J	P	B
VIRILIS PHYLAD							
D.virilis	X		X				X
D.lummei	X		X		X	X	
D.novamexicana	X		X		X	X	X
D.a.texana	X		X			X	X
D.a.americana	X		X		X	X	X
MONTANA PHYLAD							
D.montana		X	X	X	X		X
D.flavomontana		X		X	X		X
D.borealis	X		X	X			X
D.littoralis		X	X			X	

4.4.4 DISCUSSION

In order to facilitate the discussion, the results for both female songs and male aggressive songs are shown in a simplified form in Table 4-6. In the virilis phylad, males of all species produce aggressive song in the form of pulse song. Therefore the production of pulse song, in itself, is not a sex-specific character. Interestingly, in D.virilis where females produce no pulse song (although in D.virilis (brewery) they do) and in D.lummei where they produce very little, the males of these species do produce pulse song, although not very often. In the remaining species of the virilis phylad where both males and females produce pulse song it is possible to perform a rough comparison of mean interpulse intervals: D.novamexicana: 52ms(female):58ms(male); D.a.texana: 65ms(F):55ms(M); D.a.americana: 36ms(F):43ms(M). In other words, the interpulse intervals of male and female songs are roughly comparable.

In the four montana phylad species, it is only the females of D.littoralis which produce pulse song and here the mean interpulse intervals of male and female pulse songs are also very close: 45ms(F):47ms(M). The females of the remaining three species of the montana phylad produce only buzz song but males of two of the species (D.montana and D.borealis) do produce pulse song but unfortunately not in sufficient quantity for a useful analysis to be performed. D.flavomontana is unique among the species tested in that it does not produce any pulse song at all. Interestingly, like the females of these species, males of D.montana ,D.flavomontana and D.borealis also produce a buzz song. Therefore the production of buzz song is not a sex-specific character either.

Jabbing behaviour, which results in the production of buzz song, is restricted to males. It has been observed in *D.lummei*, *D.novamexicana* and *D.a.americana* from the *virilis* phylad and in *D.montana* and *D.flavomontana* from the *montana* phylad.

It could be argued that in species where both sexes produce pulse song (*D.novamexicana* ,*D.a.texana* ,*D.a.americana* and *D.littoralis*) the pulse songs of the male and female may be sufficiently different to allow the male to distinguish between the two. The comparison of interpulse intervals (see above) reveals that this is unlikely because they are probably too similar. Sufficient data was available from *D.a.americana* to perform a statistical comparison (Student's t-Test) of female song and male aggressive song. (Male aggressive song: ANOVA: $F=2.14;p>0.05$ (PL); $F=26.47;p<0.05$ (No.C/P); $F=18.12;p<0.05$ (ipi);the ipi (mean of means)=1.63 ± 0.03(0.05) log ms). The analysis indicated no significant difference between the songs, at the five percent level, in terms of pulse length ($t(15)=1.23$) , number of cycles per pulse ($t(15)=0.91$), interpulse interval ($t(16)=1.89$). The estimated intrapulse frequencies were also similar. They did differ in one respect however, and this applies also to *D.lummei* ,*D.novamexicana* and *D.a.texana* :whereas female pulse song normally consists of short bursts containing 3,4,5 or 6 pulses, male aggressive pulse song normally consists of long bursts containing around 10,20 or even 30 pulses. By contrast, both male and female *D.littoralis* produce long trains of pulses.

In conclusion, it can be said that for each species of the *virilis* group that has been tested, the song produced by the female when courted by the male is similar to the aggressive song of the male. In the virilis phylad both female song and male aggressive song are in the form of pulse song. (Although the females of the stock strain of D.virilis are silent, females of the D.virilis(brewery) strain produce pulse song as do the males of both strains). In the montana phylad species, both female song and male aggressive song are in the form of buzz song in three species (with some pulse song being produced by males of two of these species), and pulse song in one species.

Rough comparisons of male and female pulse song in the virilis phylad, and in D.littoralis, and a more detailed comparison in the case of D.a.americana, reveal that they are similar with respect to the measured song parameters. It is not possible to critically compare male and female buzz song in the montana phylad because of the unpatterned nature of buzz song, but this very irregularity of structure makes it extremely unlikely that the buzz song of the two sexes convey information relating to sex.

Female song is not a sex-specific signal.

4.5 AN ALTERNATIVE APPROACH

4.5.1 INTRODUCTION

Another approach to the problem of the function of female song is to attempt to determine its effect on the courting male: are there any differences between courtships which contain female song and those which do not? Since courtships in which the female is silent are fairly rare, the female song component of courtship must be removed artificially. This can be achieved experimentally in two ways: either the female can be prevented from producing song by the surgical removal of her wings, or the male can be deafened by the surgical removal of his aristae.

Perhaps the most important courtship parameter for the purposes of the present investigation is mating speed. If female song is important in inducing the male to continue courting then mating speed should perhaps be slower in wingless female and aristaeless male courtships. If female song provides an inhibitory or repelling stimulus to the male then wingless female and aristaeless male courtships should perhaps be faster and/or contain fewer breaks or interruptions than intact female and male courtships.

Before the results from courtships involving wingless females or aristaeless males can be properly interpreted however, it is necessary to exclude any other effects that the operations may have. The female wings may be visually attractive to the male, both as part of the general body form of the female and perhaps when they are fluttered. Perhaps most importantly, the female raises the wings into a high-V formation when she adopts the acceptance posture which may be a necessary visual cue to the male to mount the female. Silencing the female by means of wing removal will also remove these visual components of the courtship. It is therefore necessary to demonstrate that these visual cues are of minimal importance, i.e. that courtship is not light-dependent.

The surgical removal of the male aristae may affect the form of the courtship song which he produces. This would occur if the patterning of the song relies on a self-monitoring feedback system. Aberrations in the courtship song may affect various courtship parameters so it is necessary to demonstrate that aristaeless males produce normal courtship song.

The following series of experiments reports on the results of effectively removing the female song component from courtship. The experiments performed to determine the possible side-effects of the surgical operations are reported on in detail, with some interesting findings regarding the nature of the acceptance posture signal. Except where stated otherwise, the strain employed in all the experiments is D.virilis (brewery). The females of this D.virilis strain produce song during courtship, and it was preferred to strains of the other species because of its general vigour which hopefully would reduce any generally incapacitating effects which the surgical operations might have.

4.5.2 WINGLESS FEMALES

4.5.2.1 IS COURTSHIP LIGHT-DEPENDENT?

Asymmetric mass matings (see Chapter 2) were performed in complete darkness. A lamp containing a red and blue filter (wavelength 630 nm) was switched on only to allow the transfer of the males at the start of the experiment and the removal of the flies at the end. Approximately 15 mature virgin females were transferred to the mass mating flask followed by approximately 30 mature virgin males. A lid was then placed over the mouth of the flask which was then turned upside down and held steady by means of a clamp. At the end of the allotted period the flies were shaken down into the etherizer and the females were isolated.

In order to determine how many females had been inseminated they were placed separately in vials and allowed to lay eggs. The appearance of larvae indicated that mating had occured. A second method, involving the dissection of females to show the presence or absence of sperm in the spermathecae and seminal receptacle proved too unreliable and was abandoned.

Two strains were used in this investigation: D.virilis (laboratory stock) and D.virilis (brewery). D.virilis females, unlike D.virilis (brewery) females, do not produce fluttering during courtship. The flies were isolated on the day of eclosion, sexed, and vialled in groups of five. On the day of the experiment, 15 females were transferred to one bottle, and 30 males to another. The flies were then transferred to the mass mating flask. Flies used in these mass matings were 9,10 or 11 days old. The temperature was 26 ± 2 degrees Centigrade, and experiments were performed in the morning (0900-1200 Hrs) and afternoon (1400-1700 Hrs).

4.5.2.1.1 ANALYSIS AND DISCUSSION

The asymmetric mass mating technique is useful because, under normal conditions, all the females have mated within 30 minutes. This provides a useful baseline with which to compare the success of the asymmetric mass matings in the dark. For D.virilis (stock) standard asymmetric mass matings have revealed that 100 percent of sexually mature females have mated within 30 minutes (6 replicates). In these experiments, in which the females were given 30 minutes in the dark, the percentage of females mated in 4 replicates were: 100;100;94.4;81.3. Clearly courtship is not light-dependent, although visual cues probably contribute to mating success.

For D.virilis (brewery) under normal conditions, 100 percent of sexually mature females have mated within 30 minutes (3 replicates). In one replicate only 93.8 percent had mated within 30 minutes. In the dark the percentages were: 86.7;93.8;93.3;100. Again, courtship is clearly not light-dependent, although visual cues probably make a contribution to mating success.

To what extent is courtship impaired in the dark? During standard asymmetric mass matings the flies gather at the end of the flask at which the light source is placed. This positive phototaxis is not possible in the dark but the flies are not randomly distributed. This is because the flask is held upside down and the flies, which are negatively geotactic, gather on the top surface. In addition, some information is available from observations of the courtship of a white-eyed (visually impaired) developmental mutant male (D.virilis (brewery)). This male was unable to orientate properly to the rear of the female and was unable to track a moving female. However, if the female remained stationary for long enough he was able to take up a position to her rear and mount when the female adopted the acceptance posture. Thus visual cues are clearly important in locating and tracking the female once physical contact has been lost, and in performing orientation properly.

The fact that success in courtship is not light-dependent means that the raising of the wings in a high-V formation during the acceptance posture is not a necessary visual cue to the male to mount the female. Close observation of many courtships has revealed that in order for the male to respond to the signal to mount, he must have proboscal contact with the female genitalia. In all 25 observed courtships (D.virilis (brewery)) in which the adoption of the acceptance posture by the female was not immediately followed by male mounting, the male concerned did not have proboscal contact with the female genitalia. The male was normally slightly to the side of the female, licking the side of her abdomen, or sometimes at the rear but licking the anal region. From any of these positions the raised wings of the acceptance posture would be clearly in view but mounting does not occur. This can be compared to all 158 cases in which the adoption of the acceptance posture was followed immediately by mounting: in every case the male had proboscal contact with the female genitalia.

The nature of the signal which the male receives from the female genitalia is likely to be some movement of the ovipositor (vaginal) plates which is detected in some way by the male, via his proboscis (SPIETH (1968)). It is unlikely that a chemical signal is involved, at least directly, because the response is practically instantaneous. Such a close-range physical signal is useful to both the courting male and the courted female since the male is well-placed to mount as soon as he receives the signal and the female can accept the particular male whose courtship she is sampling at the time. It may also be important in a competitive situation where males are jostling for position behind the female - only the male which has ousted the contenders and can track the female closely will be in the correct position to receive the signal to mount.

The function of the high-V wing formation of the acceptance posture is probably to prevent damage to the wings as the male mounts and it also allows the male easy access. In species where no obvious acceptance posture is present the male parts the female wings with his fore-tarsi; in some species the sex combs are used for this purpose (SPIETH (1952)).

Finally, since D.virilis are as successful in the dark as D.virilis (brewery) (D.virilis females are silent) it is unlikely that males require the female song for successfully tracking the female in the dark (or in the light).

In summary, courtship in D.virilis (and probably in the virilis group as a whole) is not light-dependent. The fact that they will mate very readily in the dark means that any visual stimulation which the female, and particularly her wings, provides for the male is not crucial for a successful courtship. Most importantly, it has been shown that it is the female genitalia, and not the high-V wing formation, which provide the signal to mount which the male receives when the female adopts the acceptance posture. Therefore, the silencing of females by the surgical removal of her wings is a legitimate experimental method.

4.5.2.2 ASYMMETRIC MASS MATINGS

Four asymmetric mass matings were performed using wingless females. A control asymmetric mass mating was performed before each wingless female mass mating using intact females. On the day before each trial, 15 females were anaesthetised under carbon dioxide and their wings were removed using microscissors. The operation was performed on a porous plate which allowed the carbon dioxide to slowly filter through. The 15 control females were placed on the plate and exposed to carbon dioxide for 3 to 4 minutes.

During each trial the mated pairs were removed using a pooter, and the number of mated pairs removed in each minute of the trial was noted. The flies were kept together until all the females had been mated, or for a maximum period of 30 minutes. The temperature was 26 ± 2 degrees Centigrade and the mass matings were performed in the morning or afternoon (excluding 1200-1400 Hrs). were 10 days old.

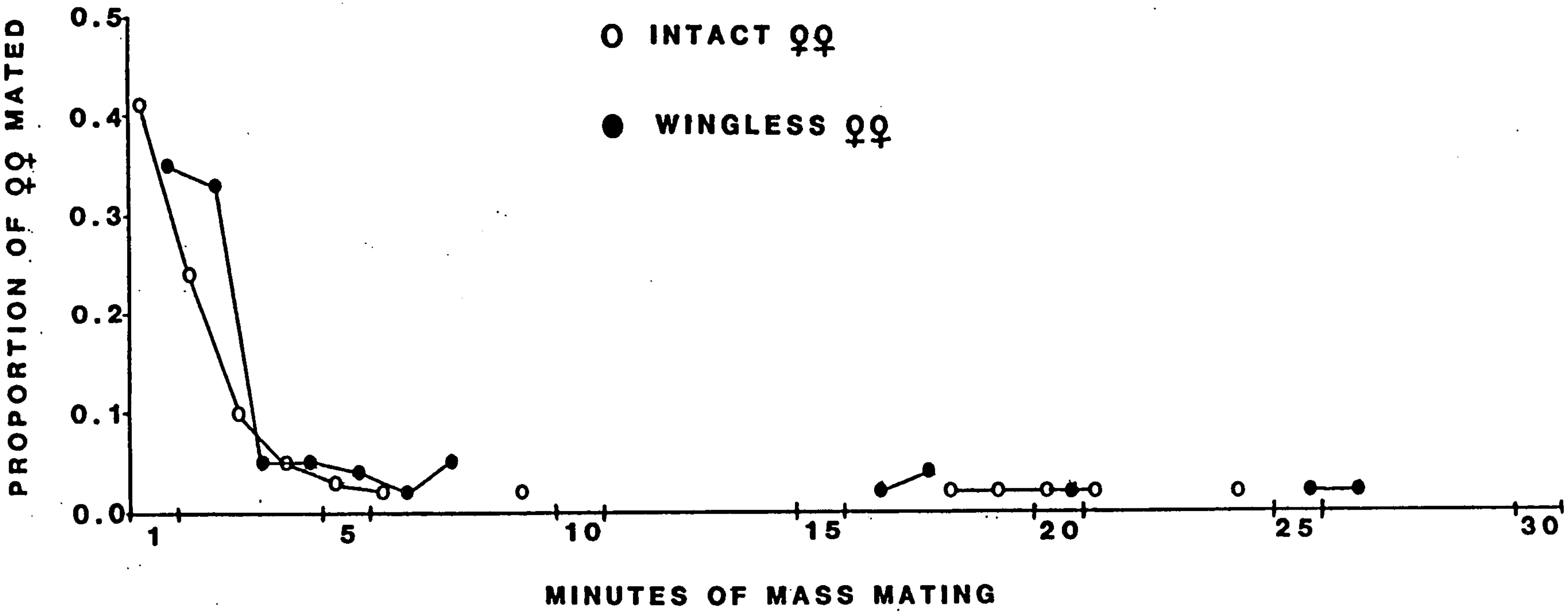
4.5.2.2.1 RESULTS AND DISCUSSION

The total percentage of females mated within 30 minutes are given in the form: INTACT:WINGLESS FEMALES - 93.3:92.9; 92.9:80; 100:93.8; 100:93.8 . On this fairly gross level it is clear that wingless females are only slightly less successful than intact females. Figure 4-5 shows the overall distribution of matings over the thirty minute period for wingless and intact females. The distributions are roughly equal.

Figure 4-5

The proportion of females which mated during each minute of asymmetric mass mating.

Combined data: intact females (4 replicates); wingless females (4 replicates).


O INTACT ♀♀
● WINGLESS ♀♀
PROPORTION OF ♀♀ MATED
0.5
0.4
0.3
0.2
0.1
0.0
1
5
10
15
20
25
30
MINUTES OF MASS MATING

The absence of female song appears to have little effect on mating speed and the distribution of the matings over the 30 minute period. In addition, wing-removal appears to have no serious side-effects.

4.5.2.3 SINGLE PAIR EVENT RECORDER ANALYSIS

The purpose of this investigation was to determine whether there are any clear differences between courtships involving wingless females (no female song) and courtships involving intact females (female song). An event recorder was used to record the following discrete male behaviours: vibration;circling;aggressive song. Those behaviors which occur for a long period of time were also recorded: orientation;mounting;acceptance posture (the acceptance posture is difficult to detect in wingless females which are unable to perform the high-V wing formation, but the abdomen depression which accompanies wing-raising is sometimes obvious.

Males and females were vialled separately in groups of five on the day of eclosion. On the day before they were to be used, females were anaesthetised and their wings removed using microscissors. The females were then vialled singly. Control females were handled in the same way but were left intact. For each mating the female was introduced into the standard cell followed by the male, about 30 seconds to a minute later. Intact female matings were alternated with the wingless female matings in order to take account of any day to day (or hour to hour) variation. Each courtship was terminated after 5 minutes if mating had not occured within that time. If there had been no orientation within 4 minutes the experiment was repeated with the same individuals. The experiments were carried out in the morning and afternoon (excluding

1200-1400 Hrs) at 26 ± 2 degrees Centigrade. The flies were 8 days old.

4.5.2.3.1 RESULTS AND DISCUSSION

Mating speed was recorded for successful courtships (INTACT FEMALES:28/31; WINGLESS FEMALES:20/31). In two of the courtships involving intact females no female song was produced and these were omitted from the analysis. Mating speed was taken as the time elapsing between the first bout of male wing vibration and mounting, excluding any intervals between bouts of courtship. In any case only 8/26 (IF) and 4/20 (WF) courtships contained any breaks in courtship at all. Since the frequency distribution is skewed, mating speed data is best characterised by the median and semi-interquartile range. A two-tailed Student's t-Test was performed on the log-transformed data (Table 4-7). This comparison of mating speed in the two groups indicates that there is no significant difference between them at the five percent level. The absence of the female song component of courtship has no significant effect on mating speed.

Table 4-7

Mating speed (the period from the first vibration to mounting, excluding breaks in courtship) in single pair courtships: intact and wingless females; intact and aristaeless males.

S.I.R.: semi-interquartile range; S.E.: standard error.

	N	MEDIAN ± S.I.R.(s)	MEAN ± S.E.	t-TESTS
INTACT FEMALES	26	17.5 ± 10.0	1.26 ± 0.08	t(44)=0.0023 p>0.05;N.S.
WINGLESS FEMALES	20	24.0 ± 16.0	1.26 ± 0.11	
INTACT MALES	15	23.0 ± 19.5	1.26 ± 0.16	t(32)=0.14 p>0.05;N.S.
ARISTAELESS MALES	19	14.0 ± 25.5	1.24 ± 0.12	

Several other behaviours were also recorded: male circling behaviour is rare. It occured in only two wingless females courtships and two intact female courtships. No male aggressive song was produced at all. The recording of mounting behaviour revealed some interesting information concerning the frequency of mounting attempts in the two groups. Under normal conditions it is very rare for the male to attempt to mount a female which has not adopted the acceptance posture; the signal which the acceptance posture provides appears to be a necessary cue to the male to mount the female. In addition, the few attempted mounts which do occur are invariably unsuccessful and the male dismounts very quickly. As would be expected then only 2 courtships involving intact females contained attempted mounts, one in each courtship.

It is of interest then that 10 of the 31 wingless female courtships contained a total of 18 mounts which failed. Without the raised-wing display of the acceptance posture it is, of course, not possible to clearly determine whether wingless females have adopted the acceptance posture or not. However, the failure of these mounts suggests either that they are attempted mounts i.e. the female has not adopted the acceptance posture, or the mount is mistimed in some way. This latter explanation is unlikely since it has been shown that the male takes his cue to mount from the female genitalia and not from the raised wings so the absence of the wings should not result in an error in the timing of the mount by the male. Why then should there be more attempted mounts in courtships involving wingless females? I think it is possible that the female wings provide some physical barrier to attempted mounts by the male. In normal courtships the head of the male lies directly beneath the wings of the female so any raising of the head, as in mounting, is physically prevented by the wings. It is also possible that the fluttering of the wings could act in this manner, both in a physical way, brushing against the aristae and head of the male whenever he raises it, and also perhaps acoustically, reminding the male of the physical presence of the wings. The male can also detect the stationary or fluttering wings visually, and this too could act to prevent mounting attempts. It should be noted however that this mechanism for preventing attempted mounts is not normally necessary since the majority (21/31) of courtships involving wingless females contain no attempted mounts at all.

Finally, the fact that wingless female courtships are similar to intact female courtships with regard to mating speed and other measured parameters is confirmatory evidence that the crucial signal of the acceptance posture is provided, not by the very obvious (to the observer) raised wing display, but by some other mechanism; this other mechanism is almost certainly a movement of the ovipositor plates which the male is able to detect via his proboscis.

4.5.3 ARISTAELESS MALES

4.5.3.1 THE COURTSHIP SONG OF ARISTAELESS MALES

The aristae are necessary in the reception of sound (MANNING (1967a)) and their removal effectively deafens the fly (SCHILCHER (1976a,b);Chapter 6). Comparison of the courtships of aristaeless and intact males should make it possible to ascertain what effects, if any, female song has upon the courting male. One problem with this technique is the possibility that it may be necessary for the male to hear his own courtship song, since the patterning of the song may rely on an auditory feedback mechanism. The idea of such a peripheral feedback loop was first suggested by BURNET, CONNOLLY and DENNIS (1971) to explain the reduced competitive mating ability and mating speed of mutant aristaeless males. In a later series of experiments BURNET, EASTWOOD and CONNOLLY (1977) recorded the courtship song of both aristaeless mutants and males which had had their aristae surgically removed. In both cases the courtship song was found to be normal and the courtship deficiencies of mutant aristaeless males were explained in terms of the pleiotropic effects of the mutation.

The experiments reported above were performed with D.melanogaster and the measurements were made on the pulse song of this species. It seemed worthwhile to discover whether the very different amplitude-modulated tone burst courtship song of D.virilis would also remain unchanged after surgical removal of the aristae. To this end the courtship song of an aristaeless male was recorded and analysed (see Chapter 2). It was found to be normal in all measured parameters: burst length is 85-155ms in intact males and

125-162ms in the aristaeless male; number of pulses per burst is 6-12 in intact males and 8-10 in the aristaeless male. The aristaeless male values for the following three parameters all fall within the ranges given for intact male courtship song: pulse length (5-10ms); number of cycles per pulse (3-6); intrapulse frequency (300-400Hz). As expected, removal of the male aristae does not affect the structure of the courtship song.

4.5.3.2 ASYMMETRIC MASS MATINGS

Two standard asymmetric mass matings were performed using aristaeless males. A control asymmetric mass mating was performed before each aristaeless male mass mating employing intact males. On the day before each trial, 30 males were anaesthetised under carbon dioxide and their aristae were removed, as close to the base as possible, by nipping them off with a pair of fine forceps. The operation was performed on a porous plate which allowed the carbon dioxide to slowly filter through. The 30 control males were placed on the plate and exposed to carbon dioxide for 3 to 4 minutes. (See wingless female mass matings for trial method).

4.5.3.2.1 RESULTS AND DISCUSSION

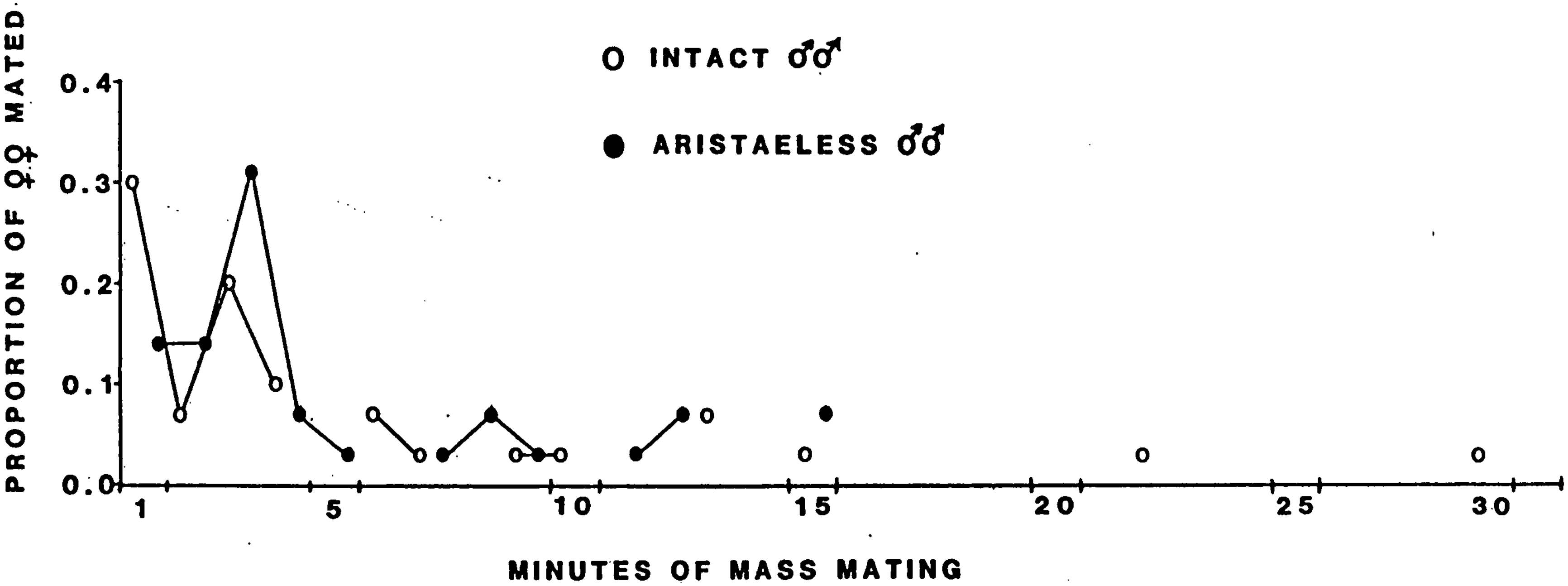
The total percentages of females mated within 30 minutes are given in the form: INTACT:ARISTAELESS MALE matings: 87.5:87.5; 100:100. The overall distribution of matings over the 30 minute period is shown in Figure 4-6.

This simple experiment demonstrates that aristaeless males are not at a gross disadvantage to intact males in terms of overall mating success and the distribution of matings over time. The effective absence of the female song component of courtship appears to have no large effect on courtship. Are there any other observable effects which can be attributed to arista-removal? SCHILCHER (1976a) has shown that D.melanogaster males increase their locomotor activity in response to courtship song. In several species of the virilis group a male has been observed to run towards a courting pair as soon as the courting male commences vibration. He may then attempt to gain access to the rear of the female himself, pushing the courting male out of the way in the process, or he may take up a position behind the courting male and begin courtship. Often, short "caravans" are formed with two or three males in a line behind the female as she moves along, frequently altering her speed and course. Aristaeless males then might be expected to be less active than intact males which might be reflected in a reduction in mating speed in mass matings. Although there are fewer aristaeless males mating in the first minute of the mass mating than intact males (Fig. 4-6), the distributions in general are about equal. It is likely that aristaeless males are not seriously handicapped in these mass matings because the levels of other stimuli (tactile, visual, chemical) are high in these crowded conditions. It is likely that these stimuli would compensate for the lack of auditory stimulation.

Fiqure 4-6

The proportion of females which mated during each minute of asymmetric mass mating.

Combined data: intact males (2 replicates); aristaeless males (2 replicates).


O INTACT ♂♂
● ARISTAELESS ♂♂
PROPORTION OF ♀♀ MATED
0.4
0.3
0.2
0.1
0.0
1
5
10
15
20
25
30
MINUTES OF MASS MATING

4.5.3.3 SINGLE PAIR EVENT RECORDER ANALYSIS

Aristaeless males are effectively deaf and so are unable to perceive the female song. A comparison of courtships involving intact and aristaeless males then is essentially a comparison of courtships which contain, or do not contain, the female song component. The purpose of these experiments was to determine whether the absence of the female song component would effect the measured courtship parameters. The procedure was essentially the same as for wingless females except that the males, instead of the females, were operated on and the aristae, rather than the wings, were removed. This operation was performed using fine forceps, and only a tiny, branchless stump of each arista remained afterwards.

4.5.3.3.1 RESULTS AND DISCUSSION

Mating speed was recorded for successful courtships (INTACT MALES: 19/31; ARISTAELESS MALES: 22/31). There were 4 intact male courtships and 3 aristaeless male courtships where no female song was produced and these were excluded from the analysis. Mating speed was taken as the time elapsing between the first bout of male wing vibration and mounting, excluding any intervals between bouts of courtship. In any case only 7/15 (IM) and 6/19 (AM) courtships contained such intervals. The median and semi-interquartile range are given for both groups of data, and a two-tailed Student's t-Test was performed on the log-transformed data (Table 4-7). This comparison of mating speed reveals no significant difference between the two groups at the five percent level. The absence of the female song component of courtship has no significant effect on mating speed.

There was little difference in the prevalence of circling behaviour in the two groups (it occured in 3 aristaeless male courtships but in none of the intact male courtships), and no aggressive song was produced in either group. There were 5 attempted mountings in the aristaeless group and 2 attempted mountings in the intact group. This small difference between the two groups in terms of the number of attempted mounts can be compared to the larger difference (18 v 2) found between wingless and intact female courtships. If it is assumed, as suggested earlier, that the wings of the female sometimes act to prevent attempted mounting, then this finding is evidence that it is the presence and movement of the wings, detected both physically and visually, rather than the female song itself, which are most important in this respect.

This series of experiments involving wingless females and aristaeless males has established that the absence of the female song component of courtship has no observable effect on either mating speed or other important courtship parameters. The consequences of this finding are discussed in the Section 4.6.

4.6 GENERAL DISCUSSION

4.6.1 INTRODUCTION

This investigation was undertaken in order to examine the phenomenon of female song in the *virilis* group in detail. The intention was to provide evidence that would either substantiate the speculations of DONEGAN and EWING (1980) or point to alternative explanations. The discussion of findings is divided into four main sections:

4.6.2 COMPARISONS WITH *ZAPRIONUS*

This consists of a comparison of the present findings with those obtained by BENNET-CLARK, LEROY and TSACAS (1980) from their detailed investigation of female song in the genus *Zaprionus*, which is closely related to the genus *Drosophila*.

4.6.3 REVIEW OF CIRCUMSTANTIAL EVIDENCE

DONEGAN and EWING (1980) present several pieces of circumstantial evidence in support of the view that female song may function both as an attractive signal and also as a sexual isolation mechanism. This evidence is re-examined in the light of the present findings.

4.6.4 THE FUNCTION OF FLUTTERING

Female song is examined not in isolation but as a component of fluttering behaviour. It is proposed that fluttering is a spacing behaviour.

4.6.5 REJECTION RESPONSES

This term has lead to much confusion in the past and has produced some misunderstanding with regard to the function of fluttering behaviour. A fresh evaluation of the function of the rejection responses is undertaken.

4.6.2 COMPARISONS WITH ZAPRIONUS

In the genus *Zaprionus*, female song is species-specific in all species and BENNET-CLARK et al (1980) suggest that it might have an important role to play in sexual isolation. By contrast, in the *virilis* group, the production of patterned pulse song is not universal and it is unlikely to function in sexual isolation (Section 4.3). Only *D.a.texana* and *D.a.americana* of the *virilis* phylad consistently produce large amounts of pulse song, and only *D.littoralis* of the four *montana* phylad species tested produces pulse song (the pulse song of *D.littoralis* is similar to that produced by *D.a.americana*). It is possible, on theoretical grounds, that the pulse songs of *D.a.texana* and *D.a.americana* are sufficiently different to function as species-isolating devices, but the ease with which crosses between these two species occur in the laboratory and the discovery of hybrids in nature weighs heavily against this possibility. In addition, it is likely that the amount of pulse song produced by females might be related to the strain rather than the species since *D.virilis* (laboratory stock) females produce no pulse song at all whereas *D.virilis* (brewery) females produce large quantities. Interstrain variation of this sort would not be expected if the female song were a species-specific signal.

In most *Zaprionus* species the female songs are also sex-specific and so may inform the male that he is courting a female. This is not the case in the *virilis* group (Section 4.4).

In the *virilis* phylad the male aggressive songs, like the female songs, are in the form of patterned pulse song. In addition, in the species where the female produces sufficient song for meaningful analysis (*D.novamexicana*, *D.a.texana*, *D.a.americana*) a direct comparison of male aggressive song and female song reveals a striking similarity with regard to interpulse interval, an important song parameter (e.g. see EWING and BENNET-CLARK (1968)). The same applies to *D.littoralis* of the *montana* phylad where both male aggressive song and female song are in the form of paterned pulse song. The males of the other three species of the *montana* phylad (*D.montana*, *D.flavomontana*, *D.borealis*) produce aggressive song which is similar to the buzz song of the females of these species. In addition, *D.montana* and *D.borealis* males also produce pulse song.

Perhaps the most important piece of evidence concerning the function of female song in *Zaprionus* species is that the structure of the female song, and the intensity of an associated behaviour called "rocking", change after copulation has occured and result in observable changes in the behaviour of the courting male. Before copulation has occured female song has a pulsed "whine" pattern and "rocking" is performed at moderate intensity. Both appear to have little effect on the courting male. After copulation, however, the pulsed pattern of the song breaks down and a continuous "whine" is produced. "Rocking" behaviour is now performed more vigorously and for longer periods. In response to this continuous "whine" and vigorous "rocking" the courtship song of the male may become erratic, extended or may cease. He may also stop courting altogether and and move away from the female.

In Zaprionus species then the females produce two types of song: an attractive song is produced by mature virgin females, and a repelling song is produced by fertilised females. To test for the possibility that females of the virilis group may produce different songs according to their reproductive state the songs of immature virgin females, mature virgin females and fertilised females (D.a.americana) were recorded and analysed (Section 4.2). The song produced by females in a non-sexual context (i.e. no males present) was also recorded to determine whether it was different from that produced by courted females. This analysis revealed that the characteristics of female song remain the same whatever the reproductive state of the female and the context of the experiment. So, unlike Zaprionus species, only one song type is produced by females in all reproductive states and there is no evidence for an attractive pre-copula song and a repelling post-copula song.

4.6.3 REVIEW OF CIRCUMSTANTIAL EVIDENCE

Since it is highly unlikely that the female song produced in several species of the virilis group is produced to attract the courting male or that it functions in sexual isolation, it is of interest to review the various pieces of circumstantial evidence which were presented by DONEGAN and EWING (1980) in support of these ideas. Such a review is made possible by the introduction of the fresh evidence which this present series of investigations has provided.

(i) There is no obvious repelling of the male

Because female song appears to have little effect on the courtship of the male it was thought that it may have an attractive function.

However, the irregular pulse song resulting from wing fluttering in D.melanogaster also appears to have little effect on the courtship of the male (CONNOLLY and COOK (1973)). Clearly, lack of obvious effect on the courting male is not direct evidence that female song is attractive.

The experiments of Section 4.2 have shown that it is highly unlikely that the female produces song in order to induce the male to continue courting. This conclusion is based on a consideration of the sexual motivation of the female producing the song. However, this experiment does not rule out the possibility that the male may find female song attractive.

One way of determining whether an actual attractive or repelling effect can be attributed to female song is to discover whether the presence or absence of female song has any effect on the outcome of courtship. Out of a total of 114 successful single-pair courtships (D.virilis (brewery)), 99 contained female song whereas 15 did not. This can be compared to the 43 unsuccessful courtships (no mating within 5 minutes), 37 of which contained female song and 6 did not. In other words, the proportion of courtships which contain female song to those which do not is roughly the same, independent of the outcome of courtship: whether or not female song is produced makes no difference in terms of courtship success. (By comparison, the data for male courtship song reveal that all of the 114 successful courtships contain male courtship song. The fact that the majority (35) of the 43 unsuccessful courtships also contain courtship song shows that although male courtship song is a necessary component of a successful courtship, it is not always sufficient (see also Chapter 3)).

In order to examine more directly the effect of female song upon the courting male, a series of single-pair courtships involving wingless females and aristaeless males (i.e. "silenced" females and "deafened" males) were performed (Section 4.5). It was shown that this artificial removal of the female song component of courtship has little effect either on mating speed or on other measured male behaviours (circling, production of aggressive song). The increase in attempted mountings by the male in courtships involving wingless females could reasonably be attributed, on the basis of the aristaeless male results, to the physical absence of the female wings rather than the absence of the female song per se.

In conclusion, it would appear that female song is neither attractive nor repelling to the courting male; or, if such effects are present, they must be of minimal importance.

(ii) The production of female song occurs both immediately prior to, and following, the acceptance posture

The female produces song right up to the adoption of the acceptance posture; if the male fails to mount the female she may lower her wings and produce song once more. The adoption of the acceptance posture indicates the female's readiness to mate, i.e. the courtship of the male has been of the correct quality and quantity to sexually arouse the female sufficiently to accept the male. The fact that the female can produce song both immediately prior to, and following, the acceptance posture was taken as good circumstantial evidence that song is produced when the female is sexually aroused. It was argued from this that one of the functions of female song may be to induce the male to continue courting, i.e. that female song is attractive.

The songs produced by the three semispecies of D.athabasca are interesting in this respect. Although MILLER, GOLDSTEIN and PATTY (1975) classify them as rejection responses, they were interested by the fact that these songs are especially noticeable at times of unsuccessful copulation attempts by the male and that mating occurs soon after they are produced. They thought it unusual that a female rejection response should be produced just prior to mating.

Clearly, the female cannot conceivably be rejecting the courting male if she is just about to accept him, or has just produced the acceptance posture. However, it must be remembered that there is no evidence that female song (fluttering), or any of the other so-called "rejection responses", indicate that the female is rejecting the male on the basis of unsatisfactory or insufficient courtship. All that can be said is that the female is able to change from the performance of rejection responses to the acceptance posture and back again very rapidly; such a system has already been discussed in Chapter 3.

(iii) Female song is produced before male courtship song

As is the case in species of the genus Zaprionus (BENNET-CLARK et al (1980)), female song is produced prior to the production of male courtship song. This fact has been used to support the idea of both a general attractive signal inducing the male to court and a possible sexual-isolation role for female song. The latter idea is particularly interesting because SCHILCHER and DOW (1977), referring to male tapping in D.melanogaster, have stressed the importance of sexual isolating mechanisms that operate as early on as possible in courtship.

It has been shown that it is highly unlikely that female song functions as an attractive signal; nor is it likely to be a sexual isolation mechanism. However, there is a more immediate reason why it tends to be produced prior to male courtship song. Close observation has revealed that fluttering behaviour (and the resultant female song) is only produced by the female in response to tactile stimulation. It is not produced spontaneously. Such tactile stimulation is provided in the courtship context by the tapping, licking and rubbing of the courting male and in the non-sexual context by one female accidentally touching or colliding with another female. Since the male must begin his courtship by tapping the female and then taking up a position to her rear where he licks her genitalia whilst rubbing the underside of her abdomen with his fore-tarsi, and since these behaviours must be performed before he produces courtship song, it is clear that female song will tend to be produced prior to the production of courtship song by the male.

(iv) "Duetting"

The term "duetting" has been used to describe the alternation of female song with male song bursts. However this term is misleading since it implies, without evidence, that female song is in some way stimulatory to the male and also that female song is produced in response to male courtship song whereas in fact (see (iii)) it is produced in response to tactile stimulation.

The phenomenon of "duetting" can be looked at in greater detail by performing a detailed survey of the 26 successful courtships (D.a.americana) analysed in Chapter 3 with regard to which male behaviours immediately precede the production of female song: in 18 it was produced after male orientation (223 records); in 19 it followed male orientation/licking/rubbing (163 records); in only 10 courtships was it produced after male orientation/licking/rubbing/vibration (57 records). This data clearly confirms that female song is produced in response to male tactile stimulation and in addition it shows that so-called "duetting" occurs only in the minority (10/23) of courtships.

Further evidence is available which demonstrates that the concept of "duetting" is misleading inasmuch as it implies that the female is "replying", or "responding", to male courtship song: aristaeless females, which are deaf, produce song in response to male courtship, as do intact females when courted by wingless males, which are silent. Finally, simulated male courtship song, which has been shown sometimes to induce females to perform the acceptance posture, i.e. is sexually stimulating to the female, does not result in female song production. (Details of these experiments are presented in Chapter 6).

The alternation of male courtship song with female song is probably due to the "see-saw" motion of the male proboscis. During each vibration the proboscal labellum is pulled down away from the female genitalia, but is replaced against the female genitalia once more at the end of each vibratory burst. It is probably this resumed contact of the male proboscal labellum with the female genitalia after each vibratory burst which stimulates the female to produce a short burst of song. In this way the "duetting" pattern is generated.

(v) The female produces a distinct repelling song

Females of the virilis phylad occasionally produce a distinct buzz song when courted by two males at the same time. Since the production of buzz song is restricted to this situation which females probably find aversive (difficulty in processing the courtship of two males simultaneously; agonistic interactions between the two males; increased physical contact with the female), and since it is not produced in normal single-pair courtships, buzz song was considered to be a repelling song. In support of this, sexually immature females of some species produce buzz song in response to male courtship. In the absence of direct experimental evidence of the effect of buzz song on the male, this circumstantial evidence seemed fairly convincing. Since pulse song is very different in structure from buzz song, it was felt that it might serve a separate, attractive, function.

However, the findings of further experiments involving the courtship of females by two males have failed to confirm the supposed special status of buzz song (Section 4.3). It has been found that females of two species (D.a.texana and D.a.americana) produce pulse song when courted by two males in addition to the occasional production of buzz song. In addition, sexually immature females (at least in D.a.americana) produce pulse song in response to male courtship. In any case, the very concept of a "repelling song" is open to serious doubt since neither song has been shown to have a repelling effect on the courting male.

4.6.4 THE FUNCTION OF FLUTTERING BEHAVIOUR

4.6.4.1 INTRODUCTION

During the course of this study of the virilis group attention has largely centered around the possible signal value of female song which is produced as a result of wing fluttering. Detailed investigations have revealed that female song is neither species-specific nor sex-specific and that the characteristics of the song remain the same independent of the reproductive state of the female so that it is highly unlikely that the song is produced in order to induce the male to continue courting. Finally, experiments involving aristaeless males and wingless females have demonstrated that the song itself has no obvious attractive or repelling effect on the courting male.

It is now clear that in order to understand the function of female song it may be necessary to examine song, not in isolation, but as the acoustic component of fluttering behaviour (which also contains visual and physical components) and also to consider the function of fluttering behaviour outside of the courtship context. With this in mind, the evidence from the present series of investigations and from other sources can now be employed in an attempt to establish the function of fluttering behaviour as a whole. It will be argued that fluttering functions as a spacing behaviour, helping to maintain the "individual distance" or "personal space" of flies. More particularly, it is a low-level signal that functions most effectively in maintaining individual distance in all-female groups and is ineffective in preventing the advances of a courting male.

Finally, male-male interactions are considered. Fluttering behaviour is an important component of such interactions and this fact lends support to the hypothesis that fluttering is a spacing behaviour. It is suggested that buzzing is also a spacing behaviour.

4.6.4.2 FLUTTERING AS A SPACING BEHAVIOUR

Fluttering behaviour in the virilis group is produced by females in all reproductive states and in both courtship and non-courtship contexts. It seems likely that a behaviour which occurs in such a diverse range of circumstances must have a general function. The fact that fluttering is always produced in response to tactile stimulation, i.e. contact with another fly, suggests that this general function may be to act as a spacing behaviour maintaining "individual distance". It is of interest that in D.melanogaster fluttering behaviour is also commonly associated with tactile stimulation (tapping and licking by the male) (CONNOLLY and COOK (1973)).

There is direct evidence that flies can and do maintain "individual distance". SEXTON and STALKER (1961) have shown that when D.paramelanica females are crowded together they assume a uniform spacing pattern which limits the number that can occupy a given area. This is achieved in two ways: firstly, they show avoidance reactions when they are within 1 to 5 mm of each other and secondly, they are able to control their immediate surroundings, at least to a limited extent, by fending off flies which approach too closely. Fending is also used to maintain "individual distance" in D.pseudoobscura (BROWN (1964)) where it is produced in response to contact stimuli. CONNOLLY (1968) has shown that flies increase the amount of preening behaviour they perform in the presence of other flies and he suggests that this may act as a visual signalling device facilitating the spacing of the flies and so reducing the possibility of accidental contact in a group situation.

Here it is argued that when a female produces fluttering behaviour, in response to contact stimuli, the acoustic and visual components of this behaviour serve to inform the fly which has approached that it has made contact with another fly and they may also signal the fly's presence to other flies in the vicinity. The actual movement of the wings outwards from the body during fluttering may provide a physical delineation of "personal space". Since the evidence presented above suggests that flies seek to avoid contact with each other, such personal advertisement acts to maintain "individual distance".

In order to understand why spacing behaviour is necessary it is important to consider the natural ecology of the flies (sources: THROCKMORTON (1982); PARSONS (1973):chapters 14 and 15). In general, species of the virilis group utilise tree sap and rotting bark as oviposition sites. Although adults may also feed at these sites, it is unlikely that the same site is used for both oviposition and adult feeding at the same time. Which particular activity is pursued is likely to be dependent upon the state (consistency, dessication, identity of fermenting organisms) of the substrate. Work on other species suggests that adults are able to utilise a much wider range of sites than are available for oviposition purposes. It is probably at the adult feeding sites where courtship and mating occur.

What is important for the purposes of the present discussion is that adults must congregate at limited substrate sites. Crowding at such sites could lead to mutual interference and increasing competition for available resources. These problems are particularly acute for females not only because oviposition sites are scarcer than adult feeding sites, but also because females must spend a large proportion of their time feeding because of the high energetic cost of egg production. There may also be a disadvantage to crowding in that contact would facilitate the transmission of parasites and disease. So, spacing devices are employed such as avoidance reactions and the protection of "individual distance" by behaviours such as fluttering, fending and walking or running away. These behaviours have the effect of minimising contact between flies and of assuring that the limited food resource is not overpopulated.

What I have attempted to establish is that it is likely that fluttering functions as a spacing behaviour maintaining "individual distance", and that this behaviour is important when viewed in the context of the natural ecology of the flies. The laboratory studies have concentrated on the function of fluttering behaviour in the courtship context and so have missed the real importance of this behaviour. However, it still remains to be explained why this behaviour is ineffective in deterring the advances of the courting male. The most likely explanation is that the sexual motivation of the courting male is at a high level, sufficient to cause him to ignore what is, in effect, only a low-level spacing behaviour which is probably most effective in all-female groups.

Evidence of high sexual motivation is provided by the vigorous and persistent tracking which the courting male must often perform.

Observations of courting flies in stock bottles often reveal prolonged chases in which one to several males form a line behind the female and follow her every movement as she runs and zig-zags from side to side. It is also worth noting that once the female is stationary and the male is courting at her rear, he is subject to a continual barrage of blows to the head as the female kicks backwards with her hind legs. As with fluttering, the male appears oblivious to this kicking behaviour.

There is another aspect to female fluttering during courtship which must be considered in relation to the natural situation: fluttering is produced not only when the female is stationary but also as she walks or runs away from the courting male (CONNOLLY and COOK (1973) report that fluttering behaviour is primarily associated with high locomotor activity in D.melanogaster). This fluttering behaviour performed whilst running from the male may warn other flies in the path so that they can take evasive action. However, one effect of this running and fluttering is probably to entice males to approach and court since any female movement is likely to be attractive to males (SPIETH (1966)). It is also likely that the courtship song produced by the tracking male also has the effect of attracting other males (see Section 4.5.3.2.1). When several males are attracted to , and begin courting, the same female, or each other, male-male interactions occur in which fluttering behaviour is involved. As will be seen, the occurence of fluttering behaviour in such interactions lends support to the idea that fluttering functions as a spacing behaviour.

As has already been described (Section 4.4), males of the virilis group produce fluttering when tapped and courted by another male and during agonistic interactions. These agonistic interactions consist of one male charging, barging or jostling another male which is either courting the female or some distance away. One or both males may produce fluttering. Males of three species also occasionally produce fluttering when courting the female, in the absence of another male. In two of these species it is almost always associated with circling behaviour.

So, as with female fluttering, male fluttering is produced in response to tactile stimulation, although it may also be produced spontaneously, e.g. whilst charging another male. There are also striking similarities in the patterning of the pulse song produced as a result of fluttering by males and females of the same species. Whether or not the precise patterning of the pulse song is crucial to its spacing signal function is unknown. However, one important difference between the fluttering behaviour of males and females in the _virilis_ phylad is that the fluttering of the male is continued for a much longer period. Female song typically consists of 3,4,5 or 6 pulses whereas male aggressive song typically contains 10 to 20 pulses,and sometimes even 30 pulses. It seems reasonable to suppose that the fluttering of the male is more persistent because this increases the effectiveness of this behaviour.

The function of agonistic behaviour is almost certainly to repel or drive off another male. Many observations have been made in the small cell of males competing with each other for the courtship position behind the female. Although part of this interaction is no doubt due to males attempting to court the female at the same time, charging and jostling are also seen. In all these interactions fluttering behaviour is very frequently produced by one or both males. In some species, fluttering behaviour is also seen as the male is circling around the female and here again it is probably associated with driving of other males from around the female.

However, male-male interactions are sometimes seen some distance from the female. Although it is not possible to investigate these interactions properly in the confines of a small cell, work with D.melanogaster where food dishes were provided in a "large" arena (aquarium or petri-dish) provides evidence that males are able to defend a food source by driving other males off (JACOBS (1960); DOW and SCHILCHER (1975); JACOBS (1978)). Various forms of "aggressive" behaviours are employed by males in these encounters including "charging" and "wrestling". Males may monopolise a food resource in this way for reasons of energy requirement and/or because females will be attracted to it.

So, although fluttering has not been shown directly to have the effect of maintaining "individual distance", the association of this behaviour with male agonistic interactions is strong circumstantial evidence that it functions in just this way. In addition, it is likely that observations of previously isolated virgin males and females in the confines of a small cell may not accurately reflect the effect that fluttering behaviour may have under more natural circumstances. In the wild, one might expect fluttering by the female to be effective as a spacing device but ineffective in preventing the advances of the courting male. Male fluttering on the other hand, because of its increased persistence and in association with other agonistic behaviours, would be expected to have some effect in repelling courting and competing males and an important role in driving off other males from the food source.

Finally, it is worth considering buzz song in the light of what has been said concerning the role of fluttering as a spacing behaviour. Although buzz song is the only form of song produced by three species of the montana phylad, females of the virilis phylad only produce buzz song when courted by two males at the same time, a situation which they may find particularly aversive. In addition, males of the virilis phylad only produce buzz song in association with jabbing behaviour. This behaviour is almost certainly associated with driving another male off, i.e. territorial behaviour as described above for D.melanogaster. It seems reasonable to suggest therefore that buzzing is a more intense or extreme spacing behaviour than fluttering, at least in the virilis phylad.

4.6.5 REJECTION RESPONSES

The female of many species of Drosophila performs several behaviours during courtship which are often termed "rejection responses". Chief amongst these rejection responses are: fluttering; kicking; fending; curling(abdomen bending); extrusion. The female may also walk or run away from the courting male, and the significance of this behaviour has been discussed elsewhere in this chapter. Decamping (flying off) usually terminates courtship and the importance of this behaviour was considered in Chapter 3. EWING (1983) has pointed out that there are many problems with the use of this term to describe female behaviour (see also Section 4.6.3(ii)). Fluttering behaviour has been re-examined in the light of new experimental findings (Section 4.6.4), and here an attempt is made to re-evaluate the functions of the other rejection responses using, where relevant, similar arguments.

Up until the paper of CONNOLLY and COOK (1973) it was generally thought that rejection responses were produced by sexually unreceptive females (immature, inseminated) or females which were receptive but found the courtship of the male inadequate or inappropriate (RENDEL (1945); SPIETH (1952,1974); BASTOCK and MANNING (1955); BURNET, CONNOLLY and DENNIS (1971); EWING (1977)). It was supposed that the function of these behaviours was to inhibit the courtship of the male, the extent of this inhibition being dependent on the particular rejection response concerned (RENDEL (1945); BASTOCK and MANNING (1955); BROWN (1964); EWING (1977)).

The work of CONNOLLY and COOK (1973) provided the rather surprising result that none of the rejection responses performed by the female (excluding decamping) has any significant effect on the courtship of the male, i.e. they are not inhibitory. Further, the behaviours are not equivalent since which particular rejection response predominates depends on the reproductive state of the female, and only extrusion (and possibly curling) is restricted to the courtship context, so it may be unwise to group these behaviours under the same heading.

Extrusion itself perhaps comes closest to being a "true" rejection response. It is performed by fertilised (and very old) females and so may be associated with the presence of well-developed eggs in the female ovaries. In addition, it is restricted to the courtship context and tends to be associated with the vibration component of male courtship. It is believed to physically prevent intromission (CONNOLLY and COOK (1973)) although COOK and COOK

(1975) suggest that it may be involved in concentrating or directing some factor which deters courting males and BURNET and CONNOLLY (1974) have found that in some strains of D.melanogaster it does appear to inhibit the courtship of the male.

In the wild it may be useful for the female to remain stationary whilst repelling and/or preventing the copulation of courting males, since she is then able to continue feeding and/or ovipositing. In addition, feeding and/or oviposition sites may be limited so it may be important that the female does not have to fly off in order to escape the attentions of males since a similar site may be difficult to find and/or already populated. Finally, remaining still may help to reduce the risk of predation which might be incurred by walking or running away from the male accross the food source.

Little is known of curling since it is only rarely performed in D.melanogaster (CONNOLLY and COOK (1973)) but, as with extrusion, it may physically prevent intromission, in this case by preventing genital contact (BROWN (1964)).

Fluttering, kicking and fending can all be produced by mature virgin (i.e. receptive) females which will eventually allow the courting male to copulate, i.e. they do not indicate a rejection of the male. Since they appear to have no inhibitory effect on the courtship of the male it has been suggested that they may function in keeping the male at a distance and so prevent copulation occuring until the female has had an opportunity to sample the courtship of the male for the purpose of species-identification (and summation of courtship stimuli? - see Chapter 3) (BURNET,CONNOLLY and DENNIS (1971); CONNOLLY and COOK (1973)).

However, as has been seen in the virilis group, there is no necessity to keep the male at bay since the female possesses a distinct acceptance signal to which the male immediately responds by mounting. The presence of the fluttering wings may help to prevent some attempted mountings (see Section 4.5.2.3.1) but this mechanism is not normally necessary. The vast majority of the other species of Drosophila also have a female acceptance posture which involves the spreading of the vaginal plates, and many also show the raising of the wings observed in the virilis group (SPIETH (1952)). So, on the basis of this evidence, it would seem unlikely that fluttering, kicking and fending are produced in order to keep the male at bay whilst the female samples his courtship. The male will not mount until he receives the acceptance signal; mounting attempts where no acceptance signal is given are unsuccessful and in any case may only be artifacts of the unnatural experimental conditions.

Clearly, as before (Section 4.6.4), it is necessary to look outside the courtship context to understand the function of these behaviours which appear to be unnecessary when examined entirely within the courtship situation. None of these behaviours is restricted to the courtship context, and it has been suggested that kicking and fending may be responses to other flies approaching too closely (BURNET, CONNOLLY and DENNIS (1971)) whilst fluttering is also produced in response to non-courtship behaviours (CONNOLLY and COOK (1973)). Indeed, SEXTON and STALKER (1961) have already shown that in D.paramelanica fending is a method of preserving "individual distance" between flies. It must also have this function in the virilis group where females in copula are frequently observed to fend off approaching flies.

Fending then, like fluttering, is likely to be a spacing behaviour which is ineffective in preventing an eager courting male from approaching, but operates in non-courtsip situations, e.g. all-female groups, to keep flies apart. One can imagine an all-female group feeding and/or ovipositing at a limited food site where such spacing mechanisms may be necessary to prevent interference and overcrowding.

Kicking is extremely similar to the motion performed with the hind legs when the genitalia are being cleaned, which occurs particularly just after copulation. It seems likely that this kicking motion, although it does make contact, is not directed at the head of the male at all but rather is an attempt to clean the genitalia which are being continually rubbed by the proboscal labellum of the male. Supportive evidence for this idea comes from the observation that the female (virilis group) often begins kicking back at the point where the male and female genitalia are in contact towards the end of copula, just before the pair separate.

One final point: in the virilis group at least fluttering, buzzing (some species), scissoring (some species), flicking and fending are produced by both the males and the females (see Chapter 3), usually in response to physical contact and/or the proximity of other flies. It is likely then that these behaviours serve the same spacing function in both sexes, probably largely by personal advertisement and physical delineation of "personal space" (particularly fending). It is also of interest that a male may also walk or run away from a male which is courting him, and males also show the "kicking" behaviour whilst cleaning their genitalia after copulation has occured.

CHAPTER FIVE

MALE COURTSHIP SONG - A COMPARATIVE APPROACH

5.1 INTRODUCTION

Courtship song is the acoustic signal which is produced as a result of male wing vibration during courtship. The courtship song of *D.melanogaster* was the first to be recorded (SHOREY (1962)) and since then the songs of many species have been recorded and analysed: e.g. members of the *melanogaster* species group (EWING and BENNET-CLARK (1968); COWLING and BURNET (1981)); members of the *obscura* species group (EWING and BENNET-CLARK (1968); MILLER,GOLDSTEIN and PATTY (1975); CHANG and MILLER (1978)); members of the *melanica* and *willistoni* species groups (EWING (1970)); members of the *funebris* species group (EWING (1979)). One general finding of these studies has been that although some courtship song characteristics are often shared by all the species of a particular species group, the song of each species is species-specific. This is good circumstantial evidence that courtship song has an important role to play in the sexual isolation of species.

Work in this field has centred on within-group comparisons of courtship song characteristics where similarities and dissimilarities are used, along with information from other sources (e.g. cytological, morphological and distribution data), to explore the evolutionary history of each group or selection of species and to establish the importance of courtship song in maintaining sexual isolation.

As EWING and BENNET-CLARK (1968) have pointed out, several factors must be taken into account when performing studies of this kind. The degree of similarity or dissimilarity of courtship song characteristics within a phylad or group may simply reflect genetic relatedness, and so indicate changes which have occured as a by-product of the adaptive process, i.e. the more similar are the courtship songs, the more related are the species. However, if courtship song functions, or has functioned, as a premating isolation mechanism, then some degree of dissimilarity may have been caused by selection pressure for such mechanisms in zones of species overlap. For instance, two sympatric species may have very different courtship songs, despite their close genetic relatedness, whilst the courtship songs of two allopatric species may be fairly similar (in accordance with the general song characteristics of the group), even though these species may not actually be very closely related (see, for example, EWING (1970) and CHANG and MILLER (1978)).

The present analysis of the courtship songs of the virilis group was undertaken with these factors in mind. Fortunately, information is available concerning the phylogeny, present day distribution and ecology of the virilis group (THROCKMORTON (1982)) and studies have also been performed concerning the sexual isolation between the species, particularly of the virilis phylad (e.g. PATTERSON,McDANALD and STONE (1947)). The recording techniques and analysis are a little different from those employed by LAKOVAARA and HOIKKALA (1979) and HOIKKALA, LAKOVAARA and ROMPPAINEN (1982) who have also examined the courtship songs of this group.

5.2 METHODS

Stocks were cultured, collected and handled in the usual way. Courtships (one female plus one male) took place in the standard cell at 26 ± 2 degrees Centigrade from 0900 to 1200 Hrs and from 1400 to 1700 Hrs. Male courtship song was recorded and analysed in the routine manner (see Chapter 2). The basic song parameters measured were: BURST LENGTH (ms); NUMBER OF PULSES PER BURST; INTRABURST FREQUENCY(Hz). The latter was determined directly using a sonagraph analysis. The measurement of some other song parameters was sometimes performed and details are given in the results section.

The courtship songs of the following species were examined:

VIRILIS PHYLAD: D.virilis; D.lummei; D.novamexicana; D.a.texana; D.a.americana.

MONTANA PHYLAD: D.montana; D.flavomontana; D.borealis; D.littoralis.

5.3 RESULTS

Oscillograms of the courtship songs of the 3 species (D.virilis; D.lummei; D.novamexicana) and 2 subspecies (D.a.texana; D.a.americana) of the virilis phylad, and 4 species of the montana phylad (D.montana; D.flavomontana; D.borealis; D.littoralis) are presented in Figure 5-1. In all cases the courtship song is produced as a result of the vibration (on the outstroke) of a single wing by the male, during courtship. The courtship behaviour was found to be similar in all the species (for a general courtship description, see Chapter 3).

Figure 5-1

Male courtship songs of the virilis and montana phylads.

(Abbreviations: see Chapter 2).

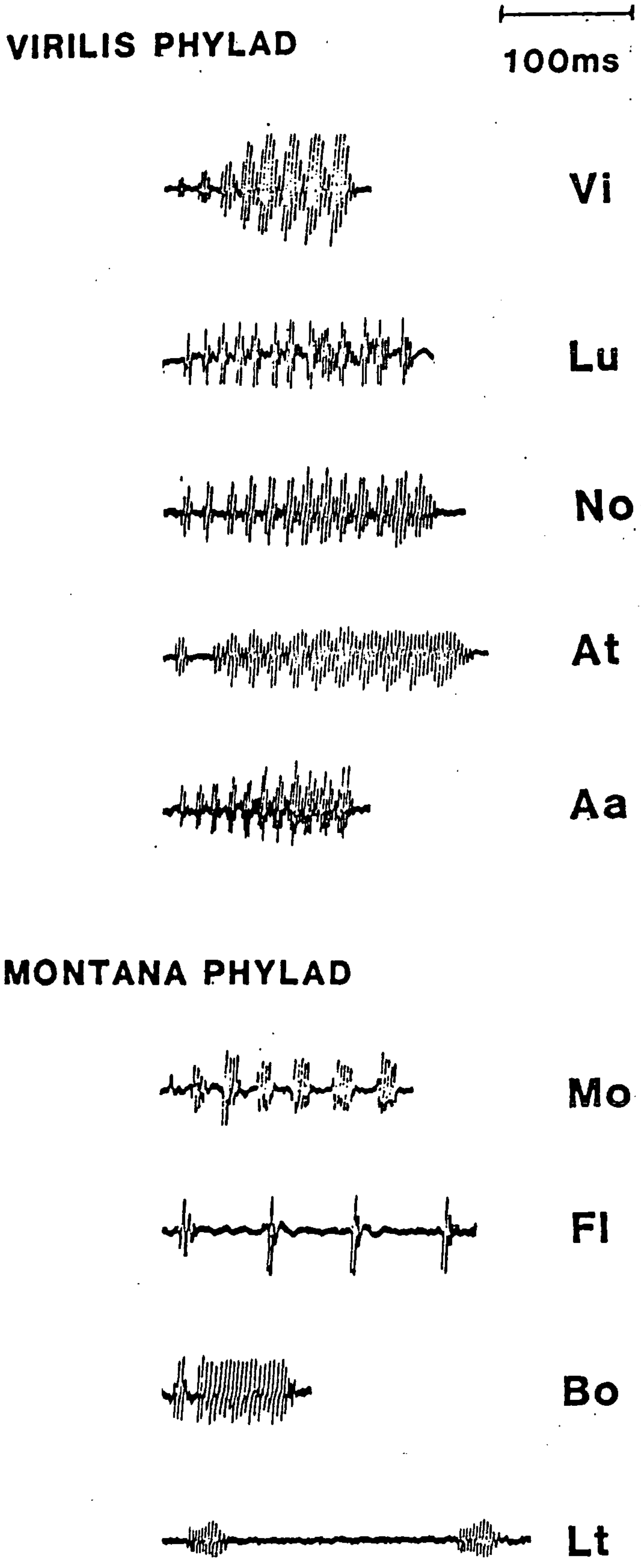

VIRILIS PHYLAD
100ms
Vi
Lu
No
At
Aa
MONTANA PHYLAD
Mo
Fl
Bo
Lt

Table 5-1

Analysis of male courtship songs of the *virilis* phylad (MEAN OF MEANS ± S.E.(S.D.)).

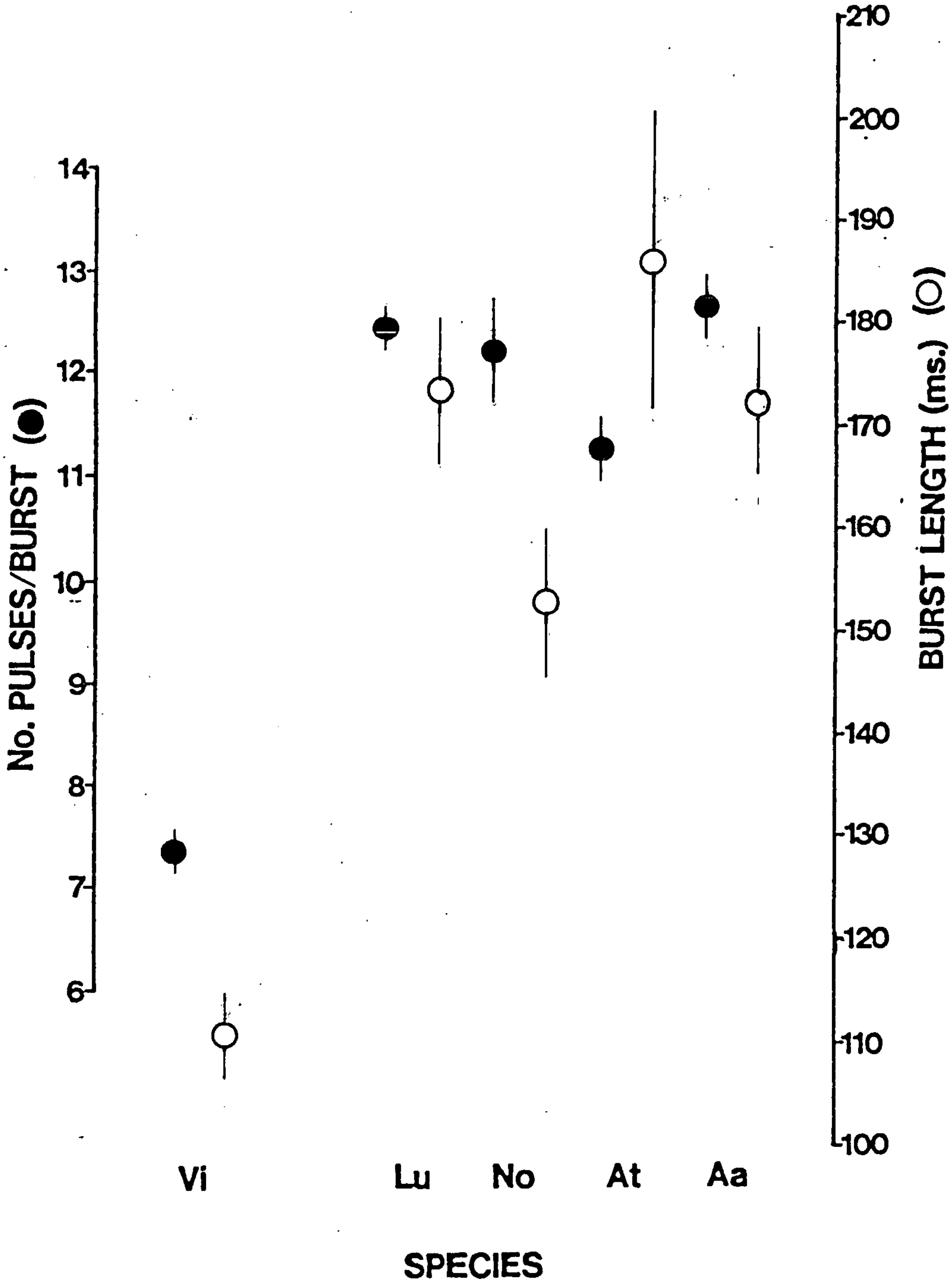
D.virilis	*D.lummei*	*D.novamexicana*
BURST LENGTH (ms)		
N=14; n=111	N=4;n=15	N=4;n=32
110.50 ± 3.72(14.0)	172.75 ± 7.33(14.66)	152.98 ± 7.37(14.74)
Range:70.0-150.0	Range:135.0-215.0	Range:107.5-210.0
No. PULSES PER BURST		
7.35 ± 0.20(0.77)	12.45 ± 0.23(0.47)	12.18 ± 0.50(1.01)
Range:5-9	Range:10-14	Range:9-16
INTRABURST FREQUENCY (Hz)		
300-400	400-500	300-400

D.a.texana	*D.a.americana*
BURST LENGTH (ms)	
N=5; n=42	N=8; n=64
185.57 ± 13.86(30.98)	172.0 ± 7.25(20.50)
Range:147.5-270.0	Range:125.0-250.0
No. PULSES PER BURST	
11.31 ± 0.29(0.64)	12.66 ± 0.27(0.76) (n=72)
Range:10-13	Range:10-16
INTRABURST FREQUENCY (Hz)	
300-400	400-500

Figure 5-2

Male courtship songs of the virilis phylad: number of pulses per burst and burst length (ms).

MEAN OF MEANS ± STANDARD ERROR.


No. PULSES/BURST (●)
14
13
12
11
10
9
8
7
6
BURST LENGTH (ms.) (○)
210
200
190
180
170
160
150
140
130
120
110
100
Vi
Lu
No
At
Aa
SPECIES

5.3.1 COURTSHIP SONGS OF THE VIRILIS PHYLAD

The courtship songs of the virilis phylad are fairly similar to each other (see Fig. 5-1). In general, each courtship song takes the form of an amplitude-modulated tone burst. A series of pulses can be recognised within each burst. The main body of the burst is preceeded by a single pulse (D.a.texana) or by 2,3 or 4 pulses (D.virilis; D.lummei; D.novamexicana; D.a.americana). These early pulses tend to be of slightly lower amplitude, and contain fewer cycles, than the pulses within the main burst.

The lower amplitude of these early pulses is probably due to the position of the vibrating wing in relation to the body during their production. Close to the body, i.e. in the early stages of the outstroke, poor resonance conditions and the different relationships of parts of the wing hinge have the effect of decreasing stroke amplitude (NACHTIGALL and WILSON (1967)). The mechanism is utilised in turning where the wing on one side is partially or wholly retracted, resulting in a decrease in stroke amplitude and turning in this direction. A gradually increasing stroke amplitude (pulse amplitude) as the wing is brought out from the body is clearly seen in the early pulses of D.virilis courtship song. The same general phenomenon can be seen in the courtship songs of the montana phylad (Fig. 5-1).

The analysis of the courtship songs is presented in Table 5-1. A One-way Analysis of Variance was performed on data from D.virilis and D.a.americana, and between-individual variation was found to be greater than within-individual variation for both song parameters (BURST LENGTH: F(13/97)= 13.24;$p<0.01$(Vi); F(7/56)=6.63;$p<0.01$(Aa); NUMBER OF PULSES PER BURST: F(13/97)=12.43;$p<0.01$(Vi);

$F(7/65)=2.2; p<0.05$(Aa)). For this reason, the mean value of the data for each individual has been treated as a data point, with the results being presented as the mean of the individual means plus or minus the standard error (standard deviation). On the reasonable assumption that a similar degree of between-individual variability is present in the courtship songs of the remaining species of the virilis group, all courthip song data has been analysed and presented in this way.

The data from Table 5-1 is presented graphically in Figure 5-2. It is clear that the courtship song of D.virilis is somewhat different from those of the other members of the virilis phylad, whose courtship songs are very similar to each other. This difference can be demonstrated statistically with a Student's t-Test comparison of the songs of D.virilis and D.a.americana: NUMBER OF PULSES PER BURST: $t(20)=8.59; p<0.001$; BURST LENGTH(ms): $t(20)=15.57; p<0.001$. Thus the courtship songs are significantly different. This finding means that courtship song can be used as a diagnostic character for D.virilis (see NEWBURY,DONEGAN and STEWART (in press)).

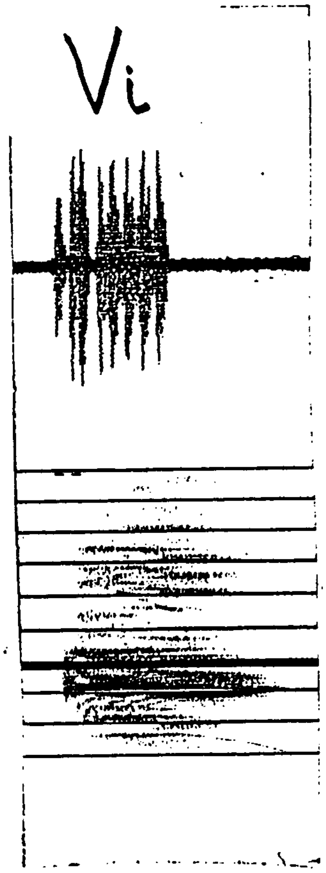
Intraburst frequency sonagrams are presented in Figure 5-3(a). For D.virilis, D.novamexicana and D.a.texana the dominant frequency band occurs between 300 and 400 Hertz. For D.lummei and D.a.americana it occurs between 400 and 500 Hertz. In all species, faint harmonics occur up to 1000 Hertz, although it should be noted that their importance is greatly exagerated by compression (selective amplification). Similar harmonics are also found in the courtship songs of the montana phylad (Figure 5-3(b)).

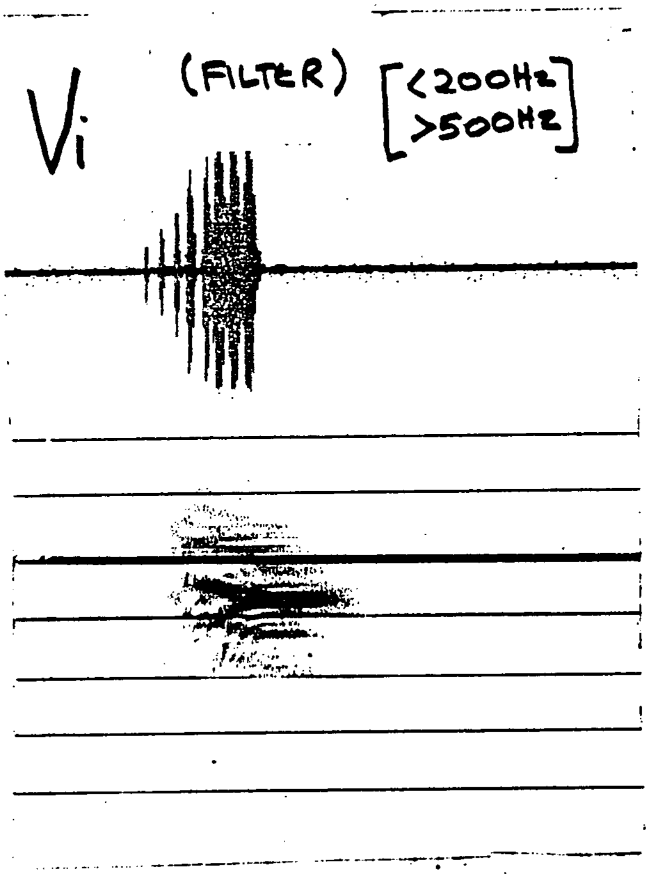
Figure 5-3(a)

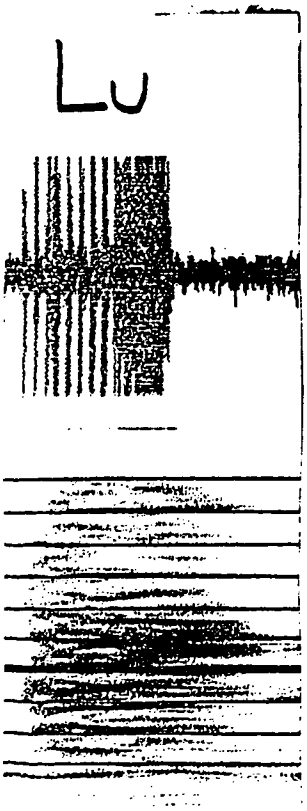
Male courtship songs of the *virilis* phylad: sonagrams.

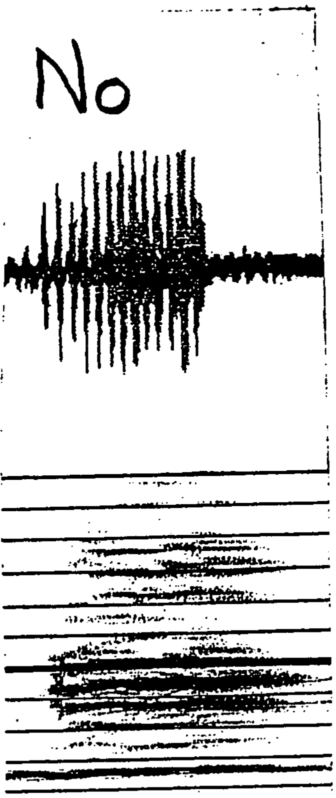
Bold line = 400Hz; interval = 100Hz.

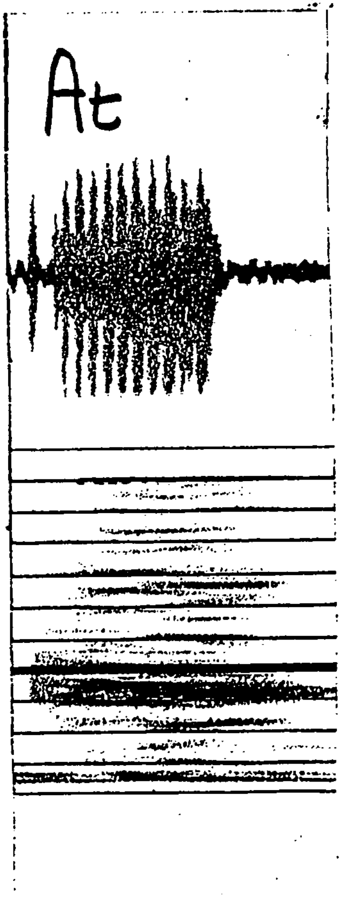
(Abbreviations: see Chapter 2).


Vi


Vi
(FILTER)
<200Hz
>500Hz


Lu


No


At

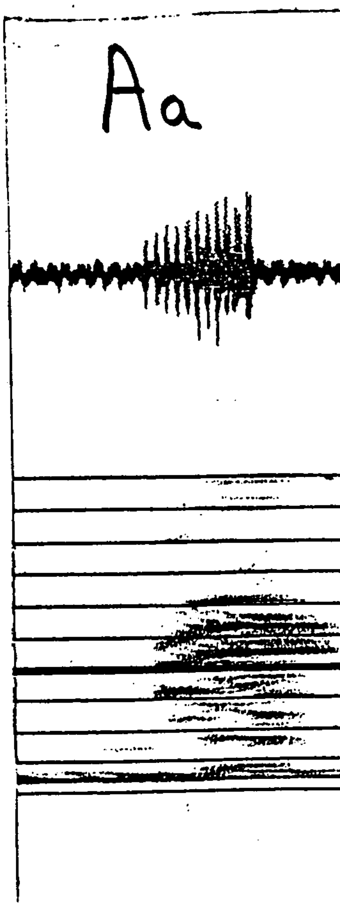
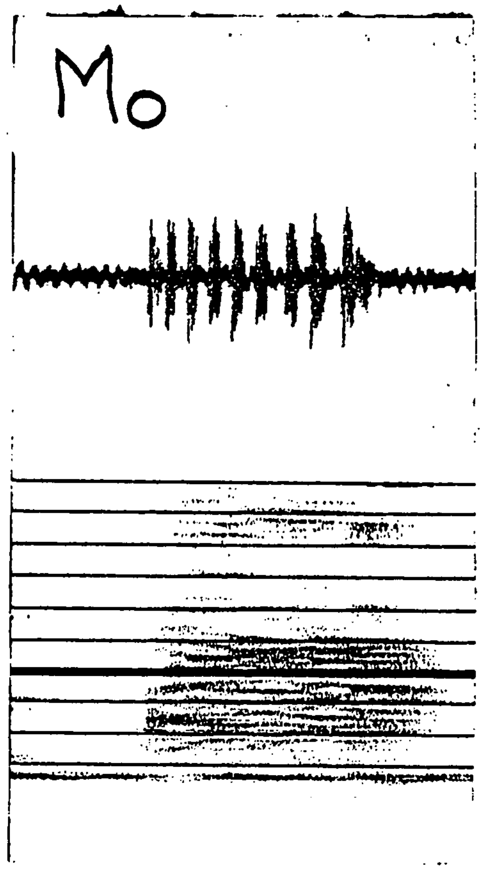

Aa

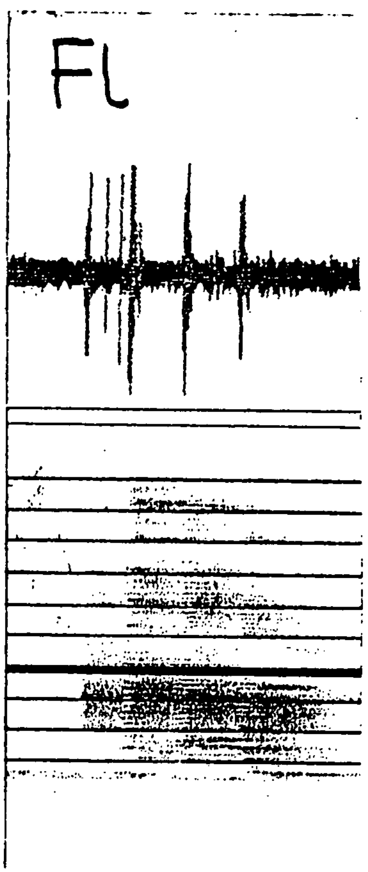
Figure 5-3(b)

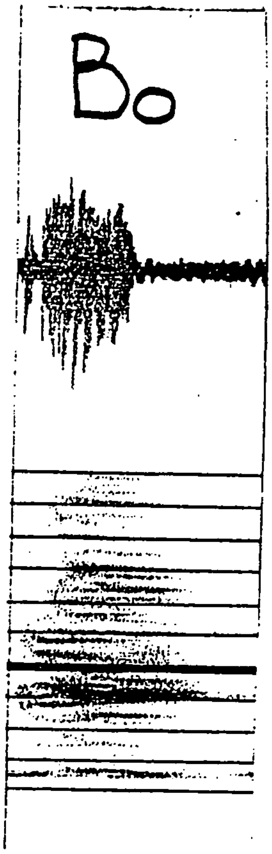
Male courtship songs of the montana phylad: sonagrams.

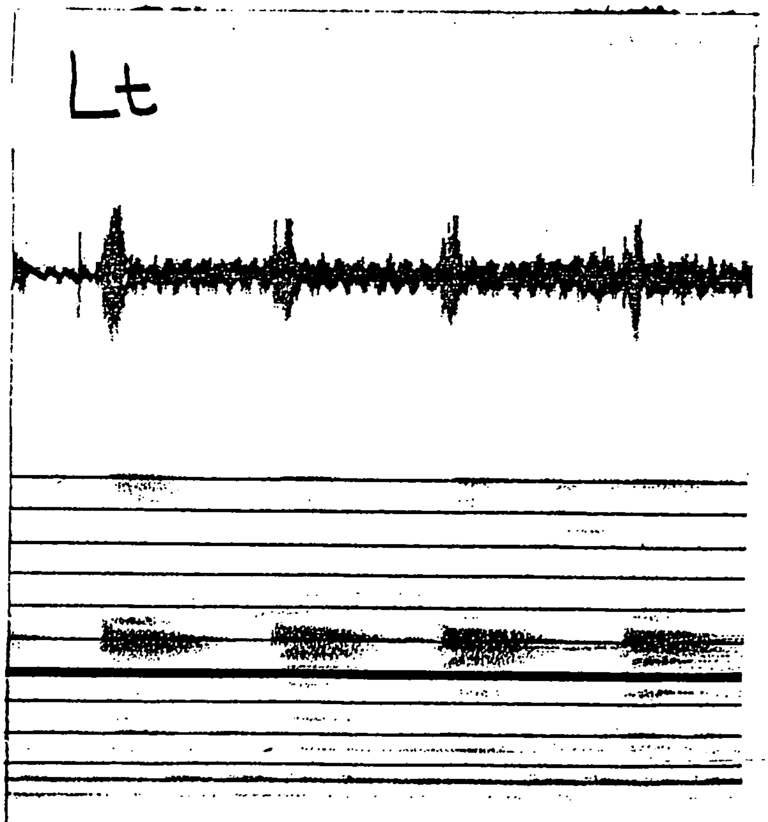
Bold line = 400Hz; interval = 100Hz.

(Abbreviations: see Chapter 2).


Mo


Fl


Bo


Lt

5.3.2 COURTSHIP SONGS OF THE MONTANA PHYLAD

The analysis of the songs (see Figure 5-1) is presented in Table 5-2, and the sonagrams are given in Figure 5-3(b).

The courtship song of D.montana consists of a train of between 6 and 10 polycyclic pulses (mean:8). The first pulse (P(1)) is of lower amplitude, shorter duration (mean:9ms) and contains fewer cycles (mean:3) than subsequent pulses (means:14ms;5). The first interpulse interval has a mean duration of 24 milliseconds which is shorter than subsequent interpulse intervals (mean:31ms). Intrapulse frequency is between 400 and 500 Hz.

The courtship song of D.flavomontana consists of a train of between 3 and 5 polycyclic pulses (mean:4). The train is, on average, a little shorter than that of D.montana song (means:193ms(Fl);211ms(Mo)) and the pulses contain fewer cycles (means:3(Fl);5(Mo)). Interpulse intervals, however, are longer than those of D.montana (means:58 to 70ms(Fl);24ms and 31ms(Mo)) and there is a progressive increase in mean interpulse interval as the train proceeds. Intrapulse frequency is between 200 and 400 Hz.

The courtship song of D.borealis is similar, in some ways, to that of members of the virilis phylad. The overall patterning, with a single isolated pulse at the beginning of the song, most closely resembles that of D.a.texana. However, the burst length is considerably shorter, and is closest to that of D.virilis (means:105(Bo);111ms(Vi)). Some amplitude modulation of the tone burst is apparent in some records, but this is by no means a consistent feature of D.borealis song. Intraburst frequency is between 300 and 400 Hz.

The courtship song of D.littoralis consists of a short duration (mean:29ms) tone burst containing, on average, 13 cycles. It is unlikely that interburst interval could have a signal function since the range is probably too great (145-330ms) and it is more likely that burst length itself is the critical parameter. Intraburst frequency is between 450 and 550 Hz, although some records were as low as 200 Hz.

Table 5-2(i)

Analysis of male courtship songs of the montana phylad (MEAN OF MEANS ± S.E.(S.D.) (N;n)).

ABBREVIATIONS:

TL: TRAIN LENGTH (ms)

No.P/T: NUMBER OF PULSES PER TRAIN

PL: PULSE LENGTH (ms)

No.C/P: NUMBER OF CYCLES PER PULSE

IPI: INTERPULSE INTERVAL (ms)

IPF: INTRAPULSE FREQUENCY (Hz)

BL: BURST LENGTH (ms)

No.C/B: NUMBER OF CYCLES PER BURST

IBF: INTRABURST FREQUENCY (Hz)

IBI: INTERBURST INTERVAL (ms)

Table 5-2(ii)

	D.montana	*D.flavomontana*
TL:	211.01 ± 15.25(30.50) (4;73)	193.48 ± 7.15(15.99) (5;49)
	Range:150.0-280.0	Range:130.0-267.5
No.P/T:	7.59 ± 0.35(0.70) (4;75)	3.94 ± 0.19(0.43) (5;49)
	Range:6-10	Range:3-5
P(1)L:	8.53 ± 0.76(1.52) (4;74)	8.78 ± 0.61(1.37) (5;49)
	Range:5.0-15.0	Range:5.0-12.5
No.C/P(1):	2.91 ± 0.42(0.85) (4;74)	2.53 ± 0.24(0.54) (5;49)
	Range:1-4	Range:1-4
IPI(1):	24.35 ± 0.71(1.42) (4;72)	57.83 ± 1.96(4.39) (5;49)
	Range:17.5-30.0	Range:50.0-70.0
REMAINING PULSES		
PL:	14.05 ± 0.80(1.60) (4;488)	9.85 ± 0.40(0.90) (5;148)
	Range:10.0-25.0	Range:5.0-20.0
No.C/P:	5.40 ± 0.36(0.71) (4;488)	3.30 ± 0.13(0.30) (5;148)
	Range:4-10	Range:2-5
IPI:	30.68 ± 0.54(1.07) (4;414)	(2) 62.10 ± 2.77(6.19) (5;49)
	Range:25.0-40.0	Range:52.5-75.0
		(3) 64.65 ± 1.63(3.27) (4;42)
		Range:55.0-77.5
		(4) 69.69 ± 1.45(4.11) (3;8)
		Range:62.5-75.0
IPF:	400-500	200-400

Table 5-2(iii)

	D.borealis	*D.littoralis*
BL:	104.91 ± 5.49(9.51) (3;53)	29.21 ± 1.66(3.32) (4;69)
	Range:90.0-130.0	Range:20.0-42.5
No.C/B:	33.58 ± 2.04(3.53) (3;53)	12.95 ± 0.23(0.46) (4;69)
	Range:27-42	Range:9-17
IBF:	300-400	450-550
IBI:		218.90 ± 19.84(39.68) (4;54)
		Range:145.0-330.0

5.4 DISCUSSION

5.4.1 PHYLOGENETIC RELATIONSHIPS

Generally speaking, the courtship songs of the *virilis* phylad species are fairly uniform in character and differ from those of the *montana* phylad species which exhibit a variety of song types (see Figure 5-1). In general, these findings, i.e. uniformity in the *virilis* phylad and diversity in the *montana* phylad,correspond with the phylogeny of the *virilis* group, as shown in Figure 5-4. The phylogeny is based on gene arrangements, karyotypes, crossabilities, proteins and morphology and is taken from THROCKMORTON (1982).

The *montana* phylad contains nearly twice as many species as the *virilis* phylad and exhibits much greater chromosomal variability (STONE, GUEST and WILSON (1960); THROCKMORTON (1982)). Species of the *virilis* phylad cross more readily with each other and produce fertile hybrids more frequently than do species of the *montana* phylad. Crosses between species of the two phylads go slightly less well than most crosses within the *montana* phylad (THROCKMORTON (1982)). In addition, electrophoretic analysis of over 360 proteins has revealed that species of the *montana* phylad have fewer proteins in common, have more proteins unique to each species and share fewer ancestral proteins than do species of the *virilis* phylad (HUBBY and THROCKMORTON (1965)).

Figure 5-4

Phylogeny of the *virilis* group; from gene arrangements, karyotypes, proteins and anatomy.

(From THROCKMORTON (1982)).

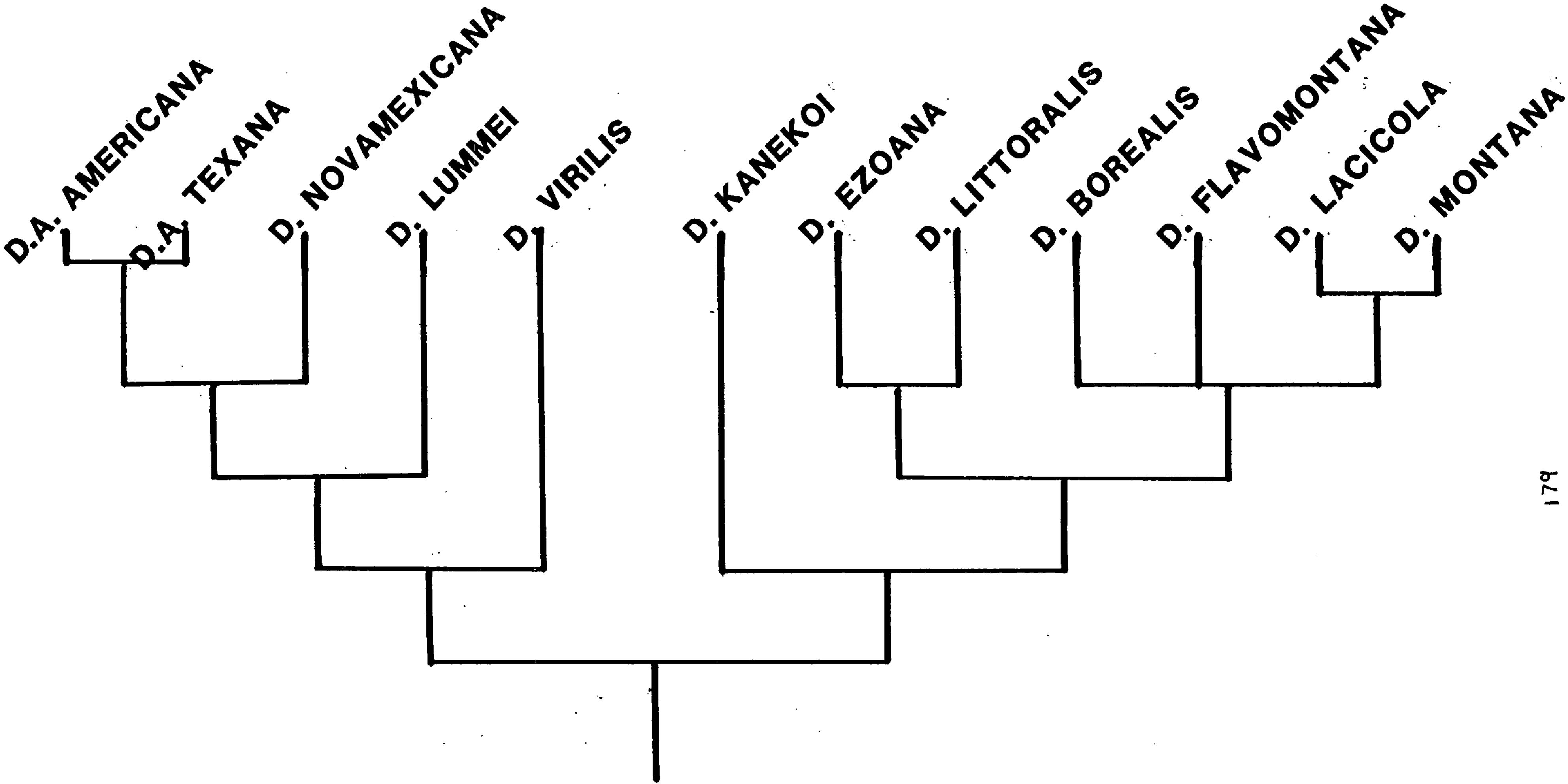
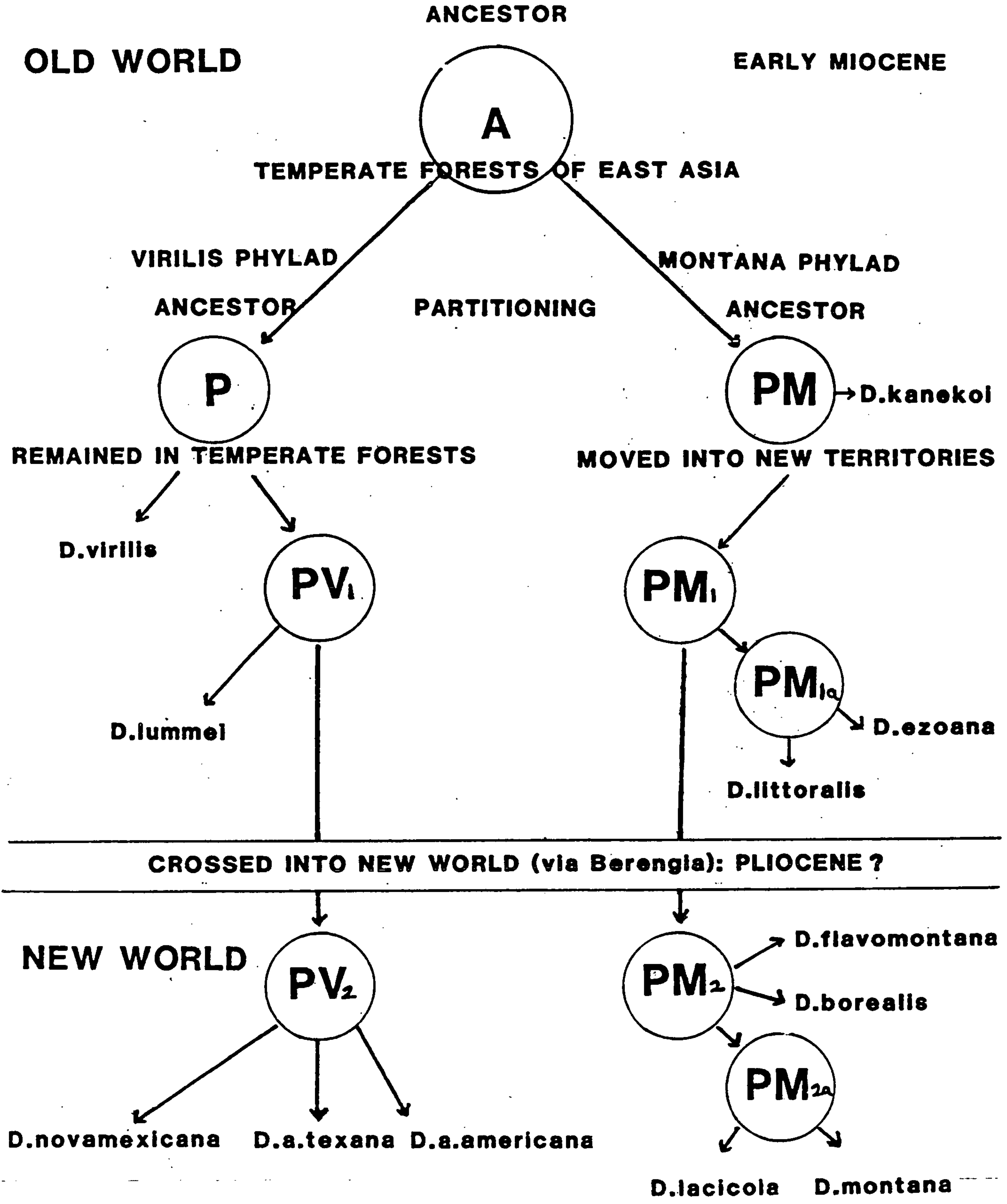

D.A. AMERICANA
D.A. TEXANA
D. NOVAMEXICANA
D. LUMMEI
D. VIRILIS
D. KANEKOI
D. EZOANA
D. LITTORALIS
D. BOREALIS
D. FLAVOMONTANA
D. LACICOLA
D. MONTANA

Figure 5-5

Model for the evolution of the virilis group; details supplied in text.

(Information taken from THROCKMORTON (1982)).


ANCESTOR
OLD WORLD
EARLY MIOCENE
A
TEMPERATE FORESTS OF EAST ASIA
VIRILIS PHYLAD
ANCESTOR
PARTITIONING
MONTANA PHYLAD
ANCESTOR
P
PM
D.kanekoi
REMAINED IN TEMPERATE FORESTS
MOVED INTO NEW TERRITORIES
D.virilis
PV1
PM1
PM1a
D.lummei
D.ezoana
D.littoralis
CROSSED INTO NEW WORLD (via Berengia): PLIOCENE ?
NEW WORLD
PV2
PM2
D.flavomontana
D.borealis
PM2a
D.novamexicana
D.a.texana
D.a.americana
D.lacicola
D.montana

A model for the evolution of the group is shown in Figure 5-5 (from information supplied in THROCKMORTON(1982)). It is likely that the group ancestor inhabited the temperate forests of east Asia in the early Miocene (c. 20 mya). Partitioning occurred, perhaps along a cool-warm or north-south gradient, which gave rise to the ancestors of the two lineages (P and PM). Both lineages expanded into the New World by way of Beringia, probably no later than the Pliocene (5-7 mya).

It is thought that the virilis phylad lineage was conservative and slow-changing, remaining within the temperate forest habitat and retaining what was probably the larval substrate (oviposition site) of the group ancestor (willow). By contrast, the montana phylad is thought to have diversified into the new territories of boreal and montane forests. This ecological shift in both climate and vegetation resulted in divergence and rapid change, with the employment of new larval substrates: alder (D.montana); aspen (D.montana, D.lacicola, D.borealis); cottonwood (D.flavomontana); sycamore (D.littoralis); birch and spruce (D.ezoana), although D.kanekoi employs willow.

The extent to which courtship song similarities and dissimilarities are explicable as by-products of evolutionary change is, of course, difficult to assess. In general one can say that the ecological conservatism and close genetic relatedness of the species of the virilis phylad are reflected in the similarity of the courtship songs of this phylad. The courtship song of D.virilis itself is distinguishable from the rest (see Fig. 5-2) and this may reflect the fact that the other members of the phylad are more closely related to each other than they are to D.virilis. The diversity of the courtship songs found in the montana phylad may, in part, be explicable in terms of the evolutionary divergence and more distant genetic relatedness of the species within this phylad.

This sort of comparison is similar to that performed by WATABE and HIGUCHI (1979) who found some degree of corespondence between spermathecal morphology and the phylogeny of the virilis group.

However, a close examination of the courtship songs from all species of the montana phylad (additional song data from HOIKKALA et al (1982)) reveals some similarity between certain species. The courtship songs of D.montana, D.kanekoi and D.ezoana are roughly similar, each consisting of a long train of polycyclic pulses (mean number of pulses: 8,9(Mo);10(Ka);13(Ez)) with short ipi's (e.g. 24ms and 31ms (Mo)), as are the songs of D.flavomontana and D.lacicola which consist of short trains of polycyclic pulses (4,5(Fl);5(Lc)) with long ipi's (e.g. 58-70ms(Fl)).

The presence of similar songs in species which are not closely related may be coincidence, or it may indicate that courtship song is a conservative character in that large changes in courtship song are not brought about as a by-product of other adaptive processes. If this latter explanation is correct, then the genetic variability which has resulted from the rapid and divergent evolution of the montana phylad must be thought of as providing only the necessary potential, or capacity, for the evolution of distinctive courtship songs. The implication of this argument is that where large changes in courtship song have occured as, for instance, in D.littoralis or D.borealis, they are the product of selection pressure for such changes. This is discussed in detail in the next section.

5.4.2 SEXUAL ISOLATION AND DISTRIBUTION

The conditions under which courtship song may change (if passive change as a by-product of other adaptive processes is minimal) require that it functions in sexual isolation, and are found in sympatry following allopatric speciation. The process of allopatric speciation (see DOBZHANSKY (1970); SHORROCKS (1978)) begins with the geographical separation of two populations which were originally one. Each of these populations gradually becomes adapted to local conditions which results in genetic change. Should the two populations (races,incipient species) come into contact once more the genetic differences may be such that the hybrids that are produced as a result of interbreeding are weak or sterile. Since this is costly in terms of wasted gametes, selection favours, and acts to strengthen, premating isolation mechanisms which act to prevent such crosses. In time, the genes which prevent interbreeding, and hence have a positive selective value in the zone of contact, gradually diffuse out into the two respective populations and two reproductively isolated species are formed.

In theory, the species-specific courtship songs of the _montana_ phylad may have evolved because of the need for effective premating isolation mechanisms between species, or incipient species, which have come into contact once more after a period of geographical isolation. Under these conditions, selection would favour both males which produced species-specific songs and females which could discriminate between them. Unfortunately there is no direct experimental evidence that females are able to use courtship song for the purpose of discriminating between males of different species in the _virilis_ group. What is available is general information on the sexual isolation existing between the species of the virilis group and also knowledge of their present day distribution.

The information on sexual isolation has been gained from two main types of experiment: "male choice" and "no choice". In "male choice" experiments 1(2) males of species A are placed with 5(10) females of species A and 5(10) females of species B for 1(3) days, after which time the females are dissected to check for insemination. It is from these experiments that the so-called "isolation index" is calculated. "No choice" experiments take one of two forms: in one type, 10(1) males of species A are placed with 10 females of species A or B; in the other type, a single male of species A is placed with a single female of species A or B. In both cases the flies are left together for 1(3) days and the females are dissected at the end to check whether insemination has occured. The results from these types of experiment are often difficult to interpret because of the lack of direct observational data on the actual mechanisms which contribute to the isolation, and also because of the clear artificiality of placing a group of flies together in an enclosed space for a number of days.

Knowledge of the present day distribution of the virilis group species is also difficult to interpret for the purposes of the present study. This is largely because there is no reliable information available on where the adult feeding and mating sites might be. Since it is at the mating sites where there will be selection pressure for distinctive courtship songs to prevent interbreeding, this gap in our knowledge is difficult to bridge. The preferred oviposition sites are known, but work on several other species suggests that whereas oviposition sites are species-specific, adults are likely to be generalists in their choice of feeding (and mating?) sites (see,for example, CARSON (1971); BEGON and SHORROCKS (1978)).

5.4.2.1 VIRILIS PHYLAD

Investigations concerning the sexual isolation existing between species of the virilis phylad (excluding D.lummei) have been performed by STALKER (1942); PATTERSON,MCDANALD and STONE (1947); SPIETH (1951); WATANABE and KAWANISHI (1979,1981). They include both "male choice" and "no choice" tests. These workers have established that isolation is either weak or non-existent, except in crosses involving males of D.virilis and D.novamexicana where isolation is strong (D.virilis (female) x D.novamexicana (male) is an exception). The strength of this isolation may in large part be attributable to discrimination against foreign females by the males of these two species (STALKER (1942); SPIETH (1951)).

That discrimination is exercised by the D.virilis male has been confirmed in a series of crosses where single pairs were allowed 10 minutes to copulate ("success") in the standard cell. The results are presented in Table 5-3. Whereas all D.virilis x D.a.americana crosses contained courtship, 5 of the 11 unsuccessful D.a.americana x D.virilis crosses contained no courtship at all. In addition, only 2 of the unsuccessful D.virilis x D.a.americana crosses contained little courtship, whereas all 6 of the unsuccessful D.a.americana x D.virilis courtships contained very little courtship by the male. So, the D.virilis male is less willing to court a foreign female than is the D.a.americana male, and even when he does so his courtship is short-lived and non-persistent.

Table 5-3

Crossings involving *D.virilis* (Vi) (combined data for *D.virilis* (1801.1) and *D.virilis* (brewery)) and *D.a.americana* (Aa).

FEMALE	MALE	TOTAL	COURTSHIP	COPN.	SUCCESS	F1	F2	COPULA TIME (mins.)
Vi	Vi	32	32	30	30/32	X	X	3.48 ± 0.10(0.50)
Aa	Aa	7	7	4	4/7	X	X	2.21 ± 0.12(0.25)
Vi	Aa	14	14	7	7/14	-	-	2.26 ± 0.12(0.33)
Aa	Vi	16	11	5	5/11	-	-	2.05 ± 0.18(0.41)

Male discrimination is likely to be based on some chemical present on the cuticle of the alien female which the male detects when he taps her. The importance of male discrimination in sexual isolation has also been identified in five sibling species of the *D.melanogaster* group (SCHILCHER and DOW (1977)). These findings are important to remember since some authors, e.g. WATANABE and KAWANISHI (1979,1981) seem to assume, without direct evidence, that discrimination is only carried out by the female.

In general then the members of the *virilis* phylad show little or no sexual isolation from each other. Where strong isolation is present, it is largely the result of discrimination on the part of the male so it is unlikely that courtship song has any important role to play in sexual isolation where it exists. This finding is expected in view of the close similarity of the courtship songs of this phylad.

The present day distribution of the *virilis* phylad is shown in Figure 5-6 (adapted from THROCKMORTON (1982)). Although *D.virilis* itself is a domestic species found throughout the world in breweries, stores and produce houses, it is endemic in China and east Asia where, in common with all the other species of the *virilis* group, it is restricted to the margins of lacustrine or riparian freshwater (species of the *virilis* group are thought to be semiobligatory commensals of the beaver, *Castor* *fiber* (Europe and Asia) and *C.canadensis* (North America) (SPIETH (1979)). All species of the phylad (except domestic *D.virilis*) are thought to utilise willow as a larval substrate but they are, in general, completely allopatric. However, *D.virilis* and *D.lummei* probably come into contact in southern Russia and Japan but it is not known how isolation is maintained here. There is also a zone of overlap between D.a.texana and D.a.americana but isolation must be weak since hybrids of the two subspecies have been found in nature (THROCKMORTON (1982) p.234)

Where species distributions of the virilis and montana phylads overlap (see Figures 5-6;5-7;5-8), e.g. D.novamexicana and D.montana, the species are thought to be ecologically isolated from each other: they employ different oviposition sites and show different adaptations to cold (THROCKMORTON (1982)). Sexual isolation studies, e.g. PATTERSON, McDANALD and STONE (1947), have shown that sexual isolation between the phylads is very strong although in "no choice" tests some heterospecific crosses are successful, particularly if D.virilis or D.littoralis females are involved.

Since, in general, the species of the virilis phylad do not come into contact in nature and are ecologically isolated from species of the montana phylad, there has been no necessity to develop effective premating isolation mechanisms. Therefore, the uniform courtship song patterning may represent the ancestral song type. The discrimination exercised by D.virilis and D.novamexicana males against foreign females may be a premating isolation mechanism which has evolved as a by-product of other adaptive processes. It is not known what mechanisms are involved in the strong isolation which has been found between members of the two phylads.

Figure 5-6

Geographical distribution of species of the *virilis* phylad.

(From THROCKMORTON (1982)).

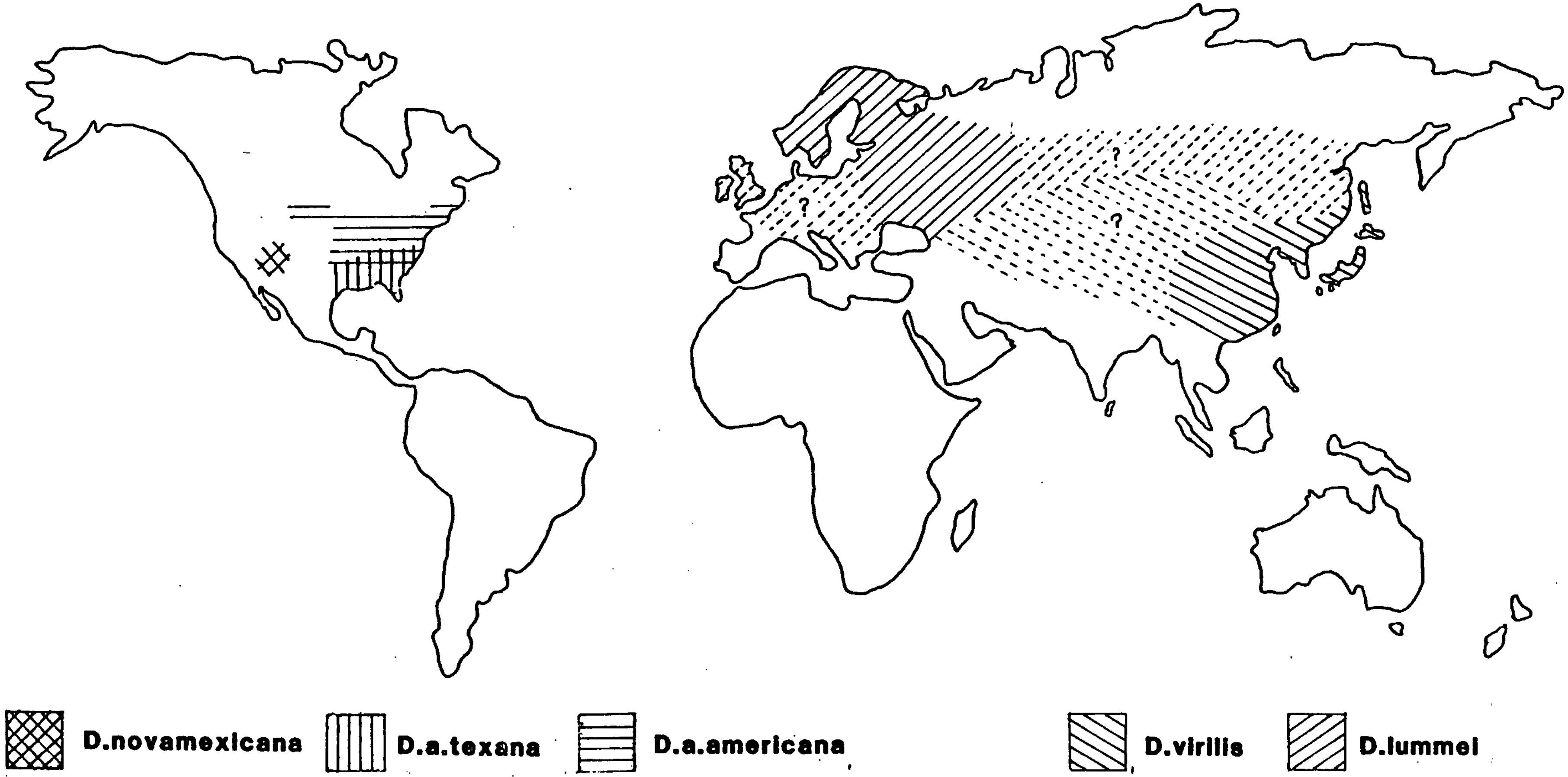
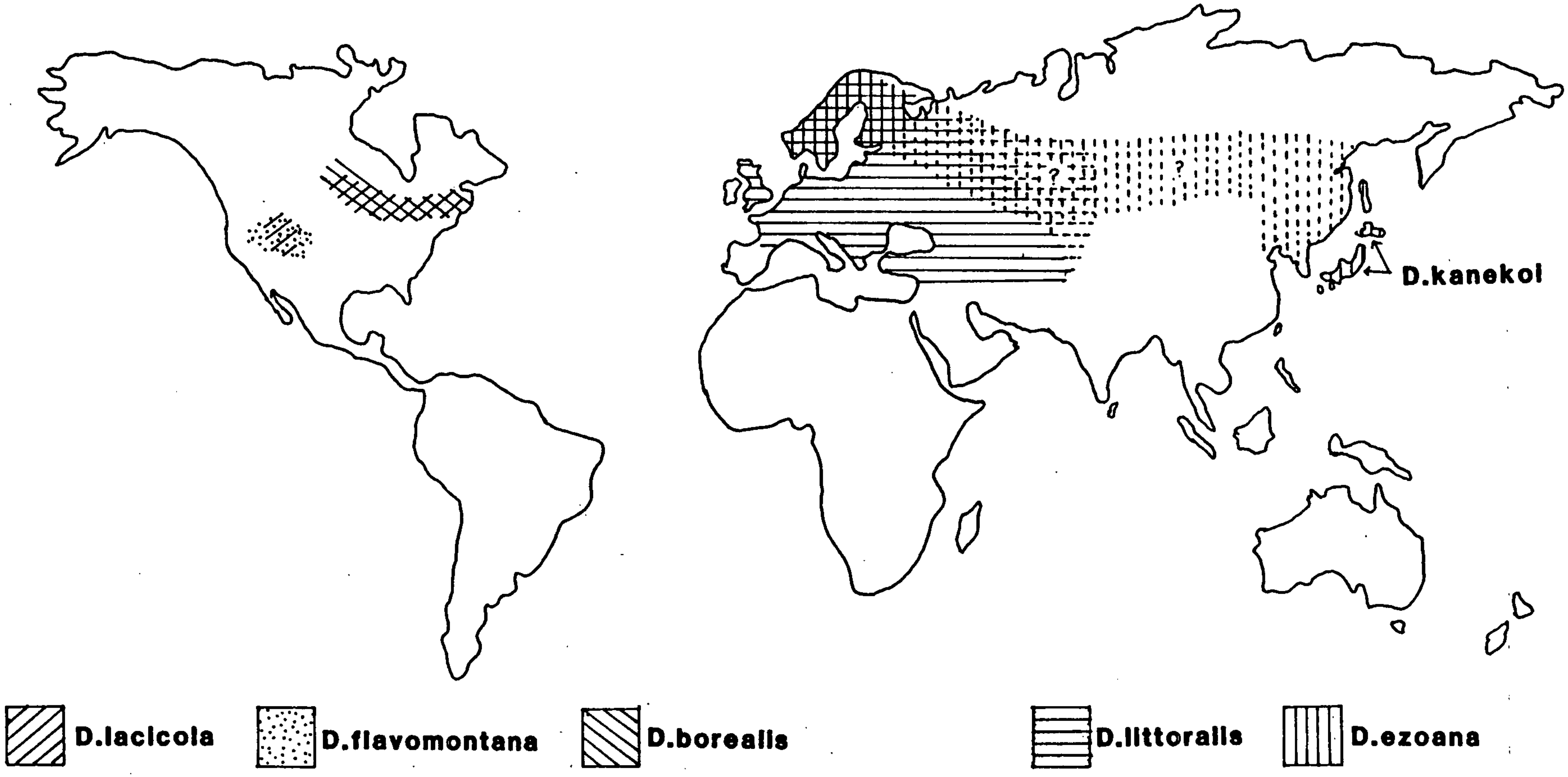
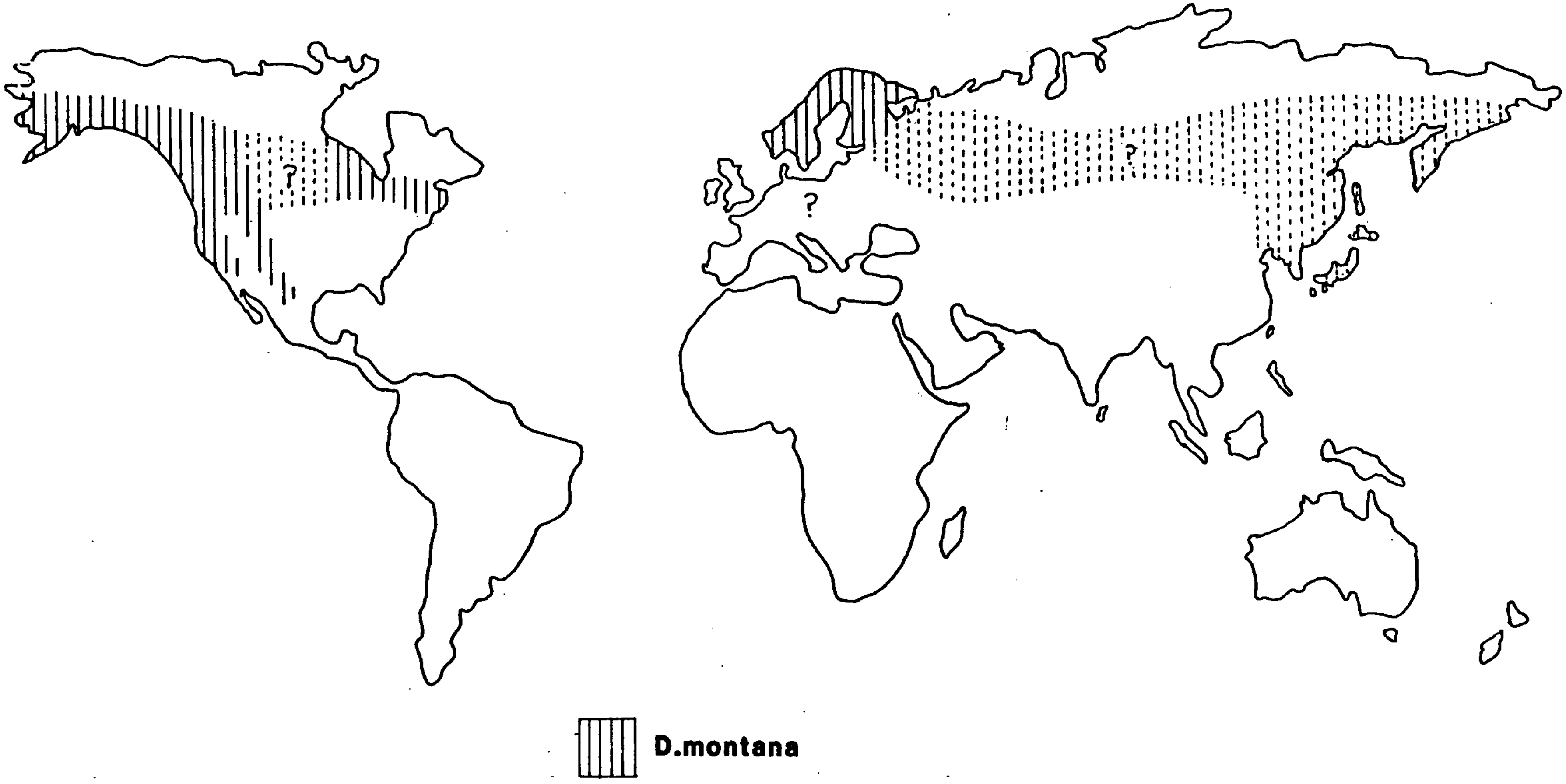

?
?
?
D.novamexicana
D.a.texana
D.a.americana
D.virilis
D.lummei

Figure 5-7

Geographical distribution of species of the montana phylad.

(From THROCKMORTON (1982)).


?
?
D.kanekoi
D.lacicola
D.flavomontana
D.borealis
D.littoralis
D.ezoana


?
?
?
?
▥ D.montana

5.4.2.2 MONTANA PHYLAD

Laboratory studies of the sexual isolation existing between species of the montana phylad (excluding D.kanekoi) have been performed by PATTERSON, McDANALD and STONE (1947); PATTERSON (1952); STONE, GUEST and WILSON (1960). Most of the information has been obtained from "no choice" tests.

Isolation between all species is very strong, although the D.flavomontana x D.lacicola crosses go moderately well, and the D.flavomontana (female) x D.littoralis (male) cross goes even better (76% of females inseminated). Unfortunately, no detailed observations have been made so it is not known whether the strong sexual isolation between species is the result of male or female discrimination, or a combination of both. For this reason, it is not possible to directly evaluate the importance of courtship song in maintaining this isolation.

A simple comparison of cross success with courtship song similarity or dissimilarity yields ambiguous results. For example, D.flavomontana and D.lacicola have similar songs and their crosses go moderately well, but D.montana and D.ezoana also have roughly similar songs but crosses invariably fail. On the other hand, D.flavomontana and D.littoralis have very different courtship songs yet the crosses between these species are more successful than any others in the montana phylad. These apparent contradictions can only be resolved by more research into the mechanisms which maintain reproductive isolation in this phylad.

The distribution of the montana phylad species is shown in Figure 5-7, and that of D.montana itself in Figure 5-8 (both adapted from THROCKMORTON (1982)). In the New World, several species appear to be sympatric to some degree. The distribution of D.flavomontana overlaps with that of D.montana and D.lacicola, whilst D.montana overlaps with D.borealis, and with D.lacicola. However, D.flavomontana employs cottonwood as an oviposition site whereas D.montana employs aspen or alder and D.lacicola employs aspen, and it exhibits different habitat preferences. D.montana, D.lacicola and D.borealis all employ similar oviposition sites (aspen) but D.montana is unlikely to come into contact with these other species since it prefers higher altitude habitats, being better adapted to cold conditions. However, D.lacicola and D.borealis are sympatric throughout their respective ranges and it is possible that they come into contact at adult feeding and mating sites. THROCKMORTON (1982) believes that the sympatry of these species may reflect the recent overlap of formerly separate ranges as the species moved into areas which had been recently glaciated. Insufficient time may have elapsed to evoke ecological displacement or exclusion. It is possible that the very different courtship songs of these two species are a result of selection for effective premating isolation mechanisms.

In the Old World, the distributions of D.ezoana and D.kanekoi may overlap in Japan, but D.kanekoi has been discovered too recently (1979) for much information to be available on its distribution and ecology, although it is thought that it employs willow as an oviposition site, whereas D.ezoana employs birch or spruce. There is extensive overlap between D.littoralis, D.montana and D.ezoana and although the oviposition sites of these species may be different (sycamore (Li)), they may come into contact at adult feeding and mating sites. Little is known of the ecology of these species in

Eurasia and more research is needed to determine whether sexual isolation mechanisms (such as courtship song?) are necessary in the wild.

5.5 CONCLUSIONS

The similarity of the courtship songs of the *virilis* phylad probably reflects both the relatively close genetic relatedness of the members of the phylad, and the absence of any selection pressure for change since the species are now largely allopatric and were probably so at their origin. The courtship song of *D.virilis* itself is distinguishable from the others but it is unlikely that females are able to exercise discrimination on this basis since sexual isolation between the species is weak or non-existent, except where the male has been shown to exercise discrimination against foreign females. It is quite possible that the amplitude-modulated tone burst patterning of the courtship song represents that of the common ancestor of the phylad.

The diversity of the courtship songs of the *montana* phylad can be attributed to both change as a by-product of other adaptive processes and to selection pressure for distinctive songs which act, or have acted, as premating isolation mechanisms in zones of species overlap. Evidence for the former assertion is provided by the large genetic variability of the species of the phylad, but the finding that some species possess moderately similar songs whereas the songs of other species are very different may indicate either that passive change has made only a minor direct contribution to song diversity, or that it has occured to widely varying degrees in different species. If one accepts the first of these explanations, then the songs of D.montana, D.ezoana and D.kanekoi may represent the ancestral song type from which the songs of D.lacicola, D.flavomontana, D.borealis and D.littoralis have evolved.

Since there is no direct experimental evidence available that courtship song functions as a sexual isolation mechanism between any species of the montana phylad, circumstantial evidence from general sexual isolation studies and species distribution data must be sought. Although it is clear that sexual isolation between species of the montana phylad is, in general, very strong, the lack of direct observational data makes it impossible to identify the mechanisms which may be involved in this isolation. Knowledge of the world distribution of the species is also difficult to interpret for although it may appear that two species are sympatric, and may even share the same oviposition sites, it is not known whether the species actually come into contact at adult feeding and mating sites. In addition, it is not known whether the species were allopatric, parapatric or sympatric at their origin. Therefore it is not possible to say whether the sexual isolation which has been recorded in the laboratory reflects the natural situation, past or present, i.e. whether it has evolved between sympatric species, or whether it is merely a by-product of the genetic differences between the species of this phylad. The evidence from the courtship songs alone cannot be used to distinguish between these two possibilities; supportive evidence is needed.

This rather unsatisfactory state of affairs can only be resolved by more detailed laboratory studies of sexual isolation, along with the identification of feeding and mating sites in the wild.

CHAPTER SIX

MALE COURTSHIP SONG - AN EXPERIMENTAL APPROACH

6.1 INTRODUCTION

The experiments reported in this chapter are all concerned with the stimulatory function of male courtship song. In Section 6.2, the effects on mating speed and mating success of artificially removing the courtship song component are examined; Section 6.3 provides electrophysiological evidence that the arista is necessary for the reception of courtship song by the female; Section 6.4 reports on recorded and simulated courtship song playback experiments.

6.2 WINGLESS MALES AND ARISTAELESS FEMALES

6.2.1 INTRODUCTION

The importance of the vibration component (courtship song) of the courtship repertoire of the male in providing sexual stimulation for the female was discussed in Chapter 3. The evidence came principally from a detailed analysis of "normal" courships involving D.a.americana. In this section, the effects of artificially removing the courtship song component are examined. The technique involves either the "silencing" of males (amputation of their wings) or the "deafening" of females (removal of their aristae). Several wingless male courtships were recorded to make certain that these males are incapable of producing courtship song. Evidence that the removal of the aristae "deafens" females will be presented in the next section.

Similar experiments have been performed using D.melanogaster (MANNING (1967a,b); COOK (1973a); EASTWOOD and BURNET (1979)) which have demonstrated that courtships involving wingless males and/or aristaeless females are of longer duration and show a lower mating success than courtships involving intact males and females. However, these findings may not be directly applicable to species of the virilis group since there are several important differences between the courtship of these species and that of D.melanogaster. One difference is that the D.melanogaster male produces two types of courtship song: sine song, which is believed to sexually stimulate the female, and pulse song, which is thought to act as a species-specific "trigger" stimulus (SCHILCHER (1976b)); species of the virilis group produce only one type of courtship song. Secondly, and perhaps most importantly, D.melanogaster courtships involve very little physical contact with the female. After the initial tap, the male faces the female and produces wing vibration, often circling around her as he does so; contact is only made again when the male passes to the rear of the female and licks her genitalia briefly before attempting to copulate (BASTOCK and MANNING (1955); BURNET and CONNOLLY (1974)). By contrast, courtship bouts in species of the virilis group are characterised by the continuous licking of the female genitalia and rubbing of the underside of her abdomen by the male; wing vibration is superimposed upon these behaviours (see Chapter 3). It is likely that such physical contact, which is absent from the courtship of D.melanogaster, provides additional sexual stimulation of the female.

For these reasons it was felt necessary to obtain independent experimental evidence for the contribution of the courtship song component to the sexual stimulation of the female in the virilis group species.

6.2.2 METHODS

The methods employed in these experiments (asymmetric mass mating; single pair event recorder analysis) have already been described (Chapter 4). The only differences are that the wings were removed from the males instead of the females, and the aristae were removed from the females instead of the males. In the asymmetric mass matings, 6 replicates were performed for each of the 2 experimental conditions. D.virilis (brewery) was the experimental animal.

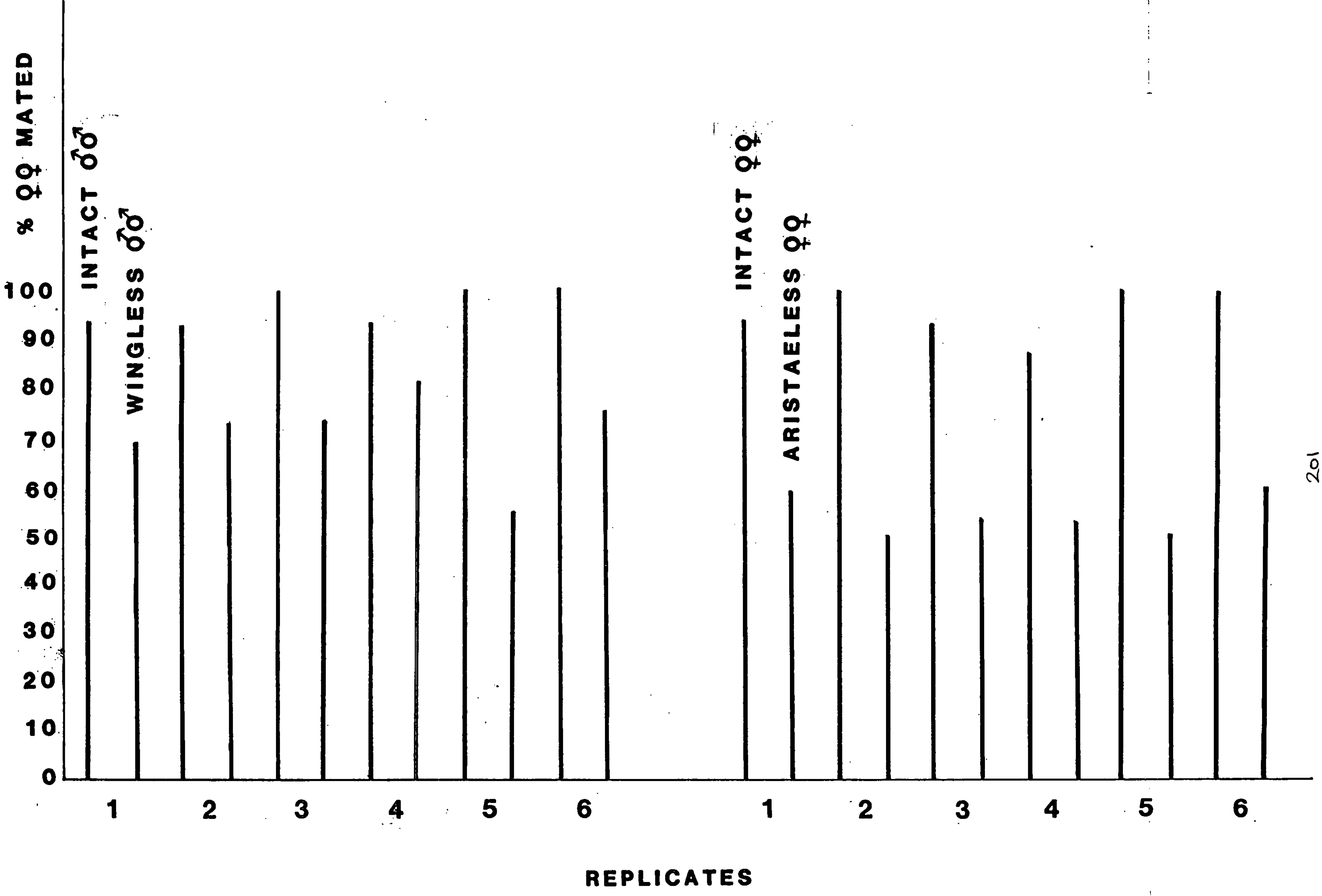
6.2.3 RESULTS

6.2.3.1 ASYMMETRIC MASS MATINGS

The percentages of females which had mated within 30 minutes are given in Figure 6-1. The data from the replicates have been combined in Figure 6-2, which shows the proportion of total matings which occured in each minute of the mass mating.

Figure 6-1

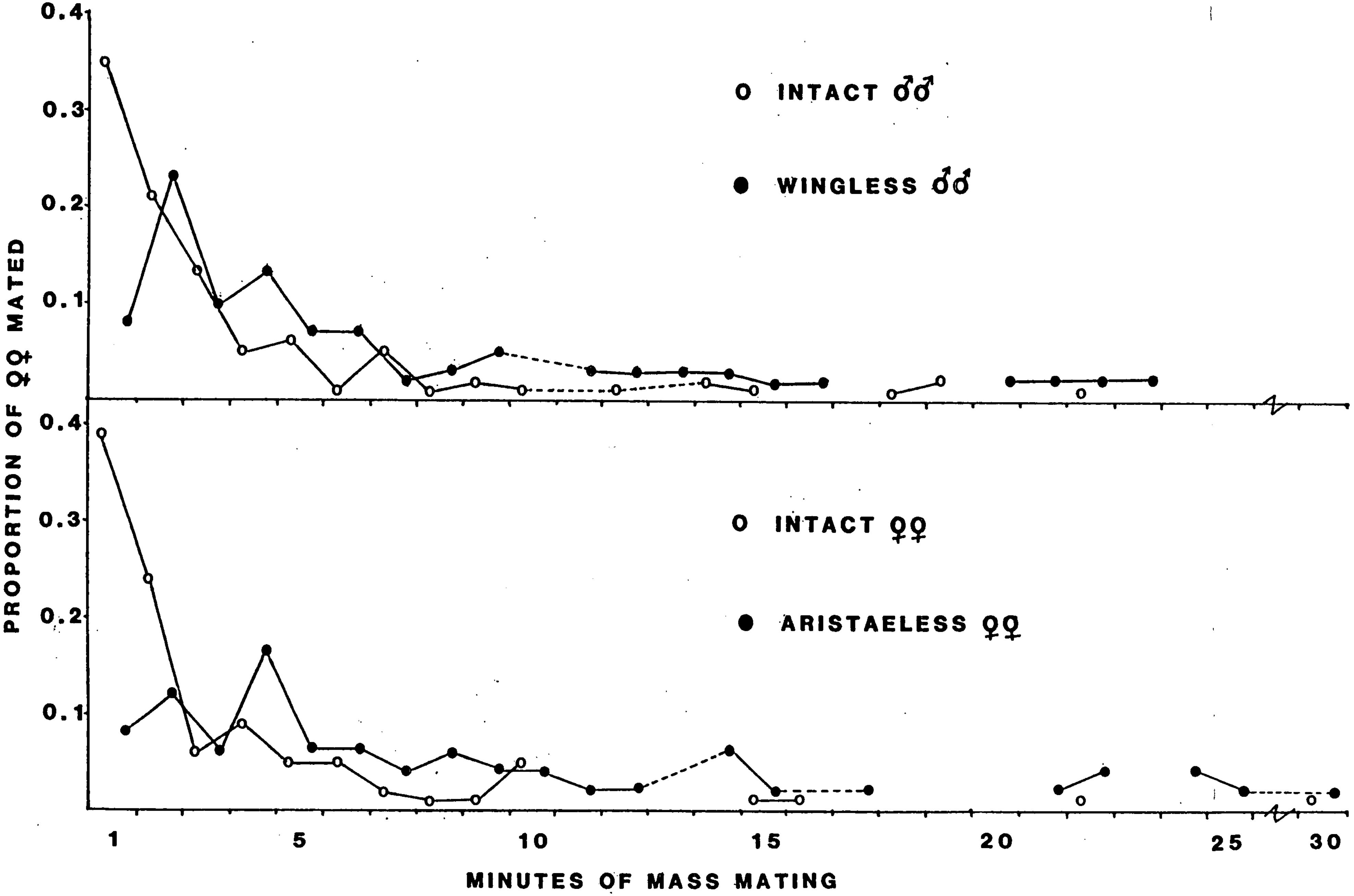
Percentage of females mated in asymmetric mass matings: intact v wingless males; intact v aristaeless females (6 replicates for each condition).


% ♀♀ MATED
INTACT ♂♂
WINGLESS ♂♂
INTACT ♀♀
ARISTAELESS ♀♀
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
1
2
3
4
5
6
REPLICATES

<u>Figure 6-2</u>

Proportion of females mated during each 1 minute period of asymmetric mass mating.

Combined data: intact v wingless males; intact v aristaeless females.


0.4
0.3
0.2
0.1
○ INTACT ♂♂
● WINGLESS ♂♂
PROPORTION OF ♀♀ MATED
0.4
0.3
0.2
0.1
○ INTACT ♀♀
● ARISTAELESS ♀♀
1
5
10
15
20
25
30
MINUTES OF MASS MATING

Analysis

A two-tailed Sign Test was performed on the data presented in Figure 6-1:

intact v wingless males : $p=0.032$

intact v aristaeless females : $p=0.032$

A significantly smaller proportion of females mate in mass matings involving wingless males and aristaeless females.

The 6 replicates in each study consist of paired controls and experimentals to take account of day to day variation. However, as can be seen from Figure 6-1, there is no evidence of consistent day to day variation, so it is legitimate to directly compare the wingless male results with the aristaeless female results, provided that the intact groups are also compared to demonstrate that there is no significant difference between them. A two-tailed Mann-Whitney U Test was employed for this purpose:

wingless males v aristaeless females : $U=2; p=0.008$

intact males v intact females : $U=11; p=0.310$

There is no significant difference between the intact groups, but the comparison of the two experimental groups reveals that courtships involving aristaeless females contain a significantly smaller proportion of matings than courtships involving wingless males.

General information concerning differences in mating speed can be obtained by comparing the number of matings occuring in the first 5 minutes with the number occuring in the subsequent 25 minutes (Chi-square Test):

intact v wingless males : Chi-square=$5.45; p<0.05$

intact v aristaeless females : Chi-square=$15.66; p<0.001$

Wingless males and aristaeless females take longer to mate than intact males and females.

More detailed information on mating speed can be obtained through an examination of the proportion of matings which occur in each minute of the test (see Figure 6-2). The largest proportion of matings occur in the first minute for intact groups (0.35,0.39), in the second minute for wingless males (0.23) and in the fourth minute for aristaeless females (0.16). In addition, the majority of matings have occurred by the second minute for the intact groups (0.56,0.63), by the fourth minute for wingless males (0.54) and by the sixth minute for aristaeless females (0.54). Therefore, as shown previously, wingless males and aristaeless females take longer to mate than intact animals, but aristaeless females take longer to mate than intact females courted by wingless males.

6.2.3.2 SINGLE PAIR COURTSHIPS

(i) Mating success

The number of courtships in which the pair mated within the 5 minute observation period is shown in Figure 6-3 for intact and experimental groups. Courtships which did not contain vibration have been omitted from the analysis, giving totals of 31 intact and 30 wingless male courtships, and 31 intact and 33 aristaeless female courtships.

Analysis

The number of successful and unsuccessful courtships for intact and experimental groups were compared using a two-tailed Chi-square Test:

intact v wingless males : Chi-square=14.65;$p<0.001$

intact v aristaeless females : Chi-square=2.25;$0.5>p>0.1$

Wingless males are significantly less successful than intact males but, although courtships involving aristaeless females tend to be less successful than those involving intact females, the difference is not significant.

(ii) Mating speed and courtship duration

Mating speed (courtship duration) was calculated as the period elapsing between the beginning of the first bout of orientation by the male and mounting (termination), excluding all breaks in courtship. This measurement has been used throughout in order to allow between-group comparisons (N.B. the more usual measurement of mating speed as the period from the first male wing vibration to mounting cannot be applied to courtships involving wingless males). The analysed data is presented in Table 6-1 and Figure 6-4.

<u>Figure 6-3</u>

Mating success of single pair courtships:

(Success = mating within 5 minutes of introduction of male)

intact v wingless males; intact v aristaeless females.

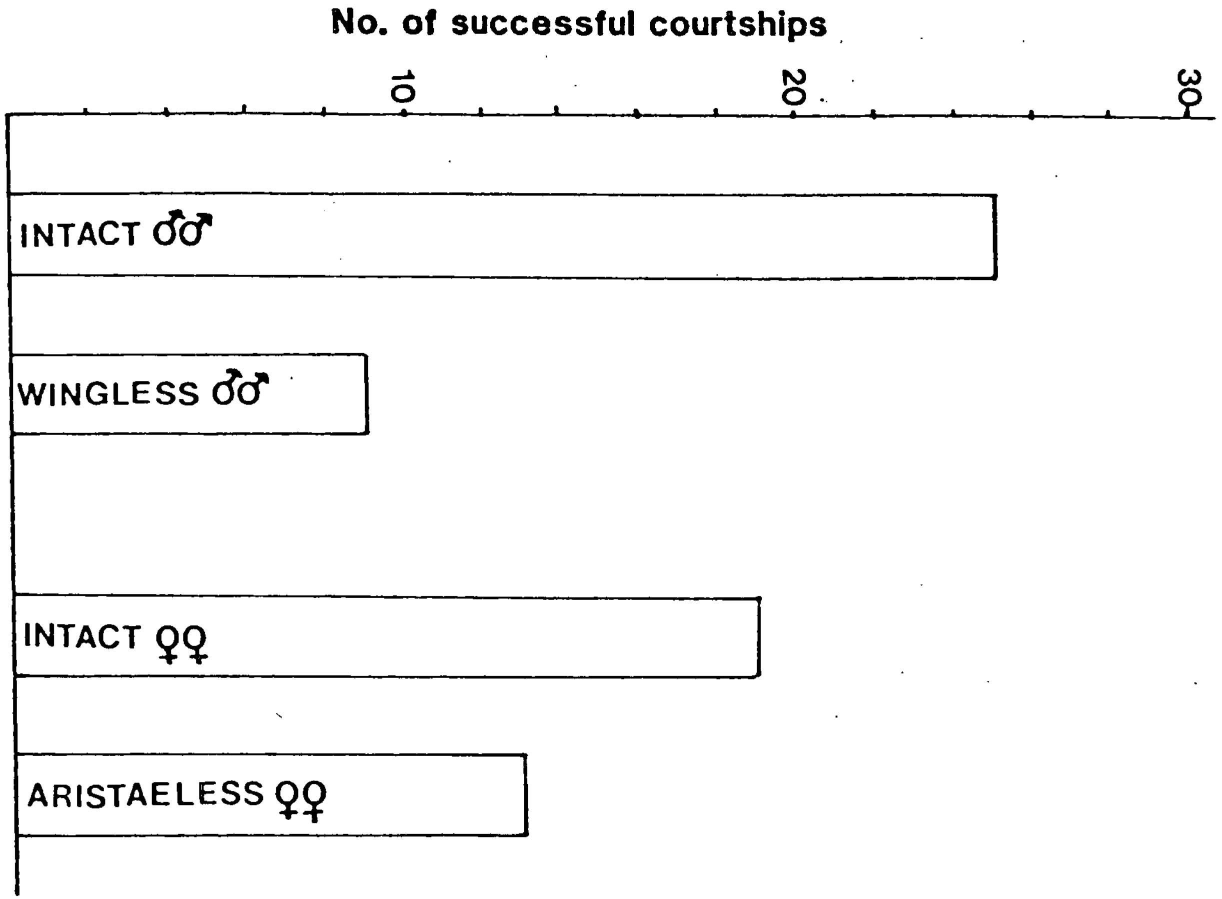

No. of successful courtships
10
20
30
INTACT ♂♂
WINGLESS ♂♂
INTACT ♀♀
ARISTAELESS ♀♀

Figure 6-4

Mating speed and courtship duration (log.seconds) of successful and unsuccessful courtships:

intact v wingless males; intact v aristaeless females.

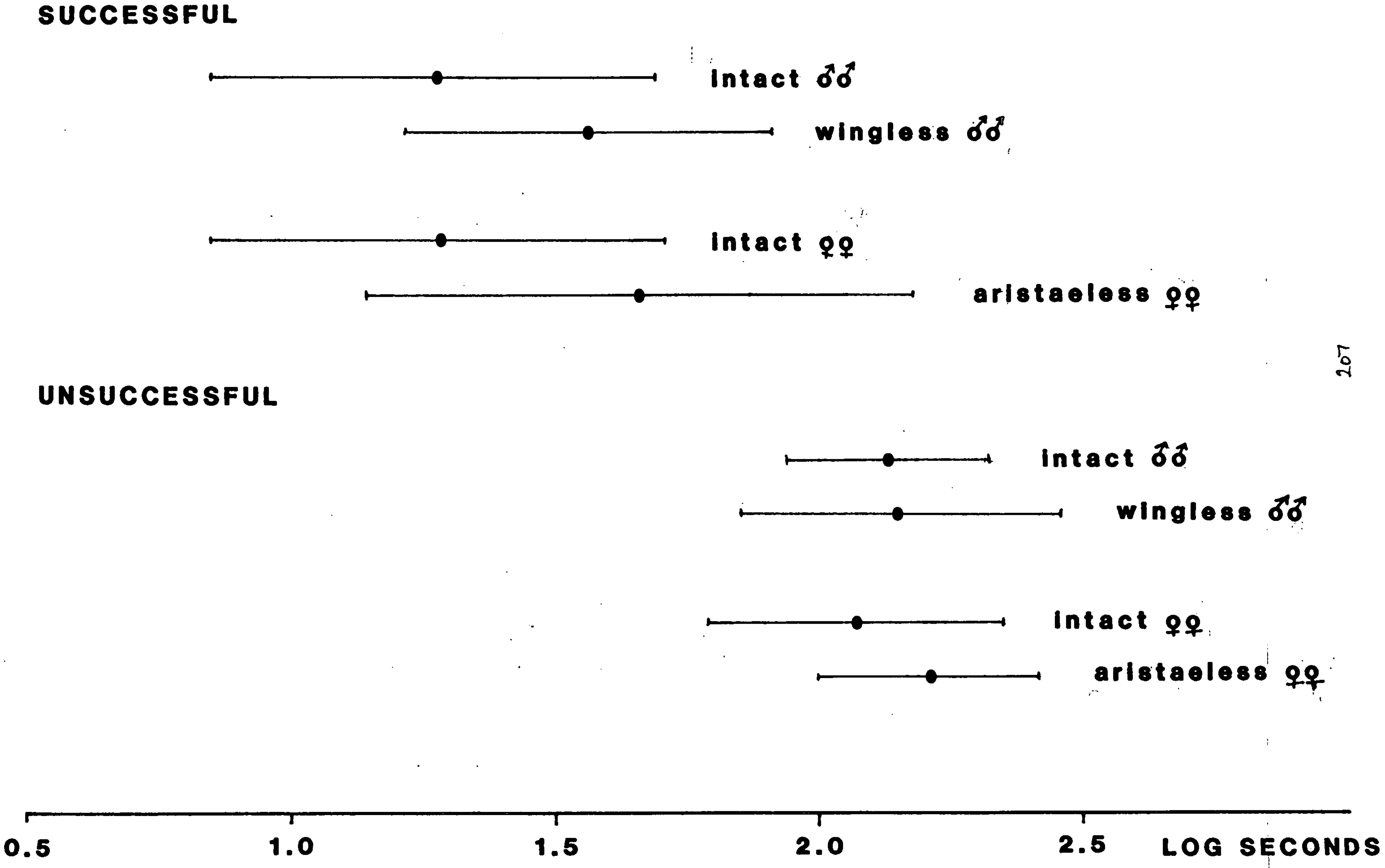

SUCCESSFUL
intact ♂♂
wingless ♂♂
intact ♀♀
aristaeless ♀♀
UNSUCCESSFUL
intact ♂♂
wingless ♂♂
intact ♀♀
aristaeless ♀♀
0.5
1.0
1.5
2.0
2.5
LOG SECONDS

Table 6-1

Analysis of mating speed of successful courtships and courtship duration of unsuccessful courtships involving intact and wingless males; intact and aristaeless females (D.virilis (brewery)). (Abbreviations: INT: INTACT; W-LESS: WINGLESS; A-LESS: ARISTAELESS).

	N	MEDIAN ± S.I.R.(s)	MEAN ± S.E.(S.D.) (log.s)
SUCCESSFUL			
INT MALES	25	17.0 ± 14.0	1.27 ± 0.08(0.42)
W-LESS MALES	9	29.0 ± 26.8	1.56 ± 0.12(0.35)
INT FEMALES	19	18.0 ± 16.0	1.28 ± 0.10(0.43)
A-LESS FEMALES	13	55.0 ± 70.0	1.66 ± 0.15(0.52)
UNSUCCESSFUL			
INT MALES	6	124.5 ± 47.0	2.13 ± 0.08(0.19)
W-LESS MALES	21	163.0 ± 60.0	2.15 ± 0.06(0.30)
INT FEMALES	12	143.5 ± 25.5	2.07 ± 0.08(0.28)
A-LESS FEMALES	20	194.0 ± 31.8	2.21 ± 0.05(0.21)

Analysis

Two-tailed Student t-Tests are used in all comparisons.

(i) Comparison of successful intact and operated groups:

intact v wingless males : $t(32)=2.07;p<0.05$

intact v aristaeless females : $t(30)=2.11;p<0.05$

Wingless males and aristaeless females take significantly longer to mate than intact males and females.

(ii) Comparison of successful intact and unsuccessful operated groups:

intact v wingless males (unsuccessful) : $t(44)=8.8;p<0.001$

intact v aristaeless females (unsuccessful) : $t(37)=8.45;p<0.001$

Unsuccessful wingless male and aristaeless female courtships contain significantly more courtship than successful courtships involving intact individuals. In other words, the lower mating success of courtships involving wingless males and aristaeless females is not due to a deficit of courtship in these groups.

(iii) Comparison of unsuccessful intact and unsuccessful operated groups:

intact (unsuc.) v wingless males (unsuc.) : $t(25)=0.20;p>0.1$

intact (unsuc.) v aristaeless females (unsuc.) : $t(30)=1.56;p>0.1$

Since there is no significant difference between the courtship duration of unsuccessful intact and operated groups, a proportion of the unsuccessful courtships involving wingless males and aristaeless females must be due to chance variation in female receptivity, i.e. they would have failed anyway, independent of the experimental manipulations, because a proportion of the females were unreceptive.

(iv) Comparison of successful intact males and females:

intact males v intact females : $t(42)=0.08;p>0.1$

(v) Comparison of successful wingless males and aristaeless females:

wingless males v aristaeless females : $t(20)=0.53; p>0.1$

There is no significant difference in mating speed between the intact groups or between wingless males and aristaeless females.

6.2.4 DISCUSSION

The purpose of this series of experiments was to investigate the importance of courtship song in relation to the other stimuli provided by the courting male. Such an investigation requires that courtships which contain courtship song are compared with those that do not. Since the latter are rare, the courtship song component must be removed artificially. In practice, this involved the use of wingless males and aristaeless females in a series of asymmetric mass matings and single pair courtships. A detailed analysis of the results has already been presented and can be summarised as follows:

(i) courtships involving wingless males or aristaeless females are less successful, in terms of the number of matings, than those involving intact individuals. This is true for both asymmetric mass matings and single pair courtships, although in the latter the difference between intact and aristaeless females is a tendency only and is not statistically significant. These differences in mating success are not due to courtship deficit in the operated groups since wingless males court persistently and aristaeless females receive persistent courtship from intact males.

(ii) where courtships are successful, wingless males and aristaeless females take longer to mate than intact individuals.

From these findings it is safe to conclude that courtship song provides a major portion of the sexual stimulation which is supplied to the female during courtship. In its absence, the other stimuli provided by the courting male (mainly tactile (licking and rubbing) and also possibly chemical and visual) are either insufficient to elicit the acceptance posture or, if they are sufficient, a longer period is required for their summation by the female (see Chapter 3 for a detailed discussion of the female courtship summation model).

Similar experiments employing D.melanogaster (MANNING (1967a,b); COOK (1973a); EASTWOOD and BURNET (1979)) have produced qualitatively similar results in that wingless males and aristaeless females have been shown to exhibit a lower mating success and mating speed than intact individuals. However, the courtship of D.melanogaster differs from that of species of the virilis group in several important respects, most notably in the amount of physical contact that occurs (BASTOCK and MANNING (1955); BURNET and CONNOLLY (1974)). Since such contact has been shown to be a source of sexual stimulation in the courtship of D.virilis (brewery), "non-contact" species such as D.melanogaster might be expected to be at an even greater disadvantage than D.virilis (brewery) when the courtship song component is artificially removed. Unfortunately, direct quantitative comparisons between these two species in terms of the mating success and mating speed of wingless males and aristaeless females are not very useful for several reasons, including differences in experimental method, strain differences (EASTWOOD and BURNET (1979)) and other factors such as sexual vigour. In addition, the relative importance to the female of other stimuli, e.g. visual stimuli, may differ in the two species.

Finally, although courtships involving wingless males and aristaeless females were assumed on theoretical grounds to be equivalent in that the courtship song component has been effectively removed from both, the results suggest otherwise. In single pair courtships, both wingless males and aristaeless females show an equivalent reduction in mating speed when compared with intact individuals, but the reduction in mating success is only significant for wingless males. Aristaeless females show a trend in this direction but the results are not statistically significant. Interestingly, COOK (1973a) reports a similar effect in D.melanogaster and suggests that aristal amputation may not exclude all the input from wing vibration, either because lower antennal structures are able to receive the signal (see also IKEDA, IDOJI and TAKABATAKE (1981); SCHILCHER (1976a)) or because it is received through other organs of the body. If this is so then aristaeless D.virilis (brewery) females may receive some acoustic stimulation, albeit from a greatly attenuated, and probably distorted, signal, and this may explain the improved mating success recorded in courtships involving such females as compared to those involving wingless males.

However, in asymmetric mass mating experiments, not only do a smaller proportion of aristaeless females mate, but they also take longer to mate, than intact females courted by wingless males. How can these findings be reconciled with the explanation offered above for the improved mating success of aristaeless females? One possibility is that the faulty sound processing system of aristaeless females may be overwhelmed by the courtship songs received from several males simultaneously. This may have a negative effect in that it may affect the ability of the female to process the other male courtship components. Intact females courted by wingless males have no such problem and probably only summate the tactile (chemical, visual?) stimulation provided by the simultaneous courtship of several males.

6.3 THE ROLE OF THE ARISTA IN THE RECEPTION OF SONG

6.3.1 INTRODUCTION

In Diptera, the antennae are the principal organs of hearing (BENNET-CLARK (1971)). The detailed structure of the antenna of a D.virilis (brewery) female is shown in Figure 6-5 (scanning electronmicrograph courtesy of Dr. Jaleel Miyan). The mechanics of the system were worked out by MANNING (1967a) from a series of behavioural experiments (with D.melanogaster) involving aristaeless females and females in which the funiculus had been immobilised with glue. The arista is moved by the sound energy of the courtship song and acts as a lever arm or "sail", causing the partial rotation of the funiculus to which it is attached. Since the funiculus is attached to the pedicel by a short stalk, its rotation directly stimulates Johnston's organ within the pedicel, resulting in the transmission of impulses via the antennal nerve to the brain. The latter has been confirmed by EWING (1978) who has made direct recordings from the antennal nerve of D.funebris.

The importance of the arista in sound reception has also been established in a series of competitive mating tests involving aristal mutants of D.melanogaster: "aristaeless" (reduced size); "thread" (no side branches); "aristaeless"/"thread" (small stump only) (BURNET, CONNOLLY and DENNIS (1971)). The relative success of the females depends upon the surface area of the arista such that wild type females are most successful whilst those with the smallest surface area ("aristaeless"/"thread") are the least successful. BURNET et al (1971) conclude that a reduction in aristal surface area has the effect of reducing the stimulation supplied to

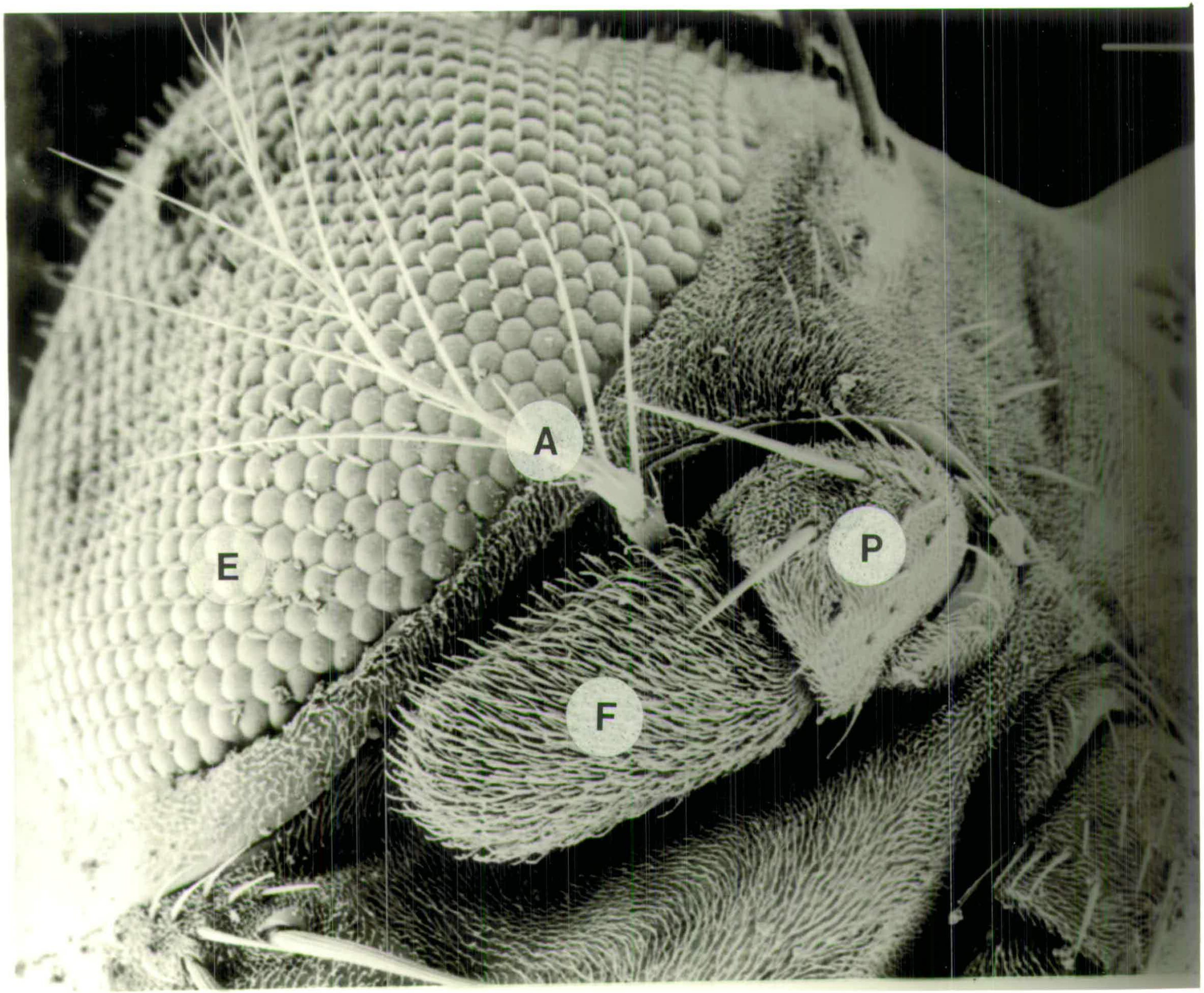
Johnston's organ. Thus mutant females suffer from sensory deficit and receive insufficient stimulation. They may also be unable to discriminate the species-specific patterning of the pulse song since the signal which is transduced is weak and easily distorted by extraneous auditory signals and random activity in the nervous system. This is another reason why the mutant females may be unwilling to mate.

The purpose of the present experiment was to record from the antennal nerve of intact and aristaeless *D.virilis* (brewery) females in order to provide electrophysiological evidence for the sensory deficit which is supposed to result from aristal amputation.

Figure 6-5

Electronmicrograph of antenna of female D.virilis (brewery).

(Abbreviations: A = arista; F = funiculus; P = pedicel; E = eye).


A
P
E
F

6.3.2 METHODS

A D.virilis (brewery) female from which the legs and wings had been surgically removed was embedded in plasticine. An unshielded tungsten electrode (tip diameter:5 microns) was then inserted, through the base of the pedicel, into the antennal nerve. The indifferent electrode was inserted into the abdomen. Recorded or simulated song (see next section) was played to the fly through a loudspeaker suspended vertically above the preparation. A Bruel and Kiaer type 4117 microphone placed next to the preparation allowed the simultaneous monitoring of song and antennal nerve responses, which were both displayed on an oscilloscope and were recorded on tape for filming and analysis. The arista of the antenna was then snipped off with a pair of fine forceps and further recordings were made.

In addition to the above, the antennal nerve response to blowing and touching of the antenna was recorded both before and after the amputation of the arista. These tests were performed both to make certain that the electrode was correctly positioned and also to ensure that the responsiveness of the preparation had not waned with time. As a control against cross-channel "talking" in the tape recorder and general electrical interference, the electrode was re-positioned in the abdomen and the preparation was once again subjected to courtship song and blowing and touching of the antenna.

6.3.3 RESULTS AND DISCUSSION

Filmed oscilloscope traces are presented in Figure 6-6. The amplitude and patterning of the courtship song are faithfully reproduced in the response of the antennal nerve (Figs. 6-6(a);6-6(b)). Since the tungsten electrode is unshielded, the activity of many fibres is picked up simultaneously and as a result individual spikes from many units are being summated to produce the "cycles" (compound action potentials) visible on the trace. In addition, it is clear from Figure 6-6(b) that there are two cycles of neural discharge for each cycle of song; this may indicate that the electrode is recording from two populations of nerve fibres, each of which fires in response to the movement of the funiculus in one direction only.

These recordings demonstrate that information concerning the burst length, number of pulses per burst, number of cycles per burst or pulse, and the intraburst frequency of the courtship song, is available to the female. Which of these parameters is actually important in providing sexual stimulation (and for species recognition?) must be determined within the central nervous system.

Figures 6-6(c);6-6(d) show the antennal nerve response to simulated courtship song for an intact and aristaless antenna, respectively. Since the other tests performed on the aristaless antenna (blowing, touching) show a strong response, i.e. there has been no large reduction in sensitivity as a result of the operation itself or the passage of time, the lack of antennal nerve response demonstrates that the arista functions in the reception of courtship song.

Figure 6-6

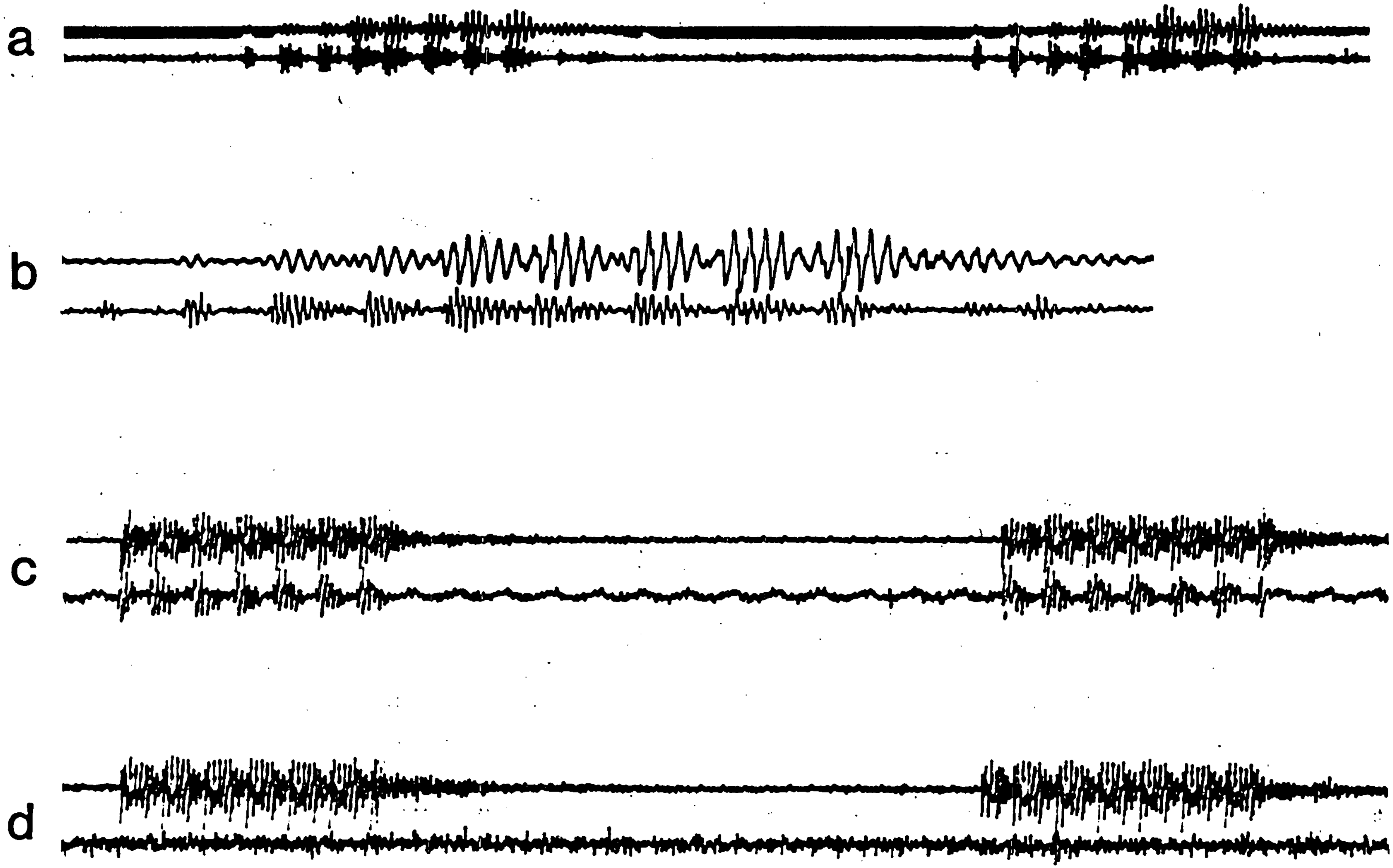
Antennal nerve recordings (bottom trace) produced in response to recorded or simulated male D.virilis courtship song (top trace).

(a) Filtered courtship song (1cm = 25ms).

(b) Expanded trace of one burst from (a) (1cm =10ms).

(c) Simulated courtship song; intact antenna (1cm = 25ms).

(d) Simulated courtship song; aristaless antenna (1cm = 25ms).


a
b
c
d

Recently AVERHOFF, EHRMAN, LEONARD and RICHARDSON (1979) have cast doubt on the audio-mechanical role of the arista and have proposed instead, on the basis of detailed electronmicrographic anatomical studies (*D.melanogaster*), that its function is primarily chemosensory. They have also performed a series of matings (*D.pseudoobscura*) with females from which the arista, or the funiculus and arista, have been removed from each antenna. In contrast to the findings of MANNING (1967a) who found no difference in mating success with *D.melanogaster*, AVERHOFF et al (1979) found that the mating success of the latter group was greater lowered with respect to that of aristaeless females. They interpret these results as indicating the overriding importance of olfactory cues in the courtship of this species.

Whilst no comment can be made on the anatomical findings until further research has been carried out, the results obtained with *D.pseudoobscura* are rather at odds with those of BROWN (1964) who has demonstrated both a reduction in mating success and an increase in courtship duration in courtships involving wingless males of this species, which indicates that courtship song is important in the sexual stimulation of the female. Finally, the electrophysiological findings presented above are further evidence that the arista performs an audio-mechanical role in the reception of courtship song.

6.4 COURTSHIP SONG PLAYBACK EXPERIMENTS

6.4.1 INTRODUCTION

Casual observations provide some support for the idea that courtship song alone may be a sufficient stimulus to elicit the acceptance posture from the female. SPIETH (1952) writes of the *virilis* group species: "Repeatedly it was observed that when a male and female of one of these species were engaged in courtship, another female in the observation cell that was not being courted but merely sitting near the courting pair gave the acceptance response every time the male vibrated his wing......Sometimes the solitary female was...... only a few millimeters away, and sometimes as far as 2 cm. away from the pair". However, in the course of the present studies this effect has been observed only rarely (in *D.lummei*).

IKEDA, IDOJI and TAKABATAKE (1981) have obtained similar results to those of SPIETH (1952) with *D.mercatorum*. They used a special "double cell" consisting of a small, circular inner cell (14mm. diameter) enclosed within a larger, circular cell (50mm. diameter): as one of the two virgin females present in the inner cell gives the acceptance response to the wing vibrations of the courting male, some or all of the virgin females in the outer cell also adopt the acceptance posture. Unfortunately, using similar apparatus, I have been unable to replicate these results (*D.virilis*; *D.virilis* (brewery); *D.lummei*; *D.mercatorum*). This failure is probably due to the differing receptivity of females of different strains since IKEDA et al (1981) have shown that the effect is very strain-specific.

An alternative technique for providing females with only the courtship song component of the male courtship repertoire is to playback recorded or simulated courtship song to a group of females in a small cell. Previous simulated song playback experiments have been performed with D.melanogaster (BENNET-CLARK and EWING (1967,1969); BENNET-CLARK, EWING and MANNING (1973); SCHILCHER (1976a,b)), with D.melanogaster and D.simulans (KYRIACOU and HALL (1982)) and with Zaprionus tuberculatus and Z.sepsoides (LEE (1983)). In these experiments (except SCHILCHER (1976a)) the stimulatory value of simulated courtship song has been calculated on the basis of the mating success or mating speed of wingless males. The advantage of using females of the virilis group species is that they exhibit a clearly recognisable acceptance posture when they are ready to accept the male, thus obviating the need for wingless males as determinants of female receptivity. The purpose of the experiments which are now described was to discover whether either recorded or simulated courtship song alone would be sufficient to elicit the acceptance posture from females.

6.4.2 METHODS

5 to 20 females of one species (usually D.virilis (brewery)) were placed in a small (2cm x 2cm x 1cm) perspex cell with a cotton mesh floor. The cell was then suspended directly above a loudspeaker, some 2cm vertically from the top edge of the loudspeaker cone. Recorded or simulated courtship song was then played to the females for varying amounts of time and the production of the acceptance posture by any of the females was noted.

The antennal nerve recording set-up described in Section 6.3 was used to determine a suitable sound intensity level for the playback experiments. It was found that although there was some antennal nerve response at an average sound intensity of 58 dB, the response peaked at an average of 75 dB (110 dB maximum). This latter average intensity was chosen as the baseline for the playback experiments, although it was varied in some trials.

6.4.3 RESULTS AND DISCUSSION

(i) Recorded song

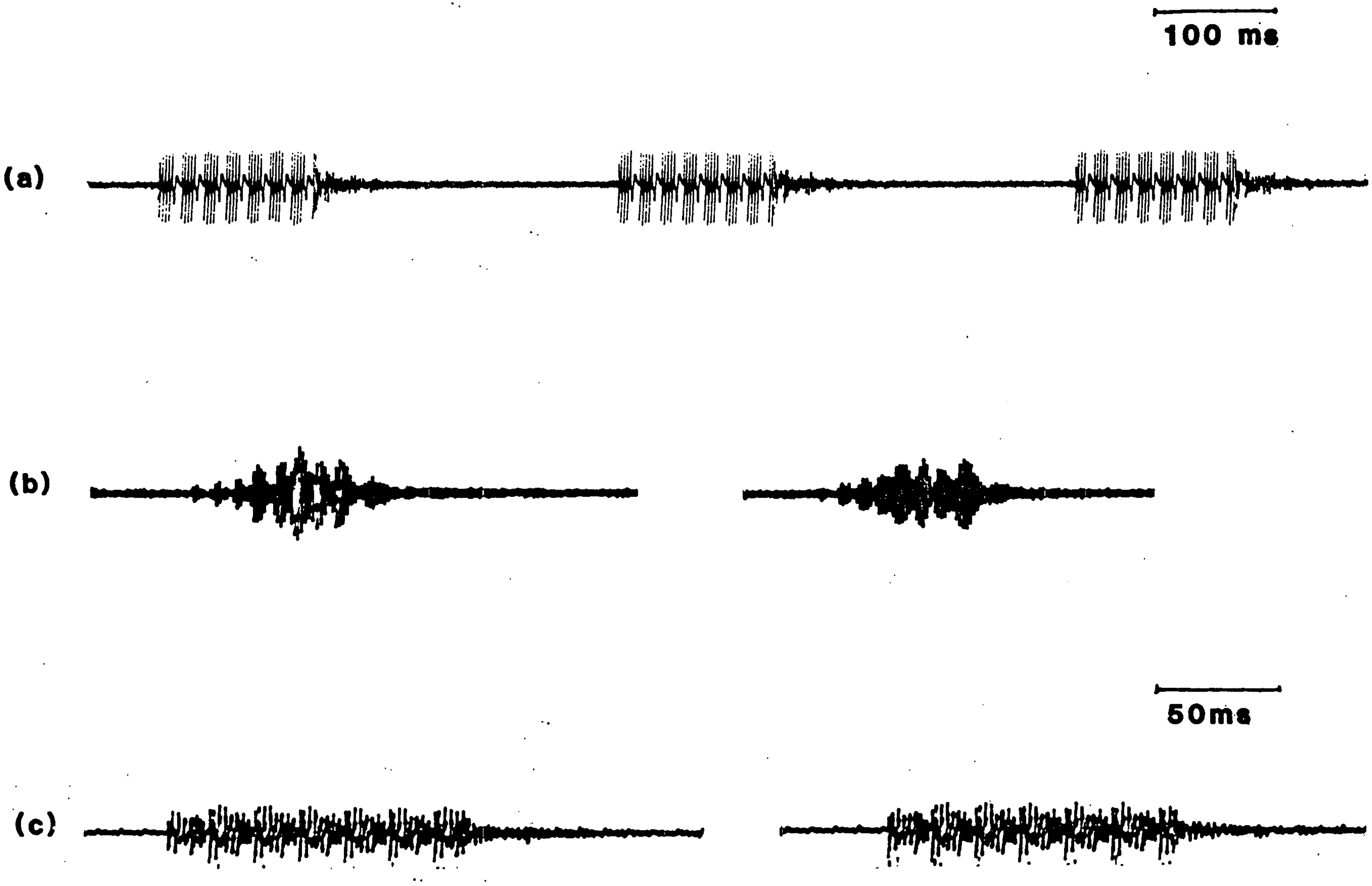
Extraneous noise was removed from the recorded courtship song by filtering out all frequencies less than 200 Hz and greater than 500 Hz. In the first series of tests, two consecutive bursts of song, which had been recorded repeatedly on tape, were played back to the females. The duration and interval between repeated bursts were varied. The results were generally poor, with only two females giving the acceptance posture in one trial (total of 6 trials). The second series of tests involved the playback of a tape loop which contained 6 consecutive recordings of a single filtered burst of courtship song. On the loop, the gap between bursts was approximately 0.9 seconds. The females were exposed to this for 30 seconds and then given a 30 seconds interval, followed by another 30 seconds "on" and 30 seconds "off", and so on. A total of 8 trials were performed but the acceptance posture was not observed in any of them.

Figure 6-7

(a) Simulated courtship song.

(b) Recorded courtship song as received through a B.and K. microphone in the position of the cell.

(c) Simulated courtship song as received through a B.and K. microphone in the position of the cell (expanded trace).


100 ms
(a)
(b)
50ms
(c)

(ii) Simulated song

The simulated courtship song of D.virilis (brewery) is shown in Figure 6-7(a). For comparison, see Figure 5-1, Chapter 5 and Figure 6-7(b), which shows two bursts of recorded (filtered) courtship song of this species as received by a Bruel and Kiaer type 4117 microphone placed in the position of the cell. Figure 6-7(c) shows two expanded bursts of simulated song as received by this same microphone (N.B. for an equivalent record of filtered courtship song see Figure 6-6(a)). The parameters of the simulated song are as follows (with those of actual D.virilis (brewery) courtship song given in brackets):

burst length:130ms (mean=126ms (85-155ms)); number of pulses per burst:8 (mean=9 (6-12)); pulse length:10-12ms (5-10ms); number of cycles per pulse:5 (3-6); intrapulse frequency:350Hz (300-400Hz); interpulse period:6ms (early pulses:5-10ms).

The females were subjected to a routine of one minute "on", one minute "off", one minute "on", etc. Each trial lasted 15 minutes.

Unfortunately, the results were inconsistent. In 13 out of 73 trials, acceptance postures were produced by one or several of the females in the cell. In one trial, 5 females produced the acceptance posture after 30 seconds of simulated song and thereafter, for a period of 2 hours, continued to respond with the acceptance posture almost immediately the simulated song was switched "on". Successes were only obtained with either D.virilis or D.virilis (brewery) females; D.lummei, D.novamexicana, D.a.texana and D.a.americana females gave no response at all.

So, although there has been some success, at the present time this system is too unreliable for use in any quantitative study.

However, given enough time it might be possible to refine the technique such that consistent results are obtainable. One approach would be to use only particularly receptive females: (i) those females which rapidly produce the acceptance posture in response to male courtship could be prevented from mating and removed for use later in song playback tests; (ii) more receptive strains? Another approach would be to alter the parameters of the simulated song: in these experiments the pulses are unshaped (no amplitude modulation) and the period between pulses is held constant, but it may be possible to produce bursts of simulated song which more closely resemble real courtship song.

Once the system could be made to work reliably, it would be possible to examine the parameters of courtship song which are important for the sexual stimulation of the female (and critical for species-discrimination?). Experiments would involve altering the parameters of the simulated song and employing females of different species in the playback cell. Female courtship summation could also be examined by varying the amount of courtship song provided and varying the intervals between bursts of song.

CHAPTER SEVEN

GENERAL DISCUSSION

Since each experimental chapter of this thesis contains a detailed discussion, only the main findings of each chapter will be summarised here, together with some ideas for future research.

Chapter 3

A general description of courtship in D.a.americana is followed by the identification and definition of important behaviours, a selection of which are then used in a detailed event recorder analysis of courtship. Each courtship is shown to consist of a series of bouts, or sequences, of courtship behaviour punctuated by periods of non-courtship behaviour. Within each sequence, the direction and frequency of the transitions between the three main male courtship behaviours reveal a predominant, hierarchical order (orientation (O) - orientation/licking/rubbing (OLR) - orientation/licking/rubbing/vibration (OLRV)) in which succeeding behavioural elements are superimposed upon, but do not replace, preceding behaviours. In addition, vibration tends to be restricted to the later sequences of courtship. This hierarchical patterning is explained in terms of a modified form of the motivational model first proposed by BASTOCK and MANNING (1955) for male courtship behaviour in D.melanogaster. It is suggested that the centres in the brain which control the various behaviours receive common excitation but have different thresholds for firing so that, as the internal sexual excitation level of the male increases, as courtship progresses, the behaviours are triggered in a set order and are superimposed upon one another.

There is great variability between courtships in terms of the number of sequences each contains, and the mating speeds. Since courtship only occurs when there is contact between male and female, one important cause of this variability is the behaviour of the female: she may remain stationary and allow the male to court, or move rapidly away and so break contact, terminating the sequence. In this way the female is able to directly control sequence length (the time available to the male to perform his courtship repertoire) and, therefore, the amount of courtship she receives. When she is satisfied with the courtship which the male has provided, she adopts the acceptance posture, a signal to the male to mount and copulate.

The decision to terminate a sequence or allow the male to continue courting, and to finally accept the male, must be based on the motivational state of the female which, in turn, is influenced by the courtship provided by the male. The most important component of the male's courtship repertoire, in terms of the sexual stimulation which it provides for the female, is wing vibration, or rather the courtship song which results from vibration. There is a direct temporal correlation between vibration and the adoption of the acceptance posture by the female, and courtships which do not contain vibration are generally unsuccessful (this topic is explored further in Chapter 6). In addition, sequences which contain vibration are longer than those which do not.

However, courtships vary greatly in terms of the number of vibrations each contains. It is suggested that this may reflect differences in the courtship requirements of individual females, i.e. each female summates the courtship of the male until the internal sexual excitation reaches some threshold level which triggers the adoption of the acceptance posture. Female courtship summation has been proposed by previous authors to explain courtship processing in D.melanogaster, and the model holds up against the recent criticisms of ROBERTSON (1982).

In the present study, the idea of female courtship summation is supported by the following findings: later sequences tend to be longer than early sequences, i.e. the female is allowing the male to court for longer as a result of the persistence and summation of the stimulation which the male provided in the early sequences; sequences which contain the acceptance posture other than the final sequence tend to precede the final sequence, i.e. once the acceptance posture has been elicited it is easier to elicit it again.

Ideas

A more detailed examination of female courtship summation.

(i) It would be useful to repeat the event recorder analysis, with greater numbers, in order to confirm the findings.

(ii) In order to look at the persistence of courtship stimulation it would be of interest to interrupt courtship immediately after the female has adopted the acceptance posture and then, after a variable interval, allow the pair to come together again and note the time taken for the male to re-elicit the acceptance posture. Perhaps a courting pair could be separated with a paint brush.

(iii) Various experimental regimes (see for example COOK (1973b); CROSSLEY and McDONALD (1979)) could be used to establish whether each female has an individual vibration requirement which remains constant over time, i.e. a set threshold value for sexual stimulation. It is likely (see COOK (1973b)) that the threshold value stabilises a few days after sexual maturity has been reached. Previous to this the threshold value is not constant, but decreases over time (see Section 2.4 and COOK (1973b)).

Chapter 6

This chapter will be discussed here since many of the findings are relevant to those of Chapter 3. The importance of courtship song (vibration) in providing sexual stimulation for the female is examined in a series of asymmetric mass matings and single pair courtships involving "silenced" (wings removed) males and "deafened" (aristae removed) females of D.virilis (brewery). Courtships from which the courtship song component has been artificially removed in this way are shown to be less successful, over the same time period, than those involving intact individuals although, in the case of aristaeless females, the decrease in success rate is a tendency only. The latter result may be caused by the failure of aristal amputation to result in the exclusion of all auditory input. However, it should be noted that recordings from the antennal nerve of aristaeless females, in contrast to those taken from the antennal nerve of intact individuals, failed to demonstrate any response to recorded or simulated courtship song. Perhaps the equipment is not sufficiently sensitive to detect a small antennal nerve response and/or natural courtship song is louder or more effective for some other reason. In addition, successful courtships involving operated individuals are of longer duration than those involving intact individuals.

These findings indicate that the male behaviours O and OLR are less effective than OLRV in providing sexual stimulation, but they may be sufficient to elicit the acceptance posture over an extended time period; i.e. in terms of the female courtship summation model, the summation by the female of low quality sexual stimulation (O, OLR) takes longer than the summation of high quality stimulation (OLRV).

Finally, attempts to elicit the acceptance posture from groups of D.virilis and D.virilis (brewery) females using only recorded or simulated courtship song have met with some success, but the results are, at present, inconsistent. If the system could be made to work reliably then a number of interesting experimental applications are possible, some of which are presented below.

Ideas

(i) The technique could be used to examine female courtship processing. Once the acceptance posture has been elicited using played-back song, the song could be stopped, for a varying interval, and then played again; the time taken to elicit the acceptance posture again after the resumption of song following a varying interval of silence would provide useful information on the persistence of sexual stimulation. Individual females could then be tested on consecutive days to determine whether the number of vibratory bursts required to elicit the acceptance posture remains constant over time, i.e. is there a fixed individual courtship requirement or threshold value?

(ii) The system could also be used to examine the role of courtship song in sexual isolation. How do females respond to the recorded or simulated songs of different species? It may be possible to identify the particular parameters of song which are important in providing sexual stimulation and for species discrimination.

(iii) Simulated song could be supplied to courtships involving wingless males to determine whether the mating speed can be restored to that of courtships involving intact individuals.

Chapter 4

When female pulse song (fluttering) was first observed and recorded in D.a.americana and some other species of the virilis group it was thought that it might be an attractive signal to the courting male, since the male appears unaffected by the song (i.e. it is not repelling), and that it might have a role to play in sexual isolation, since the song is regularly patterned. However, with regard to the former, D.a.americana females also produce pulse song outside of the sexual context in all-female groups, and, in addition, the production of pulse song is not restricted to sexually mature females; immature virgins and fertilised females also produce the song. It is unlikely that immature and fertilised females would produce a song which is attractive to a courting male, and it is also unlikely that such a song would be observed in a non-courtship context. Further evidence that female song is unlikely to be an attractive signal is provided by the finding that the artificial removal of the song component from courtship (wingless females; aristaeless males) has no effect on mating speed or mating success. This result can be contrasted with the large effect which removal of the courtship song component has upon both of these variables (Chapter 6); courtship song is undoubtedly involved in sexually stimulating the female. Incidentally, since mating speed and mating success are unaffected by the absence of female song, it is also unlikely that the song is strongly inhibitory or repelling in function; if it were, one would expect mating speed to increase in its absence.

If female song has a role to play in sexual isolation, then it should be species-specific. To test for this the songs of all species of the virilis phylad and four species of the montana phylad were recorded and analysed. D.novamexicana, D.a.texana and D.a.americana of the virilis phylad and D.littoralis of the montana phylad, regularly produce pulse song. The songs of D.novamexicana and D.a.texana, and D.a.americana and D.littoralis, are similar to each other so, in general terms, the patterning of female pulse song is not species-specific. On the other hand, the songs of D.a.texana and D.a.americana, and D.novamexicana and D.a.americana, are easily distinguishable from one another (on the basis of interpulse interval). In addition, the former subspecies are sympatric and so would perhaps have need of a premating isolation mechanism. However, laboratory studies involving crosses of both pairs have shown that sexual isolation is weak, and hybrids of D.a.texana and D.a.americana are found in nature.

Perhaps female pulse song acts as a sex-identifier. In order to test whether the song is sex-specific, within-species comparisons were made between the female songs and the male aggressive pulse songs. In all species the male and female songs are very similar, the only difference being that bursts of male aggressive pulse song tend to last longer. Therefore, female song is not sex-specific either.

Having failed to demonstrate that female song has any obvious signal value, it was decided to examine it in the context of fluttering behaviour in general. It is suggested that fluttering may function as a spacing behaviour, helping to maintain "individual distance", or "personal space", through personal advertisement and physical delineation of space. The maintenance of "individual distance" has been reported in Drosophila paramelanica (SEXTON and STALKER (1961)) and it seems likely that a behaviour which is produced in response to tactile stimulation by females in all reproductive states, and in the non-courtship as well as the courtship context, would have just such a general function. In addition, the finding that female songs are similar to male aggressive songs also points to a spacing function, since male aggressive songs are commonly associated with agonistic interactions involving the repelling, or driving off, of other males. The same arguments can be applied to the buzz songs produced by males and females of several virilis group species.

One obvious problem with this idea is that fluttering/female song appears to have no effect on the courting male. It is probable, however, that the sexual excitation of the courting male is sufficient to overcome what must be a weak spacing device. It is probably most effective between females at feeding/mating and oviposition sites in the wild, where it would act to reduce contact and the possibility of overcrowding.

Ideas

(i) Clearly it would be desirable to obtain direct empirical evidence that fluttering behaviour in general, and female song in particular, have a spacing function. One approach to this problem would be to measure the number of interactions and individual distances over time of a group of females housed in a cell. The number of females could be increased or decreased, and the number of interactions involving fluttering could be noted. The results from intact females could be compared with the results obtained in similar tests involving wingless females in order to estimate the importance of fluttering behaviour in maintaining "personal space". Aristaeless ("deaf") females could be used to examine the particular importance of the female song component. The floor of the cell could be lined with Drosophila medium in order to approximate to the natural condition, and females in different reproductive states (immature, mature and fertilised) could be tested.

(ii) It would be informative to investigate male agonistic behaviour in more detail, which in several species includes the production of a song which is similar to that produced by the females. An arena could be set up containing food dishes, to determine whether these food sources are defended, and whether males which are successful in displacing other males secure more matings with the females which are attracted to these food sources. Similar experiments have been performed with D.melanogaster (e.g. DOW and SCHILCHER (1975)) but certain features (e.g. jabbing buzz) of the aggressive behaviour of some virilis group species are previously unreported and worthy of further investigation. With regard to male aggressive pulse and buzz songs it would be interesting to discover whether wingless ("silent") males are less effective in defending food sources and displacing other males.

Chapter 5

The courtship songs of the virilis phylad species all take the form of an amplitude-modulated tone burst. They are very similar to each other, but it is possible to distinguish the song of D.virilis from the rest, and this has been used as a species diagnostic character (see NEWBURY, DONEGAN and STEWART (in press)). By contrast, the courtship songs of the four species of the montana phylad which were examined are distinctive and, therefore, species-specific. In general, the similarity of the virilis phylad courtship songs reflects the close genetic relatedness of these species, and the slow and conservative evolution which is supposed to have occured in this phylad. The dissimilarity of the montana phylad songs reflects the more distant genetic relatedness of these species and the more rapid and divergent evolution of this phylad.

The above account suggests that changes in song occur as a by-product of other adaptive processes. However, an examination of the songs of the other species of the montana phylad reveals that there are close similarities between some species. This may be due to coincidence, or perhaps passive change in song characteristics has occured to widely varying degrees in different species. A third possibility is that song is an essentially conservative character and that passive change has made only a minor contribution to song diversity. If this is the case, then large changes in song characteristics are likely to be the product of selection pressure for effective premating isolation mechanisms in zones of species overlap.

Unfortunately, there is no direct experimental evidence that courtship song functions in maintaining sexual isolation. What is available is some general information concerning the degree of sexual isolation between the species of the group, and their present day geographical distribution. If courtship song is involved in sexual isolation, then one might expect that species of the virilis phylad, and those species of the montana phylad which possess similar songs, are allopatric and show little or no sexual isolation, whereas the *montana* phylad species possessing dissimilar songs should be sympatric and exhibit strong sexual isolation. Is this the case?

Generally speaking, sexual isolation between members of the *virilis* phylad is weak or non-existent and the species are completely allopatric. Where sexual isolation is strong, it is due to discrimination on the part of the male and this mechanism has probably arisen as a by-product of other adaptive processes. The geographical distributions of *D.a.texana* and *D.a.americana* do overlap to a certain extent, but isolation must be weak since hybrids are found in nature. It is thought that *D.virilis* and *D.lummei* are probably sympatric in southern Russia and Japan but how they remain isolated is unknown since sexual isolation in the laboratory is weak. Where species of the *virilis* and *montana* phylads overlap it is thought that they are ecologically isolated (different temperature preferences; different oviposition sites) and sexual isolation in the laboratory is very strong.

In the *montana* phylad, sexual isolation is generally very strong. However, there is no simple correlation between strength of isolation and song similarity or dissimilarity. The geographical distribution of these species in the New World reveals several regions of overlap, although it is thought that most are ecologically isolated. However, since actual mating sites have yet to be found, this information must be treated with care. Interestingly, *D.lacicola* and *D.borealis* are sympatric throughout their respective ranges and have similar temperature preferences and oviposition sites. Perhaps the very different courtship songs of these two species are the product of selection for an effective premating isolation mechanism? In the Old World, several species appear to be sympatric but are likely to be ecologically isolated. However, reliable field information on these species is lacking and it may be that several species do come into contact at mating sites. It is of interest, in this context, that the courtship song of *D.littoralis* is very different from that of the other three Old World species, *D.montana*, *D.ezoana* and *D.kanekoi*, whose songs are fairly similar. Perhaps there has been selection pressure for a distinctive song in this species?

Ideas

(i) Clearly, more information is needed on the actual mating sites of the *virilis* group species, particularly the species of the *montana* phylad, since it is at the mating sites where selection for isolating mechanisms will occur.

(ii) It would be useful to examine the mechanisms involved in the strong sexual isolation of the *montana* phylad species. Tests could be performed with wingless males and aristaeless females to determine the importance of courtship song to the process of discrimination. This work would complement the simulated song experiments suggested earlier (Chapter 6).

(iii) A detailed study of *D.lacicola* and *D.borealis*, and *D.montana* or *D.ezoana* and *D.littoralis* , with respect to (i) and (ii) would be of particular interest.

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APPENDICES

Appendix (Chapter four)

SONAGRAMS: bold line = 400Hz; interval = 100Hz.

(Abbreviations: see chapter 2)

(a) Female buzz song (No).

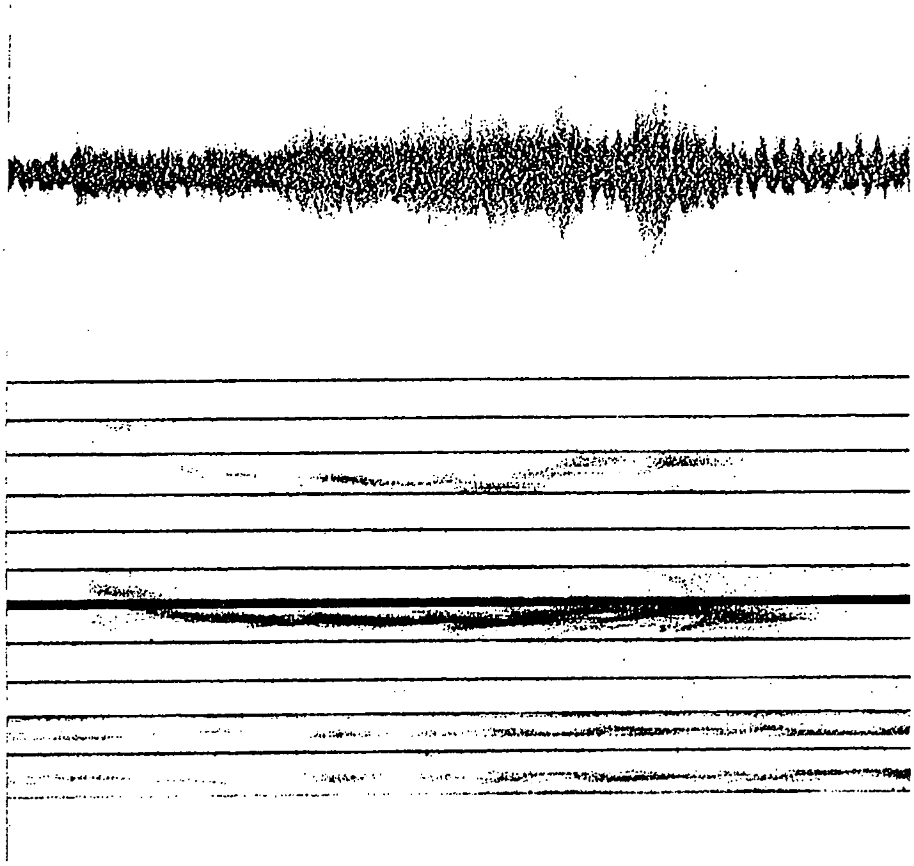
(b) Female pulse song (Aa).

(c) Male aggressive pulse song (Aa).

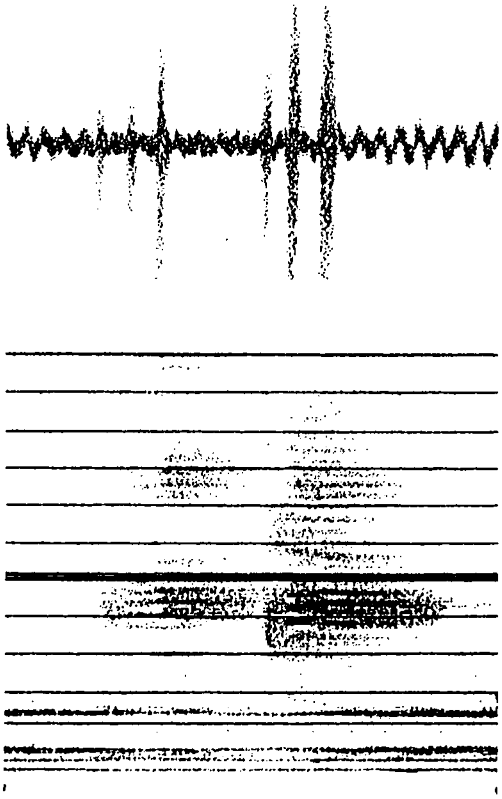
(d) Male aggressive jabbing buzz song (No).

(e) Male aggressive buzz song (Fl).

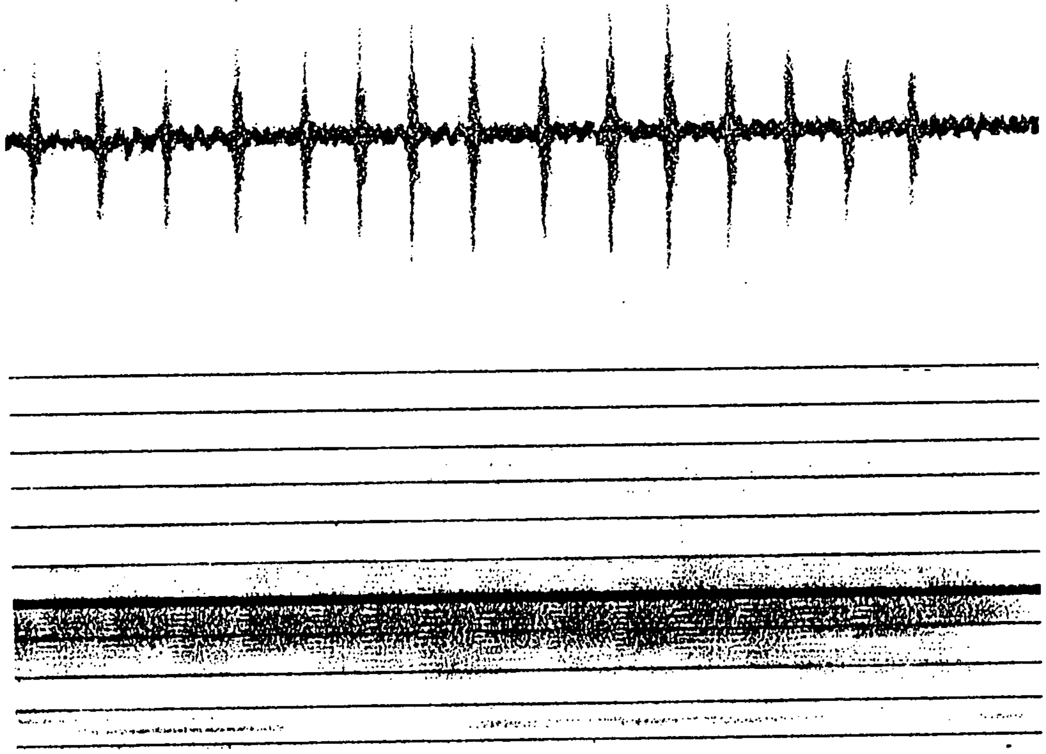
(a)



(b)



(c)



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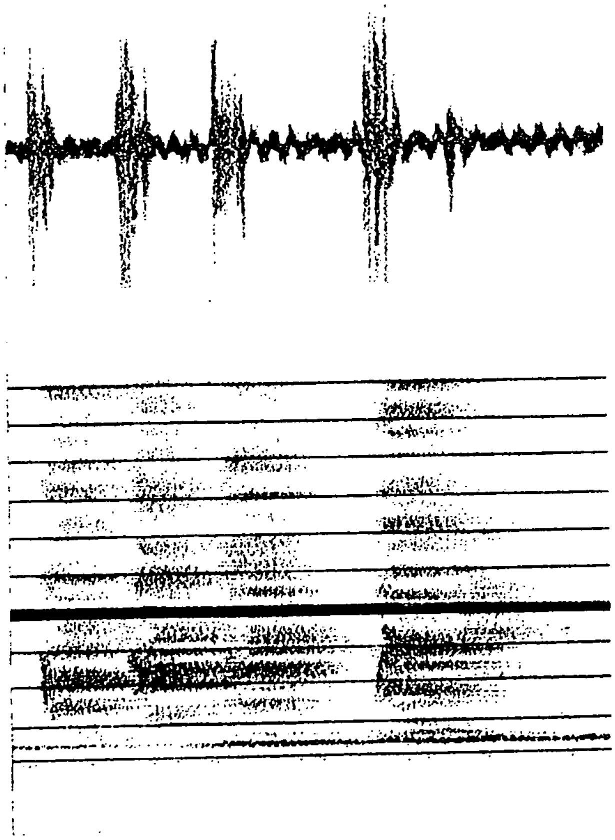

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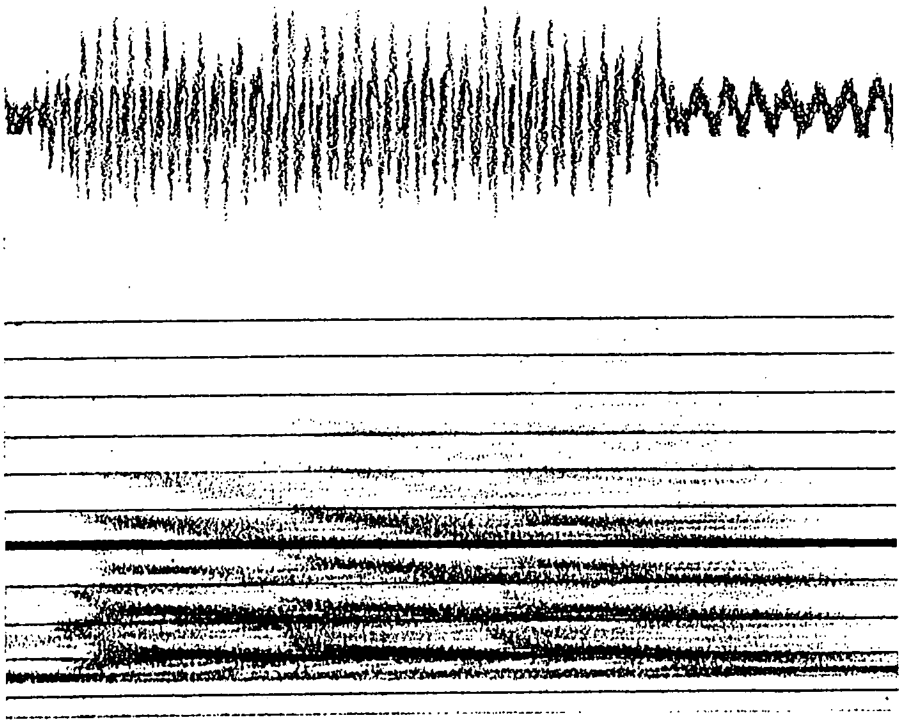

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Duetting in *Drosophila* and *Zaprionus* Species

Bennet-Clark et al. (*Anim. Behav.*, **28**, 230–255, 1980) have described male–female duetting in *Zaprionus* species and they discuss possible roles for the female songs. We have been examining sexual behaviour in the *Drosophila virilis* species group where the same phenomenon occurs. *Zaprionus* and *Drosophila* are very closely related genera and it is likely that some of the functions of female song will be common to both. The pattern of sexual behaviour in some members of the *virilis* species group makes investigation of this problem easier than in *Zaprionus*.

We have recorded the songs of eight species within the *virilis* species group of which two, *D. americana* and *D. borealis*, almost invariably performed duets. In four further species, *D. ezoana*, *D. montana*, *D. virilis* and *D. flavimontana*, females sometimes sang during courtship, while in *D. lacicola* and *D. littoralis* female song was not observed. Females of the six duetting species in addition performed 'aggressive' or 'repelling' songs. Sexually immature females produced these songs when courted, as did mature females when courted by more than one male at the same time, a situation females appear to find aversive. These songs are quite distinct from those produced during normal courtship and it therefore seems unlikely that the latter have the same repelling function. Indeed observation clearly shows that the female songs produced during courtship do not repel the males.

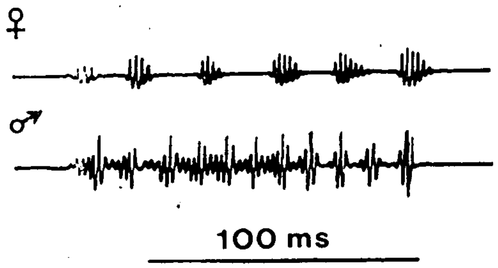

Fig. 1. Courtship songs of male and female *D. americana*.

The species that we have examined in detail is *D. americana*, whose courtship songs are illustrated in Fig. 1. One notable characteristic of male song is a rather irregular amplitude modulated tone burst, while in contrast, the female produces a more regular song consisting of trains of polycyclic pulses. Thus the female song, being more stereotyped, has more of the characteristic of a sexual isolation mechanism, a role more commonly attributed to male song. In support of this is the observation that in almost all *D. americana* courtships that we have recorded (46/48), the female started singing before the male and did so in response to male tactile stimulation. It is not until the later stages of the courtship that the male starts singing and a duet develops.

D. americana is unusual among *Drosophila* species in that males do not attempt to copulate until females signal their sexual readiness. The female acceptance posture is to spread the wings in high V formation; following which males usually, but not invariably, copulate with the female. An acceptance posture is also found in other members of this species group. If the male does not copulate but continues to court, then the female resumes her song. This provides good circumstantial evidence that one function of female song is to induce males to continue courting.

Another similarity with *Zaprionus* is that *D. americana* males continue singing after they have mounted the females. The function of this behaviour is obscure; it could act to repel other males until sperm transfer is completed rather than provide further stimulation for the females.

These observations provide some evidence to support two possible functions for female songs suggested by Bennet-Clark et al.: that they are involved in sexual isolation and that they maintain the males' sexual interest until such time as the females are prepared to mate. At the same time they refute the opinion that *Zaprionus* 'shares many of the advantages of *Drosophila* as well as offering a different and broader spectrum of behaviour'.

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Drosophila virilis and its cosmopolitan relatives in urban islands.

Key words: Drosophila virilis - Drosophila - natural populations - population structure - species distribution.

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ABSTRACT

Populations of brewery *Drosophila* have been studied for 2 years. Species distributions differ between breweries, and are very different from those found in woodland and fruitmarkets. It is suggested that breweries form "urban islands". One of the commonest species found in breweries appears to be *Drosophila virilis*, which has not previously been found in Britain. Tests on various characteristics, including courtship song, have shown conclusively that this species is a strain of *D. virilis*.

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BREWERIES AS URBAN ISLANDS

Man-made environments provide insights into the ability of organisms to adapt to unusual habitats. Some animals, such as herring gulls (Larus argentatus) have become so successful in their man-made surroundings that they are classed as pests (Monaghan & Coulson, 1977).

Breweries form an interesting habitat for study of the population structure of cosmopolitan species of Drosophila. There are at least four population structures possible.

Brewery populations could be (a) homogenous with nearby woodland populations;

(b) similar to that found in other urban habitats, such as fruit markets;

(c) on "islands", with species distributions different from that found in the above habitats;

(d) a special case of (c), with different species distributions in each brewery and little migration between breweries.

Drosophila species generally found in man-made environments tend to be tolerant of alcohol, extremes of temperature, and desiccation (Parsons & Stanley, 1981 ; Stanley et al., 1980 ; Oakeshott et al., 1982). Woodland species appear to be less adaptable. For example, although Drosophila simulans is a common species in Australian woodland, it is very seldom found in wine cellars, as it is not tolerant of alcohol (McKenzie & Parsons, 1972).

Studies on winery populations in Spain (Malpica & Vassalo, 1980 ; Monclus & Prevosti, 1978), Australia (McKenzie & Parsons, 1972), and

America (Marks et al., 1980), have shown that species distributions outside wineries are very different from those inside. D. melanogaster is the dominant "fermentation area" species in every case. D. melanogaster is also the dominant species in a British fruit market (Atkinson, 1977), but is rarely found in nearby British woodland (Shorrocks, 1975).

The above studies suggest that Drosophila populations inhabiting man-made environments are not homogenous with those in the wild. D. melanogaster, which is very resistant to alcohol, is usually the commonest species.

To determine which of the suggested species distributions applies, collections of Drosophila have been made from 13 Yorkshire breweries, with 5 being visited regularly throughout the summers of 1981 and 1982.

The habitats in which brewery flies are found are usually associated with waste products. Waste yeast, returned beer sent for destruction, dregs in returned bottles, seepage from storage vessels and spent hops have all proven to be attractive sites. In every brewery visited, flies were prevented from reaching the fermentation areas.

Permission to collect depends on an undertaking that the brewery involved remains anonymous, so each brewery has been given a code letter. Collections were made using a muslin net and a pooter. Species distributions at 11 of the 13 sites are shown in Fig. 1, which also includes a woodland site for comparison. The species distributions are interesting in three respects. First, species distributions differ from that found in fruit markets (where D. melanogaster, D. simulans, and D. immigrans are the commonest species, with D. melanogaster predominating; (Atkinson, 1977)) and nearby woodland (Shorrocks, 1975 (Fig.1); Dyson Hudson, 1954; Basden, 1954). Second, Drosophila virilis is found exclusively in breweries. It has never been found in British woodland (Shorrocks, 1975) or fruit markets (Rosewell, pers. comm.). Third, species distributions differ from brewery to brewery. Although some general trends are apparent, such as D. virilis tending to be found above bottles (Site W, Site U) this is by no means always the case (Site D, Site K). Collections R and L were both above waste yeast, and yet show different species distributions.

The above findings indicate that breweries form "urban islands" in which the *Drosophila* populations are not homogenous with either the surrounding woodland or other urban habitats. Breweries also show some isolation from each other, suggesting the population structure given in (d) above.

IDENTIFICATION OF *D. VIRILIS*

The species which was first discovered at site T in 1980 was identified as a member of the *virilis* group. The *virilis* group consists of 11 species and is divided into 2 phylads: the *montana* phylad contains 7 species and the *virilis* phylad contains 4 species, one of which is divided into 2 subspecies. Of the 4 species which occur naturally in Europe, one is a member of the *virilis* phylad (*D. lummei*) and three are members of the *montana* phylad: (*D. montana*, *D. ezoana*, *D. littoralis*). Only *D. littoralis* is known to occur naturally in Britain (Throckmorton, 1982). Professor Lakovaara, who is familiar with the four European species, has examined the morphology of the brewery flies. Whilst they differ from the European species, the brewery flies are most similar to *D. virilis*. Since *D. virilis* has never before been found in Britain, a number of tests were performed to substantiate the morphological evidence.

Three enzymes, isocitrate dehydrogenase, malate dehydrogenase and malic enzyme have electromorphs that are characteristic for each of the four European species (Lakovaara et al., 1976). Fig. 2 shows the electromorphs of these species along with those of *D. a. americana* and *D. virilis* (both *virilis* phylad species) and brewery *virilis*. Brewery *virilis* is easily distinguishable from three of the European species and from *D. a. americana* on this basis. It is similar to *D. lummei* but bears closest resemblance to *D. virilis*.

Secondly, the courtship song of brewery *virilis* was compared with the courtship songs of the *virilis* group. Courtship song is produced as a result of male wing vibration during courtship. In many species it has been found to be species-specific and so may have an important role to play in species isolation (e.g. Ewing & Bennet-Clark, 1968). The courtship songs produced by the species of the *virilis* phylad are rather similar to each other and are in the form of an amplitude-modulated tone burst. The courtship song of brewery *virilis* has a similar structure (Fig. 3(a)).

Two important song parameters are the number of pulses per burst and the burst length, measured in milliseconds. The courtship songs of the *virilis* phylad are compared with regard to these parameters in Figure 3(b). The courtship songs of *D. lummei*, *D. novamexicana*, *D. a. texana*, and *D. a. americana* are closely grouped but *D. virilis* itself is easily distinguishable from the rest. Brewery *virilis* song is placed between *D. virilis* and the other species but most closely resembles *D. virilis*. Allowing for interstrain variation, brewery *virilis* is almost certainly a strain of the *D. virilis* species. Note that while the electromorphs of *D. virilis* and *D. lummei* are rather similar, these 2 species are easily distinguishable on the basis of their courtship songs.

Finally, a series of reciprocal crosses were performed involving brewery *virilis* collected from five sites and *D. virilis* (Table 1). Brewery *virilis* differ neither from each other nor from *D. virilis* in terms of replicate success (G test on complete table: $G = 4.098$; $P > 0.995$) and all successful crosses resulted in large numbers of viable and fertile offspring. Single pair matings between *D. virilis* and brewery *virilis* from one of the sites were observed in a small cell. The flies were observed for 10 minutes. The courtship behaviour of brewery *virilis* was found to be similar in all respects to that of *D. virilis* and all crosses were successful (*virilis* male + brewery *virilis* female : 7/7 ; brewery *virilis* male + *virilis* female : 8/8). Again, large numbers of viable and fertile offspring were produced. By comparison, crosses of brewery *virilis* and *D. virilis* with *D. a. americana* were completely infertile.

The findings of these tests and the morphological examination are very good evidence that brewery *virilis* is a strain of *D. virilis*.

DISCUSSION

Breweries provide a unique environment where domestic species of *Drosophila* can thrive. The division of *Drosophila* populations into brewery "urban islands" obviously has a profound effect on the population structure of each species. This is most clearly shown for *D. virilis*, which is only found in breweries, and not in intervening woodland or fruitmarkets. However, this study does not show whether the species distributions are changing or stable. For example, *D. virilis* could be colonising Britain via breweries, or could be at the limit of its range, and only able to survive in man-made environments where it is at an advantage over native species because of adaptations such as alcohol tolerance or desiccation resistance. Collections over a long period of time would distinguish between these two possibilities.

Although this study shows that breweries are likely to be isolated from each other, it does not show why a particular species predominates at each brewery. For example, *D. melanogaster* predominates at site D, and *D. virilis* at site W, yet the micro-habitats (bottles) where these were collected are identical. There are two possible explanations for this result. Perhaps the micro-habitats important to the flies have not been correctly identified. Each species has adaptations suiting it to a particular set of as yet unknown conditions, and the numbers of the predominant species are proportional to the prevalence of these conditions. Alternatively, the species distributions of seasonal populations could be directly related to the composition of the founder populations that survive the winter. There is some evidence (unpublished data) that in some breweries, overwintering populations are rather small, resulting in small founder populations at the beginning of each season. If a small overwintering population in a particular brewery is subjected to random acting factors, such as cold or insecticide treatment, then the founder population may have a different species distribution than that of other breweries. The predominant species is therefore determined largely by chance. Further analysis may distinguish between these possibilities.

The fragmentation of Drosophila populations into isolated groups, combined with small founder populations and little migration between breweries could also result in random drift of characteristics that are not under a high selection pressure. This should lead to differentiation between breweries. Experiments are in progress to test this hypothesis.

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Table 1 Numbers of fertile crosses out of a total of ten replicates for each cross. Outbred stocks cultured from the sites given provided female and male parents, which are shown along the left-hand and top respectively. 1 virgin female was placed in a vial containing Lakovaara's malt medium (1966) together with two males. After 4 weeks at 18 C, in constant light, vials were scored for presence or absence of offspring. Reciprocals of the same cross do not differ significantly from each other (binomial probability for 1:1 summed over all crosses gives $X^2 = -2\sum \ln P = 1.628028$;$P > 0.995$) (Sokal & Rohlf, 1981).

		MALES					
	strains	B	H	K	T	W	1801
F	B	9	9	8	9	9	10
E	H	7	9	9	9	6	9
M	K	9	10	6	9	10	8
A	T	7	10	9	10	10	10
L	W	10	8	9	10	8	9
E	1801	6	9	8	8	9	10
S							

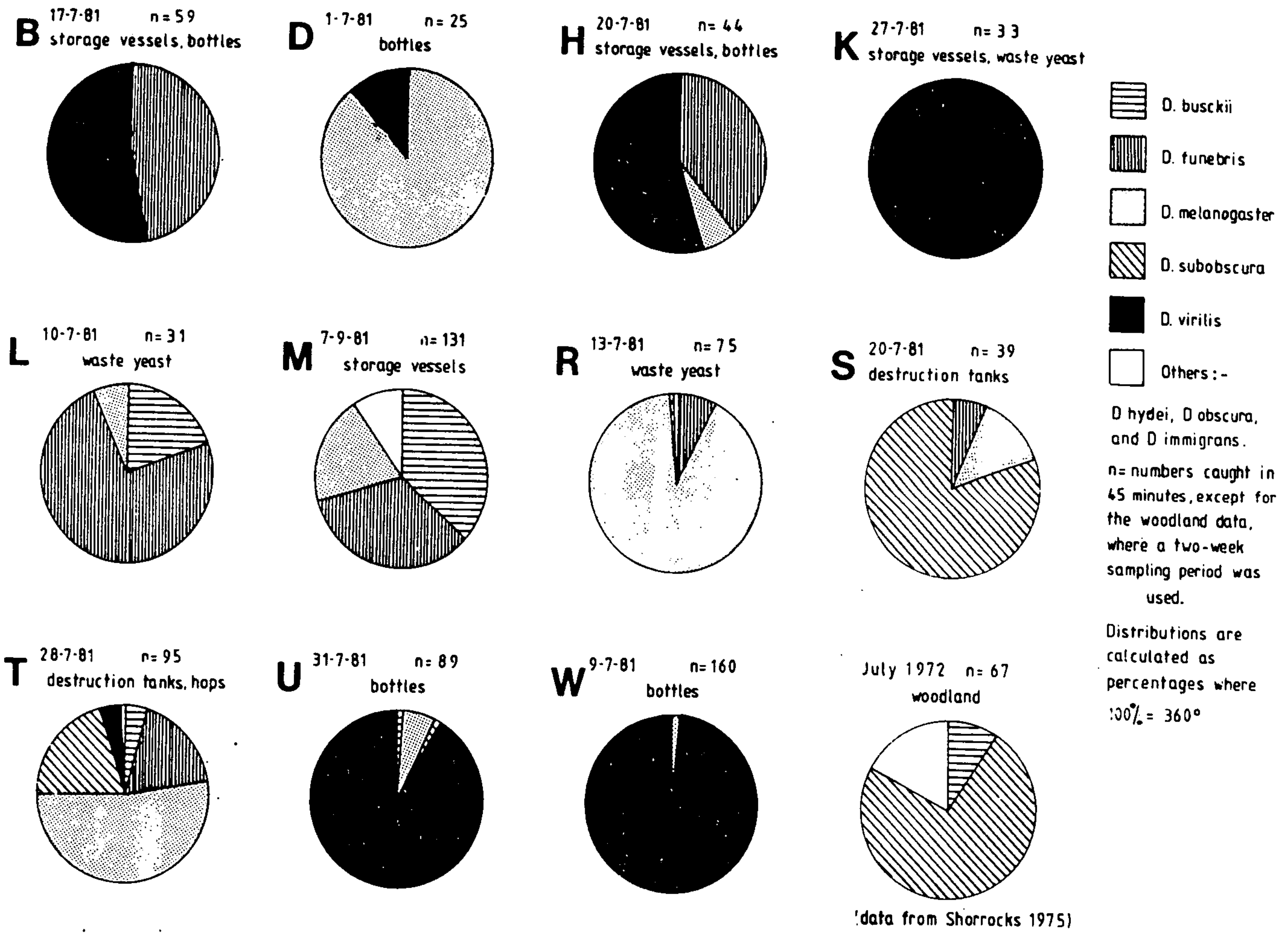

Fig. 1 Pie diagrams showing the species distributions of *Drosophila* at 11 Yorkshire breweries. A woodland site is included for comparison. Distributions at sites B, H, K, T and W were similar throughout the summer of 1981. Brief descriptions of the habitats where *Drosophila* are found are given for each site.

Fig. 2 Predominant electromorphs of the enzymes given for each of the *virilis* group species. The species and strain numbers are : *D. montana*(1200)(Mo); *D. ezoana*(1308)(Ez); *D. lummei*(1100)(Lu); *D. littoralis*(1026)(Li); *D. a americana*(1760.8)(Aa); *D. virilis* (1801.1)(Vi); brewery *virilis*(Vi(b)). Enzymes tested are : Malic enzyme(Me); Malate dehydrogenase(Mdh); Isocitrate dehydrogenase (Idh). Electrophoresis methods and staining recipes are given in Ayala et al.(1972) and Saura et al.(1973).

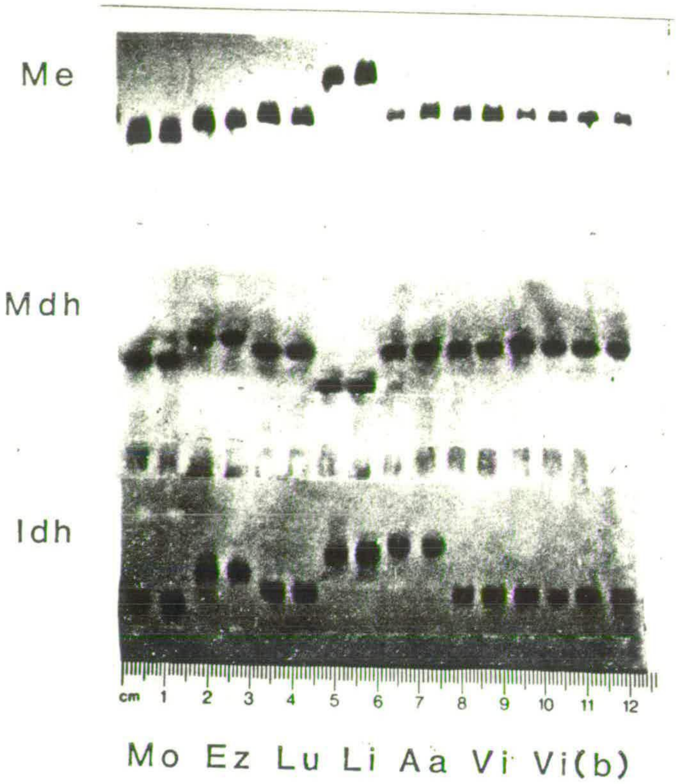
Me
Mdh
Idh
cm 1 2 3 4 5 6 7 8 9 10 11 12
Mo Ez Lu Li Aa Vi Vi(b)

Fig. 3 (a) The male courtship song of brewery *virilis*.

(b) Comparison of the male courtship songs of brewery *virilis* and the other species of the *virilis* phylad. Mean ±standard error values for number of pulses per burst and burst length (ms) are given (26 ±2 C). Abbreviations and strains : *D. virilis*(1801.1)(Vi); brewery *virilis*(Vi(b); *D. lummei*(3264.1)(Lu); *D. novamexicana*(1952.2)(No); *D. a. texana*(2320.2a) (At); *D. a. americana*(1760.8)(Aa).