AN INVESTIGATION OF MALE MATING SUCCESS IN *DROSOPHILA SUBOBSCURA* COLLIN.

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DECLARATION:

I hereby declare that this thesis has been composed by myself, and that the work described within it is my own except where stated in the acknowledgements.

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ABSTRACT

This thesis describes a number of morphological and behavioural traits that contribute to the courtship ability of a male Drosophila subobscura.

I showed that male D.subobscura feed the female with regurgitated crop contents during courtship. The male's ability to provide food depended upon his size and recent feeding history. Males who were prevented from producing a drop, or who produced poor quality or small drops had longer courtships with starved females. The starved females were more likely to move away from such males. Females that took the food had higher fecundity on a low nutrient medium. Although small males only produced small drops of food, they were better than large males at keeping up with females. Courtship feeding was described for other species within the genus.

A further material benefit females might gain by mating with certain males is a 'high quality' ejaculate. I showed that the proportion of eggs hatching and the number of offspring left by a female during her lifetime depended upon the male with whom she mated. The size of the male reproductive organs was measured to determine whether the ability to transfer a large ejaculate limited a male's fertility.

In every case reproductive organ size was scaled on wing length to give a relative organ size. The relative size of the accessory glands and seminal vesicles increased with age, but testis size was unchanged after eclosion. Females mating with males with relatively small accessory glands and seminal vesicles left fewer progeny.

The influence on courtship duration of a male's relative reproductive organ size and glycogen store was assessed. Groups of males with relatively small reproductive organs had longer courtships in single pair tests. The amount of glycogen available was important when reserves were low due to flight or a low nutrient diet. Within group comparisons using partial and multiple correlations confirmed the between group differences.

Males with low reserves of stored glycogen courted females less vigorously than did males with high reserves. There was little difference in the courtship vigour of high and low fertility males, although the low fertility males produced a drop less frequently. The conditions that might favour low fertility males retaining the drop were discussed.

The crop sizes of wild flies suggested they may be starved. There was a correlation between relative crop size and both relative accessory gland and relative testis size. Wild females mating with wild males producing drops of food may gain a high fertility mating as well as nutrients for egg production.

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Chapter 1.

GENERAL INTRODUCTION AND METHODS.

1.1. GENERAL INTRODUCTION TO THESIS.

DARWIN (1871) invoked the theory of sexual selection to explain secondary sexual characteristics, usually in the male, that were costly in terms of survival but that increased an individual's chances of acquiring mates. He envisaged two components; a battle between males for the possession of females and a process whereby females chose to mate with the 'most attractive' males. These two components of sexual selection have been termed intrasexual and intersexual or epigamic selection (HUXLEY 1938 and see BLUM and BLUM 1979, BATESON 1983, THORNHILL and ALCOCK 1983, WEST-EBERHARD 1983 and PARTRIDGE and HALLIDAY 1984 for some recent reviews). In the following account I shall draw on examples from the insects to illustrate the main points.

There are numerous examples of the importance of male competition in determining mating success, and intrasexual selection has clearly been a potent force in the evolution of male characteristics associated with their ability to locate females, to fight other males for the opportunity to mate, or to ensure that females use their sperm to fertilise eggs. For example, in the silkmoth Bombyx mori males have a highly branched antenna bearing a large battery of receptor cells that respond to extremely low concentrations of the female pheromone, bombykol (SCHNEIDER 1969). Males of the beetle Podeschnus agenor have elaborate horns that they use in battles with other males over burrows in sugar-cane to which

females come to feed and mate (EBERHARD 1979). Finally, males of the damselfly Calopteryx maculata have processes on the penis head which they use to scoop out a previous male's sperm from the female's sperm storage organs prior to depositing their own (WAAGE 1979).

There are also examples of females choosing a mate with a phenotype or resource that contributes to the female's survival or her ability successfully to rear offspring. For instance, male scorpion flies Hylobittacus apicalis collect prey items which the females feed upon during copulation (THORNHILL and ALCOCK 1983). Males with small or unpalatable offerings are rejected as mates or only allowed to copulate briefly and the female soon remates. Females that discriminate against males with inferior prey lay more eggs per unit time than females who fail to discriminate. This form of female choice may lead to male competition over resources attractive to females and presents no theoretical difficulty. However, female choice of males based upon the males' own characteristics is more contentious, both for theoretical reasons and a lack of relevant data.

Theoretical models of female choice all involve the joint evolution of a male character and a female preference for it (discussed below). However, female choice may not require this type of explanation (PARKER 1983). Females may simply be moving towards stimuli that they find most conspicuous given their sensory tuning evolved in contexts other than mate discrimination. For example, female mole crickets Scapteriscus acletus move towards singing males and the males producing the loudest songs gain the most matings (FORREST 1980). The calls of the noisiest males may propagate further and be more likely to be heard. This may simply be a case of

passive attraction of the females reflecting constraints of their nervous system or it may be that female behaviour has evolved to make matings with loud callers more likely. Unravelling the evolutionary history of such behaviours is very difficult.

The first person to produce a model of how female preferences might evolve was FISHER (1930). He argued that if there was a heritable female preference for a heritable male character advantageous under natural selection then, if non-random mating occurred with the females with the preference mating with the males with the character, sons from these matings would inherit both the preference and character genes. The preference genes would therefore spread through the population because of their association with the advantageous character genes. Once the preference genes reached a certain frequency, a new process occurs whereby females with the preference genes gain an advantage because their sons are preferred by females because the preference genes are now common. At this point FISHER claimed a 'runaway' process would occur with the male trait and female preference for it increasing in the population ever more rapidly until finally the evolution of the trait is checked by severe counter-selection on it. O'DONALD (1967,1980) produced two-locus genetic models of FISHER's process that confirmed the spread of the female preference but not the runaway process. ANDERSSON (1982a) showed that the runaway process could be triggered by a character disadvantageous under natural selection if males that were fit in other respects could afford the cost of the character.

LANDE (1981 and discussed in ARNOLD 1983) produced a polygenic model of female choice and emphasised the influence of genetic drift on the evolution of female mating preferences. He started with a

heritable male character under normalising natural selection and heritable female preferences for the character that varied between females and incurred no immediate costs or benefits. LANDE argued that, within a finite population, random genetic drift in female mating preferences produces random selective forces on the males, which in turn affects the mating preferences because of the genetic correlation between the traits. For any degree of disadvantage of the male character under natural selection, there is a corresponding strength of female preference which can maintain the male character in the population. This gives rise to a line of equilibria, the slope of which is specified by the ratio between the strength of the stereotyped female preference and the intensity of natural selection acting upon the character. Populations evolve along trajectories determined by the regression slope, B/G, where B is the additive genetic covariance between the male and female traits and G is the additive genetic variance for the male character. If the slope of the line of equilibria is greater than the genetic regression slope, then populations evolve towards the line of equilibria and the composition of the population at equilibrium depends upon its starting point. In this stable case populations can drift along the line and if they drift above or below it, selection will tend to take them back to a different equilibrium. In the unstable case the slope of the line of equilibria is less than the genetic regression slope and any drift away from equilibrium will trigger a runaway process involving evolution away from the line of equilibria. KIRKPATRICK (1982) reached similar conclusions to LANDE using a two-locus haploid model.

Female preference is selectively neutral in these models. This means that drift within a population can establish a female preference for a male trait in the absence of the trait. This pre-existing preference can then allow a disadvantageous male character to become established in the population when it appears through mutation. However, PARKER (1982) has pointed out that there are costs to being choosy, such as wasted time or a failure to mate, to which the LANDE and KIRKPATRICK models might be sensitive. If the costs of choice outweigh the benefits then, perhaps a FISHER-type process where the female preference is carried along by an initially advantageous male character is required for the evolution of female choice. A particularly important trigger for the FISHER process could be selection for reproductive isolation between speciating populations.

Although we might not expect to witness male characters evolving as FISHER envisaged (because the process is likely to be uncommon and rapid), his model does predict certain genetic correlations in extant populations. At evolutionary equilibrium further evolution of the male sexually selected character is opposed by natural selection and there is no net fitness heritability. Any heritable genetic variation increasing the value of a character that increases male mating success, will have a balancing pleiotropic negative effect on some other fitness component. For example, in sticklebacks Gasterosteus aculeatus there is heritable genetic variation for red throat colour in males, and females mate preferentially with red-throated males (SEMLER 1971). SEMLER used unpublished data to argue that red throated males were also more vulnerable to heavy predation. The assumption of zero net fitness

heritability may not be correct. Recurrent mutation, migration between locally adapted populations and temporal variation in selection could all produce some fitness heritability (reviewed in PARTRIDGE 1983). A change in the balance of the various components of fitness can change genetic variance in total fitness (LANDE 1982 discussed in ARNOLD 1983). Because net fitness is a function of mating success as well as other fitness components, a change in the net preference of females, for whatever reason, can create genic variance in total fitness. ARNOLD (1983) considers that equilibrium populations may well vacillate between the production and erosion of heritable fitness variance. So we might expect a certain amount of fitness heritability in natural populations. Where fitness heritability occurs, then heritable genetic variation affecting male mating success could be associated with high net fitness because it has sufficiently low pleiotropic negative effects on other fitness components, or because it has positive pleitropic effects on these components. One study (PARTRIDGE 1980 discussed below) suggests that male mating success in D.melanogaster might be genetically correlated with larval viability.

There are practical difficulties associated with demonstrating female behaviour that has evolved because it resulted in mating with males with particular genotypes. One of these difficulties is that female choice might be confounded by the effects of inter-male competition. PARTRIDGE and HALLIDAY (1984) illustrate this point using D.melanogaster. When courted by several males the female is more likely to mate with a large male than a small male. The authors argue that this is not evidence for female choice because the female may have a fixed probability of accepting any male. The greater

success of larger males might be because they are better at searching for or tracking females. Even if this is not the case, the louder songs produced by the larger males might be audible to the female from a greater distance. If the males most succesful in male competition for females are also the most conspicuous males, it can be very difficult to make an empirical distinction between male competition and female choice.

One case where female choice may have evolved in the way FISHER (1930), LANDE (1981) or KIRKPATRICK (1982) envisaged is in widowbirds (ANDERSSON 1982b). Males of this species defend territories in which females breed. The males have extremely long tails which they use in flight displays over their territories. ANDERSSON lengthened the tails of some males by glueing extra feathers on, others he shortened and others he cut and restored as controls. The males with the artificially lengthened tails attracted most females onto their territories and ANDERSSON was able to rule out the possible confounding effect of competition between males for high quality territories. It seems unlikely that passive attraction was involved as the displaying males were visible from over one kilometre away and females visited several males before settling on a territory.

The other major practical problem associated with demonstrating that female behaviour has evolved because it resulted in matings with males with particular genotypes is that females may gain material benefits from the male, even when they only associate for mating. I shall illustrate this problem with an example. PARTRIDGE (1980) allowed one group of female D.melanogaster to mate freely with males in a population cage ('choice' group) and took another group of females from the cage as virgins and then randomly paired these

females with males taken from the cage ('no choice' group). She then collected the first instar larvae hatching from the eggs laid by the females of the two groups and competed them against a standard competitor (a fixed number of larvae homozygous for the mutant 'sparkling'). PARTRIDGE found that more of the larvae from the pairings in which mate choice was free to occur survived to eclosion indicating that the offspring from the 'choice' group were fitter with regard to larval survival than the offspring from the 'no choice' group. KREBS and DAVIES (1981) describe PARTRIDGE's experiment but then go on to conclude; "Therefore by choosing mates, females can increase the survival of their offspring and since male fruitflies contribute only DNA to their offspring, the females must somehow be able to choose good genes." But, is it really true that male Drosophila contribute only DNA to their offspring? The DNA is transferred in male ejaculate consisting of several thousand sperm awash with seminal fluid. If female Drosophila were able to use nutrients from the male ejaculate to increase the size of the eggs they laid then the 'choice' females in PARTRIDGE's experiment may simply have been mating with the males with most nutrients to offer (PARTRIDGE personal communication). Female bushcrickets, Requena verticalis, use nutrients derived from male ejaculate to increase egg size (GWYNNE 1984), egg size is known to be correlated with larval survival in a number of insect species (CAPINERA 1979) and recently MARKOW and ANKNEY (1984) using D.mojavensis and PARTRIDGE (personal communication) using D.melanogaster have found that female Drosophila do use nutrients obtained from male ejaculate in oogenesis. As PARTRIDGE and HALLIDAY (1984) point out, to demonstrate that females are choosing males purely for the genetic consequences this has for

their offspring the effects of male competition and possible non-genetic benefits must be ruled out.

One way to reduce the effects of male competition is to eliminate male interference by observing single males with females. If some measure of courtship success in this situation is available, then it might be possible to identify male characteristics that are important during courtship. There is good evidence for Drosophila melanogaster that the duration of courtship depends upon the male's ability to stimulate the female. Male D.melanogaster vibrate their wings during courtship producing a song that the females perceive with their antennae (BENNET-CLARK and EWING 1967, EWING 1978). EWING (1964) found that courtship duration increased as more of a male's wings were removed thus reducing the intensity of the song he was capable of producing. Courtships tended to be shorter than normal if females were 'prestimulated' with artificial courtship song (SCHILCHER 1976, KYRIACOU and HALL 1982). There is also evidence that females have a 'fixed' courtship requirement in terms of the number of bouts of vibration they receive before accepting a male (COOK 1973, CROSSLEY and MACDONALD 1979). Mutant male D.melanogaster that sing less often and for shorter periods than wild-type males have longer courtships (BASTOCK 1956) and mutant males that have longer bouts of singing have shorter courtships (KYRIACOU, BURNET and CONNOLLY 1978). Also large male D.melanogaster tend to sing louder and more often than small males and have shorter courtships (PARTRIDGE, EWING and CHANDLER unpublished results). It seems to be the case in D.melanogaster that courtship duration is inversely related to the intensity or frequency of courtship stimuli provided by the male.

If female receptivity is controlled for, then measuring courtship duration will help identify male characteristics important in courtship. If female behaviour towards males is also recorded then it may be possible to determine whether females are actively discriminating against particular categories of males (PARTRIDGE and HALLIDAY 1984), although there are problems associated with using single pair tests. For instance, for female choice to occur perhaps females need to be able to compare males. As a starting point, it is useful to identify male behavioural and morphological traits important in courtship and record female responses to various males. Features concerning male interference, aggression and ability to locate females and the behaviour of females in the presence of several males can be examined and built in to later models of the mating system.

Then there is the question of non-genetic benefits. Do male Drosophila really only give females their genes or are there material benefits to be gained by mating with particular males (see above)? In this thesis I shall try to identify male traits contributing to courtship success, whether females use these traits to discriminate actively between males and what material benefits the females might gain by discriminating. The species I have chosen to work with is D.subobscura and the reasons for using this species are discussed below.

D.subobscura is a 'light dependent' species in the sense that if virgin males and females are kept together in total darkness little or no insemination occurs (PHILIP, RENDEL, SPURWAY and HALDANE 1944, RENDEL 1945, PINSKER and DOSCHEK 1980). A number of authors have stressed the importance of visual cues during D.subobscura

courtship, both in terms of the male's ability to perceive and track the female (e.g.WALLACE and DOBZHANSKY 1946, MILANI 1951b), and in terms of the female's ability to perceive visual stimuli (e.g. BROWN 1965). PINSKER and DOSCHEK (1979) found that there was a linear relationship between mating success and contrast discrimination ability. They went on to demonstrate that contour-blind males were unsuccessful with contour-blind and wild-type females even though wild-type males were succesful at light of the same wavelength (PINSKER and DOSCHEK 1980). They also showed that, although contour-blind females did mate with wild-type males, they did so at much lower frequencies than wild-type females. Clearly visual stimuli during courtship are important to both the male and female. This means that by simply watching D.subobscura courtship the observer should identify some of the more important courtship stimuli without having to smell and listen to the courtship at the same time. This does not mean that olfactory and acoustic stimuli do not play a part.

Another reason for using D.subobscura is that there are indications from previous work that female D.subobscura might actively discriminate between males and by so doing leave more progeny (MAYNARD SMITH 1956 discussed in chapters 4 and 5).

Finally, although our understanding of Drosophila ecology is very limited, more is known about the ecology of D.subobscura than most other Drosophila species. It might be possible to put the behaviour of laboratory stocks of flies into an ecological context. Furthermore, because D.subobscura occurs in the woodlands around Edinburgh there is scope for observations on wild flies both in their natural habitat and in the laboratory.

1.2. GENERAL MATERIALS AND METHODS.

1.2.1. The stock.

The stock of D.subobscura used throughout this thesis was established in 1980 from 42 males and 39 females collected in woodland at Dalkeith and Ormiston near Edinburgh. In 1981 and 1982 further collections of wild flies from around Edinburgh were incorporated into the stock.

1.2.2. Culturing and collecting the flies.

The stocks were housed in a constant temperature room at $20\pm1^{o}C$. The lights in this room were switched on at 9a.m. and off at 9p.m. giving a 12 hour light:12 hour dark daily cycle.

The stocks were maintained in population bottles and a population cage. The population bottles were one-third pint glass milk bottles containing approximately $70cm^3$ of standard cornmeal-molasses-agar drosophila medium seeded with active Baker's yeast. The adults feed and lay eggs on the medium and the larvae develop within it. The adults were transferred weekly to fresh bottles and, under these conditions, the next generation began to emerge about three weeks after the first eggs were laid. Eclosion continued for a further 2 weeks before the old bottles were discarded. The population cage contained 20 population bottles run on a five week cycle. Each week the four oldest bottles were emptied of flies, removed from the cage and replaced with four fresh bottles.

The body size of adult Drosophila is largely dependent upon the larval food supply and temperature of development. Increasing the temperature or larval density results in smaller adult size (SANG 1949, CHIANG and HODSON 1950, SOKOLOFF 1955, TANTAWY and MALLAH 1961). MCFARQUHAR and ROBERTSON (1963) found that the mean size of wild D.subobscura collected at Helensburgh in Scotland was smaller than the mean size of a population of Helensburgh flies raised in the laboratory at 18°C with unrestricted access to yeast. There was greater variation in the size of the wild adults resulting from a number of flies smaller than any found in the laboratory population. The authors argued that the differences between the laboratory and wild populations were more likely to be a consequence of differences in larval nutrition than of differences in temperature during development. They suggested that wide variation in the larval food supply was a regular feature of the environment of D.subobscura. ATKINSON (1979) separated the effects of temperature and larval nutrition on the adult body size of a population of D.melanogaster in a fruit and vegetable market near Leeds. He found that larval density was as important as temperature in explaining variation in adult wing length through a season and argued that there may be a correlation between temperature and larval crowding that tends to conceal the effects of larval competition. ATKINSON (1979) concluded that larval competition is probably commoner in the field than some studies have suggested (e.g. TANTAWY and MALLAH 1961). A recent study by GRIMALDI and JAENIKE (1984) supports ATKINSON's view and demonstrates that for four species of mycophagous Drosphila, larval competition for food is an important determinant of larval survival and adult body size.

In this thesis I used 'high' and 'low density' vials to generate variation in adult body size. Groups of adults were taken from population bottles and put into 7.5cm x 2.5cm glass vials containing about $7cm^3$ of the standard drosophila medium seeded with active Baker's yeast. The flies were left in 'low density' vials for about 24 hours and in the 'high density' vials for three or four days. In the low density vials fewer eggs were laid, there was less larval crowding and probably little larval competition for food or space and most of the flies emerging were large. In the high density vials there was greater larval crowding, larval competition was higher and many emerging adults were small.

To collect virgin flies, bottles or vials in which adults were eclosing were emptied of all adults just before artificial dawn at 9 a.m. About eight hours later any flies that had eclosed were anaesthetised with carbon dioxide and sexed under the binocular microscope. Flies whose wings had not yet unfurled (less than two hours old) were discarded. In such flies carbon dioxide anaesthetisation results in a rapid diffusion of carbon dioxide into the air bubble in the gut that is used in the process of straightening and spreading the wings (DAVID and HUOT 1973). This may rupture the gut and result in subsequent death. However, SHARP (1983) found that, after this early sensitive period, short exposure to carbon dioxide, such as required for sexing, separating or weighing flies, did not effect the longevity or progeny production of the females he tested. The sexes were stored separately in vials containing the standard drosophila medium seeded with active Baker's yeast. Females were stored in batches of five to a vial but males were stored individually to avoid any possible inhibitory effects of

previous male:male interactions on courtship activity (MAYNARD SMITH 1956). Males became sexually active when one day-old. The first females became receptive when two days old and by five days of age all females were receptive.

1.2.3. Measuring body size.

The two indices of body size used in this thesis were body weight and wing length measured from the anterior cross-vein along the third longitudinal vein to the tip of the wing. Weight and this measure of wing length are highly correlated in laboratory populations of D.subobscura (CHARLESWORTH 1978).

Lightly anaesthetised individuals were weighed on a Cahn electrobalance. A wing was removed from the fly before being measured under the 50x magnification of a binocular microscope using an eyepiece graticule. The easiest way of holding the wing for measurement was to pick it up using a piece of clear sticky tape and then to stick it onto a piece of white paper. This also meant that each wing could be filed and the size measured again at a later date if required. When a measure of body size was required before an experiment the flies were weighed the preceding evening. Wing length was measured when the flies were no longer required.

1.2.4. Courtship observation.

Courtship was observed in the constant temperature room in which the stocks were housed. Because the courtship activity of laboratory stocks of Drosophila varies through the day (e.g. EWING

1963) all courtship observation was conducted within three hours of lights-on.

1.2.4.1. The observation cells.

To observe courtship under the binocular microscope the flies were introduced into a $1cm^3$ perspex cell. The cell had a muslin floor and a small hole in the side, with a removable stopper, through which the flies could be introduced. The introduction was achieved without anaesthetising the flies by using an 'introducer' (see DONEGAN 1984 for description). The cell was illuminated by a high intensity lamp with heat filter and was observed from above.

The main cell used for observation was simply a 7.5cm by 2.5cm glass vial with a layer of damp cotton-wool at the bottom. The cotton-wool ensured that the air within the vial remained humid. The flies, first the female and then the male, were aspirated into the vial using a pooter. A foam bung was then pushed down to a height of 1.5cm above the surface of the cotton-wool and the flies were observed through a x2 magnifying lens.

A third type of cell was used for photographing courtship. This cell was simply a perspex ring with an internal diameter of 1cm, a depth of 0.2cm and with several muslin-covered air-holes. The ring was placed upon a clean glass slide, the flies were introduced through a hole in a cover resting on the cell and then this cover was carefully replaced with a clean glass cover-slip. Various combinations of transmitted light and light incident from a high intensity lamp with heat filter or from a ring flash were used. Although this small, shallow cell restricted the movement of courting

flies it did enable high magnification photographs to be taken without having to track the moving flies.

1.2.4.2. Recording courtship.

If only the courtship duration was being measured then the time between the first bout of orientation and copulation was recorded on a clock and several courtships could be observed simultaneously. For more detailed courtship recording a single pair was observed and the courtship recorded on an Apple II computer with remote keyboard, monitor and disc-drive (DEAG 1983a). The time at which a particular key on the remote keyboard was pressed was recorded on a program disc which was later read into a data file on the main EMAS computer. Using a 'keytime' programme (DEAG 1983b) the keypress-time data file could be analysed to give a basic behavioural analysis involving, for instance, latencies, frequencies, durations and rates per minute for the various behaviours. The behaviours recorded and analysed in this thesis are described in the next section.

1.3. GENERAL COURTSHIP DESCRIPTION.

The courtship behaviour of D.subobscura has been described by RENDEL (1945), MILANI (1951a and b, 1956), WEIDMANN (1951), SPIETH (1952), MAYNARD SMITH (1956), BROWN (1965) and PINSKER and DOSCHEK (1980) and their descriptions of a typical courtship sequence are summarised below.

When a male encounters a receptive female he approaches her from the side and enters the 'orientation' phase of courtship. Often at the start of this part of the courtship the male taps the female with his front legs. He may also extend his proboscis as he tracks the female maintaining his position facing her side. In contrast to many Drosophila species male D.subobscura do not sing (EWING and BENET-CLARK 1968), although they do make fast flicking movements with both wings. During orientation the male may also make 'rowing' motions with his middle legs by rotating them forward, upward and back. Eventually the male manoeuvres himself into a position in front of and facing the female. The female may then 'dance' by stepping rapidly from side-to-side and the male attempts to maintain his position at her head. Either during the dance or when the female stops the male raises his wings into the wing display. Typically this display involves raising the wings into a 'V' above the body and twisting them so that the upper surfaces face towards the female. At this stage the female may extend her proboscis and 'kiss' the male so that the labellar surface of her proboscis comes into contact with the labellar surface of the male's proboscis. The male then circles and attempts to mount. In successful copulation attempts the female stands still as the male circles rather than moving away (RENDEL

1945, MAYNARD SMITH 1956, BROWN 1965). The sequence may break down at any stage and female 'repelling' behaviours (SPIETH 1974, but see EWING 1982, DONEGAN 1984) involve the female turning, walking, running or flying away from the male, fending the male off with her legs, or lifting or curling her abdomen away from the male.

Briefly, then, the courtship appears to involve the male manoeuvering himself into a position in front of and facing the female where he can perform some or all of the various frontal display elements (the dance, wing display and kiss) that are a prelude to successful copulation attempts (BROWN 1965). The courtship behaviours I record in this thesis are orientation, frontal orientation, dance, wing display, kiss, circling, copulation, female move away, following and a category 'other' which includes all male 'non-courtship' behaviour. 'Frontal orientation' is simply defined as orientation in front of the female and 'orientation' therefore becomes orientation at the side of the female. 'Female move away' includes all the female 'repelling' behaviours but where more than one occur together in the courtship sequence, such as abdomen curling during or followed by running, then only one occurrence of 'female move away' is recorded. 'Following' includes the male walking towards a stationary female as well as following a moving female.

One other courtship behaviour was recorded; namely drop production by the male. Whilst observing courting pairs I noticed that some males appeared to be carrying a drop of fluid on their extended proboscis. In chapter 2 I describe the source of this drop, its fate during courtship and its influence on male courtship success.

Chapter 2.

COURTSHIP FEEDING.

2.1. GENERAL INTRODUCTION.

Feeding of the female by the male before, during or after copulation has evolved independently a number of times in insects. THORNHILL (1976a) has recognised three categories of food: the male himself, glandular secretions by the male and food collected or captured by the male. In this introduction I review the various examples of courtship feeding in insects within THORNHILL's (1976a) framework.

2.1.1. Cannibalism of the male.

In the Mantidae (Dictyoptera) females have been reported to eat males during or after copulation (e.g. ROEDER 1935). In Mantis religiosa the male approaches the female stealthily and, when about a length away, very rapidly leaps onto the female (ROEDER, TOZIAN and WEINST 1960). Once mounted in the right position the male is rarely molested. However, if he lands badly or is intercepted the female begins to eat him, usually starting with the head. In male mantids a nerve centre in the sub-oesophageal ganglion exerts a continuous inhibitory action upon the endogenous efferent activity in the last abdominal ganglion responsible for copulatory movements. Decapitation of the male, therefore, releases continuous copulatory movements apparently of greater intensity than those of sexually excited intact males (ROEDER et al 1960). Males in this condition

can still copulate and go on attempting to do so for several days or until the female works her way down to the last abdominal segment. FABRE (1911) noted with regard to a copulating pair "... but he had no head, no neck, scarcely any thorax! The female, her head turned over her shoulder, was peacefully browsing on the remains of her lover! And the masculine remnant, firmly anchored, continued its duty!".

THORNHILL (1976a) cites another account by FABRE (1911) concerning Carabus auratus (Coleoptera) as an example of the female devouring the male after copulation. FABRE kept 20 males and 5 females in a cage and observed that the females eviscerated the males at the end of the breeding season and consumed their abdominal contents. There was no such behaviour earlier in the summer even though copulation was observed. FABRE remarks on the fact that the males made no attempt to fight back and implies that, having mated the females and fulfilled their reproductive function, they allow themselves to be eaten. However, as he did not observe courtship or copulation at the time of evisceration, this cannot be considered an example of paternal investment. In the same account he describes similar behaviour by female crickets (Orthoptera).

Females of many of the insectivorous ceratopogonid midges eat the male during copulation. STAEGER (1839) first observed this behaviour in Mallochochelea nitida and GOETGHEBUER (1914,1921) found female Johannsenomyia nitida and J.inermis with male terminalia attached to the female abdomen and suggested that the males had been eaten during mating. EDWARDS (1920) reported that in Serromyia femorata the pair copulate with their mouthparts locked together and when copulation ends the male's body fluids are apparently sucked out

through his mouth by the female. DOWNES (1978) carried out a more detailed study and found that females pierced the male's head with their stylets and injected proteolytic saliva. As in mantids, this removes inhibition of male copulatory movements. After about 30 minutes the male is reduced to an empty, dry cuticular shell. When the female moves off the cuticle breaks at the membrane between the 6^{th} and 7^{th} abdominal segments leaving the male's torn-off genitalia attached. DOWNES considers this behaviour to be common in the Heteromyiini , Sphaeromiini and Palpomyiinae and there are isolated records in the Ceratopogoninae and Stilobezziini.

2.1.2. Glandular products.

2.1.2.1. The accessory glands.

The accessory glands are the glands associated with the genital canal and whose products are secreted into the genital ducts and affect the reproductive success of males and females in a variety of ways. With the exception of the Apterygota and the Palaeoptera they are found throughout the insects and in males have developed as outpockets of the vas deferens or seminal vesicles (mesadenia), as outpockets of the ejaculatory duct (ectadenia) or as glandular areas incorporated into the wall of the genital ducts (see LEOPOLD 1976 for review). In the species analysed the secretions typically contain a mixture of proteins, carbohydrates, occasionally lipids and, in the blattids, uric acid. Different components of the secretion influence reproductive success in a variety of ways and have been implicated in; sperm transfer, activation and storage; the prevention of female

remating through mating plugs, spermatophragma, the insemination reaction and the alteration of female behaviour; and the stimulation of egg production and oviposition. Nutrients provided by the male may also be used by the female in oogenesis and the production of fat body and hence may reduce the time and possible risk involved in collecting food (see chapter 6 for further discussion of accessory gland function).

The amount of material transferred to the female during copulation is highly variable and in some species may represent a weight loss by the male of up to 40 percent (GWYNNE 1983). A number of orthopteran (e.g. ALEXANDER and OTTE 1967, GWYNNE 1982) and neuropteran (WITHYCOMBE 1922) females have been observed to eat the spermatophore after mating, and a number of dictyopterans, orthopterans, a trichopteran and a coleopteran either partially or totally digest and absorb the spermatophore within the reproductive tract (see ENGLEMAN 1970 for review). In the cockroach, Xestoblatta hamata , females feed directly from the male's accessory glands after copulation (SCHAL and BELL 1982). In a number of lepidopteran (GOSS 1977, BOGGS and GILBERT 1979, ENGEBRETSON and MASON 1980, BOGGS 1981, BOGGS and WATT 1981), orthopteran (e.g.FRIEDEL and GILLOT 1977, BOWEN,CODD and GWYNNE 1984), dictyopteran (MULLINS and KEIL 1980, SCHAL and BELL 1982), and dipteran (MARKOW and ANKNEY 1984, PARTRIDGE personal communication) species, components of radiolabelled male ejaculate have been shown to be involved in oogenesis.

2.1.2.2. The dorsal glands.

Glands found in various parts of the male body which specifically secrete products on which the female feeds before, during or after copulation have been described in three insect orders; Orthoptera, Dictyoptera and Coleoptera.

HANCOCK (1905) first described a dorsal gland in males of the cricket Oecanthus fasciatus . The gland secretes its product into a pit on the metanotum. Once a female has located a calling male she may climb onto his back, the male raises his tegmina revealing the pit and the female then begins to feed on the secretion. After about half an hour (FULTON 1915) copulation occurs and the spermatophore is transferred. The female may carry on feeding for up to 65 minutes before dismounting (WALKER and GURNEY 1967). The female then removes the spermatophore and eats that. HOHORST (1936) found that if the female was removed immediately after copulation she proceeded to consume the spermatophore and suggested that the post-copulatory feeding may be important in preventing the female from eating the spermatophore before it is empty of sperm.

Since HANCOCK's original description (HANCOCK 1905) a number of different types of dorsal gland have been described and this type of courtship feeding, with the female mounted dorsally on the male, appears to be widespread in the Gryllidae, Tettigoniidae and Gryllacrididae (see ALEXANDER and BROWN 1963 and ALEXANDER and OTTE 1967 for references). For instance, in a North American species of Nemobius females feed from a long glandular spine at the base of the hind tibia (FULTON 1931, MAYS 1971) and in Hapithus agitator the

female, after feeding briefly from a pair of small metanotal glands, eats the male's wings (BLATCHLEY 1891, ALEXANDER and BROWN 1963). Male Isophya acuminata have two rows of hairs on the first abdominal segment connected to glands (VON ENGLEHARDT 1915) and in Discoptila fragosoi BOLDYREV (1928a) observed females licking a thick fluid secreted on the underside of the tegmina during copulation. He also reported female Bradyporus multituberculatus biting on the oversized lip projecting backward from the pronotum until it bled, and then feeding on the blood whilst the male attached the spermatophore (BOLDYREV 1928b).

ALEXANDER and BROWN (1963) argue that copulation appeared in insects at about the same time that wings originated, and that most or all pterygote insect orders passed through a stage in which the copulatory act occurred with the female mounted on the male's back. Therefore, the male pterygote ancestor probably possessed dorsal female-attracting devices, and notal flaps (and ultimately wings) may well have evolved as guides for keeping the female in position, or for use in epigamic display, or as covers for dorsal glands or simply as something for the female to chew on.

A number of cockroach species have tergal glands on the abdominal segments. For instance, the gland in Supella supellectilium is located on the seventh abdominal tergite and consists of a pit bearing setae into which glandular products are secreted. In Blatella germanica the glands open onto the seventh and eighth abdominal tergites and there are no associated setae. Males raise the wings and tegmina during courtship allowing the female to feed on the secretions prior to copulation (ROTH 1952).

In the Malacodermata (Coleoptera) glandular structures were first recorded by EVERS (1948,1956) on the elytral tips of Axinotarsus pulicarius and the frons of Malachius bipustulatus and later in other species within the group on the antennae, tibiae, palpae or thorax (MATTHES 1959,1960,1962). During courtship MATTHES (1962) observed females biting into the elytral or head glands and feeding. After repeated offerings the male mounted and copulation occurred. MATTHES (1962) described similar feeding behaviour during coupling in some chrysomelid species.

2.1.2.3. The salivary glands.

Perhaps the best known example of the use of salivary secretions during insect courtship is in the Panorpidae (Mecoptera). Males within this family attach salivary secretions to leaves and then attract females using a pheromone. The male copulates whilst the female feeds on the proteinaceous salivary pillar. During copulation the male adds to the pillar and it usually takes the female several hours to devour it (BYERS and THORNHILL 1983 and references therein).

In a number of tephritid species (Diptera) males produce a mound of foam from the proboscis as part of their courtship behaviour, e.g. Afrocneros mundus (OLDROYD 1964) and Eutreta species (STOLTZUS and FOOTE 1965). Male Rioxa pornia attract females to mating sites using odour emanating from inflated pleural regions of the abdomen. When contact is established males take several minutes to secrete a small mound of foam. Once the female starts to eat it the male mounts and copulates. The female soon finishes the mound

and the male then dismounts and takes up to 15 minutes to secrete a much larger one. The female takes considerably longer to consume this second mound allowing the male to complete copulation (PRITCHARD 1967). Directly after copulation male and female Spathulina tristis indulge in prolonged 'kissing' behaviour during which material from the male's enlarged salivary glands is transferred from his proboscis onto the female's proboscis and thence into her crop (FREIDBERG 1982).

In another dipteran, Rivellia boscii , the male mounts the female and at intervals of 3 or 4 minutes during copulation a droplet of colourless fluid appears on his proboscis. The male then lurches forward and the female takes the drop with her proboscis and eats it (PIERSOL 1907). This may be repeated many times before separation and PIERSOL assumes that the fluid originates in the salivary glands. If the female does not take the drop the male sucks it back in.

A similar behaviour has been reported by WHEELER (1924) for Cardiacephala myrmex . Males step from side-to-side in front of the female, swaying the abdomen towards her and striking it down on the leaf. The female eventually turns up the tip of her abdomen and bends her head back. The male mounts and is able to place a drop of food from his proboscis onto the female proboscis whilst achieving intromission. The female then staightens her body and copulation ensues for about 15 minutes. During this period the male frequently extends his proboscis and deposits a drop of fluid onto the female's eye (he cannot reach her proboscis). She wipes the drop off with her fore-legs and then draws them over her proboscis. This may be repeated " a dozen or more " times. WHEELER concludes that the drop is regurgitated from the crop and that copulation is probably ended

when the female perceives the male's supply of liquid food to be exhausted and kicks him off. Neither he nor PIERSOL (see above) provide evidence supporting their conclusions with regard to the origin of the drop. In both cases the source is probably the crop and therefore these two examples should be included in the 'collected food' category. However, for want of further information they are both included in this section in line with THORNHILL's 1976a classification.

2.1.3. Collected food.

2.1.3.1. Prey.

The presentation of a prey arthropod to the female before copulation occurs in three insect families; the Panorpidae and Bittacidae (Mecoptera) and the Empididae (Diptera).

THORNHILL has undertaken detailed studies on a number of mecopteran species (see BYERS and THORNHILL 1983 for references). Having found a dead arthropod, male Panorpa feed briefly, then stand by it and disperse a sex pheromone and attempt to mate with any female that may be attracted to feed. Within the bittacids, males once again acquire a prey arthropod and feed upon it briefly. If the prey is unsuitable (perhaps unpalatable or too small) the male discards it and searches for another. Otherwise, he undertakes a series of short flights holding the prey between his hind-legs. Between flights he hangs by his fore-legs from a leaf or twig and disperses pheromone. A female may be attracted and whilst she feeds upon the dead arthropod held by the male, he copulates. At the end

of copulation they struggle for what is left of the offering, in most cases the male retaining possession. Once again he feeds and either discards the arthropod and searches for another, or retains it for another mating attempt. Large prey arthropods may be used in up to 3 successive copulations.

Within the Empididae males advertise and present their prey offerings in a variety of ways. The "balloons" carried by males were first described by OSTEN-SACKEN (1877) and were later found to be used, not as aeronautical surf-boards on which to cavort among the sunbeams as MIK (1888) suggested, but as wrappings for prey items or as courtship offerings in their own right. KESSEL (1955) reviewed the literature and suggested an evolutionary sequence, stages of which can be seen in various extant species. His first stage was represented by species in which the males carry no offering and are occasionally eaten themselves. The next involved species in which the male first catches a prey item, then searches for a female, presents it to her and copulates as she eats it. In other species the males fly in swarms advertising their prey offerings. The female approaches and enters the swarm, and feeding on the prey item and copulation with one of the males takes place in flight or after landing on nearby vegetation. In the fourth stage, the prey is pasted with shiny, viscid globules or silken threads probably produced by the anal glands, and the fifth includes species in which this wrapping is elaborated into the "glistening white balloon" from which the flies get their common name. The male and female couple in flight, the balloon is passed to the female and whilst she lands and feeds on the prey within, the male copulates. In later stages the prey becomes smaller and more dried-up and a number of species

construct balloons containing inanimate objects or no prey at all. The balloon is still transferred to the female and she manipulates it during copulation. Perhaps a final stage in the sequence is reflected by a number of species in which the males carry nothing at all but simply fly around with their unusually large, pennate legs hanging down (KESSEL and KESSEL 1961).

There is no evidence that males without gifts occasionally fall victim to females and DOWNES (1970) and ALCOCK (1973) question KESSEL's sequence. They argue that the initial importance of the prey offering to the male and female was probably its nutritional value to female fecundity and survival. In some arctic species the only food females obtain are the courtship offerings of males (DOWNES 1970).

2.1.3.2. Plant food.

There are two examples of males presenting females with plant food during courtship, one in the hemipteran Stilbocoris natalensis and one in wasps of the sub-family Thynninae (Hymenoptera).

Male S.natalensis depulp a dried fig using their front legs and then carry the seed around firmly fixed by their stylets to the tip of the rostrum (CARAYON 1964). On finding a female they present the seed during an elaborate courtship, finally removing it from the rostrum and injecting it in a number of places with saliva. CARAYON states that this serves to digest part of the seed and make it more attractive to the female. Eventually the female approaches the male and plunges her buccal stylets into the seed. When she has a firm hold the male slowly swings his body up against hers and, on

achieving intromission, relinquishes the seed. The female carries on feeding and copulation may last from 3 to 4 hours.

Female wasps of the sub-family Thynninae burrow to locate hosts. Their legs are modified to serve this function and they have no wings. As they feed on nectar and honey-dew, they are totally dependent on males to provide food and this the males achieve in a variety of ways (GIVEN 1953 and ALCOCK 1981a and b). Usually the males transport the female in coupled flight from the oviposition site to feeding sites. Associated with this, females have strengthened genital segments and mouthparts modified for gripping the male. Males have modified genital claspers and organs, and tend to be much larger than the females. Once at the feeding site females of several species feed directly, but more often the male feeds for a few minutes before moving off to a secluded spot to feed the female. The male regurgitates a drop of food which in some species is held using specialised mouthparts whilst the female feeds on it. In other species the male deposits the drop on the tip of his abdomen where it is accessible to the female in the typical flight position; in other species the drop is deposited on the substrate. Alternatively, males of a number of species have a "concave excavation" on the underside of the head, formed from the labiomaxillary fossa, in which they can carry a large food bolus. In species where this is well-developed females are rarely carried to feeding sites and the males transport all the food to them. Finally, in one south Australian species the male excretes a drop of viscous fluid from the tip of his abdomen and the female consumes this drop from her flight position.

2.1.4. The evolution of paternal investment in insects.

TRIVERS (1972) suggested that female choice may play a role in selecting for increased male parental investment, where parental investment is defined as "any investment by the parent in an individual offspring that increases the offspring's chance of surviving (and hence reproductive success) at the cost of the parent's ability to invest in other offspring". THORNHILL takes up this theme and argues that nuptial feeding in bittacids has probably evolved by female preference for greater male nutritional investment (THORNHILL 1974,1980 and 1983) and suggests that female choice for greater male parental investment may well have been the selective context for the evolution of all types of male investment patterns in insects (THORNHILL 1976a). If this is true of nuptial feeding then we might expect:

1) the nutriment provided by the male contributes to the number or survival of the offspring produced by the female, either directly or indirectly by reducing the energy she might spend or risks that might be taken searching for food, at some cost to the male;

2) females discriminate between males on the basis of the food they provide (or some correlate) prior to, during (length of copulation) or after (remating) copulation.

The contribution of male-derived nutrients to offspring number or survival will be discussed in the next chapter. The influence of the offering on female mating behaviour has only been rigorously examined by THORNHILL (1976b). He showed that female Hylobittacus

apicalis discriminated against males with small prey in two ways: they either will not mate at all, or if they do only copulate briefly (few sperm are transferred) and remate readily.

In various Panorpa species males compete for dead arthropods and, if none are available, secrete salivary masses. THORNHILL (1979) showed that females mated more readily with males that guarded larger arthropods, more readily with males guarding arthropods than those with salivary masses and, finally, more readily with males guarding salivary masses than those with nothing at all. However, in this example he allowed males to compete for access to the food items and, therefore, cannot be sure that the females were choosing males on the basis of their food item rather than another correlate of aggression. Similarly, the males who did not secrete salivary masses may have been deficient in some other way. If there is a good correlation between the trait the female is using (e.g.body size or courtship vigour) and the quantity or quality of food the male is offering this distinction may be trivial, but it is important to recognise it. For instance, GWYNNE (1982) showed that heavier male Conocephalus nigropleurum have larger spermatophores and females given a choice between two singing males moved towards and mated with the heavier male. Females move towards the louder song of larger males (GWYNNE 1982) and GWYNNE's (1984) statement that "females prefer to mate with males able to supply larger spermatophores ..." is misleading on two counts. Firstly, females move towards recordings of several males singing in preference to single males (MORRIS, KERR and GWYNNE 1976) and so, in the field, may approach a group of small males rather than a solitary large male. Secondly, large males will have small spermatophores if they have recently

mated (FEAVER 1977). Apart from being less efficient than choosing males directly on the basis of their nutritional offering, female choice for male body size could not be the selective context favouring an increase in male nutritional investment from, say, 2% to 40% of body weight (this is the range of spermatophore sizes produced by males of different katydid species (GWYNNE 1983)). To increase male nutritional investment, females would need to discriminate against males offering fewer nutrients. This might be achieved if the female remated when she was hungry and the first male's sperm were displaced. Selection might then favour males who provided more food at mating and increased the interval to the female's next mating. However, it may not always be possible for females to assess directly a male's nutritional contribution and selection will favour females that use phenotypic characters correlated with the value of a male's offering.

Other studies provide only anecdotal evidence. CARAYON (1964), for example, states that male S.natalensis rarely court without seeds and when they do are unsuccessful, but provides no data nor says whether these same males are successful with seeds. A similar situation exists within the extensive literature on courtship feeding in birds (reviewed by LACK 1940, ROYAMA 1966 and SMITH 1980). Although it has been suggested that the ability of a male to provide food during courtship may be important in determining whether pairs form (e.g.NISBET 1973) or break-up (e.g.TASKER and MILLS 1981), the importance of male food provisioning to pair formation and maintenance has not been tested.

In this chapter I identify the source of the drop produced by male D.subobscura during courtship and show that a male's ability to

provide food can be an important determinant of his courtship success.

2.2. THE ORIGIN AND FATE OF THE DROP.

2.2.1. Introduction.

The two likely sources of the drop observed during D.subobscura courtship are the salivary glands and the gut. In species in which salivary secretions are utilised during courtship the males often possess particularly well-developed salivary glands (e.g. FREIDBERG 1982 and BYERS and THORNHILL 1983 and references therein). In Drosophila these glands are relatively poorly-developed and there is no sexual dimorphism in their size. Therefore, the drop probably originates in the gut.

To establish the source of the drop and its fate during courtship, males were fed stained yeast cells before introduction to virgin females. The courtship was observed and then both sexes were dissected and scored for the presence of stain. Both fed and starved females were used to determine whether starved females took the drop more frequently than fed females and so indicate whether or not the drop may be of nutritional value to the female. In a separate experiment the behaviour of courting pairs was observed to provide detail on when the drop appeared and what happened to it during courtship.

2.2.2. Materials and methods.

2.2.2.1. Stained yeasts.

The sexes were isolated at eclosion and aged separately in food vials (see General methods). Males were stored individually and when 4 days old were transferred to vials containing the standard drosophila food medium, but with a drop of a solution of stained yeast cells added instead of the usual active Baker's yeast. This solution was prepared by gently heating active Baker's yeast cells in a solution of distilled water to about 60°C to deactivate the cells without rupturing them. The solution was centrifuged and the supernatant discarded. The dead yeast cells were then stained with crystal violet for 1 minute, washed in distilled water, fixed in Gram's iodide for 1 minute and, finally, washed twice more in distilled water. At each step the solution was centrifuged and the supernatant discarded. The stained cells were then given to the males in a distilled water suspension.

The following day individual males were introduced to either a "fed" or a "starved" 5 day old virgin female in a 1 cm^3 perspex cell (see General methods) and the courtship observed. "Fed" females were left in standard vials with active Baker's yeast for the full 5 days. The "starved" females, in contrast, were stored in vials containing only damp cotton-wool once they were 3 days old.

After courtship both sexes were dissected in saline and examined. Initially, they were examined under the x40 objective of a monocular microscope to look for individual stained yeast cells. However, many cells had been digested and the stain released and,

although it was possible to identify stain particles, it was very much easier to see the stain under a low power microscope or with the naked eye. The purple colouring of stained areas was readily visible, even in the fed females where the amount of stained food ingested was often a small proportion of the total crop volume. In these cases, the recently ingested stained yeast was localised around the opening of the duct into the crop and little mixing had occurred.

In a control experiment 20 females were fed stained yeast cells when 4 days old and the next day courted with 5 day old virgin males given the normal active Baker's yeast. After courtship both sexes were dissected to see if any food had passed from the female to the male.

2.2.2.2. Courtship observation.

Single virgin 5 day old males were introduced to a 5 day old virgin female in a 1 cm^3 perspex cell. The courtship was observed down a binocular microscope and recorded on the Apple keyboard (see General methods).

2.2.3. Results.

2.2.3.1. Stained yeasts.

In the courtships with both the fed and starved females the median number of drops produced by each male was one and in only three courtships did the males fail to produce a drop (figure 2.1). The males produced a total of 49 drops in the 40 courtships with the

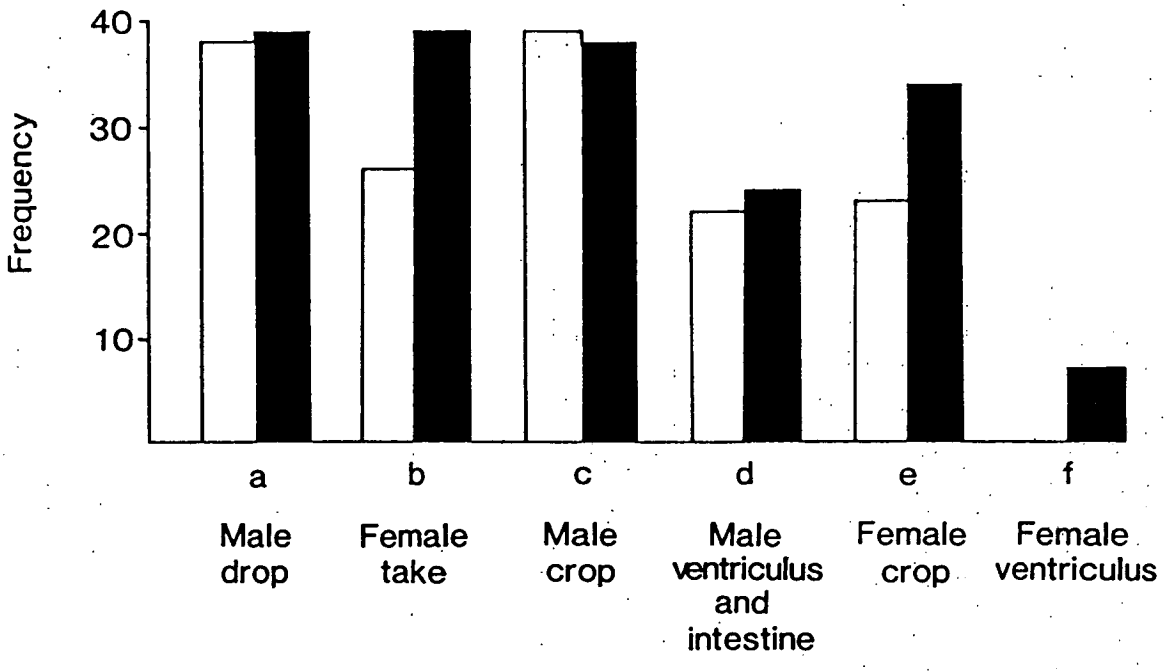

Figure 2.1. The frequency of food transfer with fed ☐ and starved ■ females. a) shows the number of courtships in which the male produced a drop. b) shows the number of courtships in which the female was seen to take the drop. c) to f) shows the regions of the fly where stain was scored upon dissection. N for each group = 40.

starved females (range 0 to 3 drops per courtship) and a total of 60 drops in the 40 courtships with the fed females (range 0 to 4 drops per courtship). The difference in total number of drops produced is not significant (χ^2=0.55, p>0.1).

Significantly more starved females than fed females were observed to take at least one drop during courtship (χ^2=11.8, p<0.001). The starved females took 45 of the 49 drops produced and the fed females took 31 of the 60 drops produced. The starved females took a significantly higher proportion of the drops available (χ^2=18.76, p<0.001) indicating that the drop may have nutritional value.

Stain was found in the male crop, ventriculus and intestine, and in the female crop and, in a few starved females, the ventriculus. Stain was more often recorded from the starved females' crops than from the fed females' crops (χ^2=11.8, p<0.001). No stain was found in the salivary glands, nor recorded from the 20 unstained males courted with the stained females in the control experiment.

The highly elastic crop is used to store collected food (figure 2.2) and periodically portions are expelled back into the oesophagus and through the cardia into the ventriculus for digestion. Once food is through the cardia, the stomadeal valve and cardiac sphincter prevent it returning from the mid-gut. Therefore, the data suggest that during coutship males are regurgitating a part of their crop contents which females are consuming. The food passes into the female crop or straight into the ventriculus for digestion in the case of some starved females.

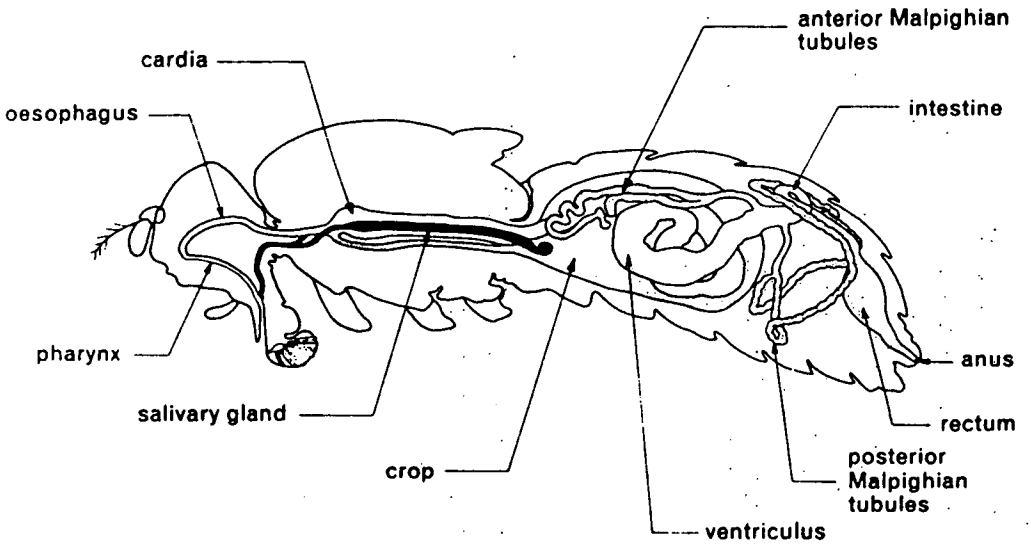

Figure 2.2. The diagram of the digestive system of Drosophila melanogaster is taken from Demerec (1950).

2.2.3.2. Courtship observation.

In this experiment the courtships of 39 pairs of well-fed virgin 5 day-old males and females were recorded. Only one pair failed to mate within the 15 minute observation period. A total of 51 drops were produced by the males in 30 of the 39 courtships, and of these 33 were taken. The median number of drops produced in each courtship was one as was the median number taken. The greatest number of drops produced by a single male was 5 during a 4 minute 2 seconds courtship. 3 of these were taken by the female and 2 taken back in by the male.

The sequence diagram depicting the frequencies of behavioural transitions (figure 2.3) shows that the drop was produced most frequently during frontal orientation. Once it appeared it was carried on the male's extended proboscis (figure 2.4a) until the female either took it (figure 2.4b) or decamped and the male sucked it back in, or it was lost on the wall of the cell (2 occasions). Just before the drop appeared on the male's proboscis contractions could be seen ventrally in the anterior region of his abdomen.

The female took the drop with her proboscis from the male's proboscis most frequently after the wing display or, if she took the drop earlier in the courtship sequence, the male performed the wing display as she took it. After the wing display or the female taking the drop, the male circled and attempted to mount (figure 2.3). On 31 of the 33 occasions that males performed the dance or wing display with the drop the female took it. On 1 occasion she started to dance again without taking it, on the other the male circled, mounted and

Figure 2.3. The sequence diagram depicts the frequency of behavioural transitions during courtship. The widths of the arrows are proportional to the frequency of the transitions. A width of 1mm represents 10 transitions. Only frequencies greater than 2 are drawn.

The behaviours shown are other (OT), follow (F), female move away (X), orientation (O), frontal orientation (OF), dance (DA), wing display (WD), orientation with drop (ODR), frontal orientation with drop (OFDR), dance with drop (DADR), wing display with drop (WDDR), kiss (K), circle (C) and copulation (COP). 39 courtships were observed.

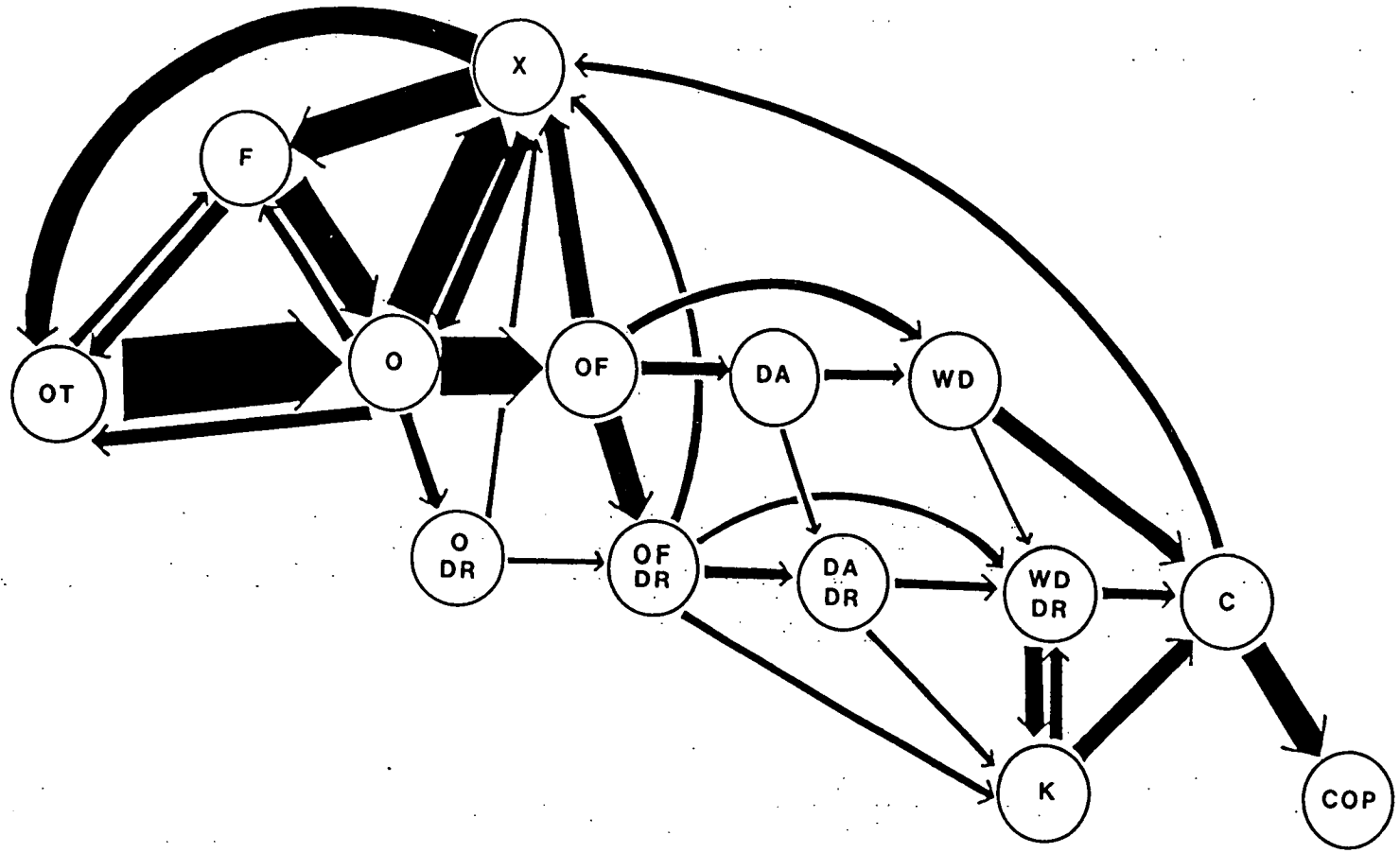
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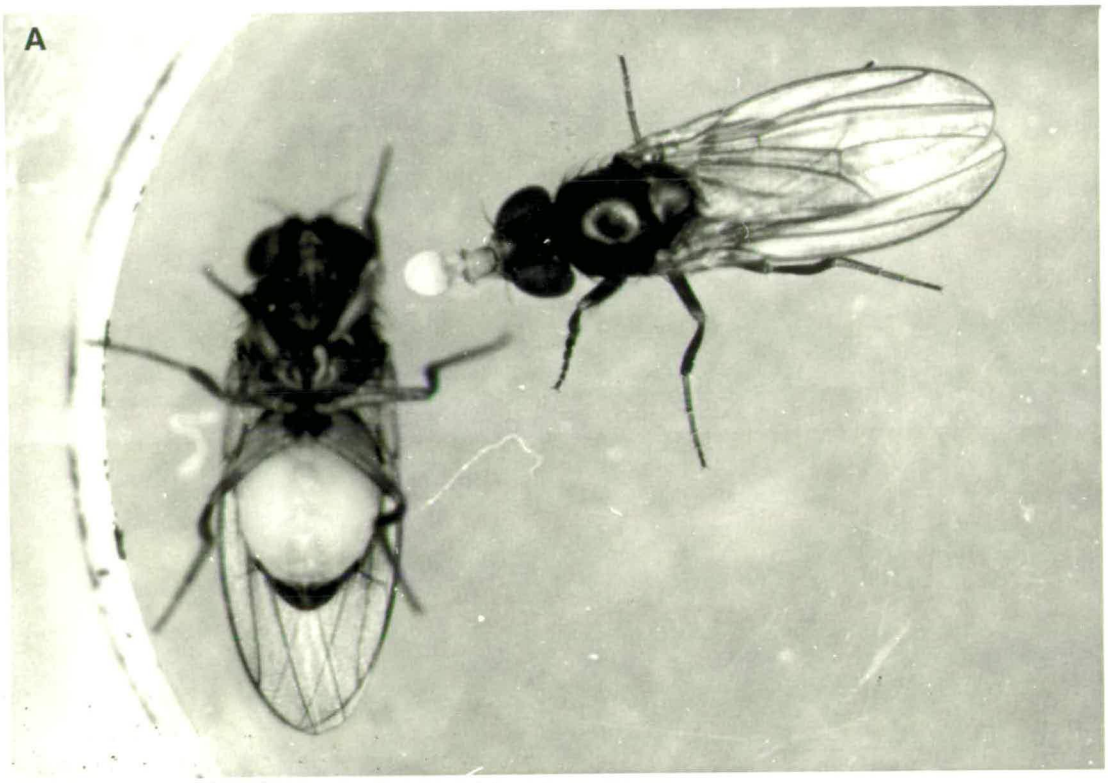
Figure 2.4. A) A male is carrying a drop on his extended proboscis during orientation. The female is standing upside down on the underside of the roof of the shallow cell. B) A few seconds later the female is on the floor of the cell and is taking the drop from the male. Part of the drop can still be observed on the end of the male's proboscis just in front of the female's antennae on the photograph. The drop is being held below the female's head where she can easily take it with her proboscis. Both photographs are printed to a 16x magnification.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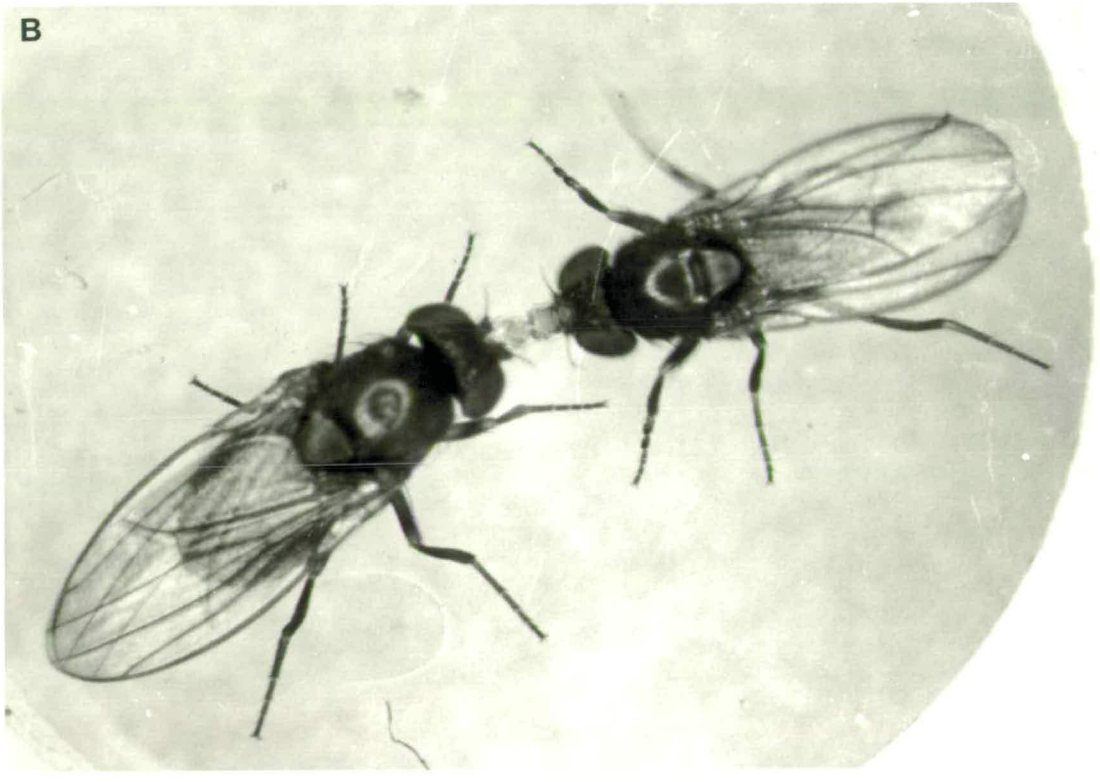

B

achieved intromission before sucking the drop back in.

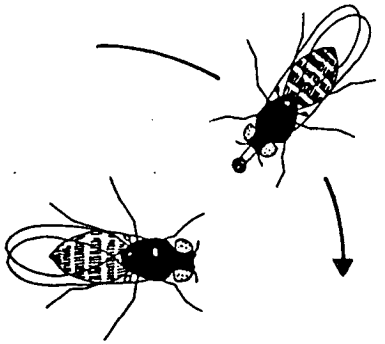
Usually, once the proboscises had touched, no more was seen of the drop. However, it was occasionally still visible on the female's proboscis whilst the male was circling. The handling time for some of these drops was several seconds and the male was copulating before the female had ingested it. Whilst manipulating the drop on her proboscis the female stood still whether she accepted the male or rejected him by kicking. The length of time required for the manipulation may have depended on the viscosity or size of the drop.

In summary, males often regurgitated a part of their crop contents during courtship and carried it on their extended proboscis until it was taken by the female or she decamped. The female took the drop with her proboscis usually after the dance and wing display and the male then circled and attempted to mount (figure 2.5).

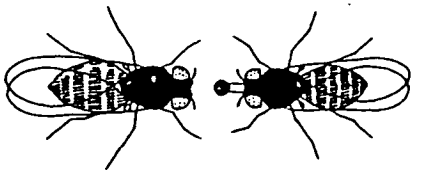
On the 33 occasions that the female took the drop and the male circled, only 25 resulted in copulation. How does this compare with copulation attempts when no drop was produced? The median duration of the 30 courtships in which a drop was produced was 57.3 seconds (range 9.6 to 242.7 seconds). That of the 9 courtships in which no drop was produced was 109.8 seconds (range 30.6 to 233.2 seconds). The difference between the courtship times of the two groups is not quite significant ($W=511$, $p=0.064$). The sample size is small and there is considerable overlap. In any case, the comparison throws no light on the importance of the drop *per se* in courtship. It may be that males who do not produce a drop are deficient in some other aspect of courtship or perform less vigorously.

Table 2.1 compares the number of times a female moved away from a particular male behaviour with and without the drop. There is no

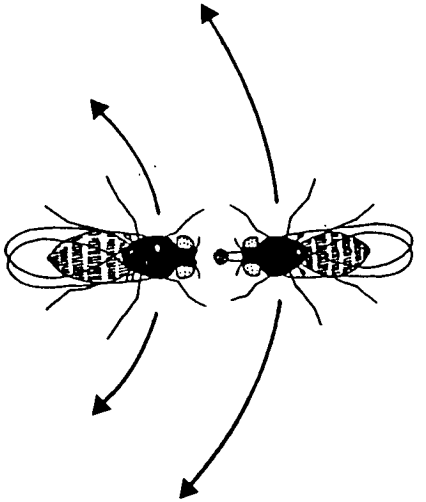
Figure 2.5. The diagram illustrates a typical courtship sequence involving the drop. The male is on the right except in figure 6 where he is shown circling behind the female. The arrows indicate the direction of movement. The drop is represented by the small, stippled circle.



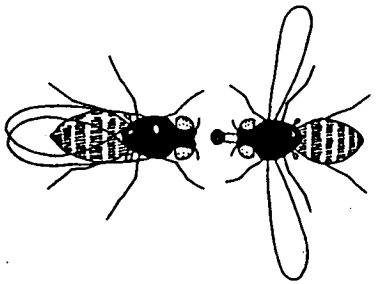
1. Proboscis orientation



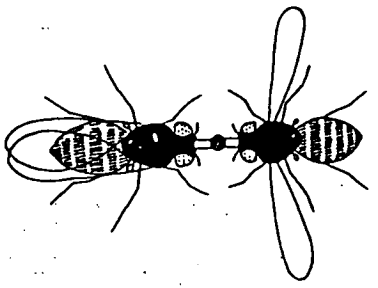
2. Head-to-head



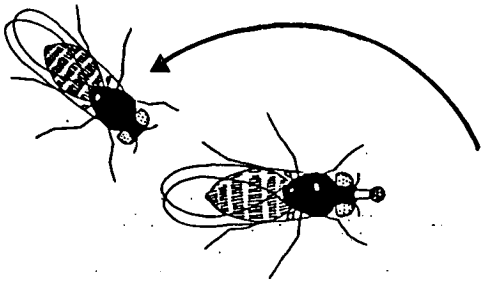
3. Dance



4. Wing display



5. Proboscis touching



6. Male circles

TABLE 2.1

Female "repelling" responses to male behaviours with and without the drop.
Data from 38 courtships.

	Male carrying drop			Male without drop		
		Followed by female repelling response			Followed by female repelling response	
Behaviour	Frequency	Frequency	%	Frequency	Frequency	%
Orientation	9	5	56	103	64	62
Frontal orientation	33	10	30	47	25	53
Dance	20	0	0	15	0	0
Wing display	33	0	0	16	0	0
Circle	33	8	24	16	3	19

difference between the success rate of male circling and attempted copulation after producing a drop compared to circling without producing one. There is an indication that females were more likely to move away from a male performing frontal orientation if he did not carry a drop, but the difference is not significant at the 95 percent level ($X^2=3.25$, $0.05<p<0.1$). Again, the comparison is not particularly useful as the female may be turning away for other reasons than the absence of a drop. For instance, it may be that the appearance of a drop simply reflected the length of time frontal orientation had proceeded, which, in turn, may be inversely correlated with the likelihood of a female moving away.

So, although its clear that males are regurgitating crop contents during courtship and females are consuming them, it is not clear whether this food presentation influences a male's mating success. Do males who regurgitate food and present it to the female increase their chances of mating? Experiments designed to answer this question are reported in the next two sections of this chapter.

2.2.4. Conclusions.

1) Males frequently regurgitated food stored in their crops when courting females.

2) The drop was carried on the extended proboscis until it was taken by the female or she decamped.

3) The female took the drop with her proboscis directly off the male's proboscis and he then circled and attempted to copulate.

4) Males could produce a single drop repeatedly or several drops successively. The number taken by a female during a courtship ranged

from 0 to 3 and copulation did not always follow the female taking the drop.

5) Starved females took a higher proportion of available drops suggesting that the drop is of nutritional importance to the female.

6) The food taken from the male appeared in the female´s crop or ventriculus.

2.3. THE INFLUENCE OF MALE ABILITY TO PRODUCE THE DROP ON COURTSHIP SUCCESS; SEALED MALES AND MALE SIZE.

2.3.1. Introduction.

Having shown that males are feeding females during courtship, the next step was to demonstrate the influence a male's ability to produce the drop had on his courtship success. If the drop does affect a male's chances of mating then males who are unable to produce one, but whose courtship is otherwise identical to normal males, should suffer a reduction in courtship success. In the first experiment reported in this section males were physically prevented from producing a drop by sealing their proboscises with glue. Two methods were used to check that the rest of the males' courtship was unaffected by the sealing:

1) the frequency of various courtship behaviours was scored for each courtship,

2) fed and starved females were used.

If the drop is only of immediate nutritional value and is not being used by the female as an indicator of further benefits (such as fertility - see chapter 6), then the courtship success of sealed males with fed females should be affected to a much lesser extent, if at all, than it is with the starved females.

Another reason for using starved females is that the ecological literature suggests that food may be diffuse in the wild (e.g. BEGON and SHORROCKS 1978). A comparison of crop sizes (chapter 3) shows that females collected from the field more closely resemble the laboratory starved females than they do the fed females in the amount

of food stored in their crops. Using starved females in the laboratory may more accurately reflect the importance of drop production to males in the wild.

An alternative approach to artificially sealing a male's proboscis is to compare males who vary in either the frequency with which they produce the drop or in the size of drop produced. The first comparison was approached experimentally using starved males and the results are reported in the next section of this chapter. To examine the influence of drop size, categories of males who produce drops of different sizes are required. One way to achieve this might be to alter the viscosity of the fluid fed to the males, but this would probably influence other drop characteristics such as odour. It seemed likely that small males would carry smaller drops than large males. This assumption was supported by casual observation and by photographically measuring the diameter of drops produced by males of different weights.

Having shown that small males carry smaller drops than large males, a further insight into the importance of drop production as part of a male's courtship repertoire was gained by comparing the effects of sealing the male proboscis and starving the female on the courtship success of large and small males.

2.3.2. Materials and methods.

2.3.2.1. Sealed males with fed and starved females.

The sexes were isolated at eclosion and aged separately. When they were 4 days old the males were anaesthetised and the proboscises

of half of the males were sealed. With the right degree of anaesthetisation they protruded their proboscises making it possible to apply a tiny drop of cyanoacrylate glue to the tip using a fine copper wire. The glue spread and dried on contact and, with the careful application of a small enough drop, the males were still able to retract and extend the proboscis. The time of application was important. Immediately after the sealing males spent some time grooming the proboscis and, if they were introduced to virgin females, this interfered with courtship. The males were unable to feed and so, if left too long before being used for courtship, moved around mopping at the substrate with their proboscises. If the sealing was done just before lights off and the males left overnight they appeared to court normally the following morning. The control males were returned to their vials without sealing but after a similar period of exposure to the carbon dioxide anaesthetic.

The technique for starving the females was the same as that described earlier in the chapter. The courtship recording, however, was much cruder. The Apple keyboard was unavailable when this experiment was done and, instead, observations were noted down whilst observing individual pairs in vials containing damp cotton-wool through a x2 magnifying lens. The pairs were observed for up to 10 minutes and the duration of courtship to the nearest 15 seconds and the number of bouts of orientation and frontal display were recorded. Although the technique was crude, no bias was introduced and there were some differences between treatments that were sufficiently marked to show up with the sample sizes involved (16 or 17 pairs per treatment). The data was gathered over 3 mornings observation in "sets". A set consisted of one pair from each treatment (except for

the final set consisting only of a pair from each of the sealed male treatments). The order in which the pairs were observed within each set was randomly assigned.

Whilst observing pairs during experiments reported in the first section, I noticed that the crops of females became greatly distended as the morning progressed and their spontaneous locomotor activity declined (CONNOLLY 1966). This meant that the "fed" females used early in the morning period were not the same as those used later on. Consequently, in this and future experiments, all fed females were transferred to damp cotton-wool vials at lights on and then used as required during the morning.

2.3.2.2. Male body size and drop size.

To relate drop size to body size, large and small males were photographed courting virgin females. The large and small males were collected from low and high density vials (General methods). A small, shallow cell (General methods) was used to eliminate the need to track the courting pair and minimise the amount of focusing required. The photographs were taken down a Wild binocular microscope using a combination of transmitted light and ring flash. Ilford XP1 film was used because it is both fast, and so less light incident on the cell is required, and fine-grained, allowing accurate measurement of the drop on enlarged prints. The film was taken at a x9 magnification, developed using the Ilford XP1 developing kit and printed to a X27 magnification for measurement.

The wings were removed from experimental males after courtship and measured under a binocular microscope. This not only provided a

check on the accuracy of the photographic technique, but also allowed the size of the drop as measured on the photograph to be translated into a real size using a conversion factor determined for each male from the wing measurements. Only photographs of males where the drop could be clearly seen were used. It proved difficult and time consuming to obtain good photographs of males carrying the drop mainly because:

1) The environment within the cell was dry. When the humidity was raised the flies courted more readily, but the condensation on the underside of the cover-slip prevented photographs from being taken.

2) There was little room for the male to manoeuvre himself into position in front of the female and courtship bouts were frequently interrupted by one or other sex bumping into the cell wall.

Consequently, the sample size is small but does provide objective data on a phenomenon that is clear when flies of different sizes are observed.

2.3.2.3. The importance of the drop during courtship to males of different sizes.

At eclosion a large number of males were collected from low and high density vials. When they were 4 days old they were weighed and the following morning a range of large and small males were observed courting 5 day old females in single pairs in damp cotton-wool vials for up to 30 minutes. There were 3 treatments:

1) Normal males with fed females.

2) Sealed males with fed females.

3) Normal males with starved females.

The males were sealed and the females starved as previously described. The number of pairs mating and the time from the first bout of orientation to copulation for the mating pairs was recorded. For each treatment the data set was made up of two mornings' observation.

2.3.3. Results.

2.3.3.1. Sealed males with fed and starved females.

Observation revealed that, although sealed males were unable to produce the drop, they were able to extend the proboscis and perform the other courtship behaviours. Table 2.2 shows that sealed males orientated, performed frontal display and mated as quickly as normal males when courting fed females. Apart from not producing a drop, they appear to court normally and fed females apparently do not discriminate between them and normal males. However, this was not the situation with the starved females. Although, once again, there was no difference between the sealed and normal males in the frequency with which they orientate towards the female, the sealed males took longer to mate than the normal males ($U = 1$, $p<0.02$) and fewer mated within the observation period (Fisher exact test, $p<0.001$). The poor mating success of the sealed males with starved females seemed to occur for two reasons: firstly, the sealed males were less likely than normal males to go on from orientation to perform frontal display with starved females ($U=11$, $p<0.002$); secondly, their frontal displays were more likely to be unsuccessful ($\chi^2=14.17$, $p<0.001$). When the courtship of sealed males with starved

Table 2.2. The table summarises the courtship success of normal and sealed males with both fed and starved females.

a) is the number of pairs observed. b) is the proportion of pairs mating within the ten minute observation period. c) is the duration of courtship. d) is the number of orientation bouts per minute. e) is the number of frontal display bouts per minute. A frontal display bout is defined as an occurrence of frontal orientation, dance, kiss or wing display where the behaviour, or any combination of these behaviours, is preceded and succeeded by some other behaviour. f) is the ratio of orientation to frontal display bouts. g) is the proportion of frontal displays that were followed by mating.

For c) to f) the median value is given with the range of values in parentheses. U is the Mann-Whitney statistic.

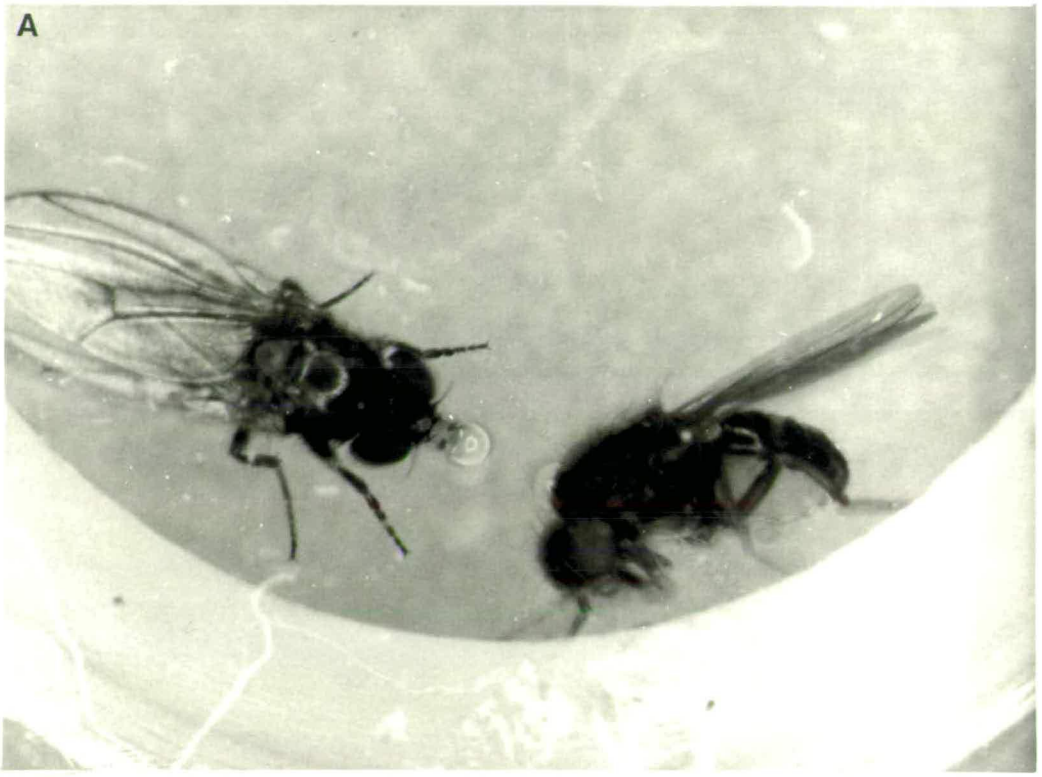
	Starved female		Fed female		
	1 Normal male	2 Sealed male	3 Normal male	4 Sealed male	Comparisons significant at $p<0.05$ (two-tailed)
a) N	16	17	16	17	
b) Pairs mating (%)	94	18	81	76	1 $x^2 = 16.31$, $p < 0.001$ 2 x 3 $x^2 = 10.92$, $p < 0.001$ 4 $x^2 = 9.56$, $p < 0.01$
c) Courtship duration (mins)	2.00 (0.30-10.00)	8.80 (7.00-10.00)	3.00 (1.00-10.00)	3.50 (0.30-10.00)	1 $U = 1$, $p < 0.02$ 2 x 3 $U = 3$, $p < 0.05$ 4 $U = 3$, $p < 0.05$
d) O bouts (min^{-1})	0.90 (0.13-2.00)	0.75 (0.38-1.56)	0.66 (0.30-1.50)	0.66 (0.18-2.75)	
e) FD bouts (min^{-1})	0.50 (1.33-4.00)	0.13 (0.00-0.66)	0.33 (0.00-1.20)	0.35 (0.00-2.00)	1 $U = 21$, $p < 0.002$ 2 x 3 $U = 63$, $p < 0.02$ 4 $U = 87$, $p < 0.05$
f) O:FD	1.0 (1.0-4.0)	5.0 (1.6-14.0)	2.0 (1.0-4.5)	2.5 (1.0-5.5)	2 x 1 $U = 11$, $p < 0.002$ 3 $U = 63$, $p < 0.02$ 1 x 4 $U = 67$, $p < 0.05$
g) No. of FD's successful (%)	65	11	59	72	1 $x^2 = 14.17$, $p < 0.001$ 2 x 3 $x^2 = 12.58$, $p < 0.001$ 4 $x^2 = 19.59$, $p < 0.001$

females was compared to that of normal males with fed females the same significant differences were found, and the same trend was apparent when comparing the sealed males' courtship with fed and starved females.

There was a non-significantly higher rate of orientation with starved females. If there was a real effect, it may have been partly due to the greater activity of starved females (CONNOLLY 1966) making it more likely that they would be encountered by males and courtship initiated and also courtship bouts terminated.

2.3.3.2. Male body size and drop size.

Photographs of a large and a small male carrying drops are shown in figure 2.6. Table 2.3 lists the measurements taken from the photographs obtained of six different courting males. The drop was assumed to be spherical and its volume estimated using the formula $4/3\pi r^3$. For two males where the whole wing was not visible on the print with the drop, wing length was measured on other prints of the same male. The Pearson correlation between the length of the wing measured under the microscope and taken from the print was 0.9929. Consequently, calculating the drop size by taking measurements from the print and using a conversion factor obtained from a comparison of the wing measurements should provide an accurate estimate of the size of the drop carried by each male. The relationship between the drop diameter obtained in this way and male wing length is shown in figure 2.7. The Pearson correlation of 0.9956 is significant ($p<0.01$) and so the diameter of the drop produced does increase with increasing male body size.


A


B

TABLE 2.3

The size of the drop produced by males with different wing lengths.

Wing length mm	Wing length measured from print mm	Drop diameter measured from print mm	Estimated true drop diameter mm	Estimated true drop volume mm^3
1.53	41	7.7	0.283	0.095
1.56	42	7.7	0.281	0.093
1.58	43	8.0	0.294	0.106
1.81	50	9.8	0.353	0.184
1.81	50	9.7	0.346	0.174
1.88	51	10.0	0.369	0.210

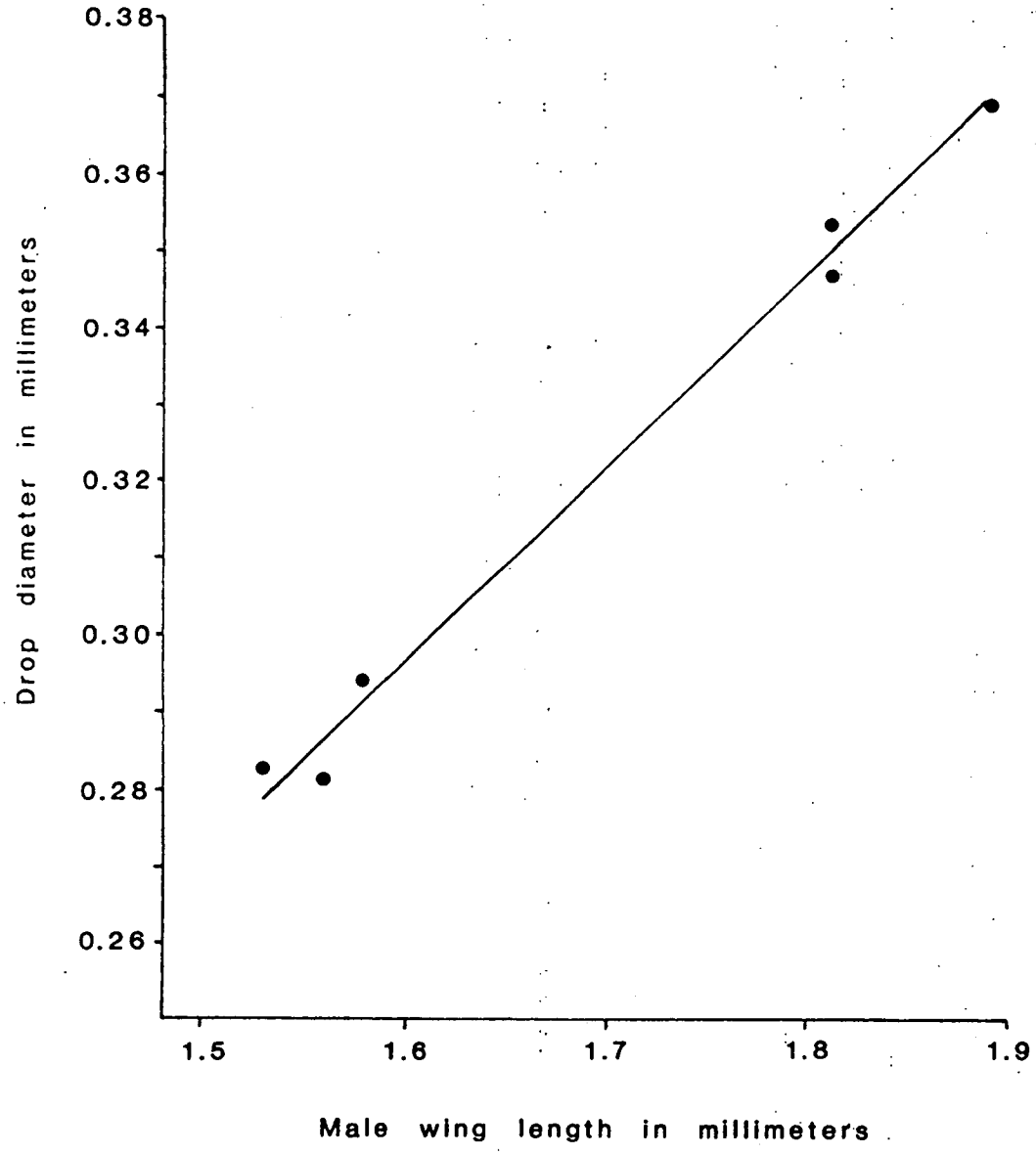


Figure 2.7. Drop diameter is plotted against male wing length. The equation for the fitted regression line is y = 0.25x - 0.11.

2.3.3.3. The importance of the drop during courtship to males of different sizes.

There were no significant differences between the two replicates for each treatment in the duration of courtships indicating that there was no day-to-day variation and so the data were pooled for each treatment (normal, $z=-0.45, p=0.326$; starved female, $z=-0.68, p=0.2483$; sealed male, $z=-0.61, p=0.2709$). The courtship durations are plotted against male weight for each of the 3 treatments in figures 2.8, 2.9 and 2.10. The time taken by normal males to mate with normal females shows considerable variation and bears no relationship to male body weight. However, there does appear to be an increase in the duration of courtship of large sealed males with normal females, and an increase in the courtship duration of small males with starved females.

If the courtship durations are divided into 2 categories, fast (<15 minutes) and slow (>15 minutes), and the males classified as large (>1 mg) or small (<1 mg), then a simple chi-squared test comparing the distributions of the points in the 4 quadrants can be carried out. When this was done it showed that there was no difference between the large and small males in the proportion of courtships that were fast with normal females ($\chi^2=0.06$, $p<0.9$). Large sealed males had a significantly higher proportion of slow courtships with normal females than small sealed males ($\chi^2=8.77$, $p<0.01$). This was due, not to an increase in the success of the small males ($\chi^2=0.08$, $p<0.8$), but to a decrease in the success of the large sealed males compared to normal males ($\chi^2=8.1$, $p<0.01$). Conversely, small males had a significantly higher proportion of slow

Figure 2.8 **Normal males with fed females.**

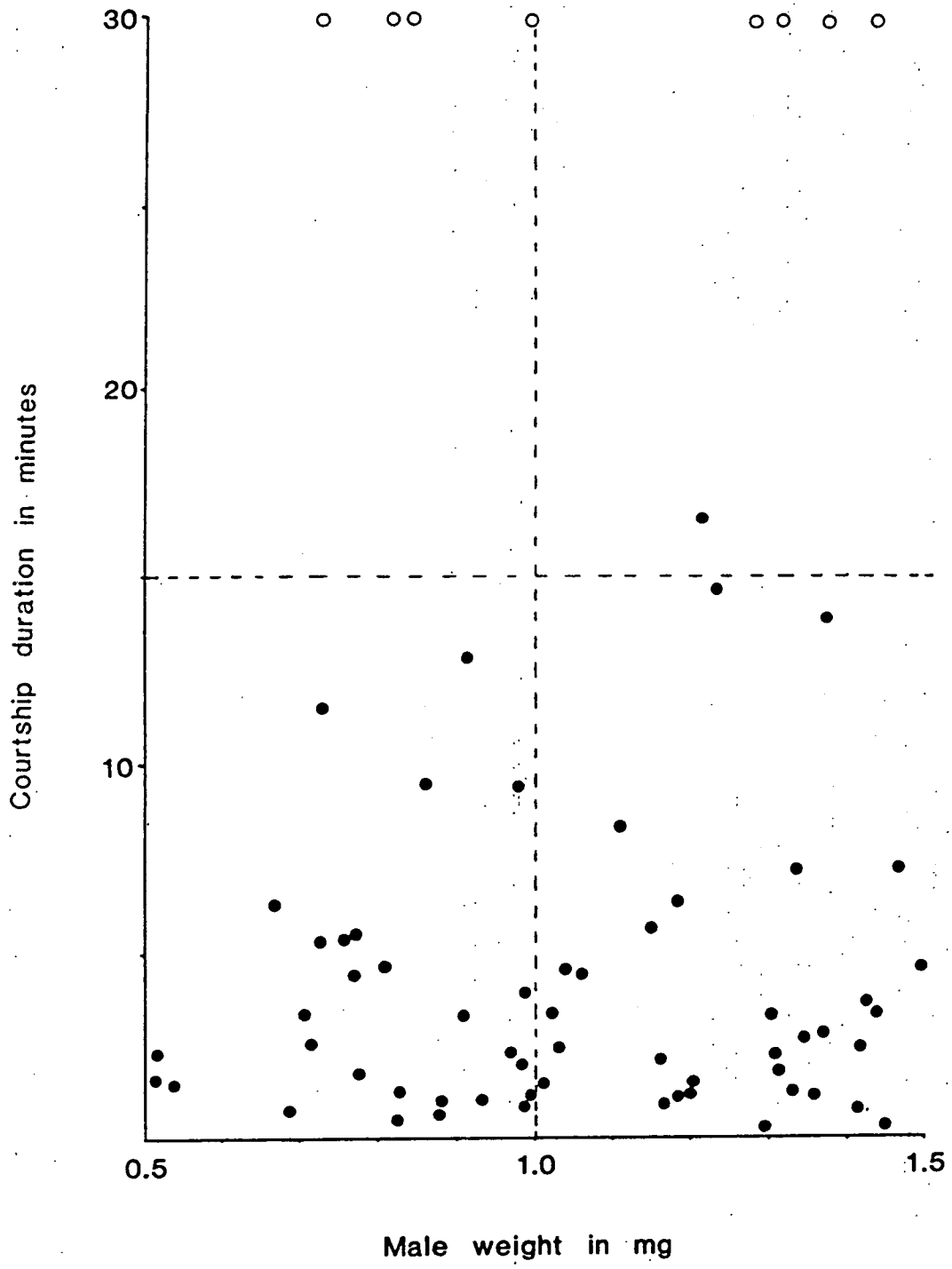


Figure 2.9 Normal males with starved females.

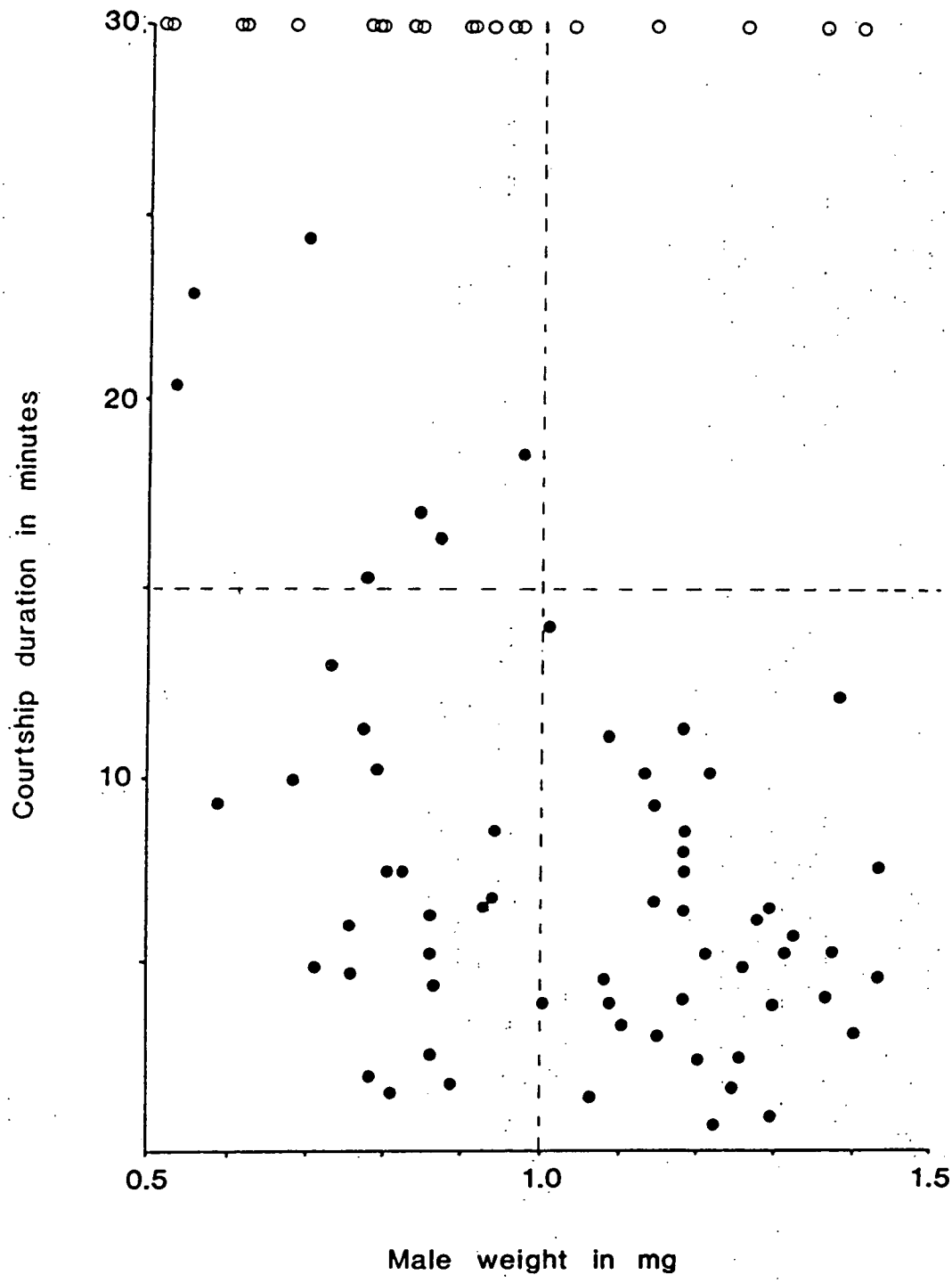
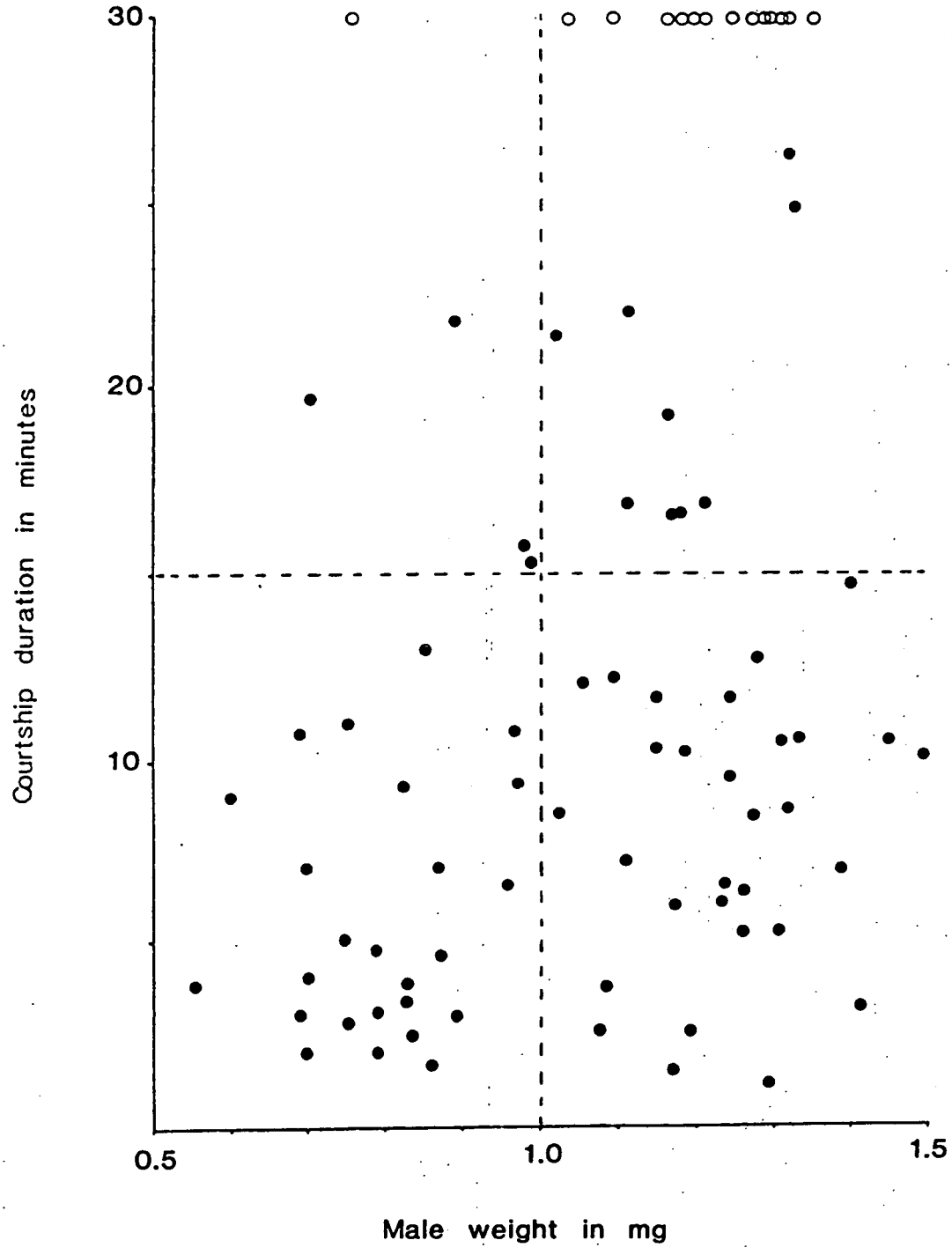


Figure 2.10 Sealed males with fed females.



courtships with the starved females than did the large males (χ^2=13.49, p<0.001), and this was due to a decline in small male success (χ^2=10.99, p<0.001) rather than an increase in large male success (χ^2=0.06, p<0.9).

From the first experiment reported in this section it was concluded that there was no difference between the courtships of sealed and normal males with fed females. The results just reported show a decline in the courtship success of large males with fed females when the proboscis is sealed. There are two points to make regarding this discrepancy. Firstly, there was a decline in the courtship success of sealed males with fed females in the first experiment, but it was not significant and was small by comparison to the decline observed with the starved females. Secondly, size extremes were collected for the experiment just described. The largest male is nearly 3x the weight of the smallest. It is towards the extremes that the effect is most marked (see figure 2.10).

2.3.4. Conclusions.

1) Males who were artificially prevented from producing the drop but otherwise courted normally, had significantly reduced mating success with starved females.

2) This decline in success resulted from a lower frequency of going on from orientation to frontal display and the frontal displays, once achieved, leading to copulation less often.

3) The size of the drop is correlated with male wing length.

4) Sealing the proboscis reduced the courtship success of large, but not small, males with fed females.

5) Small males, but not large, had significantly reduced mating success when the females were starved.

2.4. THE INFLUENCE OF MALE ABILITY TO PRODUCE THE DROP ON COURTSHIP SUCCESS; FED AND STARVED MALES.

2.4.1. Introduction.

The previous section examined the effect the absence of a drop and, indirectly, drop size had on a male's courtship success with fed and starved females. The sealing of the male's proboscis with glue could be viewed as a drastic and highly unrealistic experimental approach. Is there likely to be any variation in male ability or propensity to produce the drop in the wild and, if there is, what are the contributing factors likely to be?

One possible source of variation between males is in the amount of food stored in their crops. If the male has none he cannot regurgitate a drop. Perhaps, males with less food stored will produce the drop less frequently or produce smaller drops. In this section I present the results of an experiment in which some males were starved before being introduced to virgin females. The courtship of these males and controls was recorded in detail and the frequency and duration of various behaviours (such as drop production) and their influence on courtship speed was compared.

2.4.2. Materials and methods.

Males and females were separated at eclosion. The females were collected from standard population bottles and were of similar size. Large and small males were collected from low and high density vials. The flies were aged as previously described and "fed" flies were left

in yeasted vials until the morning of testing. "Starved" females were transferred to vials containing damp cotton-wool on the third day and "starved" males were transferred on the fourth day about 20 hours before they were tested. The males were transferred later because they die more quickly when deprived of food. Individual pairs of 5 day old virgins were observed through a x2 magnifying lens in damp cotton-wool vials with two inch bungs pushed down to leave a half inch gap for the flies. The courtship behaviour was recorded on the Apple keyboard for up to 3 minutes and after courtship the males' wings were removed and measured.

There were four treatments:

1) Fed male with Fed female;

2) Fed male with Starved female;

3) Starved male with Fed female;

4) Starved male with Starved female.

Within each treatment there were two categories; large males and small males. The influence of male size in these experiments was examined by testing for differences between these groups. The males used as "large" were the ones collected from the high density vials and the "small" those collected from the low density vials. If, after the wings had been measured, it was found that any "large" flies were,in fact, small, they were included in the "small" group for analysis and <u>vice versa</u> for any large flies occurring in the "small" group. This happened on two occasions. A large, fed male courtship with a starved female was transferred to the small male group, as was a large, starved male courtship with a fed female. Large males were defined as males with wing length greater than 1.72 mm and small males as 1.72 mm or less. The mean size and associated

confidence limits for each group are shown in table 2.4. Not surprisingly within each of the four treatments the large male group had significantly longer wings than the small male group (in each case $t>6.0$, degrees of freedom 18-23, $p<0.001$). There were no significant differences in wing length between the small male groups. However, the large starved male group used with the starved females had significantly longer wings than the large fed male group used with the fed females ($t=3.23$, degrees of freedom=21, $p<0.01$). As no comparisons were to be made between the starved male:starved female treatment and the fed male:fed female treatment (two variables involved) this was not an important difference. There were no other significant differences in wing length between the large male groups.

The data were gathered over several mornings' observation in fed and starved male sets (see section 2.3.2.1.). The experimental design was such that any day to day variation would not produce systematic differences between groups, only noise within. Comparisons were made between groups or treatments holding constant all but one of the three treatment variables (fed/starved female, fed/starved male, large/small male). The courtship times, frequency and rate at which the different groups of males performed various behaviours was contrasted. For some behaviours the median bout duration and the proportion of total time spent performing the behaviour by the males within different groups was compared. The median durations were used because the distribution of bout durations was often skewed in one or other of the groups. There was no evidence that bout duration was influenced by the length of courtship.

Table 2.4. A summary of the courtship behaviour for the eight groups is given. The number of times a behaviour occurred during courtship was measured as was the rate at which the behaviour occurred within a courtship and, for some behaviours, the median bout duration within a courtship and the proportion of a male's total courtship time that was spent performing that behaviour. For each group a median value is given with the range of values in parentheses.

TABLE 2.4

			Mean wing length mm	Number of courtships	DROPS							COURTSHIP TIMES		
					Total number			Median number per courtship		Median rate of production min^{-1}	Median duration carried secs.	Pairs mating within 3 minutes	% mating	Median duration secs.
					Produced	Taken	% Taken	Produced	Taken					
Fed male	Fed female	Large small	1.77±0.02	13	24	6	25	1.4 (0-6)	0.3 (0-2)	1.2 (0.0-6.4)	4.3 (2.3-7.9)	10	77	85.9 (9.3-180.0)
		Small male	1.64±0.03	11	18	5	28	1.6 (0-5)	0.4 (0-1)	1.0 (0.0-3.9)	5.6 (2.6-10.5)	10	91	103.0 (9.1-180.0)
	Starved female	Large male	1.79±0.03	12	11	10	91	0.9 (0-2)	0.8 (0-2)	0.3 (0.0-6.6)	2.8 (1.9-7.4)	7	58	122.7 (9.0-180.0)
		Small male	1.64±0.02	13	16	14	88	1.2 (0-2)	0.9 (0-2)	0.7 (0.0-4.7)	2.6 (1.1-50.1)	6	46	180.0 (9.7-180.0)
Starved male	Fed female	Large male	1.81±0.03	9	9	1	11	0.9 (0-3)	0.1 (0-1)	0.4 (0.0-4.6)	5.9 (3.8-7.4)	8	89	47.8 (12.9-180.0)
		Small male	1.59±0.02	11	1	1	(100)	0.0 (0-1)	0.0 (0-1)	0.2 (0.0-4.5)	5.0	8	73	99.2 (11.0-180.0)
	Starved female	Large male	1.83±0.03	10	13	6	46	1.2 (0-3)	0.5 (0-2)	0.7 (0.0-3.9)	4.3 (2.7-6.4)	2	20	180.0 (15.2-180.0)
		Small male	1.64±0.05	10	6	3	50	0.3 (0.2)	0.1 (0-2)	0.1 (0.0-2.9)	5.0 (2.0-5.4)	5	50	157.0 (7.7-180.0)

Table 2.4 (continued)

			ORIENTATION				ORIENTATION IN FRONT				FOLLOW			
			Frequency per courtship	Rate min^{-1}	Duration secs.	Proportion of total time	Frequency per courtship	Rate min^{-1}	Duration secs.	Proportion of total time	Frequency per courtship	Rate min^{-1}	Duration secs.	Proportion of total time
Fed male	Fed female	Large male	2.9 (1.0-10.0)	3.1 (0.7-8.5)	4.2 (1.8-77.6)	0.27 (0.14-0.90)	1.7 (1.0-7.0)	2.3 (0.4-6.4)	2.1 (0.5-45.0)	0.10 (0.01-0.50)	2.0 (0.0-6.0)	2.0 (0.0-4.8)	5.5 (1.5-15.9)	0.15 (0.00-0.37)
		Small male	2.2 (1.0-8.0)	2.9 (0.5-6.6)	3.7 (2.0-12.2)	0.24 (0.06-0.56)	1.9 (1.0-4.0)	1.3 (0.5-6.6)	2.7 (0.9-96.9)	0.17 (0.01-0.85)	0.3 (0.0-7.0)	0.1 (0.0-4.3)	5.1 (4.1-10.4)	0.01 (0.00-0.47)
	Starved female	Large male	1.8 (1.0-7.0)	1.5 (0.3-6.7)	8.8 (3.4-56.8)	0.42 (0.23-0.89)	1.5 (0.0-4.0)	1.6 (0.0-7.8)	2.4 (1.7 22.7)	0.11 (0.00-0.56)	0.4 (0.0-3.0)	0.1 (0.0-2.8)	4.8 (4.0-7.4)	0.00 (0.00-0.34)
		Small male	2.3 (1.0-5.0)	1.7 (0.3-6.2)	6.6 (2.0-84.0)	0.25 (0.04-0.76)	1.9 (0.0-3.0)	1.0 (0.0-6.2)	4.8 (1.7-76.1)	0.22 (0.00-0.45)	0.8 (0.0-4.0)	0.3 (0.0-2.4)	6.4 (1.2-54.7)	0.03 (0.00-0.32)
Starved male	Fed female	Large male	3.3 (0.0-5.0)	2.0 (0.0-8.1)	2.7 (1.7-60.6)	0.40 (0.00-0.90)	1.9 (0.0-3.0)	1.5 (0.0-4.6)	1.9 (1.6-5.7)	0.06 (0.00-0.44	1.7 (0.0-4.0)	1.1 (0.0-6.4)	4.2 (1.4-12.2)	0.13 (0.00-0.29)
		Small male	3.2 (1.0-14.0)	3.8 (1.0-5.4)	5.0 (3.1-24.9)	0.40 (0.16-0.69)	2.9 (1.0-8.0)	1.8 (1.0-5.4)	2.4 (1.5-16.1)	0.12 (0.03-0.48)	1.6 (0.0-7.0)	0.7 (0.0-2.8)	6.7 (4.0-13.3)	0.04 (0.00-0.42)
	Starved female	Large male	7.5 (1.0-14.0)	3.0 (1.7-4.7)	4.1 (3.4-9.4)	0.33 (0.15-0.54)	3.5 (0.0-6.0)	1.6 (0.0-3.9)	1.9 (1.2-9.9)	0.05 (0.00-0.76)	3.5 (0.0-11.0)	1.4 (0.0-3.7)	6.7 (1.9-19.1)	0.22 (0.00-0.59)
		Small male	3.5 (1.0-14.0)	2.8 (0.7-7.8)	3.3 (2.3-11.6)	0.23 (0.07-0.61)	2.5 (1.0-8.0)	1.5 (0.3-7.8)	2.8 (0.6-76.0)	0.20 (0.02-0.86)	1.5 (0.0-14.0)	0.4 (0.0-4.7)	3.9 (2.0-16.0)	0.09 (0.00-0.35)

TABLE 2.4 (continued)

			DANCE				OTHER				WING DISPLAY		CIRCLE		FEMALE MOVE AWAY	
			Frequency court^{-1} ship	Rate min^{-1}	Duration secs.	Proportion of total time	Frequency court^{-1} ship	Rate min^{-1}	Duration secs.	Proportion of total time	Frequency court^{-1} ship	Rate min^{-1}	Frequency court^{-1} ship	Rate min^{-1}	Frequency court^{-1} ship	Rate min^{-1}
Fed male	Fed female	Large male	0.9 (0.0-5.0)	0.7 (0.0-6.4)	3.5 (2.0-6.8)	0.04 (0.00-0.27)	0.4 (0.0-3.0)	0.0 (0.0-1.7)	10.4 (2.5-45.1)	0.01 (0.00-0.73)	1.1 (0.0-2.0)	1.4 (0.0-6.4)	1.0 (0.0-2.0)	1.0 (0.0-6.4)	1.8 (0.0-9.0)	1.7 (0.0-4.8)
		Small male	1.3 (0.0-2.0)	0.7 (0.0-5.0)	4.2 (1.3-7.4)	0.06 (0.00-0.30)	0.2 (0.0-4.0)	0.1 (0.0-1.3)	14.7 (13.0-62.6)	0.03 (0.00-0.81)	1.1 (0.0-3.0)	1.0 (0.0-6.6)	1.1 (0.0-2.0)	0.7 (0.0-6.6)	1.2 (0.0-7.0)	0.9 (0.0-3.6)
	Starved female	Large male	0.7 (0.0-2.0)	0.3 (0.0-5.4)	2.9 (2.2-4.6)	0.01 (0.00-0.25)	0.4 (0.0-2.0)	0.0 (0.0-0.7)	8.5 (7.1-115.1)	0.00 (0.00-0.64)	1.0 (0.0-2.0)	0.8 (0.0-6.7)	0.9 (0.0-2.0)	0.9 (0.0-6.7)	0.5 (0.0-6.0)	0.2 (0.0-2.8)
		Small male	1.0 (0.0-3.0)	0.3 (0.0-4.4)	2.2 (1.7-5.1)	0.01 (0.00-0.26)	0.6 (0.0-2.2)	0.3 (0.0-2.2)	39.1 (8.7-109.7)	0.05 (0.00-0.69)	0.7 (0.0-2.0)	0.4 (0.0-6.2)	0.8 (0.0-2.0)	0.4 (0.0-6.2)	1.2 (0.0-5.0)	0.4 (0.0-4.7)
Starved male	Fed female	Large male	0.9 (0.0-2.0)	0.5 (0.0-4.6)	2.8 (2.1-5.1)	0.04 (0.00-0.27)	0.7 (0.0-2.0)	0.5 (0.0-1.5)	6.3 (5.7-31.8)	0.05 (0.00-0.56)	1.1 (0.0-2.0)	1.4 (0.0-4.6)	1.0 (0.0-2.0)	1.3 (0.0-4.6)	2.3 (0.0-4.0)	1.1 (0.0-6.4)
		Small male	0.9 (0.0-3.0)	0.6 (0.0-5.4)	2.7 (1.4-7.1)	0.02 (0.00-0.21)	0.4 (0.0-5.0)	0.0 (0.0-1.7)	12.0 (7.8-28.4)	0.02 (0.00-0.52)	1.1 (0.0-2.0)	0.7 (0.0-5.4)	0.9 (0.0-1.0)	0.6 (0.0-5.4)	2.2 (0.0-15.0)	1.7 (0.0-5.0)
	Starved female	Large male	1.2 (0.0-4.0)	0.6 (0.0-3.9)	2.9 (1.1-5.6)	0.03 (0.00-0.08)	1.2 (0.0-4.0)	0.4 (0.0-1.3)	13.7 (2.9-30.2)	0.05 (0.00-0.68)	0.9 (0.0-3.0)	0.4 (0.0-3.9)	0.7 (0.0-2.0)	0.3 (0.0-3.9)	3.5 (0.0-13.0)	2.0 (0.0-4.3)
		Small male	0.5 (0.0-3.0)	0.1 (0.0-5.8)	4.2 (2.8-6.2)	0.00 (0.00-0.34)	0.5 (0.0-5.0)	0.1 (0.0-1.7)	19.7 (5.1-47.3)	0.02 (0.00-0.53)	1.0 (0.0-3.0)	0.5 (0.0-7.8)	0.5 (0.0-2.0)	0.1 (0.0-7.8)	2.5 (0.0-11.0)	1.4 (0.0-3.7)

Comparisons between groups were made using the Mann-Whitney U test from an SPSS package. This test only requires ordinal measurement and makes no assumptions about the distribution of the data. When the sample size was greater than 20, z values corrected for ties are given, and all the probabilities are two-tailed unless otherwise stated. When a large number of comparisons are being made there is a danger that some of the significant differences revealed may simply be a result of chance and careful interpretation is required.

2.4.3. Results.

Table 2.4 summarises the courtship data for each of the eight groups. I shall present below all the significant differences between groups as well as some of the non-significant differences where these are useful for the discussion. Any comparisons not reported were not significant at a two-tailed probability of 0.05.

2.4.3.1. Drop production.

Table 2.4 summarises the drop production data for each group. There were no significant differences between large and small fed males in either the number of drops produced per courtship or the rate of production with either fed (table 2.5; a, b) or starved females (c, d). Nor were there any significant differences in the number of drops produced or the rate of drop production of the large (e, f) or small (g, h) fed males with the fed compared to the starved females.

TABLE 2.5

Group comparisons of drop producion using the Mann-Whitney test.
Z is the standard normal variable and p is the two-tailed probability.

	Constant conditions	Drops produced	Comparison	z	p
a)	Fed male, Fed female;	number;	Big c.t., Small males:	-0.30	0.77
b)	Fed male, Fed female;	rate;	Big c.t., Small males:	-0.49	0.62
c)	Fed male, Starved female;	number;	Big c.t., Small males:	-1.13	0.26
d)	Fed male, Starved female;	rate;	Big c.t., Small males:	-0.08	0.93
e)	Big Fed male;	number;	Fed c.t., Starved females:	-1.60	0.11
f)	Big Fed male;	rate;	Fed c.t., Starved females:	-0.85	0.40
g)	Small Fed male;	number;	Fed c.t., Starved females:	-0.58	0.56
h)	Small Fed male;	rate;	Fed c.t., Starved females:	-0.61	0.54
i)	Starved male, Fed female;	number;	Big c.t., Small males:	-2.66	0.008
j)	Starved male, Fed female;	rate;	Big c.t., Small males:	-2.36	0.018
k)	Starved male, Starved female;	number;	Big c.t., Small males:	-1.45	0.15
l)	Starved male, Starved female;	rate:	Big c.t., Small males:	-1.35	0.18
m)	Starved males;	number;	Big c.t., Small males:	-2.82	0.005
n)	Starved males;	rate;	Big c.t., Small males:	-2.55	0.011
o)	Big starved males;	number;	Fed c.t., Starved females:	-0.56	0.58
p)	Big starved males;	rate;	Fed c.t., Starved females:	-0.17	0.87
q)	Small starved males;	number;	Fed c.t., Starved females:	-1.70	0.09
r)	Small starved males;	number;	Fed c.t., Starved females:	-1.41	0.16
s)	Small male, Fed female;	number;	Fed c.t., Starved male:	-3.07	0.002
t)	Small male, Fed female;	rate;	Fed c.t., Starved male:	-2.54	0.011
u)	Small male, Starved female;	number;	Fed c.t., Starved male:	-1.84	0.07
v)	Small male, Starved female;	rate;	Fed c.t., Starved male:	-1.62	0.10
w)	Big male, Fed female;	number;	Fed c.t., Starved male:	-1.33	0.18
x)	Big male, Fed female;	rate;	Fed c.t., Starved male:	-1.18	0.24
y)	Big male, Starved femlae;	number;	Fed c.t., Starved male:	-0.70	0.48
z)	Big male, Starved female;	rate;	Fed c.t., Starved male:	-0.33	0.74
i)	Small male;	number;	Fed c.t., Starved male:	-3.66	0.0003
ii)	Small male;	rate;	Fed c.t., Starved male:	-3.25	0.001
iii)	Big male;	number;	Fed c.t., Starved male:	-0.53	0.60
iv)	Big male;	rate;	Fed c.t., Starved male:	-1.22	0.22

Compared with small starved males, large starved males with fed females produced significantly more drops per courtship at a higher rate (i, j). With starved females the differences were not significant (k, l) but, overall, large starved males produced more drops per courtship at a higher rate than small starved males (m, n). There was no difference in the number of drops produced or the rate of drop production of large (o, p) or small (q, r) starved males with fed compared to starved females.

Compared to small fed males, small starved males produced fewer drops per courtship at a lower rate with fed females (s, t), but with starved females the differences were not significant (u, v). There were no differences in the drop production of large fed compared to large starved males (w, x, y, z). Overall, compared to small fed males, small starved males produced fewer drops per courtship at a lower rate (i, ii). There were no differences between the large fed compared to the large starved males (iii, iv).

2.4.3.2. Drops Taken.

The rate at which females take the drop will obviously depend partly on the rate at which males produce them. So, as well as comparing the number taken per courtship, differences in the proportion of available drops taken by males within the various groups were tested for using χ^2 for two independent samples.

There was no difference between the large and small male groups within any treatment in the number of drops taken per courtship or the proportion of available drops taken. Significantly more drops were taken per courtship from fed males than from starved males by

both fed (z=-2.34, p=0.019) and starved (z=-2.21, p=0.027) females and the proportion of available drops taken was higher in both cases. Starved females took significantly more drops per courtship than fed females from fed (z=-2.26, p=0.024) males and the proportion of available drops taken was higher and the starved females tended to take more drops from starved males (z=-1.93, p=0.053) although the difference was not significant.

The colour of the drops produced by fed males was similar to the colour of a thick solution of Baker's yeast or to the full crops of dissected flies. The drops produced by many of the starved males were much clearer and less viscous. When these males were dissected their crops often contained little of the opaque, yeast solution, but had large bubbles of a translucent fluid. This was probably water taken up from the damp cotton-wool. Presumably, the drops they produced were also watery and of lower nutritional value to the female. This might explain why a lower proportion of the drops produced by starved males were taken by both fed and starved females.

2.4.3.3. Drop duration.

A summary of the length of time males within the different groups carried the drop is given in table 2.4. The median drop durations for each male within one group were compared to those of another using the Mann-Whitney U test.

The drop was carried for longer by large (z=-2.09, p=0.036) and small (z=-2.11, p=0.035) fed males when they courted fed females than when they courted starved females. This was not entirely due to the starved females taking a higher proportion of drops because there was

also a significant difference between the fed and starved females in the "drop" to "kiss" duration ($z=-1.96$, $p=0.050$). There were no significant differences with the starved, large ($z=-1.57$, $p=0.12$) and small ($z=-0.36$, $p=0.72$) males courting fed compared to starved females.

2.4.3.4. Courtship duration.

A summary of the courtship data is given in table 2.4. A lower proportion of small fed males mated with starved females compared to fed females (Fisher exact test, $p<0.05$). There was no difference in the number of large fed males mating with the fed compared to the starved females (Fisher exact test, $p>0.05$). Large starved males had significantly longer courtships with starved females than with fed females ($z=-2.16$, $p=0.032$) and a lower proportion mated (Fisher exact test, $p<0.05$). Small starved males did not suffer a decline in mating success with starved compared to fed females ($z=-0.91$, $p=0.37$).

2.4.3.5. Orientation.

A summary of the courtship behaviours performed by the various groups is given in table 2.4. Large, starved males had a higher frequency of orientation than large, fed males ($z=-2.84$, $p=0.004$), the bouts were shorter ($z=-2.41$, $p=0.016$) and a lower proportion of time was spent in orientation ($z=-1.98$, $p=0.048$) with starved females. There was no difference in the rate of orientation ($z=-1.42$, $p=0.156$).

2.4.3.6. Orientation in front.

Small males spent a greater proportion of their time orienting in front of females than large males. This is true of both fed males ($z=-2.04$, $p=0.041$) and males courting starved females ($z=-2.10$, $p=0.036$) and tends to be the case for starved males ($z=-1.87$, $p=0.061$) and males courting fed females ($z=-1.80$, $p=0.072$).

2.4.3.7. Following.

Compared to small starved males, large, starved males spent a greater proportion of time following starved females ($z=-2.06$, $p=0.040$). Compared to large starved males, large, fed males followed starved females less frequently ($z=-3.16$, $p=0.002$), at a lower rate ($z=-2.92$, $p=0.003$) and spent a lower proportion of their time following ($z=-3.01$, $p=0.003$).

Large, fed males have more bouts of following with fed females than with starved females ($z=-2.04$, $p=0.042$), follow fed females at a higher rate ($z=-2.05$, $p=0.041$) and spend a greater proportion of their time following fed females ($z=-2.05$, $p=0.041$). Large, starved males had more bouts of following with starved females than with fed females ($z=-2.15$, $p=0.032$).

2.4.3.8. Dance, circle, wing display and other.

There were no significant differences between the groups for any of these behaviours.

2.4.3.9. Female "move away".

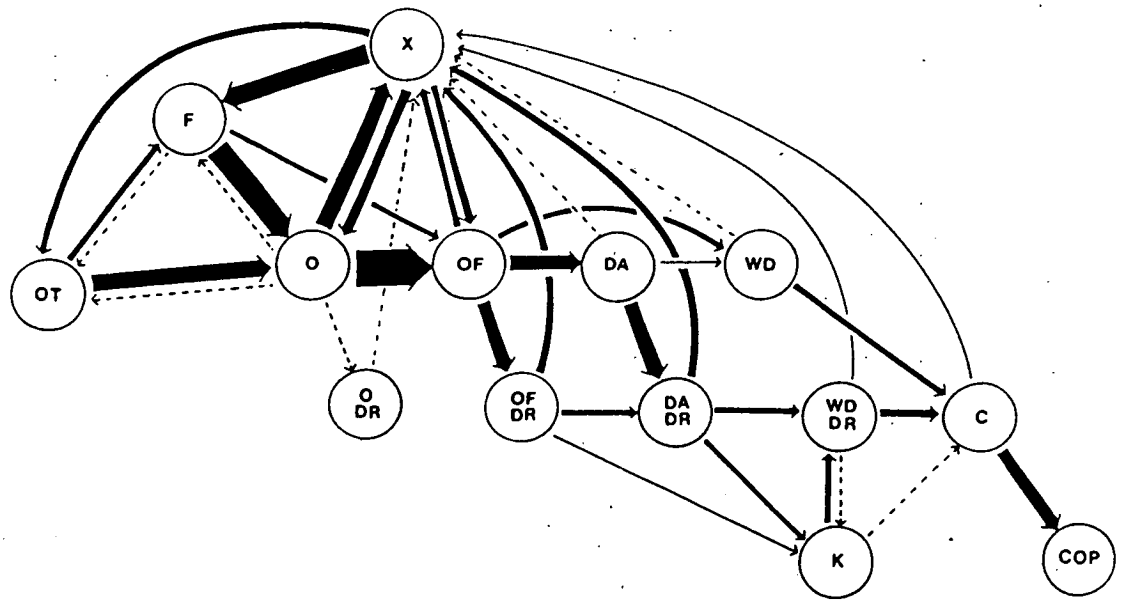
Fed females moved away more times per courtship than starved females from fed large males ($z=-1.99$, $p=0.047$) and at a higher rate ($z=-2.32$, $p=0.021$). There was no difference in the frequency with which fed compared to starved females turned away from small fed males (frequency, $z=-0.30$, $p=0.77$; rate, $z=-0.38$, $p=0.70$). Starved females moved away more frequently from large, starved males than they did from large, fed males ($z=-2.74$, $p=0.006$) and at a higher rate ($z=-2.56$, $p=0.011$). The difference between the small fed and starved males was not significant (frequency, $z=-1.30$, $p=0.19$; rate, $z=-1.09$, $p=0.27$). There was no difference in the frequency with which fed females moved away from fed compared to starved males, nor in the frequency with which fed and starved females moved away from starved males.

The sequence diagrams (figures 2.11 to 2.14) summarise the frequencies of the behavioural transitions for each group. A number of the trends already discussed are apparent , such as the higher frequency with which fed females compared to starved females moved away from large, fed males with drops. Starved males produced more drops during frontal orientation than during the dance with fed females but not with starved females. This difference is significant ($\chi^2=8.87$, $p<0.01$) and does not occur with fed males. In section 2.4.3.2. it was argued that females took a lower proportion of the drops available from starved males than from fed males. It was suggested that this was probably due to the drops of starved males having a greater water content and being of less nutritional value.

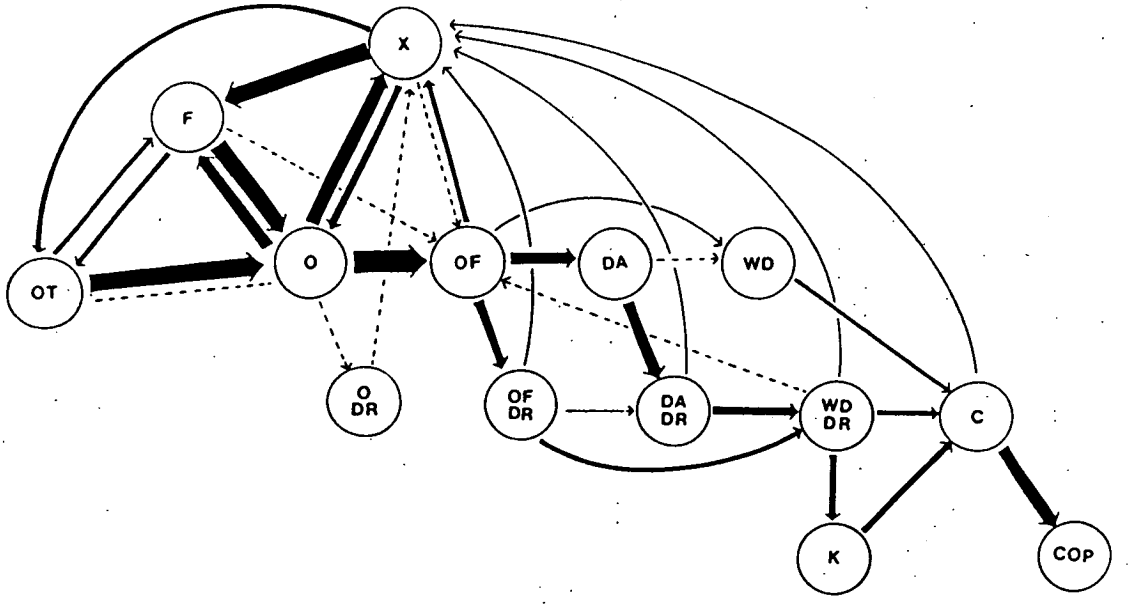
Figures 2.11, 2.12, 2.13 and 2.14. The sequence diagrams depict the frequencies of behavioural transitions during courtship for large and small fed and starved males with fed and starved females. The widths of the arrows are proportional to the frequency of transitions. A width of 1.6mm represents 10 transitions. A single transition is represented by a dashed line and a single solid line represents a frequency of two.

The behaviours shown in each diagram are other (OT), follow (F), female move away (X), orientation (O), frontal orientation (OF), dance (DA), wing display (WD), orientation with drop (ODR), frontal orientation with drop (OFDR), dance with drop (DADR), wing display with drop (WDDR), kiss (K), circle (C) and copulation (COP).

Figure 2.11. Fed males with Fed females.

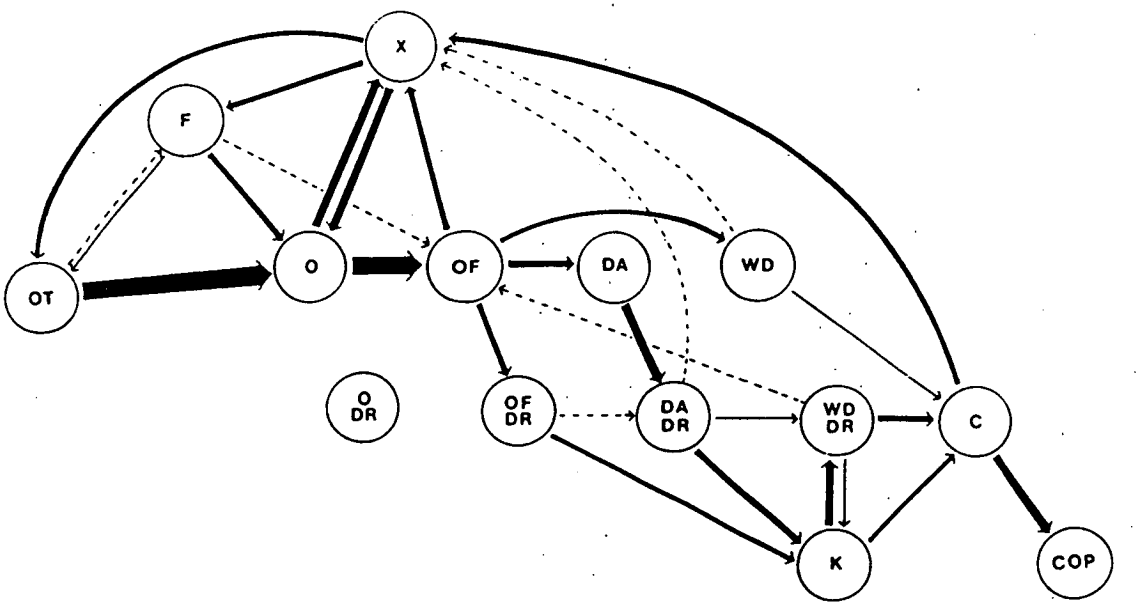


Large males.

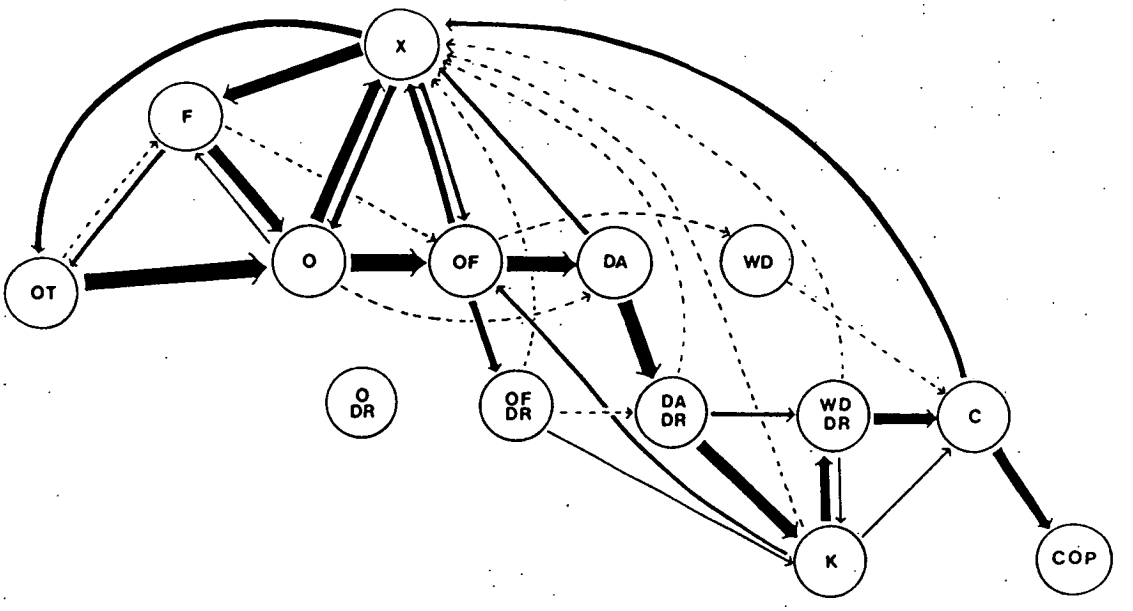


Small males.

Figure 2.12. Fed males with Starved females.

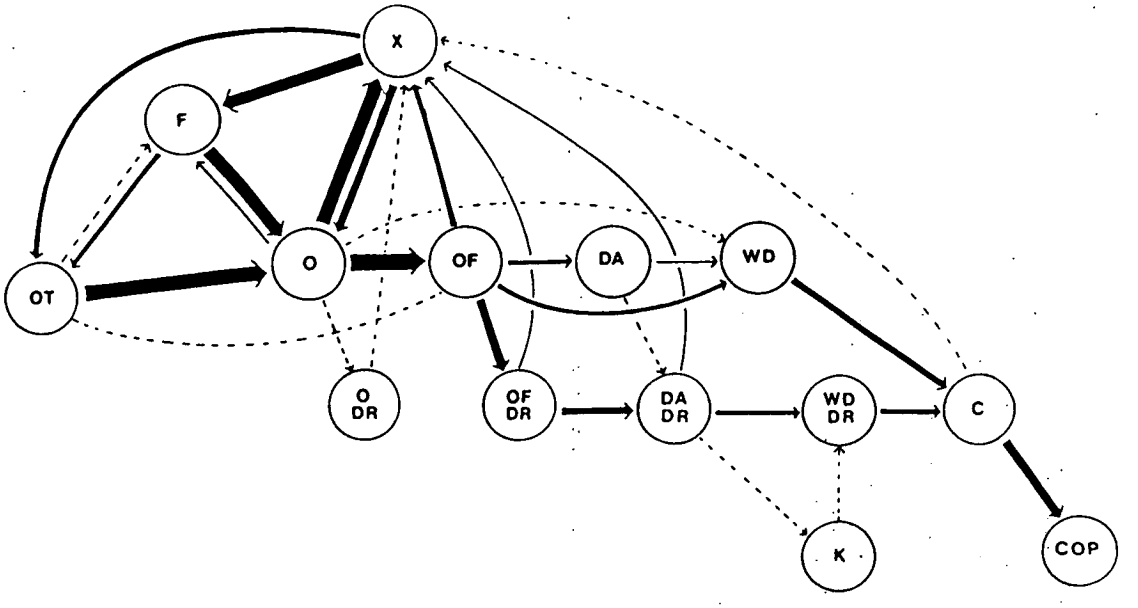


Large males.

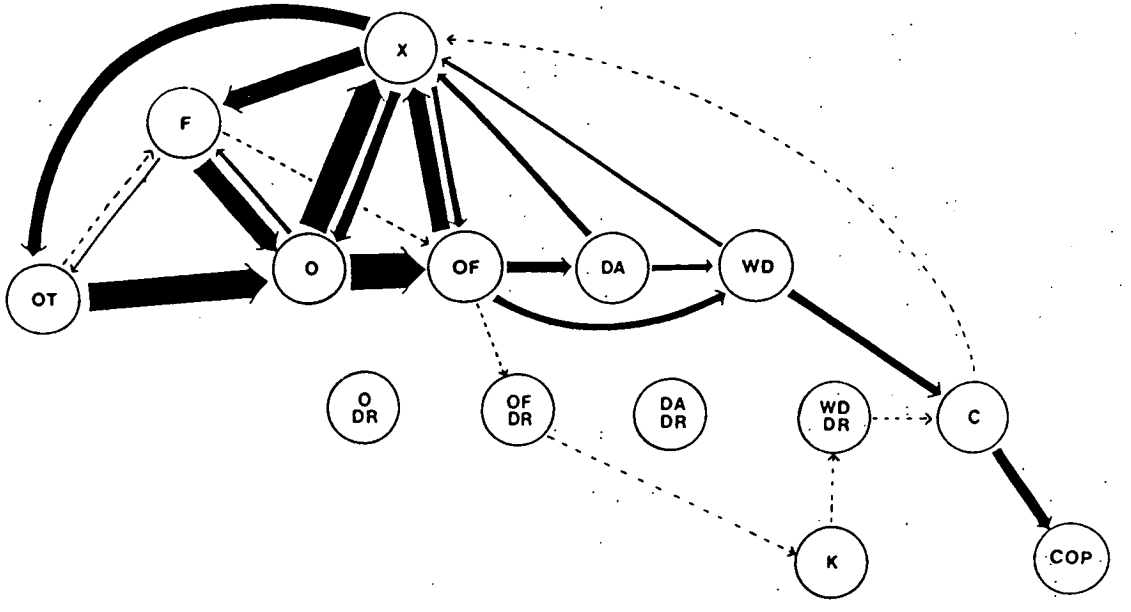


Small males.

Figure 2.13. Starved males with Fed females.

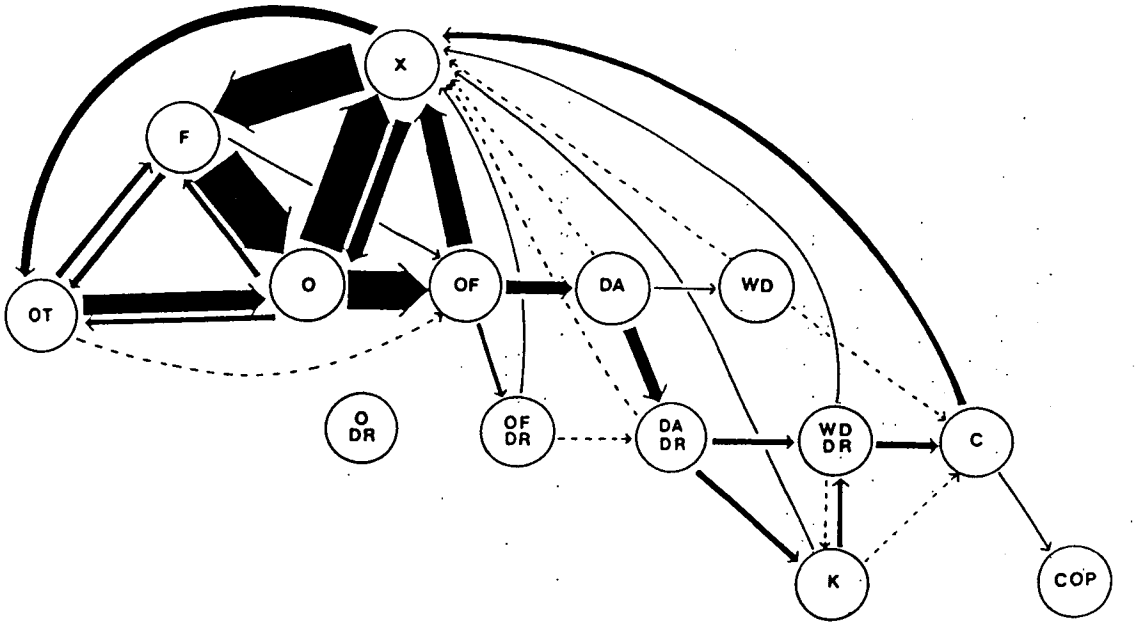


Large males.

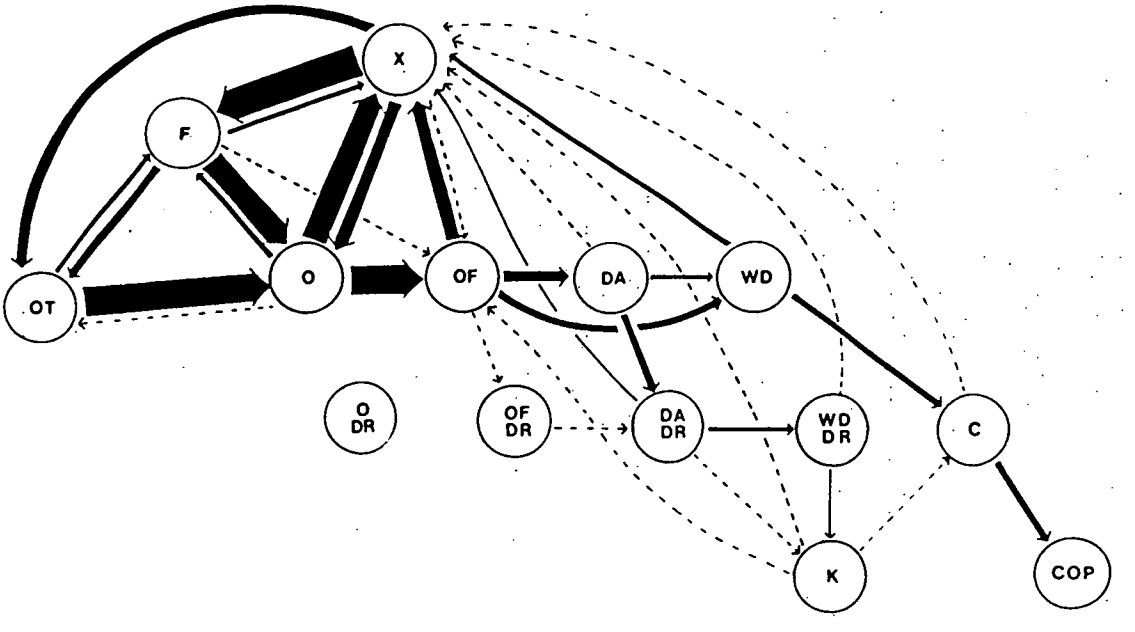


Small males.

Figure 2.14. Starved males with Starved females.



Large males.



Small males.

An alternative explanation is that the starved males lost their drops before the females had a chance to take them, i.e. they were not available. However, it is clear from the sequence diagrams that this only occurred twice and on both occasions involved fed males (figures 2.11 and 2.12). A male "wing display with drop" behaviour was followed by frontal orientation. The males lost or sucked the drop back in without an intervening female 'repelling' response.

Table 2.6 gives the frequencies with which particular behaviours occurred within the groups and how often each was followed by a female "rejection" response. The sample size is such that few meaningful comparisons can be made, however, there are some interesting points. On the occasions that a female took the drop the only groups in which she moved away before the male circled were the small, fed males with starved females and the large and small starved males with starved females. The result was that in these 3 treatments copulation was less likely to follow the kiss than it was in the other treatments. Although, there were no differences in the frequency with which fed or starved females turned away from large as opposed to small fed male behaviours involving the drop, starved females turned away from fewer large male behaviours involving the drop than did fed females ($\chi^2=4.40$, $p<0.05$) and specifically from fewer bouts of frontal orientation and the dance involving the drop than fed females ($\chi^2=4.35$, $p<0.05$). There was no difference with the small, fed males ($\chi^2=0.003$, $p>0.1$).

Table 2.6. The table shows the frequency of the various courtship behaviours within each group (the upper figure in a pair) and the number of times the courtship behaviour was followed by 'female move away' (the lower figure in a pair). Data is given for female move away (X), other (OT), follow (F), orientation (O), frontal orientation (OF), dance (DA), wing display (WD), orientation with the drop (ODR), frontal orientation with the drop (OFDR), dance with the drop (DADR), wing display with the drop (WDDR), kiss (K), circle (CI), copulation (COP), a total for orientation, frontal orientation, dance and wing display (O,OF,DA,WD) with and without the drop and a column indicating the frequency of kiss and the number of times kiss was not followed by copulation.

TABLE 2.6

			N	Median courtship duration	Frequency of behaviour. Number of times female 'moved away'. X	OT	F	O	OF	DA	WD	O DR	OF DR	DA DR	WD DR	K	Ci	COP	O,OF DA,WD Without drop	With drop	Total K. K But no cop.
Fed male	Fed female	Large Male	13	85.9	36	8	28	48	37	15	6	1	12	16	10	6	14	12	106	39	6
								14	4	1	1	1	6	7	2	0	2		20	16	1
		Small male	11	103.0	24	4	30	36	23	11	3	1	7	12	11	5	12	10	73	31	5
								12	3	0	0	1	2	2	2	0	2		15	7	1
	Starved female	Large male	12	122.7	18	5	8	29	18	7	3	0	4	8	9	10	11	7	57	21	10
								7	4	0	1	0	0	1	1	0	4		12	2	3
		Small male	13	180.0	30	8	14	33	24	16	1	0	5	14	11	14	12	8	74	30	14
								11	5	3	0	0	1	1	1	4	4		19	3	8
Starved male	Fed female	Large male	9	47.8	21	7	15	29	15	3	6	1	7	6	4	1	9	8	53	18	1
								12	3	0	0	1	2	2	0	0	1		15	5	0
		Small male	11	99.2	47	12	21	51	33	11	11	0	1	0	1	1	9	8	106	2	1
								23	15	5	3	0	0	0	0	0	1		46	0	0
	Starved female	Large male	10	180.0	63	17	47	74	32	13	2	0	3	11	8	6	7	2	121	22	6
								34	19	1	1	0	2	1	2	2	5		55	5	4
		Small male	10	157.9	45	16	32	53	28	9	8	0	1	6	3	3	6	5	98	10	3
							3	21	11	1	3	0	0	2	1	2	1		36	2	2

2.4.4. Conclusions.

1) Starved females took more drops per courtship than did fed females and they took a higher proportion of the drops available. Fed males carried the drop for shorter periods with starved females than with fed females, partly as a consequence of the difference in proportion taken, but also because when the drop was taken it was taken sooner after its appearance by the starved females.

2) Large and small fed males produced the drop at the same rate with fed and starved females.

3) The large, fed male courtship success was the same with fed and starved females. Although starved females moved away from fewer large, fed males with drops and the large, fed males spent less time following, they spent more time in orientation with the starved females.

4) Fewer small fed males mated within the observation period with starved females than mated with fed females. The drop durations were shorter with the starved females but the males were less likely to circle and copulate when the female took the drop.

5) Starvation reduced the frequency of drop production only of small males. However, the drops produced by starved males appeared to contain little yeast and both fed and starved females took a lower proportion from the starved males than they did from the fed males.

6) The large, starved male courtship success with fed females was no different to that of large, fed males with fed and starved females, but the starved males took longer with starved females. The starved females moved away more often than they did from large, fed males

with the result that large, starved males spent more time following and less in orientation. Also, circling and copulation were less likely to occur when the female took the drop than they were with large, fed males.

7) Starvation made no difference to the courtship success of small males with either fed or starved females.

8) Starved males produced more drops during frontal orientation than during the dance with fed females, but not with starved females. There was no difference with fed males.

2.5. DISCUSSION.

In this chapter I have shown that male D.subobscura regurgitate a portion of their crop contents during courtship and females take this drop of food directly from the male's proboscis and consume it. That this drop is of nutritional value is suggested by the fact that the drop originates in the male crop, passes into the female crop and ventriculus and is taken more frequently by starved females than by fed females. Females do not always take the drop, nor does copulation necessarily occur when they do, despite male attempts to mount. So how important is the drop in determining a male's courtship success?

When males were artificially prevented from producing the drop by sealing their proboscises with glue, they still orientate and perform frontal display as often as normal males with fed females and their mating success is the same. However, the sealed males had reduced mating success with starved females, apparently because the sealed males went on from orientation to perform frontal display less often, and the frontal displays, once achieved, were less likely to lead to copulation. This suggests that if a starved female does not perceive the drop the male is less likely to be able to position himself in front of her and, when he does, his display is more likely to be rejected. It also suggests that the possession of a drop is not an important male attribute with fed females.

If the absence of a drop affects male mating success, then, so too might drop characteristics such as size or quality. The diameter of the drop was shown to be highly correlated with a measure of male size. One might, therefore, predict that small males would suffer

reduced mating success with starved females compared to their performance with fed females. Large males should be less affected, if at all. This turned out to be the case; small males had reduced courtship success with starved females, but large male courtship success was unaffected.

There was no difference between large and small male courtship success with fed females. With regard to the drop this was, perhaps, to be expected. Although larger males produce larger drops, the initial sealing experiments (using medium-sized males) showed that male inability to produce a drop made no significant difference to mating success with fed females, so why should drop size be important? In a later experiment when a range of large and small males had their proboscises sealed, the sealing made no difference to small male courtship speed with fed females, but increased the time it took large males to achieve copulation. Fed females do discriminate against large males unable to produce the drop suggesting that; a) the drop is a particularly important component of a large male's courtship repertoire but not a small male's, and b) large males are inferior to small males in some other aspect of courtship.

When the behaviour of males with fed and starved females was recorded in detail it was found that large and small males produced the drop at the same rate with fed females and a similar proportion were taken from each. They produced drops at an equivalent rate with starved females, but a higher proportion were taken. As before there was no significant difference in the mating success of large males with fed compared to starved females, even though the starved females moved away from a lower proportion of large male behaviours involving

the drop. The proportion of small males mating with the starved females was lower than the proportion mating with the fed females. The small males produced drops at the same rate with the starved females, more were taken and they were taken sooner, however, the starved females moved away more often than the fed females once they had taken the drop making it less likely that the male circled and attempted copulation.

I attempted to affect drop production by starving males. The starvation reduced the rate of drop production of small, but not large, males. Starvation also appeared to affect drop quality. There were two pieces of evidence for this; 1) the drops looked "watery" and the crops contained little yeast but bubbles of a clear fluid which was probably water taken up from the damp cotton-wool in the vial, 2) both fed and starved females took a lower proportion of the drops from starved males than they did from fed males.

Although small, starved males produced fewer drops than small, fed males with fed and starved females, their courtship success with these two groups was the same as that of the small, fed males. Earlier in this discussion I argued that the production of a drop was particularly important to large males, but not the small. That a decline in the rate of production makes no difference to the success of small males is consistent with this idea. So too is the observation that starvation significantly reduced the rate of drop production by small, but not large, males, although this may simply reflect the effect of starvation on males of different sizes. With fed females the mating success of large starved males was not significantly different from that of large fed males. However, starvation did increase the courtship duration of large males with

starved females. Starved females moved away from large starved males more frequently than from large fed males and took fewer drops. The large, starved males spent more time following and when the female did take the drop, she was more likely to move away with the result that the male was less likely to circle and copulate.

In summary, males that cannot produce a drop have poor mating success with starved females, as do small males who produce small drops, large starved males who produce poor quality drops and also, with fed females, large males who cannot produce a drop. The production of a drop does influence a male's chances of mating in certain circumstances and it appears to do so in two ways; firstly, by contributing to a male's chances of achieving frontal display and, secondly, by determining how successful that display is likely to be. I will discuss each of these in turn.

To perform the frontal display a male must be able to position himself in front of the female. He can do this if she stands still or by walking backwards in front of her. If there is an obstruction in front as she walks along, the female will tend to change direction to avoid it and the male must then move laterally to hold his position. So if the female is moving, the male must constantly be walking backwards and sideways to keep in front of her (RENDEL 1945) and, if he fails, then the courtship must start again with following or orientation. Starved females show greater locomotor activity than fed females (CONNOLLY 1966) and so it is likely that male tracking is particularly important with starved females. It is possible that, especially in the context of hungry females being intercepted by males as they approach an aggregation site (see next chapter), the production of a yeasty drop slows the female down, stops her or makes

it less likely that she will turn away or decamp and so makes it easier for a male to position himself in front of her. The degree to which the drop can do this may depend on how "attractive" it is and this will be influenced by the drop's size and quality and the nutritional state of the female. Supporting this hypothesis are the observations that males who do not produce drops achieve frontal display less readily, that starved females turn away less often than fed females from large, fed males with drops, but more often from large males producing watery drops than from large males producing yeasty drops. Also, occasionally starved females were observed to turn and move towards males with a drop. Why don't starved females move away from small males producing small drops more often than they do from large males?

In chapter 4 I present a theoretical argument which predicts that small males are able to change direction faster for any given speed than large males and so are better able to maintain their position in front of the female and I provide empirical support for this idea. The drop may be less important to small males in the keeping-in-front stage because they are physically better equipped than large males to track the female. This would explain why sealing the proboscis reduced large, but not small, male courtship success with fed females.

As well as being important in influencing a male's ability to achieve frontal display (for whatever reason) the drop also appears to be important in determining how successful that display is likely to be. With starved females those males with drops have greater success than those without, those males with large drops greater success than those with small and those males with yeasty drops

greater success than those with watery drops. This seems to occur because females move away from the males who produce less attractive drops before the males can circle and attempt to copulate. In section 2.2.3.2. I reported some casual observations suggesting that females remained stationary whilst handling the drop and that larger or more viscous drops might take longer to consume. If this is the case it would provide a mechanism by which the quality or size of the drop provided by a male could influence his chances of attempting to mount and hence copulation. Starved females would be more likely to move away before the male mounted when they took either a small or watery drop requiring little handling time or no drop at all.

RENDEL in his 1945 paper on D.subobscura, whilst ignorant of the drop's existence, commented that " The function of the courtship seems to be to fix the attention of the female sufficiently to hold her still until the male can copulate." If courtship is viewed in this way then the drop, whilst playing a minor role with fed females, could be important in slowing down or stopping the hungry and more active starved females. If the drop was more "attractive", the likelihood of the female moving off before or after taking it might be reduced.

In the laboratory starved females discriminate against males unable to produce a drop or who produce nutritionally inferior drops. Is such female discrimination likely to occur in the field and, if it does, is it likely to be an important determinant of wild male mating success? Observations on this question and on the nutritional significance of the drop to wild females are discussed in the next chapter in conjunction with descriptions of courtship feeding in other Drosophila species.

Chapter 3.

THE NUTRITIONAL SIGNIFICANCE OF COURTSHIP FEEDING.

3.1. INTRODUCTION.

In the last chapter I described numerous examples of insect species in which the males provide nutrients for females before, during or after mating. In this chapter I will consider the nutritional significance of the nutriment provided by the male. Do females take the nutrients simply because they happen to be there, or do the nutrients given by the male at mating actually increase the fitness of the receiving female? An extreme example of females benefiting from nuptial feeding by males is illustrated by the Thynninae (reviewed in chapter 2). Females of many species within this group would be unable to feed at all without males to transport them to feeding grounds or bring them food.

There are a number of reports in the literature concerning the effects of nuptial feeding by males on female fitness. BENTUR and MATHAD (1975) found that females of the cricket Plebiogryllus guttiventris survived longer during periods of starvation if they had access to males and received spermatophores from them. LANDA (1960) observed that egg maturation was more rapid and the volume of fat body greater in Melolontha melolontha females with the most and the oldest spermatophores in the genital tract. He argued that the nutrients gained from the partially digested spermatophores accounted for the better physiological condition of the females that had mated more often. Radiolabelling experiments in a number of insect groups have demonstrated that male-derived nutrients are incorporated into

female somatic and reproductive tissue (reviewed in chapter 2) and a few workers have demonstrated that the nutrients provided by the male at mating are not only used by females but also increase female reproductive success.

THORNHILL (1976) found that female H.apicalis receiving larger prey items from males laid more eggs. He also argued that female survivorship might be increased if the females received more food from males at mating because the females would need to spend less time foraging and so would be exposed less often to the risk of mortality associated with foraging in this species (THORNHILL 1980). Female Panorpa who mated with males providing nuptial gifts laid more eggs per unit time than females involved in forced copulations (THORNHILL 1984).

Females of the cockroach, X.hamata, obtain urates by feeding from the uricose glands of males they have just mated with (SCHAL and BELL 1982) and these male-derived urates represent a significant input to the females' nitrogen pool, particularly if the females are kept on a nitrogen deficient diet. If females have access to uric acid after mating the preovipositional period is shortened. Oothecae collected in the field tend to have high levels of uric acid and yet wild females tend to feed on nitrogen-poor foods and rarely forage on uric acid. SCHAL and BELL (1982) suggest that the urates provided by males might be an important source of nitrogen for females in the field. Males have low nitrogen requirements yet feed on bird and reptile droppings rich in uric acid, and their uricose glands hence swell in size. An interesting consequence of this male behaviour is that, if no sexually receptive females are available to accept the male's accessory secretion, the accumulation of extracellular uric

acid results in increased male mortality (HAYDAK 1953, MULLINS and COCHRAN 1973). SCHAL and BELL (1982) also argue that the long copulation in this species allows the female to empty her crop of plant material before feeding from the male's uricose glands.

Bushcrickets probably have limited access to high protein food sources in the field (GWYNNE in press). GWYNNE (1984) counted and weighed the eggs laid by female Requena verticalis allowed to eat, over a period of 14 days, 0, 1, 3 or 7 spermatophylaxes produced by males. The females were kept on a low protein diet. Females eating more spermatophylaxes not only laid more eggs but also the eggs they laid were heavier. Larger egg size correlates with several components of offspring fitness in insects (CAPINERA 1979). In the field female bushcrickets have been observed to mate up to 12 times and hence receive 12 spermatophores (GWYNNE in press).

Female D.pseudoobscura[1] and female D.mojavensis (MARKOW 1983) kept with males left more progeny than females mated once at the beginning of adult life. PARTRIDGE and ANDREWS (unpublished) obtained a similar result using D.melanogaster and in addition found that the difference in number of progeny left by females with or without access to males was greatest if the females were kept on a low nutrient medium. Recently seminal feeding has been found in D.mojavensis (MARKOW and ANKNEY 1984) and D.melanogaster (PARTRIDGE unpublished) and it may well be that females of a number of Drosophila species are able to use nutrients derived from male ejaculate to increase their egg production. In this chapter I report the results of an experiment designed to test whether female D.subobscura that take a drop from males during courtship have greater fecundity on a low nutrient medium than females denied access

[1](TURNER and ANDERSON 1983)

to a drop.

In the last chapter I showed that in the laboratory starved females discriminated between males on the basis of the food they offered during courtship. Are females likely to be starved in the wild? Both BOULETREAU (1978) working with D.melanogaster and BEGON and SHORROCKS (1978) working with D.subobscura and D.obscura have suggested that flies might find it difficult to obtain food in the field. In Drosophila the size of the crop reflects the amount of food stored. I compare the crop sizes of D.subobscura collected in the field with the crop sizes of laboratory flies of known nutritional background.

Finally, having examined various aspects of courtship feeding in D.subobscura, it would be interesting to know if courtship feeding occurs in any other Drosophila species and, if so, whether this throws any light on the evolution of courtship feeding in D.subobscura. Both WEIDMANN (1950) and BROWN (1964,1965) described fluid exuding from the proboscises of courting males in a number of obscura group species. BROWN (1964) also noticed that male D.pseudoobscura sometimes extruded a cloacal drop during courtship and SPIETH (1952) described prolonged proboscal contact between male and female D.pseudoobscura during courtship. I obtained stocks of D.pseudoobscura and D.obscura and observed the courtship of virgins.

In his 1952 paper SPIETH observed the courtships of species from groups throughout the genus and described proboscis extrusion during the frontal display in several species groups. His only observations of fluid exudation were the production of anal drops by D.nebulosa and, in a later paper (1966), a number of Hawaiian species. I describe courtship observations on stocks of D.nebulosa

and several species exhibiting frontal displays and discuss SPIETH's observations on the Hawaiian species. Unfortunately, many Hawaiian species cannot be cultured in the laboratory and courtship observations must be conducted on wild flies either in the field or collected from the field.

3.2. MATERIALS AND METHODS.

3.2.1. The contribution of the drop to female fecundity.

Virgin males were collected from standard culture bottles and aged for five days in vials containing standard drosophila medium. Virgin females were collected upon eclosion from vials containing a low nutrient charcoal medium (DAVID and CLAVEL 1965). Inseminated females from the standard population bottles had been allowed to lay in these vials for 36 hours some weeks previously. The low nutrient charcoal medium was used to minimise possible variation in larval nutrition and hence potential variation in the amount of larval fat body carried over into the adult female. The females were aged for five days in vials containing the low nutrient medium before being introduced to a male in a vial containing damp cotton-wool. The proboscises of half of the males had been sealed the previous evening (see chapter 2) so that these males were unable to transfer any crop contents during courtship. The courtship and mating of individual pairs were observed and then the mated females put into vials containing the low nutrient charcoal medium for two days before being transferred to fresh medium for a further two days. The numbers of eggs laid in each vial were counted.

3.2.2. Crop size.

Wild flies were collected at the Dalkeith oak wood in September 1982 using a banana bait covered with fine muslin to prevent the flies feeding. As the flies arrived they were pootered into vials

containing moist cotton-wool. These vials were then placed in ice and darkness. At the laboratory the flies were stored in the fridge before being identified, sexed and dissected. The crops were drawn using a camera lucida and the area of the drawings obtained was measured using a planimeter (see chapter 6). One wing from each fly was measured under the binocular microscope.

The crops of five day-old virgin males and females were also measured. One group of flies had been left in the vials containing standard drosophila medium seeded with active Baker's yeast for the full five days (fed group). Flies in the other group were transferred to vials containing only damp cotton-wool 36 hours before dissection in the case of females and 24 hours before dissection in the case of males (starved group). Hence the fed and starved flies used in this chapter were treated in the same way as the fed and starved groups used in chapter 2. Wing length measurements were obtained for only half of the flies within the laboratory groups.

3.2.3. Courtship feeding in other Drosophila species.

3.2.3.1. The obscura species group.

The outbred D.pseudoobscura stock was an inversion stock kindly sent to Linda Partridge in 1979 by Chuck Taylor and the flies used in this study were maintained in standard population bottles at 20°C. The outbred D.obscura stock was started in 1980 from flies collected at Ormiston and Dalkeith and subsequently maintained in standard population bottles at 20°C. For the courtship observations single pairs of virgin five day-old flies were observed in a 1 cm^3 perspex

cell under a binocular microscope. Males had been provided with stained yeast cells the evening before. Ten pairs were observed for each species and after courtship the females were dissected and examined for stain.

3.2.3.2. Other species groups.

Several species were obtained from the Drosophila stock centre at the University of Texas and were then maintained at 25°C in standard population bottles. For the courtship observations the flies were treated and observed as above with one difference; three day-old flies were used. The species tested were: D.nebulosa (H29.2 El Salvador), D.fumipennis (H331.1 Trinidad), D.willistoni (1156.4 Florida), D.cardini (2395.6 Peru), D.prosaltans (H436.5 Colombia) and D.takahashii (3146.13 Luzon, P.I.).

3.3. RESULTS.

3.3.1. The contribution of the drop to female fecundity.

The numbers of eggs laid by females during the first two day period after mating and the subsequent two day period are plotted in figure 3.1 for the females mated to the normal and to the sealed males. All of the females mated to the normal males were observed to take a drop from the male during courtship. There was considerable variation in the numbers of eggs laid by different females within the groups. However, the females that took a drop laid significantly more eggs over the first two days after mating than the females denied access to a drop (median test; $\chi^2=6.06$, $p<0.02$). The two groups did not differ in the number of eggs laid over the second two day period (median test; $\chi^2=0.06$, $p>0.8$). The results suggest that females on a low nutrient medium can use the drop obtained during courtship to increase egg production. The histograms (figure 3.1) suggest that the early difference between the egg production of the 'drop' and 'no drop' groups arises because the onset of oviposition is later in the 'no drop' group.

3.3.2. Crop size.

Histograms of the flattened, cross-sectional crop areas of flies from the fed, starved and wild groups are given in figure 3.2 for the females and figure 3.3 for the males. From the figures and the statistics presented in table 3.1 it is clear that that the fed groups have larger crops than the starved groups, and that the wild

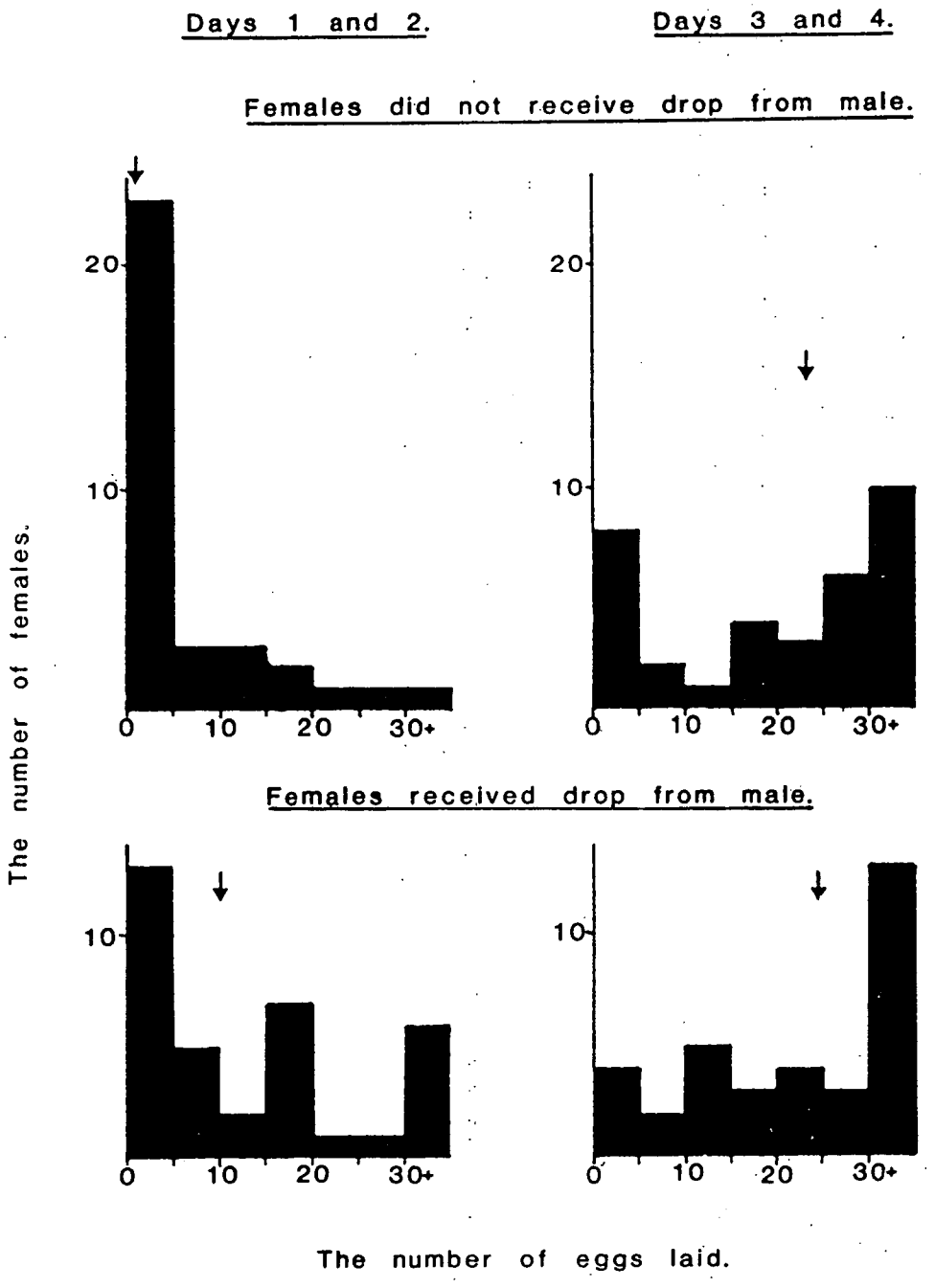
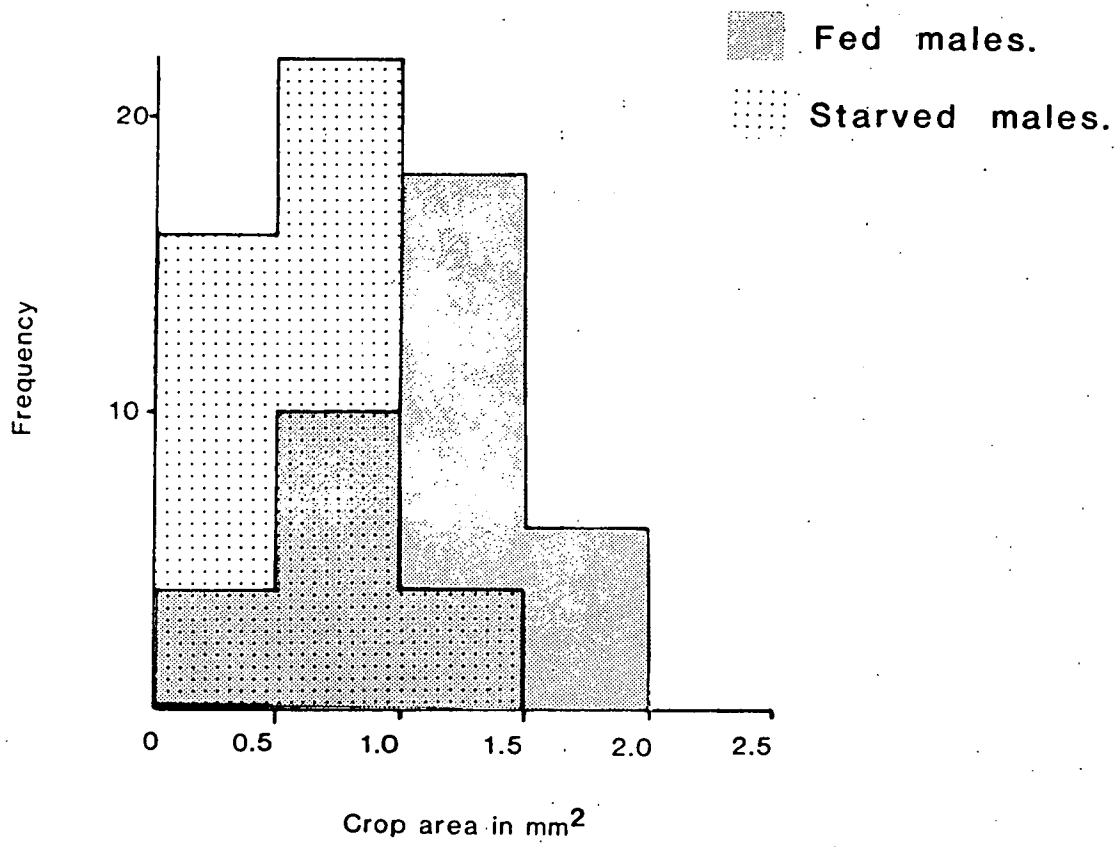


Figure 3.1. The histograms show the number of eggs laid by females receiving drops from the male during courtship and the number of eggs laid by females unable to receive a drop from males whose proboscis had been sealed. The plots on the left show the number of eggs laid over the first two day period after mating and the plots on the right show the number of eggs laid over the second two day period. The arrows indicate the medians for the different groups.

Figure 3.2. Male crop area.



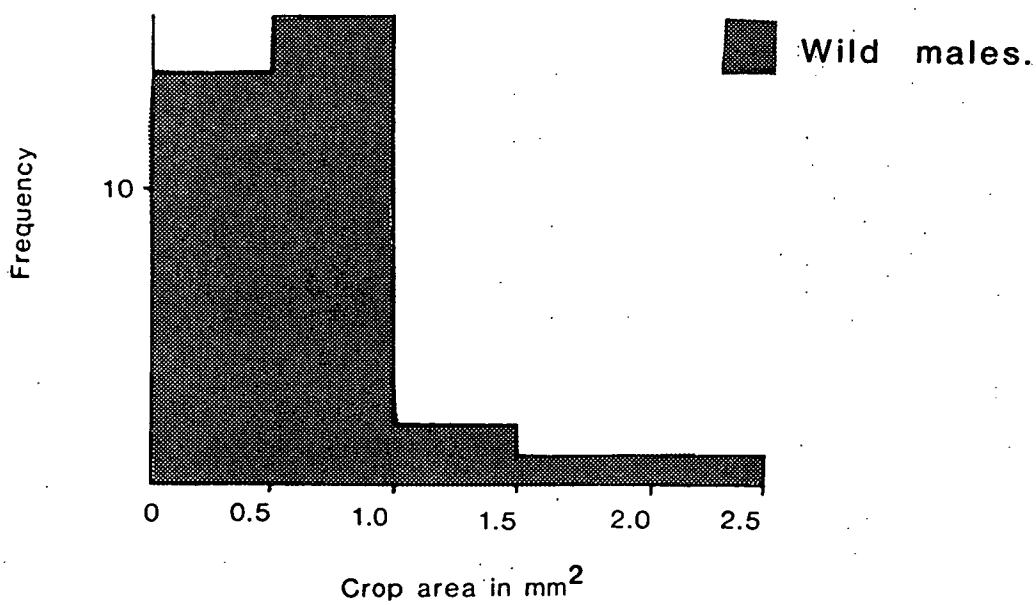
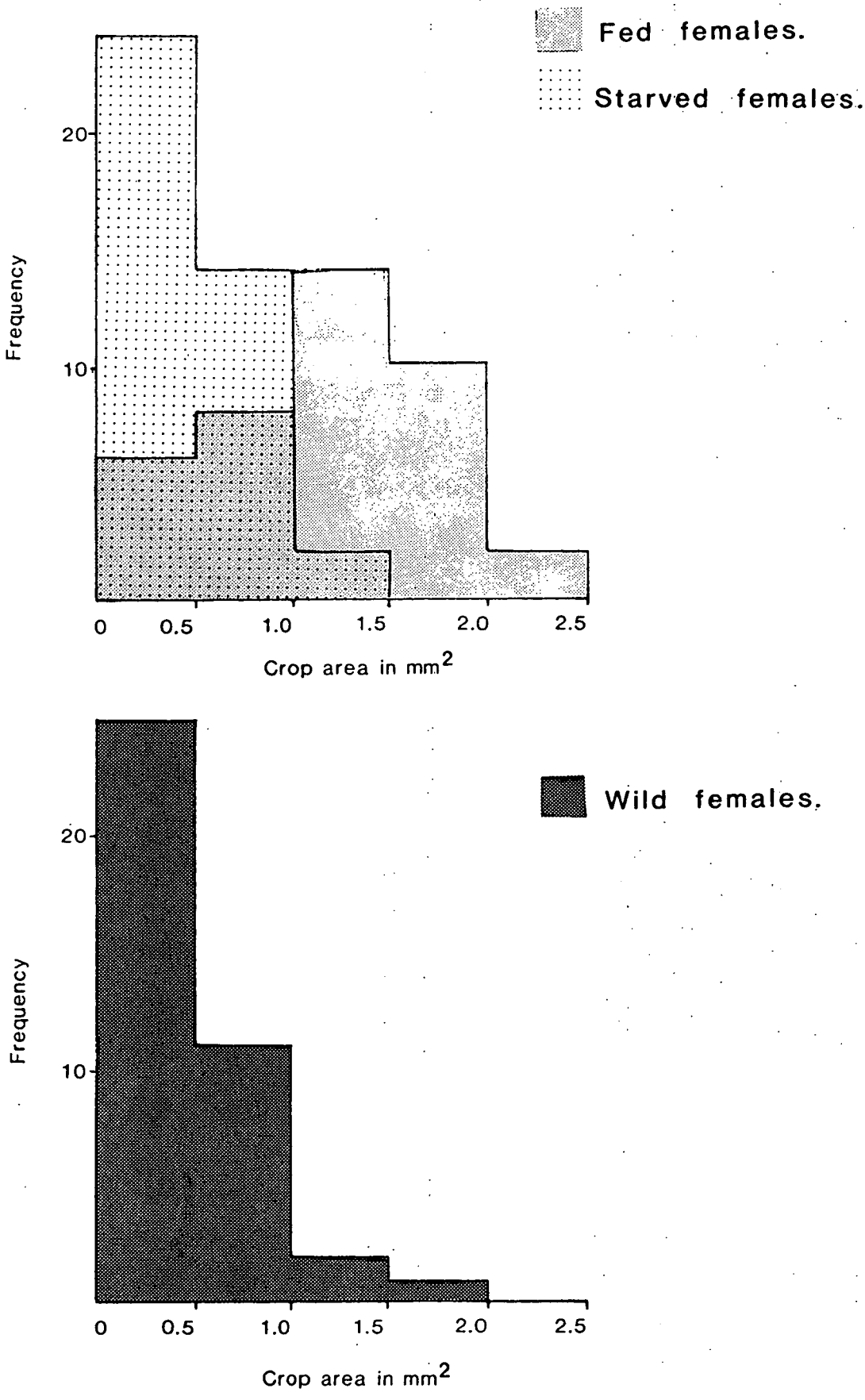


Figure 3.3. Female crop area.



males and females have crop sizes more closely resembling the starved than the fed groups.

Within the groups wing length was significantly correlated with crop area for the three male groups and for the wild females, but not for the laboratory fed or starved females (table 3.1). The fitted regression lines of crop area on wing length for the three male groups are plotted in figure 3.4 with the wild female fitted line for comparison. This plot, taking into account any between group differences in wing length, again reveals that the wild males resemble the starved laboratory males.

3.3.3. Courtship feeding in other Drosophila species.

3.3.3.1. The obscura species group.

In five of the ten D.pseudoobscura courtships observed stained yeast cells were recorded from the female crop after courtship. Observation revealed that D.pseudoobscura males regurgitate part of their crop contents onto the substrate directly or diagonally in front of the female. The pool produced may be quite large and take several seconds to secrete. If the female steps forward and feeds from the pool the male circles and attempts to copulate. If she moves away the male may consume the food and then follow. The food seems to be produced later in the courtship after a series of bouts of vibration have proved unsuccessful. In the few courthips observed the female did not take the drop directly from the male's proboscis. On one occasion a male produced a cloacal drop.

TABLE 3.1

Comparisons between groups of laboratory and wild flies of crop area (median test) and wing length (t-test).

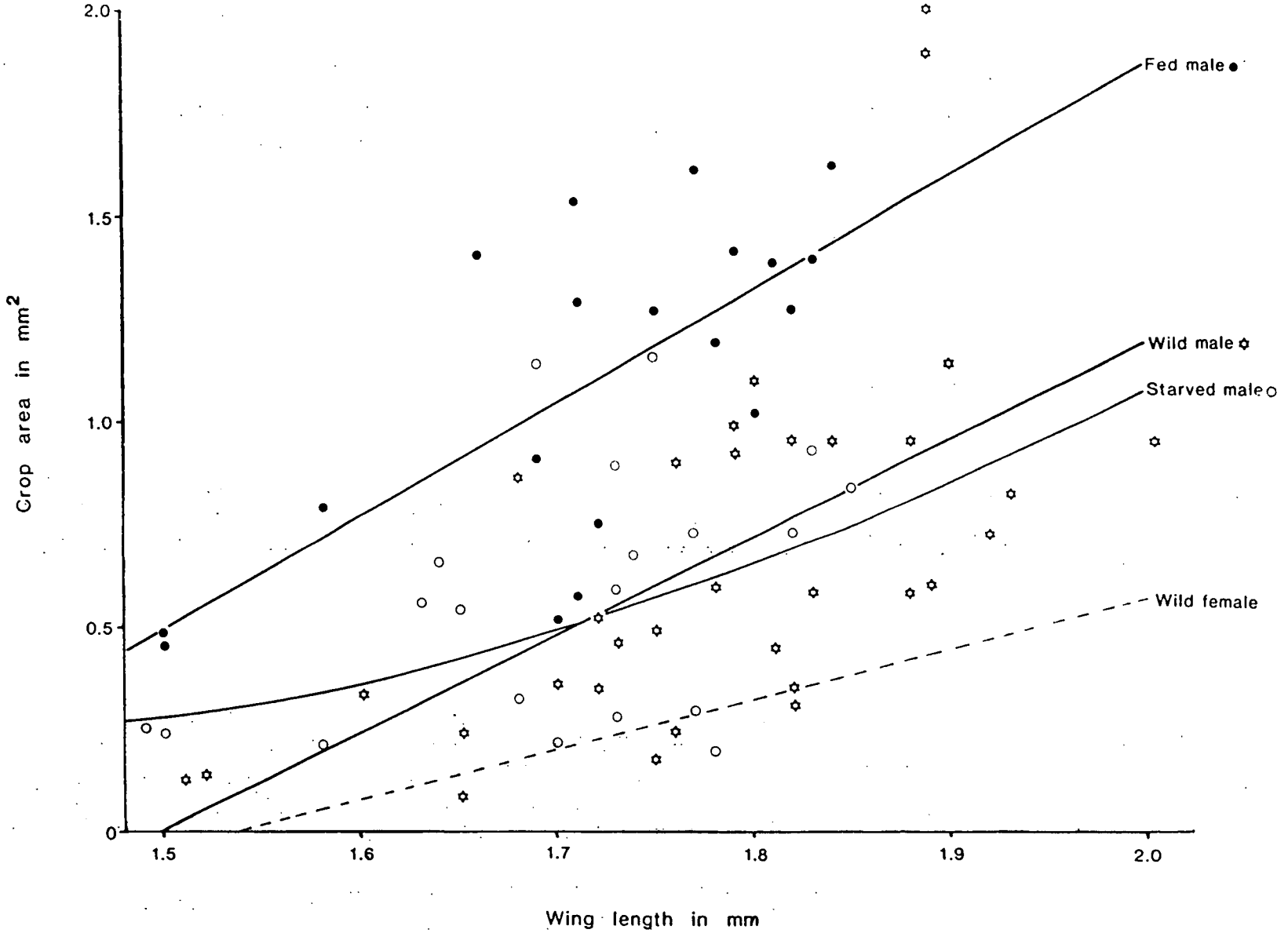
Sex	Group	N	Median crop area mm^2		N	Mean wing length mm	
Male	Fed	39	1.27		19	1.72	
	Fxst		χ^2=5.80	p<0.02		t=0.56	p=0.58
	Starved	40	0.56		20	1.70	
	StxW		χ^2=0.00	p>0.1		t=2.70	p=0.01
	Wild	33	0.59		33	1.78	
	FexW		χ^2=5.31	p<0.05		t=1.98	p=0.054
Female	Fed	40	1.16		20	1.93	
	FexSt		χ^2=12.1	p<0.001		t=0.76	p=0.45
	Starved	40	0.44		20	1.95	
	STxW		χ^2=0.01	p>0.1		t=0.96	p=0.34
	Wild	40	0.44		40	1.97	
	FexW		χ^2=8.02	p<0.01		t=1.32	p=0.20

The relationship between wing length and crop area

Sex	Group	N	Pearson Correlation coefficient
Male	Fed	19	0.6993***
	Starved	20	0.4600*
	Wild	33	0.5976***
Female	Fed	20	-0.2651
	Starved	20	0.0318
	Wild	40	0.3707*

*p<0.05 **p<0.01 ***p<0.001

Figure 3.4. Crop area is plotted against wing length for the fed, starved and wild male groups. The filled circles represent fed males, the open circles represent starved males and the stars represent the wild males. The fitted regression lines are also drawn along with the fitted regression line for the wild females for comparison. The equations for the regression lines are: fed male, $y = 2.75x - 3.63$; starved male, $y = 0.004x^{2.9}$; wild male, $y = 2.41x - 3.61$; wild female, $y = 1.27x - 1.97$.


2.0
1.5
1.0
0.5
0
Crop area in mm²
1.5
1.6
1.7
1.8
1.9
2.0
Wing length in mm
Fed male
Wild male
Starved male
Wild female

A drop was produced in three of the ten D.obscura courtships observed and was held on the male's proboscis before being taken directly from his proboscis by the female and consumed. Stain was recorded from the male and female crops.

Although no other obscura group species were tested the descriptions of fluid exuding from the male proboscis during courtship for several other species (WEIDMANN 1950, BROWN 1965) suggest that courtship feeding may well be widespread within the obscura species group.

3.3.3.2. Other species groups.

Male D.nebulosa were observed to curl the tip of their abdomens towards the female's head during courtship and pulsate an anal drop (figure 3.5). They also waved the wing behind the drop in a manner that suggested that the male might be directing odours from the drop towards the female. The drop was coloured indicating that it was at least partly made up of the stained gut contents. In a very rapid movement the drop appeared to enlarge just before being deposited by the male on the substrate in front of the female prior to circling and attempted copulation. In two of the ten courtships observed the female stepped forward and took the drop off the substrate using her proboscis. In one of these cases stain was found in the female crop but was much less obvious than the stain in the crops of the obscura group species. It was also noticeable that the production of the anal drop by the male during the abdomen curling and wing waving sometimes elicited a feeding response (mopping movements of the proboscis against the substrate) in the female.

Figure 3.5. The photographs show male D.nebulosa curling their abdomen, with a drop extruded from the tip, towards the female. One wing is waved above the curled abdomen and may be directing odours from the drop towards the female. Figure A is printed to a magnification of 24x and figure B to a magnification of 30x. Both photographs were taken by H.Piggins.


A


B

There was no evidence of courtship feeding in the other Drosophila species tested.

3.3.3.3. The Hawaiian species.

Table 3.2 lists the Hawaiian species for which SPIETH (1966,1978) has described frontal displays involving either the proboscis or exaggerated abdominal movements. Over half of the species observed have frontal displays including either one or other of these elements and a number of species include both. Fluid exudation by males was only observed in a few cases (discussed below). However, bearing in mind SPIETH's failure (1952) to observe fluid exuding from the proboscises of courting male D.suboscura and D.pseudoobscura, either because of stock differences or his technique of observation, further study might reveal drop production in some of the Hawaiian species. In the following account I shall summarise a number of Spieth's observations that strongly suggest courtship feeding and variations thereupon occur in a number of Hawaiian species.

In the large picture-winged adiastola sub-group SPIETH observed the courtships of 13 species (1966,1978) and described a basic pattern of display to which they all conformed. Typically, after initial display bouts performed at the rear of the female, the male may circle to the front and engage in a frontal display. One component of this display involves the male extending his proboscis towards the female's face with the labellar lobes fully opened. "He attempts, by repeated thrusts of the extended proboscis, to induce the female to extend her proboscis and bring her opened labellar

TABLE 3.2.

The species of Hawaiian Drosophila for which SPIETH (1966, 1978) has described frontal displays involving movements of the male's proboscis or exaggerated male abdominal movements.

Male proboscidean movements at side or in front of female

Picture wings	D.adiastola group - 16 species
	D.picticornis
	D.spectabilis
	I.clavisetae
Spoon tarsii	D.disticha
Modified mouthparts	D.comatifemora
	D.tendomentum
Unplaced species	D.atroscutellata

Male exaggerated abdominal movements at side or in front of female

Picture wings	D.adiastola group - 16 species
	D.crucigera
	D.engyochrocea
	D.grimshawii
	D.pilimana
	D.pilimana-like
	D.picticornis
	I.clavisetae
Spoon tarsi	D.disticha
	D.dasycnemia
	D.sordidapex
Bristle tarsi	D.perissopoda
	D.petalopeza
Modified mouthparts	D.comatifemora
	D.eurypeza
	D.ischnotrix-like
	D.tendomentum
Scaptomyza-like	D.nasalis
Unplaced species	D.achyla
	D.atroscutellata
	D.imparisetae
	D.incompleta

lobes into full contact with his. A reluctant female typically responds with pseudo-feeding movements by thrusting her proboscis against the substrate ... A receptive female will 'kiss' the male and he then circles to the rear ..." (SPIETH 1978). In D.truncipenna within the group, the labellar lobes of the male and female may remain in contact for about 60 seconds (EHRMAN 1978). Male Idiomyia clavisetae have a greatly enlarged proboscis in which the lateral width of the labellar lobes presented to the female during frontal display is as wide as the body (SPIETH 1966).

Other picture-wing species show similar proboscidean displays. In addition male D.spectabilis regularly draw their fore-tarsii between their labellar lobes and then extend and straighten the fore-legs pointing them forward with the tips directed at the female. Male D.tendomentum tap their moistened fore-tarsii on the substrate in front of the female and male D.pectinitarsus have a 'comb-like' appendage on the fore-tarsus which they regularly moisten during courtship by drawing it between the labellar lobes of the proboscis. SPIETH (1966) argued that this repeated licking of the comb indicated that either an airborne or contact chemical stimulus was being transmitted to the female. A number of species arc to and fro in front of the female (e.g. D.tendomentum). Male D.picticornis move from side to side in front of the female 'to prevent her from escaping' (SPIETH 1966). When she stops the male lowers his head and taps the end of his proboscis against the substrate before circling. No mention is made of whether any fluid is left on the substrate.

As well as performing the proboscidean movements described, males of the adiastola sub-group also curl the abdomen up into the air and pulsate an anal droplet (SPIETH 1966). In a number of

species in other groups the abdomen is curled to such an extent, either laterally, over or under the body, that the tip of the abdomen is brought over or beside the male's head or thorax. In a number of species, for instance D.disticha, the proboscis is extended and simultaneously the abdominal tip is brought towards the female's face. In I.clavisetae, whilst the male's greatly enlarged labellar lobes are presented to the female, the abdomen is curled up and over the male's head, the tip reaching a point directly above the male's eyes. The tip of the rectum is then everted to form a tube-like papilla and "... long abdominal hairs serve a fan-like function to direct labile materials derived from the rectal extrusion towards the female's sense receptors." (SPIETH 1966). The wings are held in a stationary position in such a way that they might "... conceivably serve as a baffle to prevent stray air currents from dispersing volatile substances ..."

A number of species drag the tip of their abdomen across the substrate and deposit a thin film of fluid. In D.grimshawi the thin film of moisture left is 0.5 to 1 cm long (SPIETH 1966). In cells or bottles this 'laying down of scent' in D.crucigera and D.engyochracea attracted other individuals, both males and females, and enabled the displaying male to court virgin females. Males and unreceptive females were aggressively chased away. Spieth (1966) considered that this scent deposition involved a sexual pheromone. However, it may simply have involved gut contents as appears to be the case for D.nebulosa.

3.4. CONCLUSIONS.

1) Virgin females that took a drop from the male during courtship laid more eggs over the following two days than the females denied access to the drop.

2) Wild males and females had crop sizes that resembled the starved laboratory groups.

3) Within the obscura species group courting male D.pseudoobscura and courting male D.obscura regurgitate crop contents which the females consume.

4) Within the willistoni species group courting male D.nebulosa produce an anal drop which the females consume.

5) SPIETH's courtship descriptions (1966,1978) suggest that courtship feeding occurs in a number of Hawaiian species.

3.5. DISCUSSION.

THORNHILL and ALCOCK (1983) conclude at the end of their discussion of nuptial feeding in insects (p.390) that, for the most part, the challenge of showing that females do make adaptive discriminations based on male offerings remains to be met. In the first chapter I showed that the drop production of wild-type male D.subobscura was influenced by male body size and recent starvation. The body size of wild males certainly varies as does the fullness of their crops, and the crop sizes of many wild males resemble the crop sizes of the laboratory starved males (this chapter). I also showed that laboratory wild-type females did discriminate between males on the basis of the food they offered and that the discriminating females tended to be the starved females. The crop sizes of wild females resemble this starved group closely. Finally, I have shown that females taking a drop lay more eggs when kept on a low nutrient medium than females not receiving a drop. The potential certainly exists for adaptive female choice based on male food offerings in the field, but is this choice likely to occur and, if so, how did it evolve?

Adult Drosophila are attracted to the yeasts and bacteria associated with the fermentation of decaying plant materials. Resources rich in microorganisms, such as decaying fungi, are patchily distributed in time and space and the flies must search them out as they become suitable for feeding or oviposition. Adults of most species tend to visit these sites for only short periods of time during the dawn and dusk activity periods before scattering into secluded nooks in the surrounding vegetation (SPIETH 1974). A number

of field workers have observed that there is often a scarcity of males collected in retainer traps compared to open traps (e.g. BASDEN 1954, SHORROCKS 1970,1975). Flies on and around the bait are collected using open traps whereas retainer traps only gather flies moving onto the bait. The scarcity of males in retainer traps suggests that whilst females are concentrated on the bait, males are distributed on and around the bait and field observations support this view. BEPPU and TODA (1976) found that most of the species they trapped and observed in a forest near Hokkaido in Japan showed an excess of males around the bait and an excess of females on the bait. BASDEN (1954) collecting at Dalkeith in Scotland also mentioned that "... males were resting on the outskirts of the bait" and I have observed similar distributions of male D.subobscura around natural resources at Dalkeith as well as around baits (unpublished personal data). Finally, this distribution of males around as well as on the resource appears to be most exaggerated in many of the Hawaiian species (SPIETH 1974) where males only feed for a short period before moving into the surrounding vegetation where they take up station and await approaching females. In many species females land off the resource before approaching with a series of short flights or by walking (SPIETH 1974, BEPPU and TODA 1976, unpublished personal observation). Males on the surrounding vegetation have the opportunity to intercept and court the females before they reach the bait.

If males intercept females as they approach the aggregation site, the production of a yeasty drop by the male, whether from the crop or hind-gut, could have evolved as a mechanism for stopping a hungry female as she approached the resource by eliciting a feeding

response thus giving the male a chance to court. Certainly in the case of the obscura group species the regurgitated crop contents would produce odours similar to those attracting the females to the resource. In the last chapter I showed that starved female D.subobscura were less likely to move away from males producing a drop than from those without one and SPIETH (1978) observed that the repeated thrusting of the male's proboscis towards the female in the adiastola sub-group induced a 'pseudo-feeding response' in the female. Similarly the production of an anal drop by male D.nebulosa elicits a feeding response in the female and females consume the deposited drop (this chapter). Female Drosophila can be observed consuming faecal deposits outside the courtship context and JACOBS (1978) described male D.melanogaster in an arena defending faecal deposits as well as food sources. It seems likely that some of the anal droplets of the Hawaiian species, rather than consisting of putative sexual pheromones, simply consist of gut contents emitting odours resembling those of the food source towards which the female is moving. This needs to be tested using the stained yeast technique successfully applied to D.nebulosa.

Males that produce a yeasty drop perhaps gain the time and opportunity to court a female. Females may gain nutrients for egg production more quickly than they would by feeding for themselves or perhaps without an associated risk of predation on the feeding site (SPIETH 1974). However, although it may benefit females to take the drop and males to produce a drop, a male does not necessarily benefit from giving the drop up to the female. Sexual conflict over the male's nutritional contribution may well occur (e.g. TRIVERS 1972, MAYNARD SMITH 1977,1982 and PARKER 1979) and the propensity of a male

to give up his food offering in the field will be influenced by many unknown variables such as the probability of mating if the female takes the drop and the probability of mating if she does not, and the contribution the drop makes to the number of offspring the male will father with that particular female weighed against the reduction in the number of offspring he will leave in future matings. We might expect that males under certain conditions or in certain species might find ways of producing yeasty odours without actually giving much food away (equally we would expect females to recognise and discriminate against such males). Viewed in this light the behavioural and morphological adaptations of a number of the Hawaiian drosophilids become particularily interesting if, as seems likely, it turns out that they involve the presentation of yeasty odours. For instance, the proboscidean moistening of the fore-tarsii of D.spectabilis before waving the fore-tarsii in the female's face and the elaborate combs of male D.pectinitarsus may serve to bombard the female with yeasty odours without involving the regurgitation and potential loss of food. The production of anal drops may serve the same function. Nothing is known of the relative nutritional merits of a drop from the crop or from the hind-gut but it does seem likely that giving up a faecal drop would represent less of a nutritional loss to a male. A number of the Hawaiian species extended their proboscis towards the female whilst producing an anal drop. For instance, male I.clavisetae present their enlarged proboscis to the female whilst directing odours towards her from the anal drop carried over the head (SPIETH 1966 discussed in this chapter). It would be interesting to take a species in which males produce both crop and anal drops, such as D.pseudoobscura, and examine the conditions under

which one or other drop was produced. For instance, does the type of drop produced depend on the male's nutritional status? Is the nutritional value of the two drops to the female different? Are a male's chances of gaining a mating greater for one or other type of drop production and how is this influenced by female nutritional status?

The extent to which males of different species feed females will depend upon the species' nutritional ecology. Unfortunately, little is known about the feeding habits of adult Drosophila. BEGON (1982) reviewed the available literature and concluded that whilst adults of tropical Drosophila species tended to share with larvae the same, often rich, yeast sources, adults of temperate species did not feed at breeding sites but utilised diffuse sources of yeast, such as leaf surfaces and aphid honeydew. Still less is known about the nutritional status of wild flies. Two relevant papers were mentioned in the introduction to this chapter. BEGON and SHORROCKS (1978) remarked that wild D.subobscura and D.obscura collected from a woodland near Leeds had very little food in their crops (also this chapter). BOULETREAU (1978) assessed the reproductive condition of wild female D.melanogaster collected in the south of France during the summer and autumn and found that there was little active vitellogenesis in the wild females probably because they were short of food.

In an environment in which food is scarce the nutrition derived from males might be particularly important to females and consequently they might remate frequently in order to obtain more nutrients. However, if females are remating often and hence female availability and sperm competition are high, selection may favour

males who invest little in a single mating but mate with as many females as possible. MARKOW and ANKNEY (1984) argue that for female D.mojavensis in the Sonoran desert, resources may be in short supply and females remate daily in order to gain nutrients from males. However, they also found that males mating seven females within the space of two hours suffered no reduction in fertility and so appeared to be transferring little ejaculate at each mating. It seems likely that nutrient transfer by males at mating will be described for other species throughout the genus and the variations observed, along with further work on the species described in this thesis and observations on adult nutritional ecology, should lead to a greater understanding of the evolution of courtship feeding in Drosophila.

Chapter 4

MALE SIZE AND AGILITY.

4.1. INTRODUCTION.

In many species large males are more successful at obtaining matings than small males (see DAY, MILES, PILKINGTON and BUTLIN in press for some recent references). In some cases their success in direct competition for females might be due to their skill in combat, in others it might be because they are better able to attract and court females, or it may be a combination of the two. For example, PARTRIDGE and FARQUHAR (1983) found that large male D.melanogaster were more successful at gaining matings under competitive conditions in the laboratory. The mating advantage of the larger males was probably partly attributable to their success in aggressive encounters with small males and partly to their superiority in courtship when assessed in a single pair situation. Song production (BENNET-CLARK and EWING 1967) and wing area (EWING 1964) are known to contribute to male courtship success in D.melanogaster and larger males have larger wings and sing more often (PARTRIDGE, EWING and CHANDLER unpublished).

It is not surprising that large males are more likely to win fights nor is it surprising that they present a greater stimulus to the female during courtship. However, there are a few cases where body size is an important component of male mating success but it is not the largest males who are the most successful. When mating pairs and non-mating individuals of the milkweed beetle, Tetraopes tetraophalmus, were collected in the field, although the mean size of

the maters and non-maters was the same (for both males and females), the variance about the mean was significantly smaller for the mating individuals (MASON 1964, SCHIERING 1977). The reasons why males and females of intermediate size were most likely to be mating in the field were not known.

Sometimes it is the small males that are the most successful at gaining matings. In moorhens, Gallinula chloropus, males do most of the incubation and the body condition of the males at the start of the breeding season is an important determinant of their ability to incubate for long periods. The males in best condition are the fattest males who also tend to be the smallest. Females compete for the opportunity to pair with these small, fat males (PETRIE 1983).

Similarly, it is the smallest male seaweed flies, Coelopa frigida, that have the greatest mating success when field conditions are simulated (DAY, MILES, PILKINGTON and BUTLIN in press), even though larger males sire most of the progeny in laboratory 'choice' experiments (BUTLIN, READ and DAY 1982). The reasons for this reversal in mating advantage are unknown.

In chapter 2 I showed that larger males had greater courtship success with starved females and this was probably because they produced larger drops of food during courtship. If all males were prevented from producing drops then the small males had greater courtship success than the large males. These findings suggested that small males, although inferior when it comes to offering food, perform some other aspect of courtship better than large males. In this chapter I shall test the hypothesis that small males are better able to keep up with the female during courtship, particularly during the dance, because they can change their direction of movement more

quickly than large males. First I shall present the theoretical reasons why this should be so.

The theory.

If a body of mass m is accelerated from rest then its velocity v after time t is simply;

$v = a \cdot t$ where a is a constant acceleration.

But $a = \frac{F}{m}$ where F is a constant force;

therefore $v = \frac{F}{m} \cdot t$

and so $t = v \cdot \frac{m}{F}$.

If we assume that the shape of animals of different sizes remains the same (isometry) then

because m is proportional to L^3 where L = length

and F is proportional to L^2 (the cross-sectional area of the muscles involved)

then t is proportional to $\frac{L^3}{L^2}$ if v is constant.

Therefore t is proportional to L

or t is proportional to $m^{0.33}$.

So larger animals take longer to reach a given velocity. Similarly, when a body of mass m travelling at velocity v is brought to rest the work done in stopping it is;

$$\text{Work done} = \frac{1}{2} m \cdot v^2 .$$

But Work done = F . D where F = a constant force and D = stopping distance;

therefore $F \cdot D = \frac{1}{2} m \cdot v^2$.

Because m is proportional to L^3

and F is proportional to L^2 ,

then in isometric animals travelling at the same velocity

L^2. D is proportional to L^3 ;

so D is proportional to L

or D is proportional to $m^{0.33}$.

For any given speed heavier animals will travel a greater distance before stopping.

So if the various assumptions of the model are met larger males should take longer to accelerate to and decelerate from any given speed. Simple biomechanics also predicts that the top speed of isometric animals is independent of their body size (HILL 1950, MCNEILL ALEXANDER 1971). Therefore, if male D.subobscura are running at top speed during the dance then males of different sizes should be travelling at the same speed. When the dancing female reverses her direction of movement larger males will take longer to slow down from this speed and so will be more likely to 'overshoot' the female and therefore more likely to lag behind her than smaller males. In this chapter I use film of males courting both females and a model female to measure the top speeds of dancing males of different sizes and the degree to which they lag behind the female during the dance. The model female is used to control for any possible differences in female behaviour towards males of different sizes.

4.2. MATERIALS AND METHODS.

4.2.1. Courtship observation and filming.

Virgin five day-old males and virgin three day-old females aged separately on standard drosophila medium seeded with active Baker's yeast were used for all the experiments. Using young females ensured that a high proportion had low receptivity and meant long courtships could be observed.

In the first experiment the courtship of single pairs was filmed in a shallow perspex cell. The cell was 3mm deep and the circular area available to the flies had a diameter of 3cm. A sheet of clear perspex covered the cell and a Super 8 cine camera with magnifying lenses was mounted vertically above it. A combination of transmitted and incident light ensured that the flies' silhouettes were readily visible during the film analysis. The film used was a Kodak black and white surveillance film (MFA 464, ASA 200) and 18 frames were taken each second. The evening before filming the males were anaesthetised and the weight of each recorded.

In the second experiment individual males were filmed courting a 'model female'. The 'model' was a female glued by her thorax to a length of thin but rigid tungsten wire. The legs and wings of the anaesthetised female had been removed to prevent her from interfering with the free movement of the wire. The observation chamber described above was used and the perspex cover was raised slightly to allow free movement of the wire bearing the female. The female was slung under the wire in such a way that she was just off the floor of the observation chamber when the wire was moved from side to side.

The movement of the wire was driven by an electric drill and figure 4.1 shows how the circular motion of the drill was translated into a sideways movement of the wire bearing the female. The diagram is explained in the figure legend. The distance travelled by the model in one sideways movement was 0.75 cm and there were two movements each second (54 cycles per minute). These values for the speed and distance of the model's movement were based on observations of females being courted during the first experiment. The electrobalance for weighing flies was unavailable at the time of this second experiment and so the wing length of each male was measured after courtship. Wing length is highly correlated with weight in D.subobscura (CHARLESWORTH 1978).

4.2.2. Film analysis.

A Super 8 film projector and a silvered glass mirror were used to project the film onto the underside of a glass table. Images of the courting flies were clearly visible on a piece of tracing paper laid on the table and could be copied. For each male measures of his maximum angular lag and top speed during the dance were recorded.

The angular lag was measured as the angle between the line joining the centres of gravity of the male and female and the longitudinal axis of the female (θ in figure 4.2). Clearly the angle between the longitudinal axes of the male and female depends upon the rotation of the male about his centre of gravity and is less useful as an indicator of the distance, w, males must move their centre of gravity to achieve the frontal position (figure 4.2). A fly's centre of gravity was estimated from the positions of the feet of a standing

Figure 4.1. The diagram illustrates how the model female was driven. The filled circles represent pivots. An electric drill turned the pulley wheel A which drove the pulley wheel B. The circular motion of the connecting rod C was translated by the universal joint D into a lateral movement of the sliding rod E within the holding slots (hatched bars). The sliding rod E pushed the lever pivoting about F resulting in a sideways movement of the female glued to the piece of wire in the observation cell G.

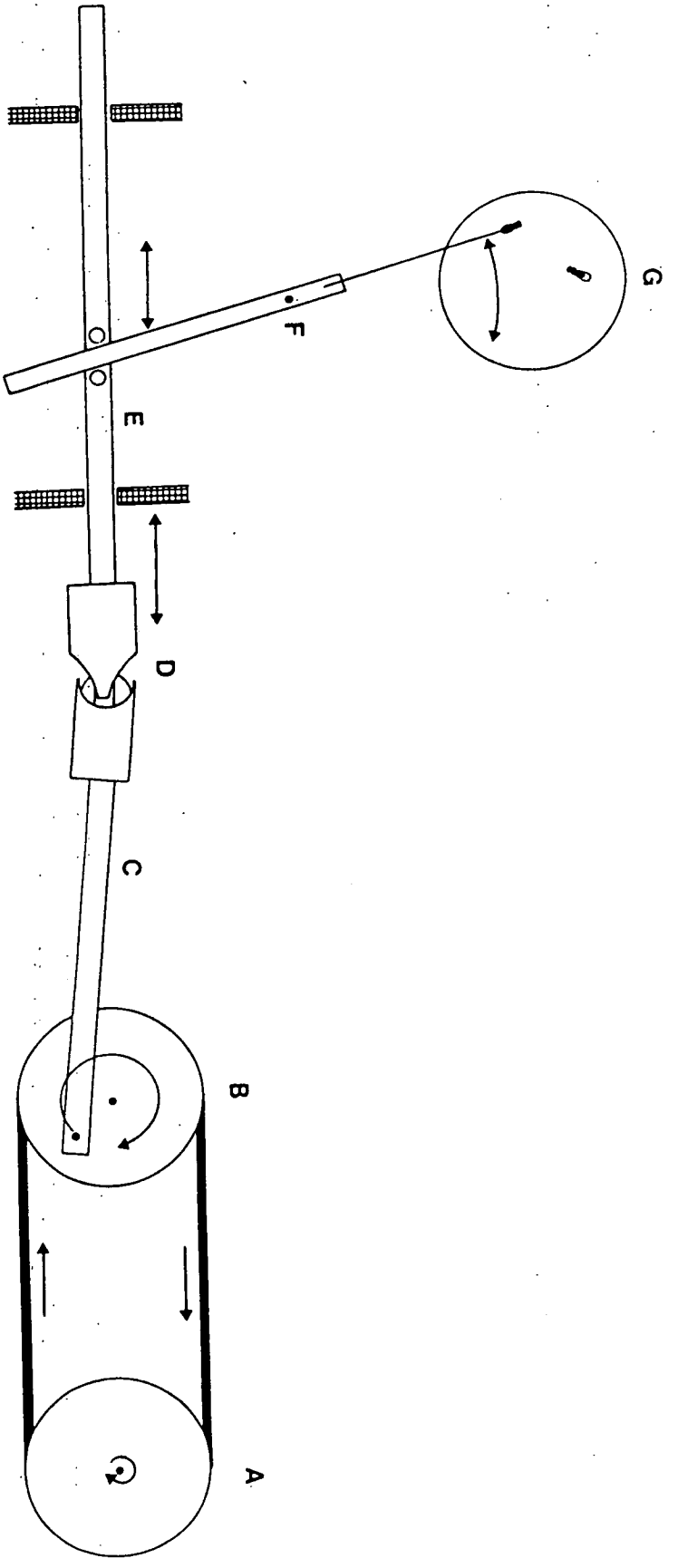
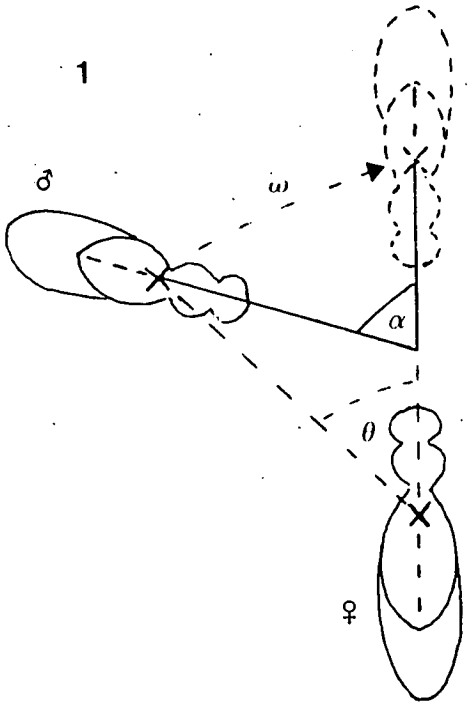

G
F
E
D
C
B
A

Figure 4.2. The diagram shows the angle, θ, used to measure the extent to which the male lagged behind the female during the dance. The crosses mark the centre of gravity of each fly and the dashed silhouette is the position the male is moving towards. w is the distance the male must move his centre of gravity to reach the frontal position. The angle subtended by the longitudinal axes of the two flies depends upon the rotation of the male about his centre of gravity (a and b in 1 and 3).


1
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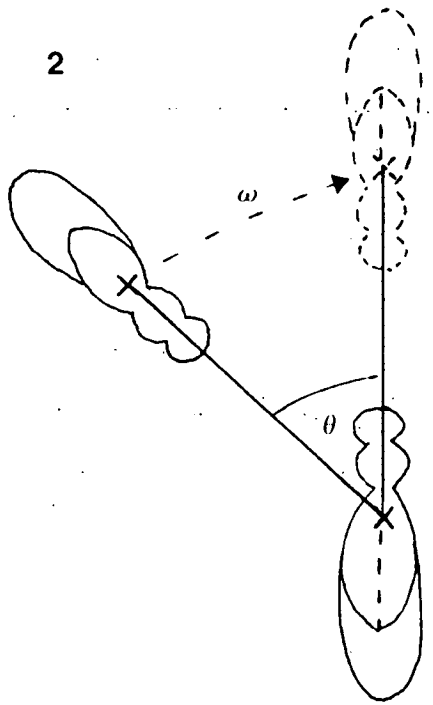
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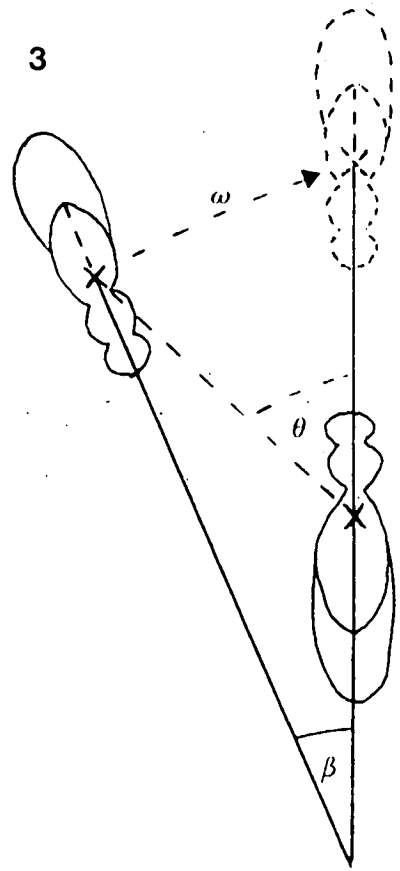

3
ω
θ
β

fly. The maximum angular lag was simply the largest value of θ occuring during a single dance movement where a dance movement was defined as a sideways movement of the female (with the male in attendance) bracketed at the beginning and end by a reversal in direction. The angular lag then becomes a measure of the 'overshooting' of the male incorporating both a deceleration of the male at the end of the previous movement and an acceleration at the beginning of the present movement. For each male the maximum angular lag was measured for five dance movements and the median value was used in the analysis.

The velocity of a male could be estimated from the distance his centre of gravity moved between successive frames and the top speed of the male was simply recorded as the greatest velocity achieved during a single dance movement. The top speed of each male was recorded in five dance movements and the median value was used in the analysis.

4.3. RESULTS.

4.3.1. The dances using normal females.

Figure 4.3 is copied from a tracing made of the later stages of a dance sequence. It shows that the position of the male relative to the female changes as the female moves and the male tracks her (this will become clearer later on). The male stayed fairly close to the frontal position and in frame 24 the female extended her proboscis towards the male, the male then performed the wing-display and in frame 36 the male left the female with a drop on her proboscis and circled. Figure 4.4 is again a copy of tracings taken from the later stages of a dance involving a different male and female. The male stayed close to the frontal position until frame 16. Between frames 13 and 16 the female reversed her direction of movement and the tracking male overshot. From this stage on the male failed to get back in front of the female and in frame 30 she has moved away and he is stationary. The two figures illustrate the importance to male D.subobscura of being able to position themselves in front of the female to prevent her moving away and give themselves the opportunity to perform the behaviours preceding circling and attempted copulation (see general courtship description).

In figures 4.5 and 4.6 the longitudinal axes of the male and female have been plotted for successive frames of two dance sequences involving two different pairs. The positions of the male and female in a particular frame are joined by a dotted line. When the female reversed her direction of movement a fresh series of plots was started below the old series. The pair drawn in figure 4.5 continued

Figures 4.3 and 4.4. The tracings are taken from a Super 8 film of two courtship sequences; one ends in circling (4.3) and in the other the female moves away (4.4). The numbers are the frame numbers in the sequence and the male's body is shaded black for identification. The cross in each frame is a fixed point of reference. The film speed was 18 frames a second.

Figure 4.3.

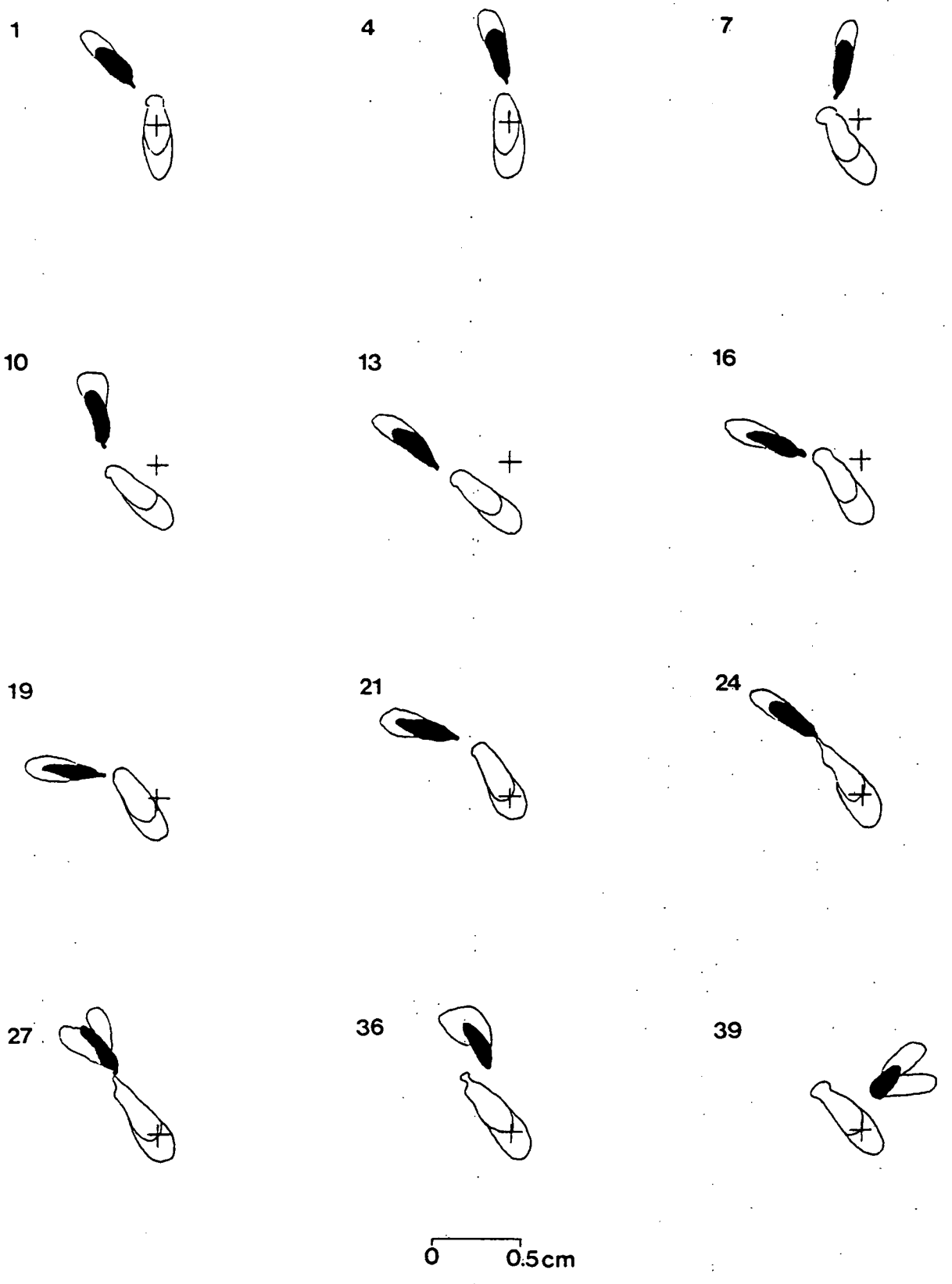
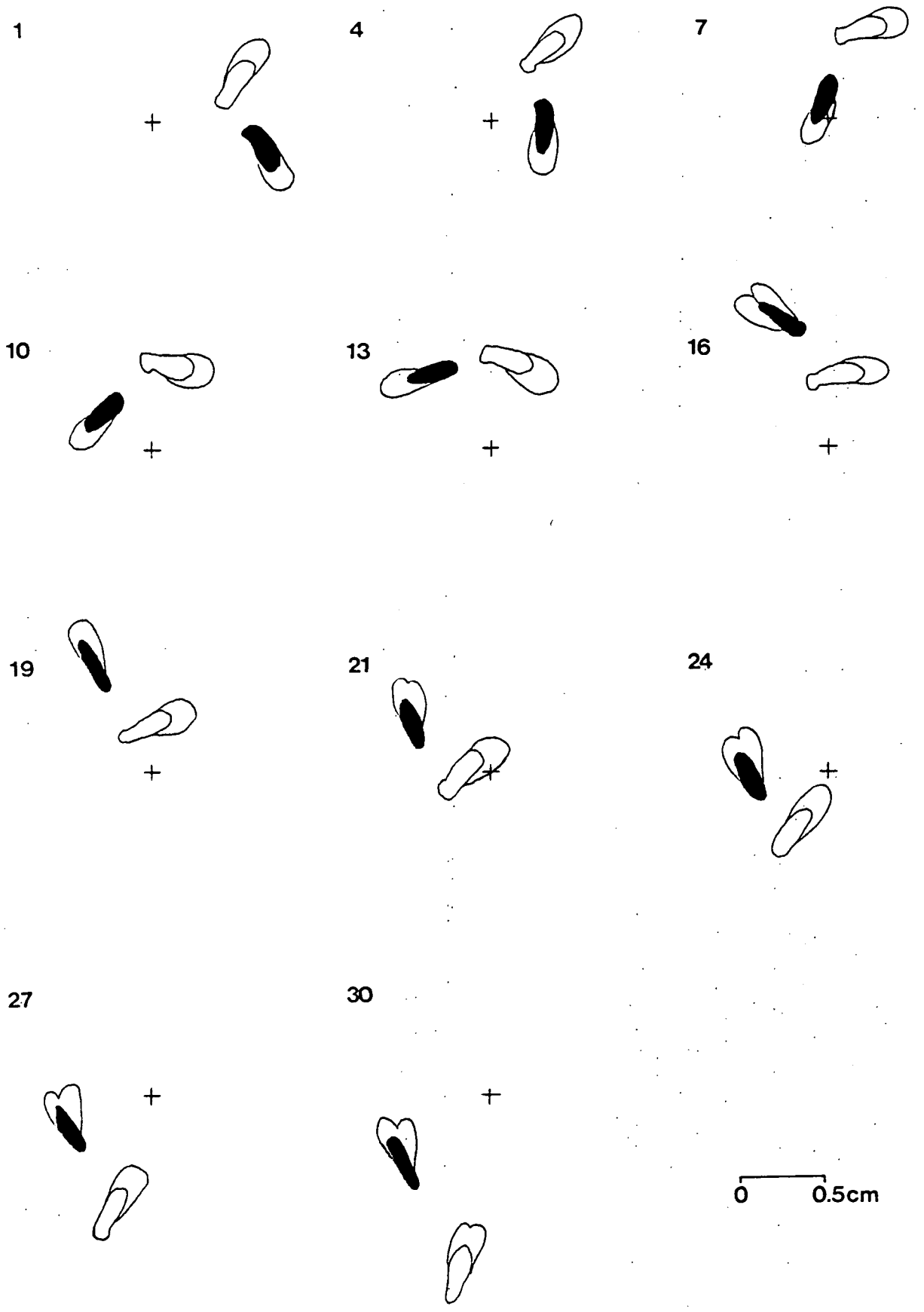


Figure 4.4.



Figures 4.5 and 4.6. The tracings are taken from a Super 8 film and show the positions of two pairs during a dance sequence. The longitudinal axis of the body of each fly is drawn for successive frames and the positions of the male and female within a frame are joined by a dashed line. The numbers are frame numbers and the arrows indicate the direction of movement of the female. A new series is drawn under the old series each time the female reversed her direction of movement. The film speed was 18 frames a second.

Figure 4.5.

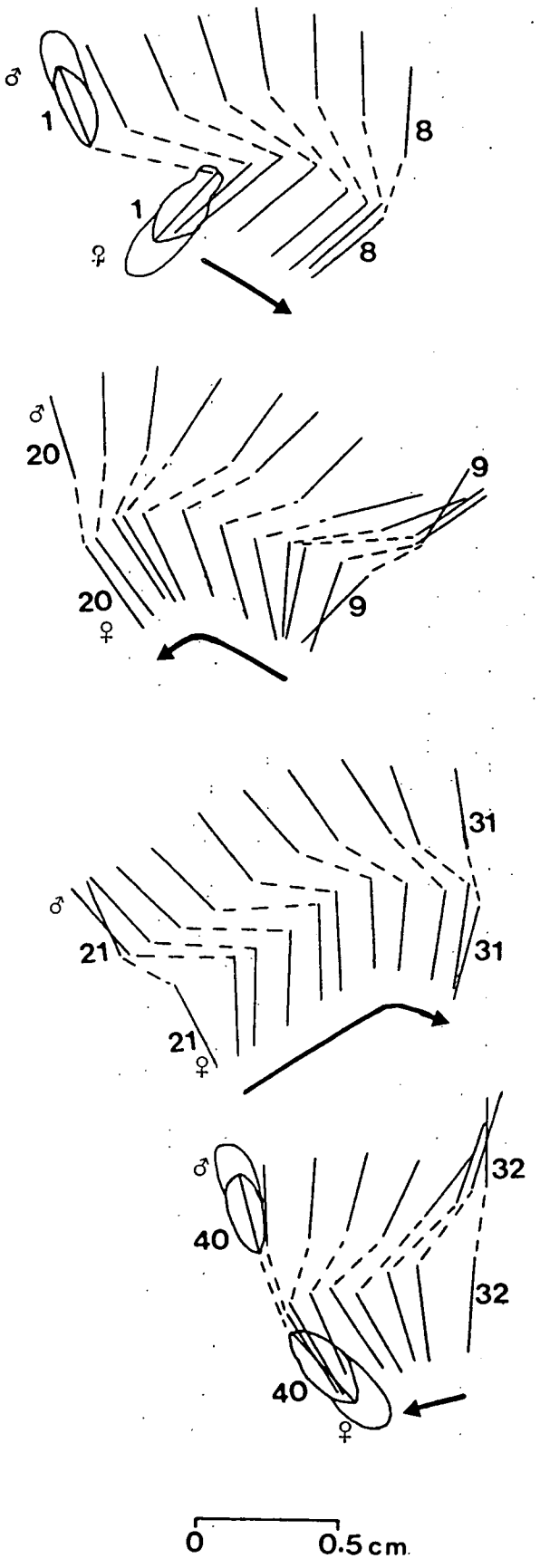
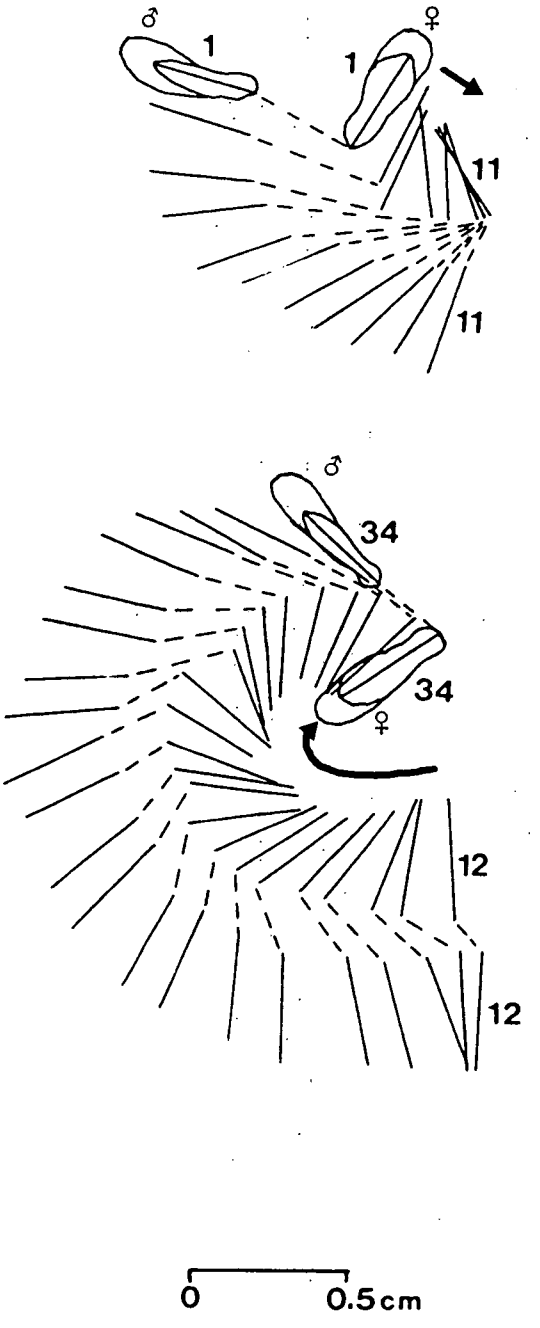


Figure 4.6.

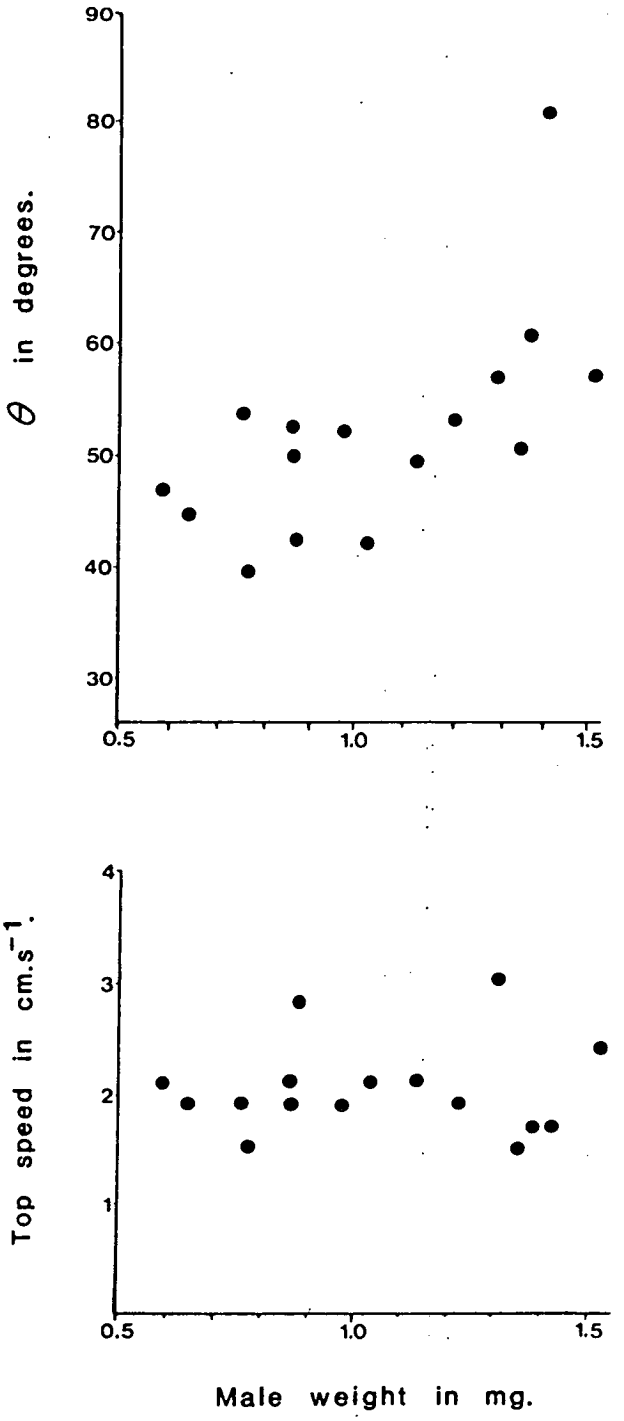


dancing briefly after frame 40 before the female stopped and the male performed the wing display and circled. The female in figure 4.6 continued to move away from the male and in frame 38 the male stopped following her.

Both figures show that the male really is tracking the female and moving towards the frontal position rather than anticipating any female movement. As the female moves she appears to 'drag' the male around with her. As the female moves forwards the male moves backwards and as the female moves backwards the male moves forwards. Both figures and figure 4.6 in particular illustrate that by turning away from the male as she moves the female can force the male to describe a wide arc in his attempts to keep up. Consequently, a female might be travelling much more slowly than the male and so be able to reverse her direction of movement faster.

In figure 4.7 the median maximum angular lag and median top speed for each male are plotted against body weight. The correlation between body weight and median maximum angular lag of 0.6340 is significant at $p<0.01$ but there was no correlation between top speed and body weight ($r=0.0637$, $p>0.1$). That larger males lagged behind the female more than the smaller males yet ran at the same top speed suggests that these top speeds really do represent the maximum velocity which males can achieve during the dance rather than lesser velocities determined by the movements of the female. As theory predicts males of different sizes run at the same top speed when tracking the female during the dance but larger males lag further behind.

Figure 4.7. The influence of male body size on the maximum angular lag (θ) and male top running speed during the dance, males with females.



4.3.2. The dances using the model.

Figure 4.8 is copied from tracings of consecutive frames of a dance sequence in which a male was tracking the model. This particular male was carrying a drop as he danced. Once males were attracted to the model they would typically dance persistently and the dance durations were much longer than those with normal females. This adds weight to the argument that it is the female that determines the course and duration of the dance. It was often laborious and time consuming to get the male interested in the model and a number of males failed to court at all.

In figure 4.9 the median maximum angular lag and top speed of males dancing with the model are plotted against wing length for each male. As before there was no correlation between top speed and body size ($r=0.0160$, $p>0.1$) but there was a significant correlation between median maximum angular lag and wing length ($r=0.7237$, $p<0.01$). The large males lag behind the model more than the small males.

It is possible that the lag observed in these experiments is a result of differences between males in the time it takes them to react to the female slowing down or stopping. There was no evidence of this in the present study but by filming at a faster speed it should be possible to make accurate measurements of the time taken by large and small males to accelerate to or decelerate from particular speeds. The 18 frames per second used in this study was insufficiently fast to enable these measurements to be made.

Figure 4.8. The tracings are taken from a Super 8 film of a male courting a model female. The male (body shaded black) is carrying a drop. The female is glued to a piece of wire. The numbers are the frame numbers and the bar in each frame is a fixed point of reference. The film speed was 18 frames a second.

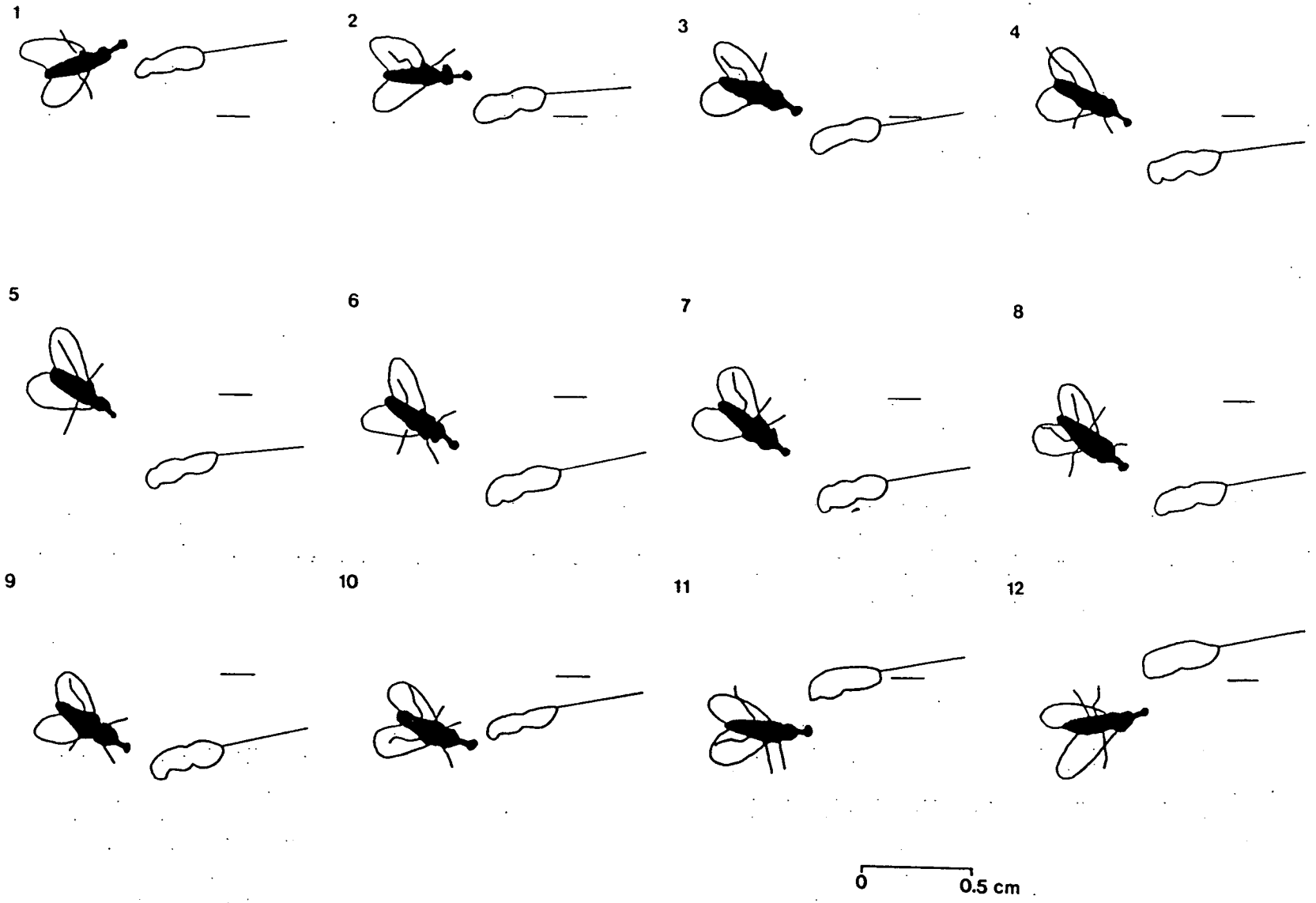
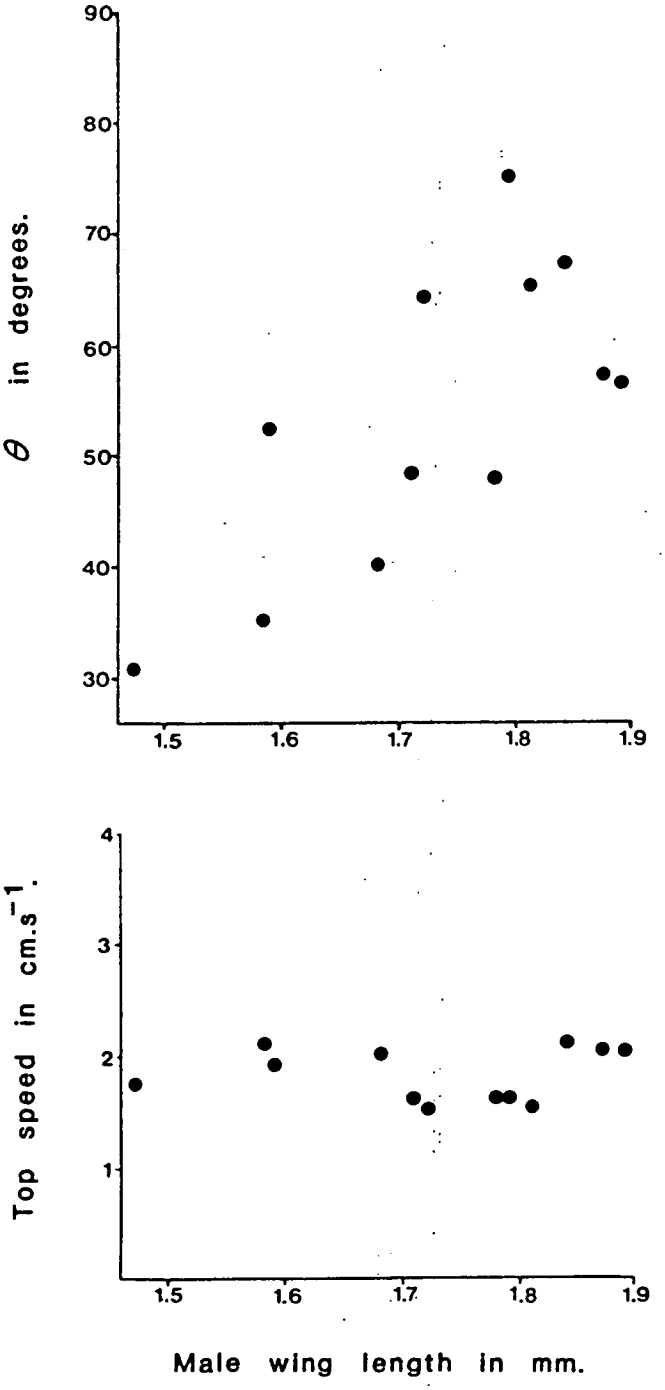

1
2
3
4
5
6
7
8
9
10
11
12
0
0.5 cm

Figure 4.9. The influence of male body size on the maximum angular lag (θ) and male top running speed during the dance; males with a model female.



4.4. DISCUSSION.

The observations reported in this chapter fit the predictions of the simple biomechanical model outlined in the introduction. The fastest speed at which males run during the dance is independent of their body size but larger males tend to lag further behind the female presumably because they take longer to accelerate to or decelerate from a given speed. It appears that small males are more agile than large males. However, a male's ability to track a moving female depends not only on his agility but also on the extent to which the female moves away from him. The greater inertia of larger males is compensated by their ability to carry larger drops of food during courtship making it less likely that the female will turn or move away from them (chapter 2). The relative importance of agility or drop production to male courtship success in the field will, in part, depend upon the nutritional state of wild flies (chapter 3). Males with empty crops will be unable to produce a drop and their agility may be particularly important. If females are starved then the emphasis might be on a male's ability to produce attractive drops. It seems to be the case that in D.subobscura larger males might have more to offer females but smaller males are better equipped to present what they have.

Although in this study male tracking ability was measured during the dance, the same arguments are applicable to more general cases of males tracking females. Most studies of Drosophila courtship use single courting pairs confined in a small chamber which restricts the flies' movements and makes detailed observation easier (e.g. BASTOCK and MANNING 1955). Under such conditions females are

unable to get away from males and any influence of male tracking ability on courtship success is greatly reduced if not eliminated. If groups of flies are observed in larger chambers where movement is less restricted then male D.melanogaster spend a large proportion of their time following or orienting on the female and courtship bouts are frequently short and are usually terminated by the female moving or flying off (EWING and EWING 1984). Female 'moving off' was distinguished from male following when the male seemed unable to keep up with the female and stopped pursuing her (L.EWING personal communication). In the EWINGS' study the male most likely to eventually copulate with a female was simply the male that had spent most time courting that particular female. Clearly if certain males were better at tracking the females as they moved these males would tend to be involved in more courtship with that female and so would have a greater chance of mating. It seems likely that male tracking ability will be important in many Drosophila species where females are approached and chased by males as they arrive at aggregation sites.

Male agility depends not only on body size but also on other factors, such as muscular efficiency, which together make up what MAYNARD SMITH (1956) has termed 'athletic ability'. MAYNARD SMITH (1956) found that the males from an inbred line of D.subobscura had lower mating success than the males from an outbred line apparently because the inbred males were unable to keep up with the female during the dance. The females mating with the inbred males left fewer progeny than those females mating with the outbred males and MAYNARD SMITH suggested that, if there was a sufficient correlation between the athletic ability and fertility of wild males, selection

would favour females who used the dance to discriminate between males. Certainly the turning of the female during the dance and her reversals of direction of movement seem ideally suited to 'testing' a male's ability to keep up. In the next three chapters I examine variation in wild-type male fertility and its influence on male courtship success and female reproductive success.

Chapter 5.

MALE FERTILITY AND FEMALE REPRODUCTIVE SUCCESS.

5.1. INTRODUCTION.

Earlier in this thesis I reviewed examples of insect species in which males provide females with nutrients at mating (Chapter 2). In a number of cases it was apparent that the nutrients given to the female by the male were used in oogenesis and increased female fecundity. Many authors argue that it would benefit females to mate with males providing more or better quality nutrients as this would be likely to enhance the females' reproductive success. By the same argument, females should benefit by mating with males who enable them to avoid wasting nutrients. This could be achieved by mating with males of higher fertility, thereby ensuring either that fewer sterile eggs are laid or that females need to remate less often and so incur fewer of the costs associated with mating, such as the time involved and possibly an increased risk of predation. In a widely quoted paper (TRIVERS 1972) three theoretical criteria for female choice of males were:

1). Ability to fertilise eggs.

2). Quality of genes.

3). Quality of parental care.

Whilst the quality of genes and parental care have received considerable attention in theoretical treatments of female choice, the importance of a male's ability to fertilise eggs (beyond ensuring that the partner is the right species, sex and sexually mature) has been largely neglected, with a few notable exceptions (e.g. MAYNARD

SMITH 1956).

One example where the fertility criterion might be important is in bird leks. Males of a number of bird species display in communal arenas or leks. Females arrive to be mated before departing to lay a clutch of eggs and raise a brood. There is no paternal investment beyond the male's sperm (so the argument goes) and, therefore, if females are choosing between males any benefit obtained must be purely genetic (for example, see BRADBURY and GIBSON 1983, but also AVERY 1984 and PARTRIDGE and HALLIDAY 1984). However, the quality and quantity of sperm transferred by a male does influence the likelihood of fertilisation and embryonic death (see AVERY 1984 for bird references). Variation in male fertility has been reported for a number of bird species, including some lekking birds, and is often correlated with male age (e.g. ENG 1963). In many lekking species the successful males are often the older males and so potentially the most fertile (AVERY 1984). Females mating at leks may be mating with the males of highest fertility.

In some lekking species the males that court most vigorously obtain the most matings (PAYNE and PAYNE 1977). As part of his 'sexual competence' hypothesis TRIVERS (1972) suggested that a male's sperm supply should be correlated with his courtship vigour and that, if this was the case, selection should favour females that were aroused by vigorous courtship. This idea was not a new one. I have already mentioned (chapter 4) MAYNARD SMITH's 1956 study in which he found that female D.subobscura mated to hybrid males obtained by crossing two inbred lines had a higher proportion of eggs hatching than the females mated to inbred males. The low fertility inbred males had low mating sucess, apparently because they were unable to

keep up with dancing females. MAYNARD SMITH suggested that if there was a sufficient correlation between the fertility and athletic ability of wild males selection would favour females who used the dance to discriminate between males.

It is difficult to demonstrate in a natural population that females choose males of higher fertility and have a greater proportion of their eggs fertilised as a result. However, there are a few studies that bear on the problem. When given a choice between recently spawned males of low fertilty and unspawned males, female lemon tetra Hyphessobrycon pulchripinnis spend a greater proportion of their time near the unspawned males (NAKATSURU and KRAMER 1982). Females had witnessed the preceding spawning events and there were no apparent differences in the appearance or behaviour of the spawned compared to the unspawned males. GIBSON and JEWELL (1982) tested if ewes given a choice of tethered rams preferred to mate with the rams with higher quality semen. There was no evidence of any relationship between a male's courtship vigour and his semen quality, and females did not mate preferentially with particular males. GIBSON and JEWELL argued that in sheep, and perhaps other ungulates, repeated mating might serve to ensure fertilisation.

Two studies support the idea that courtship vigour might be related to sperm supply. HALLIDAY (1976) has shown that the rates at which a male newt, Triturus vulgaris , performs various courtship elements during the static display phase of a sexual encounter are correlated with the number of spermatophores produced during the encounter. In turn, females are more likely to pick up the spermatophores deposited later in the succession. However, it is not known whether selective picking up of later spermatophores has any

fertilisation benefit for females. Similarily, RUTOWSKI (1979) has shown that there is a correlation between the courtship persistence and the size of the spermatophore subsequently transferred in male checkered white butterflies Pieris protodice. It is not known whether more persistent courters have a greater chance of mating, although this seems likely, or whether females obtaining larger spermatophores fertilise a greater proportion of eggs. PRAKASH (1967) reported an association between male mating speed and fertility in D.robusta. However, in this case the association arose because the faster mating males obtained more mates and therefore left more progeny.

The number of progeny left by female D.melanogaster is known to be influenced both by the age of the males they mate with (KVELLAND 1965, LONG, MARKOW and YEAGER 1980) and by whether or not the male has recently copulated (KVELLAND 1965, MARKOW, QUAID and KERR 1978). In a competitive situation older males tend to have greater mating success (LONG et al. 1980) and virgins are more successful than recently mated males (MARKOW et al. 1978). Neither study detected any differences between the groups for a number of courtship parameters measured. Furthermore, MARKOW et al. (1978) found no difference in the courtship durations of the virgin and experienced males in a single pair situation. This suggests that male interference might be partly responsible for the effect observed in the competitive situation. A criticism can be levelled at the assessment of male fertility in these and similar studies. Unlike MAYNARD SMITH (1956) they did not count the number of eggs laid and the number hatching, but simply scored the number of progeny surviving to eclosion. Apart from possible differences in larval

viability that may occur, no account is taken of possible effects on female egg-laying rate. Factors in the seminal fluid are known to stimulate female egg production (see CHEN 1984 for review). Also, female Drosophila have recently been shown to use nutrients derived from male ejaculate for oogenesis (MARKOW and ANKNEY 1984, PARTRIDGE personal communication). Younger or depleted males may simply be transferring fewer nutrients, although it is doubtful that this alone could account for the observed differences in progeny production in D.melanogaster. In the next three chapters I shall extend MAYNARD SMITH's study (1956) on the inbred lines of D.subobscura and test the sexual competence hypothesis (TRIVERS 1972) using wild type flies. By mating with a particular male is a female likely to have a greater proportion of the eggs she lays fertilised? If so, what is the variation in male fertility due to and do high fertility males enjoy greater courtship success, either because they court more vigorously or because females discriminate between them? Finally, if females do discriminate between males of high and low fertility what cues do they use? In this first chapter I present evidence that the proportion of eggs hatching for a particular female is influenced by the wild type male she mates with. I also present preliminary evidence that males of higher fertility have faster courtships in single pair tests.

5.2. MATERIALS AND METHODS.

For the first experiment wild flies were collected using a banana bait put out in the open oak wood at Dalkeith in October 1982. The bait was positioned soon after dawn and the flies arriving were pootered up before they could feed and were stored in vials containing damp cotton-wool and placed in ice and darkness. At the laboratory male D.subobscura were identified and separated. No copulation was observed in the collecting vials. At lights on (artificial dawn) the males were each put with a single virgin five day-old female. For each male the duration of copulation was noted and the male was then put with a second female. Once again copulation duration was recorded. The males were then stored individually in food vials liberally supplied with active Baker's yeast for one week. At the end of this period they were again mated to first one and then a second five day-old virgin female.

After copulation all the females were stored individually in 5 x 1.2 cm vials containing a charcoal drosophila medium (ROSE 1979) and a drop of a suspension of living yeast dried on the side of each vial. The size of the yeast drop put into each vial was standardised (drops falling from a hypodermic needle connected to a reservoir) and was more than the female could consume in 24 hours. The females were transferred to fresh vials daily without anaesthetisation. The old vials were kept and three days later (eggs take about 24 hours to hatch) the number of eggs laid in the vial and the number that had hatched were counted and recorded. This procedure continued until the females were lost or dead. Dead females were removed from the vial and their wing length measured. Often damage to the wings made

measurement impossible. A better technique might have been to remove a wing from each female at the start of the experiment.

It proved very difficult to obtain matings with the males recently brought in from the wild so a second experiment was done using five day-old virgin wild-type males from laboratory cultures. The males were collected from 'high' and 'low' density vials to provide a range of sizes. On the evening of the fourth day all the males were weighed and a range of males of different weights put aside for the experiment the following morning. In this experiment some index of male courtship ability was required. Most five day-old virgin females would be highly receptive and differences in male courtship ability may not be reflected in a range of courtship durations. Longer courtships might be obtained by using younger females, however some females might not then mate within a reasonable period. For these reasons each male was introduced first of all to a three day-old virgin female. The time to mounting was recorded and the flies were separated before copulation occurred (or had only been going on for less than 5 seconds). If a male had not mounted within a 30 minute cut-off period he was removed anyway. Each male was then put with a virgin five day-old female and left until copulation occurred. The courtship time and duration of copulation were recorded. At the end of each male's first copulation this procedure was repeated; first, a three day-old female and separation, then a five day-old female and copulation. At the end of the morning there were four courtship durations for each male; one with a three day-old female before and after copulation and one with a five day-old female before and after copulation. There was also a record of copulation duration with each of the five day-old females. Using three day-old

females was, perhaps, an unnecessary complication and rather than exaggerating differences between males may well have obscured differences to the extent that a proportion of the three day-old females were unreceptive.

The mated females were once again stored individually and maintained in the manner described earlier. Records of the daily egg and hatch counts were kept for each female and when the females died their wings were removed and measured. A bad batch of food used between days 106 to 110 may have accounted for the high death rate at this time and makes the lifespan data unreliable. The productivity data is not seriously affected because by this time all the females were laying very few or no eggs.

5.3. RESULTS.

5.3.1. Wild caught males.

I have already mentioned that few of the males caught in the wild mated when brought into the laboratory without a period of acclimatisation. I will discuss some of the data obtained using these males in the section on egg-laying and progeny production. The rest of the analysis concerns the data obtained using the laboratory stocks of wild-type flies.

5.3.2. The duration of copulation.

Two males with sterile first matings, three males with sterile second matings and three males with sterile first and second matings were excluded from the figures and analysis. A sterile mating may well result from abnormal coupling and clearly if no ejaculate is transferred in the first mating then this will affect the quantity available for the second mating. Similarly, a sterile second mating may reflect some abnormality in the first mating, for example an injury to the male. The duration of copulation is plotted against male wing length for both the first and second copulations of the laboratory males in figure 5.1. In both cases the duration was inversely correlated with male weight (1^{st} mating; $r=-0.6094$, $p<0.001$; 2^{nd} mating; $r=-0.6320$, $p<0.001$). There was no significant relationship between the duration of copulation and female wing length (1^{st} mating; $r=0.3431$; $p>0.1$; 2^{nd} mating; $r=-0.0930$, $p>0.01$). The difference between a male's first and second copulation is

Figure 5.1. The duration of copulation is plotted against male weight for a virgin five day-old male's first copulation and a second copulation starting within two hours of the end of the first.

The difference between a male's first and second copulation is plotted against male weight in figure 5.2.

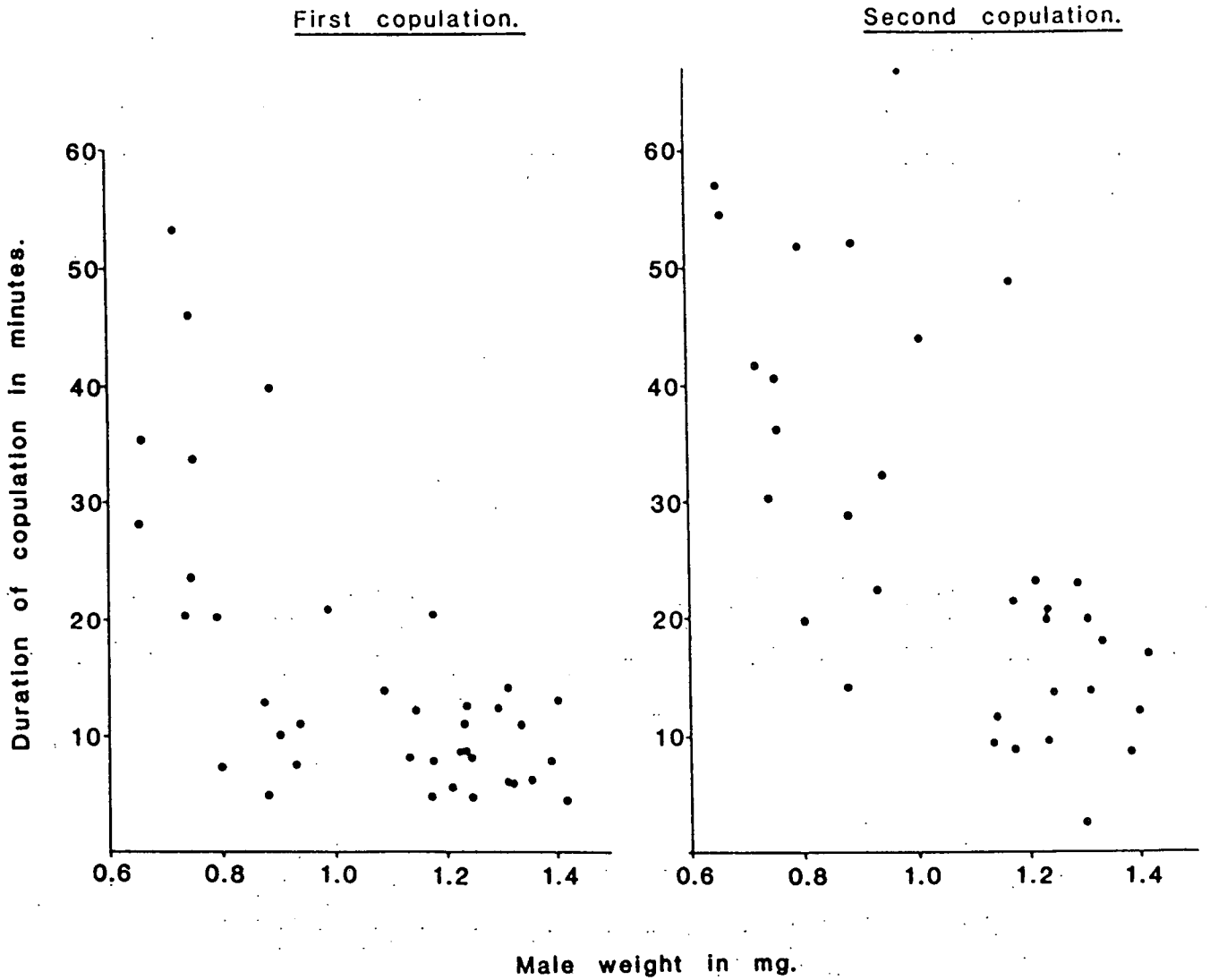

First copulation.
Second copulation.
Duration of copulation in minutes.
60
50
40
30
20
10
0.6
0.8
1.0
1.2
1.4
Male weight in mg.

Figure 5.1.

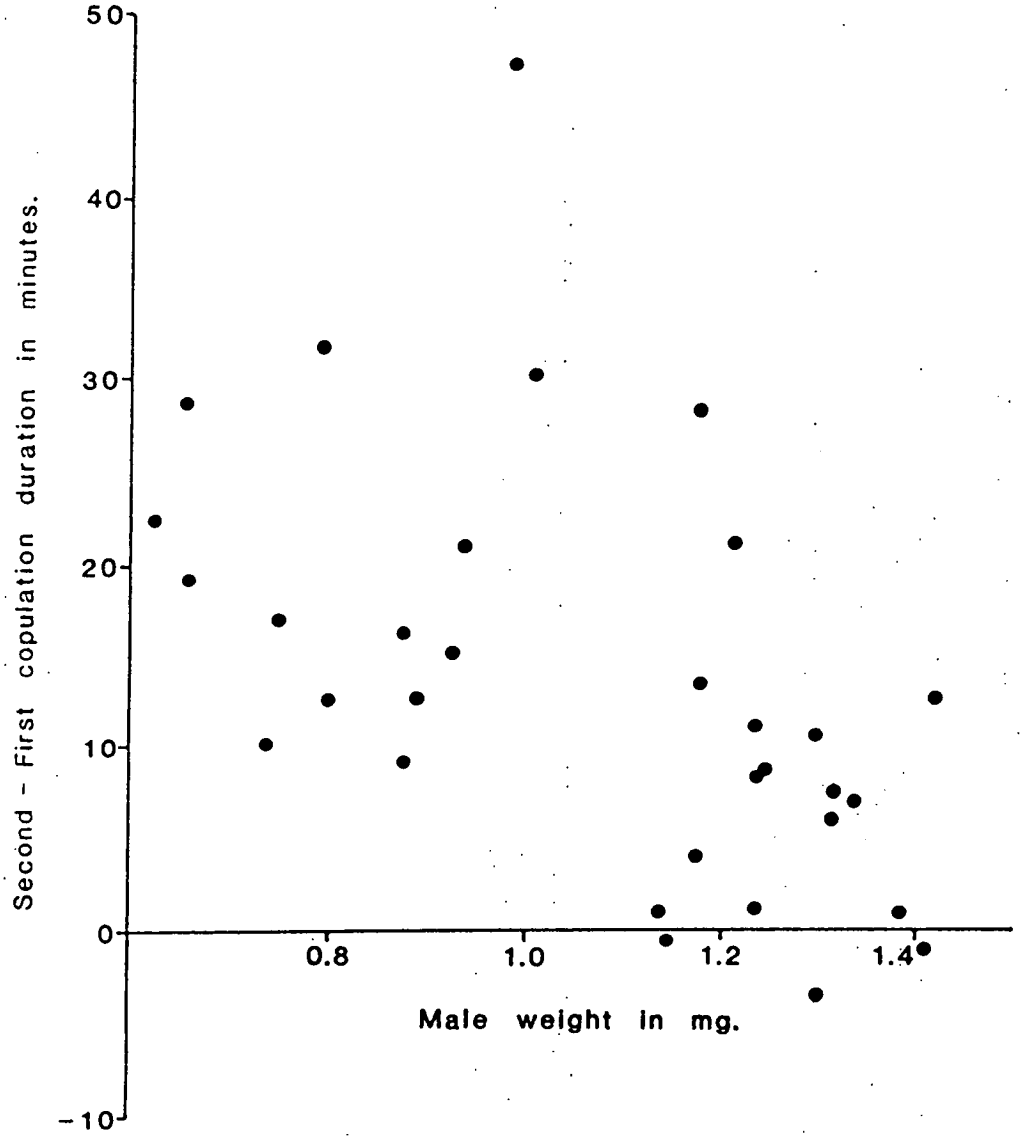
plotted in figure 5.2. The second copulation tended to be longer than the first (sign test; $z=4.42$, two-tailed $p<0.0001$) and the increase in duration was inversely correlated with male body weight ($r=-0.5382$, $p<0.001$). In summary, smaller and recently mated males tended to copulate for longer.

5.3.3. Patterns of egg-laying and progeny production.

There were only three cases where a wild male mated with two females soon after capture and then with two more females after a week's isolation with an abundant supply of yeast. The egg-laying and progeny production curves for the females mated to these three males at the start and the end of the week are presented in figures 5.3 to 5.5. In these and all similar figures in this chapter the filled circles represent the first female to be mated and the open circles the second female. There was considerable day to day variation in the egg-laying rate and so a daily mean for each ten day period is plotted. These means are joined by straight lines to make it easier to distinguish between the plots and visualise the broad patterns of egg laying and progeny production. Solid lines are drawn between the 'eggs laid' means and dashed lines between the 'eggs hatched' means.

The data for these three wild males represent no more than a pilot study but are included to illustrate a number of points. Firstly, there may well be variation in the fertility of wild males; contrast the number of eggs hatching for the females mated with male a4 with those hatching for the females mating with male a9. All the females used were reared and maintained under similar conditions and

Figure 5.2. The influence of male weight on the difference between the durations of his first and second copulations.

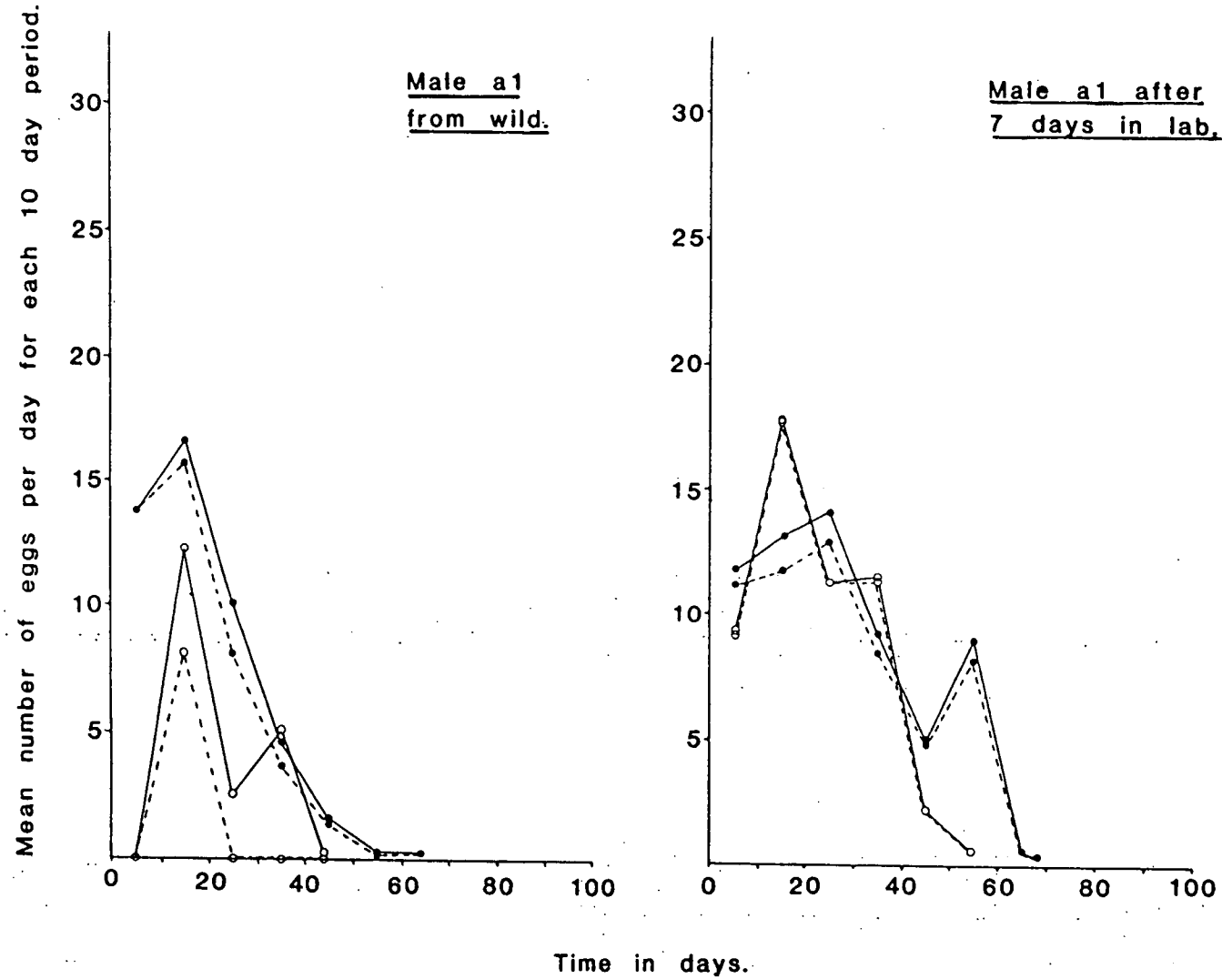


Figures 5.3 to 5.8. Each plot shows the mean number of eggs laid daily over a 10 day period and the mean number hatching daily for the same period. The solid lines join the 'eggs laid' means and the dashed lines join the 'eggs hatched' means. The filled circles represent the first female to be mated by that male and the open circles the second female to be mated.

Figures 5.3 to 5.5 are plots for three wild males mated with two females upon arrival in the laboratory and with two more females after a week housed in the constant temperature room with an abundant supply of yeast.

Figures 5.6 to 5.8 are plots for several of the five day-old males used in the main experiment.

Figure 5.3.


Male a1
from wild.
Male a1 after
7 days in lab.
Mean number of eggs per day for each 10 day period.
0
5
10
15
20
25
30
0
20
40
60
80
100
Time in days.

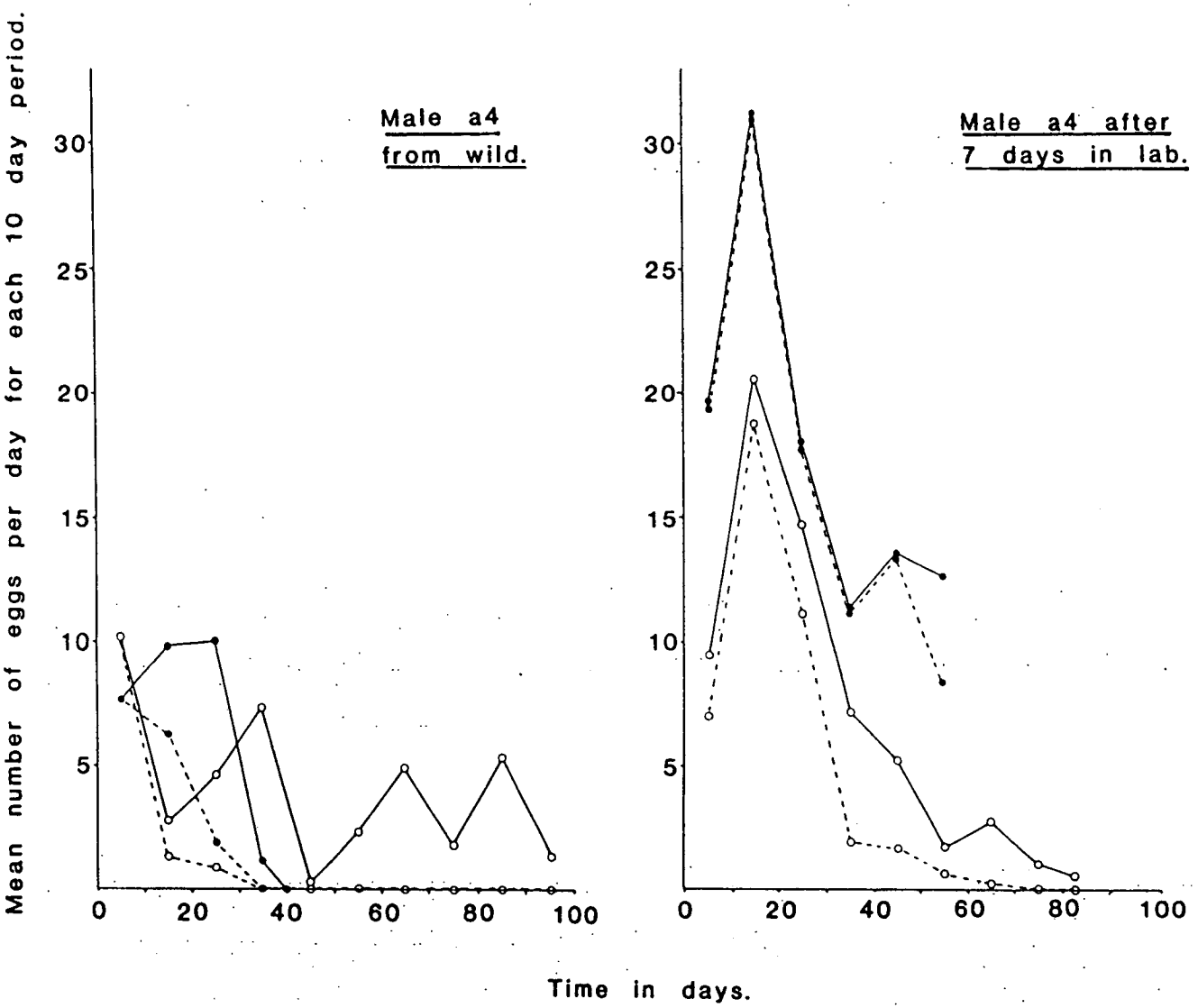

Male a4
from wild.
Male a4 after
7 days in lab.
Mean number of eggs per day for each 10 day period.
30
25
20
15
10
5
0
20
40
60
80
100
Time in days.

Figure 5.4.

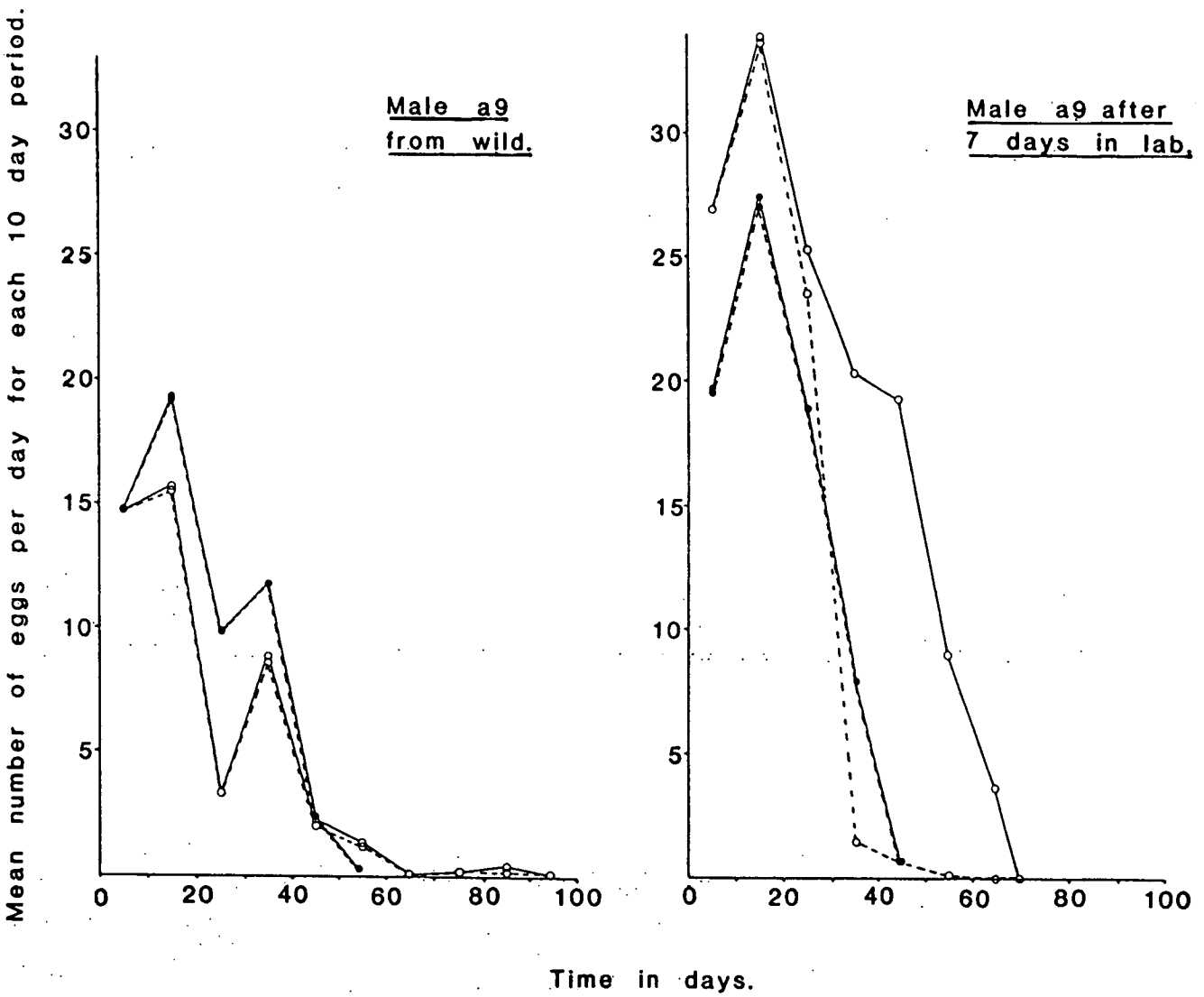
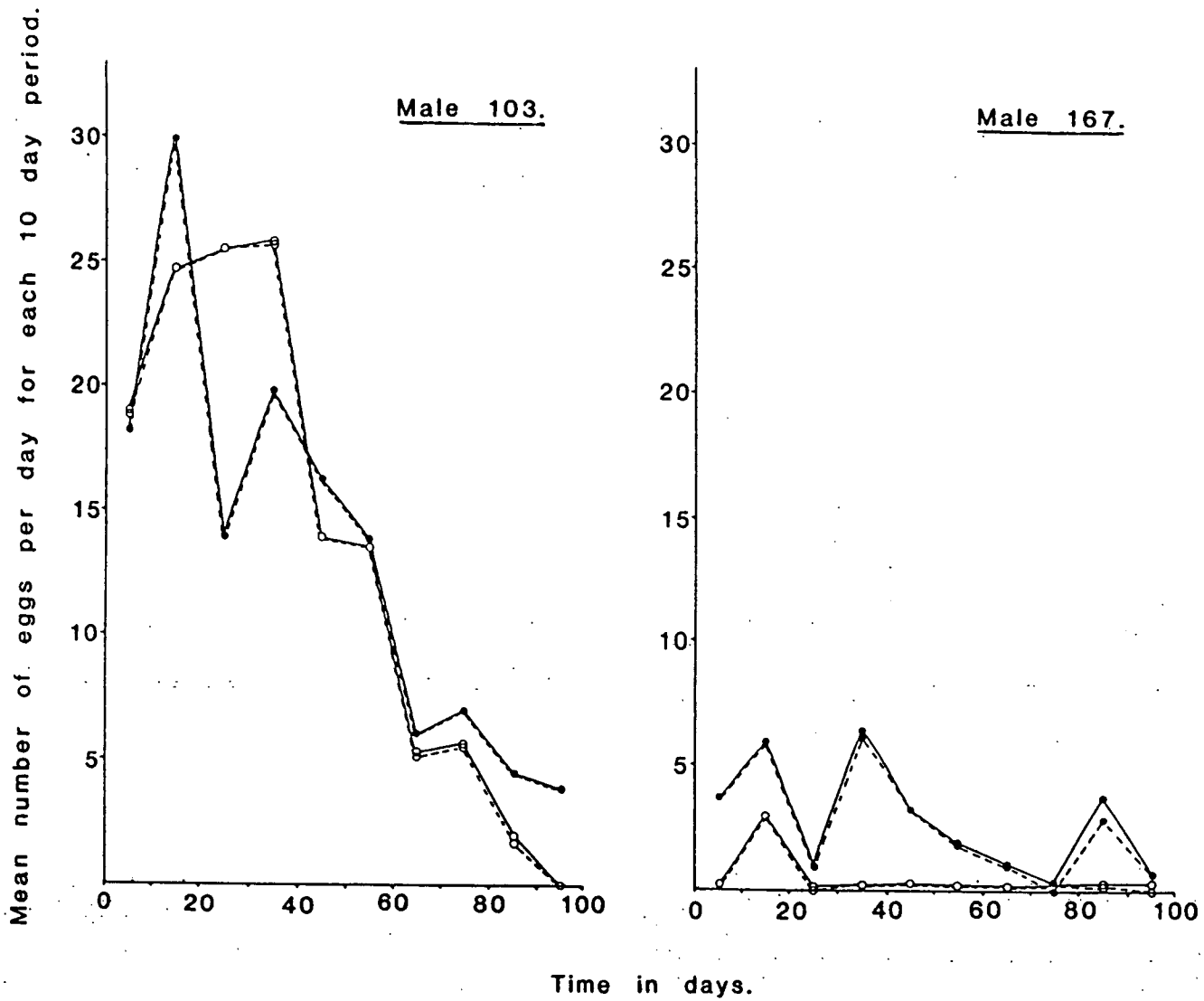

Male a9
from wild.
Male a9 after
7 days in lab.
Mean number of eggs per day for each 10 day period.
30
25
20
15
10
5
0
20
40
60
80
100
Time in days.

Figure 5.5.

Figure 5.6.


Male 103.
Male 167.
Mean number of eggs per day for each 10 day period.
30
25
20
15
10
5
0
20
40
60
80
100
Time in days.

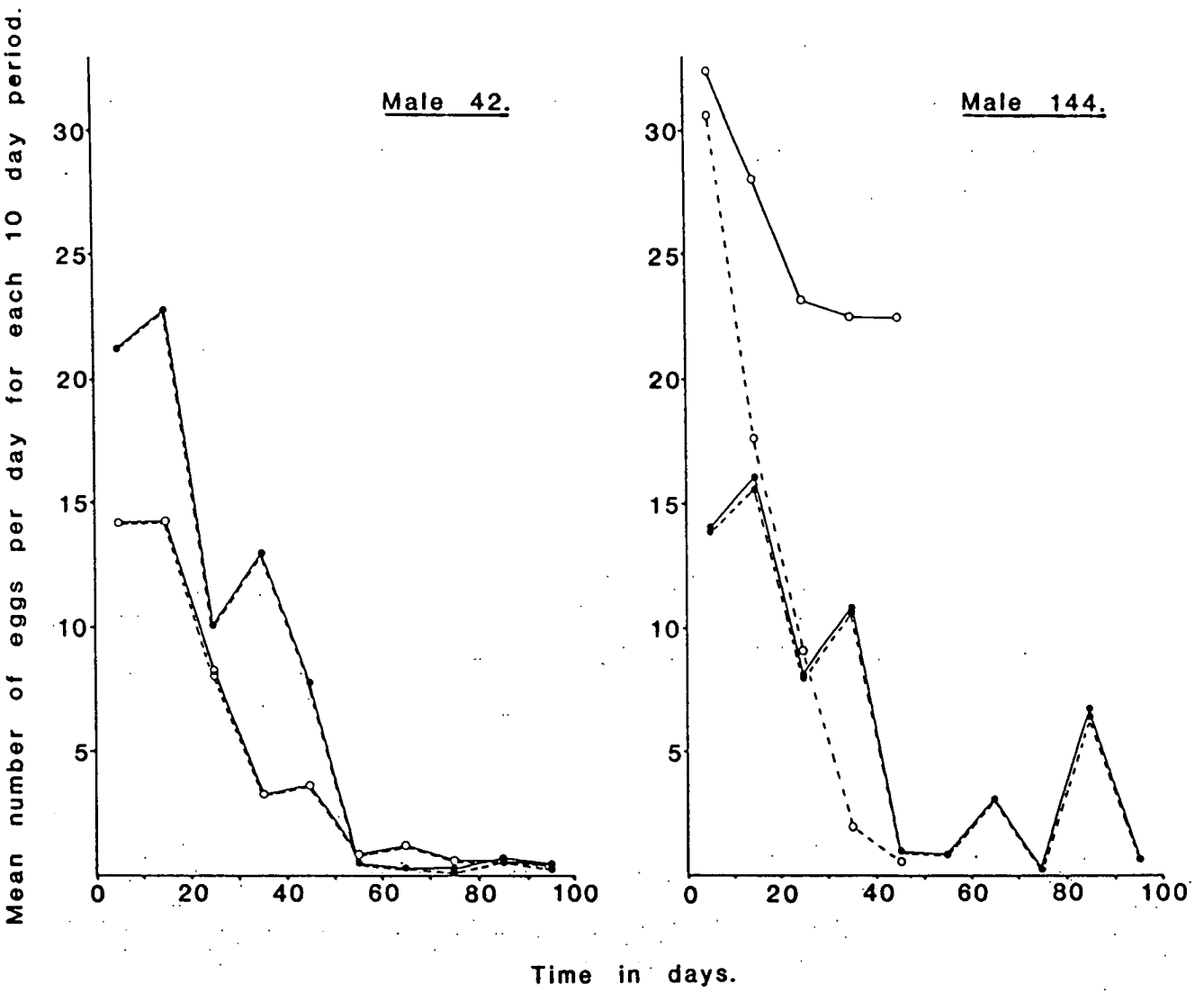

Male 42.
Male 144.
Mean number of eggs per day for each 10 day period.
30
25
20
15
10
5
0
20
40
60
80
100
Time in days.

Figure 5.7.

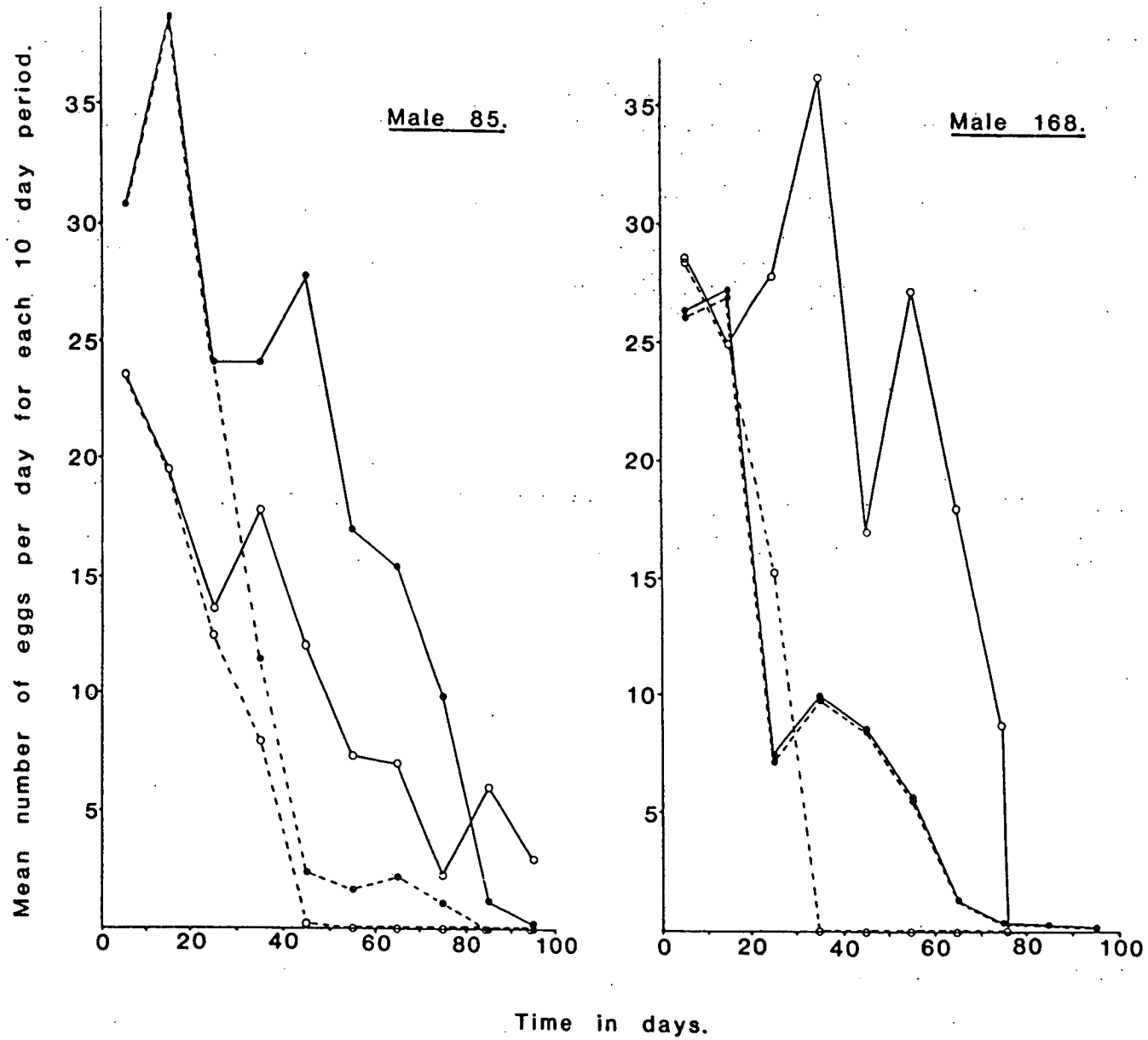

Male 85.
Male 168.
Mean number of eggs per day for each 10 day period.
Time in days.
0
20
40
60
80
100
5
10
15
20
25
30
35

Figure 5.8.

were randomly assigned to each male. Secondly, in all three cases, the two females mated to the male at the end of his week of isolation lay more eggs and leave more progeny than the two females mated at the beginning of the week. This again suggests that there may be a male effect upon the number of progeny a female will leave during her lifetime. This hypothesis was tested in the main experiment reported in the rest of this chapter.

The ten day totals of the number of eggs laid and hatching for each female in the main experiment are presented in the tables in appendix A. Figures 5.6 to 5.8 show the female egg laying and progeny plots for a number of males. The females mated to males 103, 167 and 42 laid few sterile eggs during their lifespans and sperm may never have been limiting. The rate of egg laying and progeny production declined gradually from an early peak. There is considerable variation between females with regard to the numbers of eggs laid and the number hatching. The plots for males 144, 85 and 168 illustrate cases where large numbers of sterile eggs were laid and sperm may well have been limiting. The second female mating with male 144 laid many more eggs than the first female, but left fewer progeny. The plots for male 85 show that a sudden increase in the egg laying rate was not accompanied by an increase in the progeny production (days 40 to 50 for female 1, and days 30 to 40 for female 2). The same is true for the second female mated to male 168. In these cases sperm availability may well have been limiting, but what is the evidence that this variation in egg hatchability is at least partly due to the males?

Data on the lifetime productivity of the females used in the experiment are presented in table 5.1. Females that were lost whilst

Table 5.1. The table summarises the lifetime productivity data for the two females mated to each male. In the longevity column, L is the day after mating on which a female was lost, and D the day on which a female died. The 'total' column represents the sum of the first and second females' productivity.

TABLE 5.1

		1st female					2nd female						
Male number	Male weight mg	Wing length mm	Copulation duration min. secs.	Number of eggs Laid	Hatched	Longevity Days	Wing length mm	Copulation duration mins. secs.	Number of eggs Laid	Hatched	Longevity Days	Total number of eggs Laid	Hatched
2	0.818	1.95	37.07	296	291	L 40	2.00	60.46	536	408	L 22	832	699
3	0.934		38.24	723	718	D112	1.97	71.02	336	273	D106	1059	991
7	0.754		19.32					34.59	574	564	D 87		
9	0.780	1.91	20.03	517	95	D 40	1.85	59.54	107	97	D 71	624	192
25	1.238	1.89	29.08	1162	712	D 88		44.50	169	168	D 40	1431	980
29	1.212		8.42	676	667	D107							
33	1.172		32.20	1088	555	L 78	1.92	30.25	715	423	D100	1803	978
41	0.756		33.57	75	73	L 50			58	1	D108	133	74
42	0.794	2.00	20.13	773	766	D109	1.93	51.48	475	472	L111	1248	1245
44	0.718	2.01	13.06	1397	1354	D110		41.40					
49	0.750	2.06	23.37	1362	1358	D109							
63	1.234		12.35	1018	894	D110	1.92	20.44	455	449	D 40	1473	1343
70	1.302		5.56	505	492	D 61		2.28	522	512	D 81	1027	1004
71	1.292	1.91	12.45	1661	1363	D110	1.83	23.09	615	613	D 39	2276	1976

TABLE 5.1 (continued)

		1st female					2nd female						
Male number	Male weight mg	Wing length mm	Copulation duration min. secs.	Number of eggs Laid	Hatched	Longevity Days	Wing length mm	Copulation duration mins. secs.	Number of eggs Laid	Hatched	Longevity Days	Total number of eggs Laid	Hatched
85	1.172	2.02	4.44	1895	1124	D107	1.99	8.37	1130	639	D109	3025	1763
87	0.878		12.52	1086	997	L101	1.77	28.48	9	9	D 43	1095	1006
92	0.928	1.95	7.31	486	470	D107	1.83	22.34	138	137	D 30	624	607
102	1.384	1.96	7.37	588	586	L 68	1.99	8.29	776	666	D 107	1364	1252
103	1.314	1.98	5.25	1339	1339	D107	2.06	13.56	1359	1354	D106	2696	2691
108	1.246	1.76	4.50	421	3	D109	1.92	13.35	310	9	D107	731	12
121	0.900	1.88	10.10	224	223	D 40	1.88		305	2	D108	529	225
122	0.984		20.52	869	860	D105		68.06	834	494	L 51	1703	1354
124	0.938	1.88	11.04	239	232	D108		32.12					
127	0.752	1.89	46.05	282	273	D 75			524	422	L 52	806	695
143	1.136	1.98	8.15	934	889	D107		9.13	1032	713		1966	1602
144	1.144		12.09	618	606	D107	1.99	11.29	1286	599	D 51	1904	1205
145	1.092		13.57	37	26	D106		43.54	123	120	L 29	160	146

TABLE 5.1 (continued)

		1st female					2nd female						
Male number	Male weight mg	Wing length mm	Copulation duration min. secs.	Number of eggs Laid	Hatched	Longevity Days	Wing length mm	Copulation duration mins. secs.	Number of eggs Laid	Hatched	Longevity Days	Total number of eggs Laid	Hatched
147	1.178		7.58	518	515	D 88	1.99	21.29	1038	689	D 95	1556	1204
149	1.232	2.00	8.52	1037	1028	D101	1.94	20.00	1329	1206	D 90	2366	2234
164	0.888	2.20	39.48	31	31	D 47		52.08	1554	664	D 82	1585	695
166	0.726	2.07	53.11	705	539	D 86							
167	0.878	1.86	5.05	282	268	D105	1.95	14.15	52	45	D105	334	313
168	0.656	1.94	28.21	877	865	D105		56.55	1987	688	D 81	2864	1553
171	1.174		20.28	248	215			48.39	982	655	D106	1230	870
173	0.660		35.21	1499	1383	L 78		54.24	540	527	D105	2038	1910
182	1.356	2.00		1164	887	D86							
183	1.310		14.00	996	876	D104	1.90	19.53	1269	703	D103	2265	1579
186	1.406		13.02	351	324	D 44		12.02	235	152	D 92	586	476
187	1.418	1.89	4.33	244	243	D 47		17.07	1383	586	D106	1627	829

still producing progeny at a high rate are excluded. So too are pairs in which either a male's first or second mating proved to be sterile (see section 5.3.2). Table 5.2 provides some summary data and comparisons for the two groups of females. The wing lengths of the females in the two groups were not significantly different ($t=0.72$, $p>0.1$), nor was there any difference in lifespan ($z=-1.36$, $p>0.1$). In both groups there was a significant correlation between the number of eggs laid and the number hatching, and both these variables were significantly correlated with female wing length. There was no significant correlation between male weight and the number of eggs laid by either the first or second female. There was a significant correlation between male weight and the number of progeny left by the second female. There were no significant correlations between copulation duration and the number of eggs laid (1^{st} female; $r=-0.0991$, $p>0.1$; 2^{nd} female; $r= -0.0040$, $p>0.1$) or hatched (1^{st} female; $r=-0.0749$, $p>0.1$; 2^{nd} female, $r=-0.2101$, $p>0.1$).

The first females to be mated did not lay any more eggs than the second females (paired sample test; $t=0.08$, $p>0.1$) but did leave significantly more progeny ($t=2.29$, $p<0.05$). Although, there was no difference between the two females with regard to the initial egg hatchability (in all but 4 of 56 values for the two females egg hatchability during the first 10 days was between 98 and 100%), the first ten day period during which the proportion of eggs hatching fell below 90% occurred significantly earlier in the second females' lifespan (sign test, $p=0.02$). Further evidence that there was a male effect on the number of eggs hatching, but not the number laid, comes from testing for correlations between the two females mated to each male. The number of eggs laid by each of the two females mated to a

TABLE 5.2

A summary of the productivity of the first and second females mated by males, and some factors influencing that productivity.

	First female mated					Second female mated				
	Mean wing length in mm ±95% c.l.	Median longevity in days	Mean number of eggs ±95% c.l. Laid	Hatched	% Hatched	Mean wing length in mm ±95% c.l.	Median longevity in days	Mean number of eggs ±95% c.l. Laid	Hatched	% Hatched
	1.94±0.03	105	737±153	623±135	85	1.93±0.03	101	739±168	486±108	66
Range	(1.76-2.07)	(40-112)	(31-1895)	(3-1383)	(1-100)	(1.77-2.06)	(30-111)	(2-1987)	(0-1354)	(0-100)
N	23	31	38	38	38	19	28	33	33	33

Pearson correlations

Eggs laid with eggs hatched;	r=0.9249, n=38, p<0.001	Eggs laid with eggs hatched;	r=0.7948, n=33, p<0.001
Female wing length with eggs laid;	r=0.5524, n=23, p<0.001	Female wing length with eggs laid;	r=0.5805, n=19, p<0.01
Female wing length with eggs hatched;	r=0.6663, n=23, p<0.001	Female wing length with eggs hatched;	r=0.5789, n=19, p<0.01
Male weight with eggs laid;	r=0.1721, n=38, p>0.1	Male weight with eggs laid;	r=0.2180, n=33, p>0.1
Male weight with eggs hatched;	r=0.1386, n=38, p>0.1	Male weight with eggs hatched;	r=0.3521, n=33, p=0.05

particular male were not significantly correlated ($r=0.2620$, $p>0.1$). This is to be expected if the number of eggs laid depends largely upon female size and the females were randomly assigned to each male. However, there was a significant correlation between the number of eggs hatching during the first female's lifetime and the number hatching during the second female's lifetime ($r=0.6336$, $p<0.01$). There was no correlation between the lifespans of the two females mated to each male ($r=-0.1666$, $p>0.1$). Clearly the number of progeny a female leaves during her lifetime is influenced by both the identity of the wild-type male she mates with and by whether he has just copulated.

5.3.4. Male courtship ability.

The courtship durations of each male with the four females are given in table 5.3. The males had longer courtships with the second five day-old female than with the first (sign test; $z=3.12$, $p=0.0018$). That there was no difference with the three day-old females (sign test; $z=0.5$, $p=0.62$) was probably due to the high proportion of unreceptive females in this group. Overall the courtships with the second female were longer (sign test, $z=2.74$, $p=0.0064$). When, in a control experiment, males courted for the first time later in the morning there was no difference between the courtship durations of these males and the males' first courtships in the main experiment (for each group, $n=25$; Mann Whitney; $z=0.7$, $p=0.48$). Within each of the female groups the male courtship times were ranked (table 5.3). Kendall's coefficient of concordance was calculated for the four ranks obtained for each male. The result was

Table 5.3. The table gives each male's courtship duration with four different females, the male's courtship rank with each female and his overall courtship rank. The lowest rank was assigned to the shortest courtship. A score of 30+ indicates that the male had not mounted when he was removed 30 minutes after the start of courtship.

TABLE 5.3(i)

Male number	Courtship duration in mins. secs.				Male courtship rank with				Total
	1st female		2nd female		1st female		2nd female		
	3 day	5 day	3 day	5day	3 day	5 day	3 day	5 day	
2	30+	18.12	30+	80.05	35	38	32.5	41	146.5
3	8.36	17.10	30+	8.21	22	37	32.5	28	119.5
7	30+	10.07	6.47	18.05	35	33	15	35	118
9	2.07	2.13	30+	6.04	14	19	32.5	23	88.5
29	30+	1.05	4.00	6.30	35	13	13	24	85
33	1.20	0.10	30+	2.25	9	7	32.5	14	62.5
41	30+	2.09	30+	10.00	35	18	32.5	30	115.5
42	16.50	26.25	30+	4.44	26	40	32.5	21	119.5
49	30+	5.29	2.10	28.50	35	29	11	38	113
63	30+	1.59	18.02	0.05	35	17	22	2	76
69	23.14	0.05	30+	2.30	28	3	32.5	15	78.5
71	0.05	1.10	4.58	5.41	1	14	14	22	5.1
87	30+	2.18	0.20	3.05	35	20	2	16	73
92	14.05	9.39	0.40	10.30	24	32	3	31	90
102	4.42	0.10	0.43	0.37	19	7	5	4	35
103	30+	5.10	17.40	0.05	35	28	21	2	86
104	0.35	0.05	30+	14.15	3	3	32.5	32	70.5
107	2.35	0.43	3.04	1.32	15	10	12	6	43
108	9.35	2.25	6.15	0.05	23	21	17	2	63
121	30+	33.04	30+	42.12	35	41	32.5	40	148.5
122	4.55	0.57	1.37	1.40	20	12	7	8	47

TABLE 5.3(ii)

Male number	Courtship duration in mins. secs.				Male courtship rank with				Total
	1st female		2nd female		1st female		2nd female		
	3 day	5 day	3 day	5day	3 day	5 day	3 day	5 day	
124	1.15	1.47	0.15	1.53	8	16	1	9	34
127	30+	0.55	30+	21.23	35	11	32.5	37	115.5
143	30+	11.04	25.47	30.29	35	34	23	39	131
144	4.28	4.50	12.31	7.21	18	27	20	26	91
145	1.25	2.42	0.45	7.46	10	23	6	27	66
147	16.18	16.20	30+	16.25	25	36	32.5	33	126.5
149	0.10	8.20	1.43	17.22	2	31	10	34	77
162	0.45	3.50	30+	2.01	6	25	32.5	11	74.5
164	2.50	0.05	30+	4.51	16	3	32.5	19	70.5
166	0.45	0.05	30+	1.59	6	3	32.5	10	51.5
167	0.45	8.03	10.36	2.05	6	30	19	12.5	67.5
168	1.50	1.40	6.01	3.50	12	15	16	18	61
169	30+	4.37	1.40	1.21	35	26	8.5	5	74.5
170	30+	12.14	30+	9.54	35	35	32.5	29	131.5
171	0.42	18.24	30+	1.37	4	39	32.5	7	82.5
173	8.05	0.05	30+	3.33	21	3	32.5	17	74.5
182	2.00	0.32	1.40	20.58	13	9	8.5	36	66.5
183	3.25	0.10	9.10	2.05	17	7	18	12.5	54.5
186	18.40	2.39	0.40	7.16	27	22	4	25	78
187	1.40	3.10	30+	4.36	11	24	32.5	20	87.5

significant (W=0.39, χ^2=63.17, p=0.01) indicating that the rankings obtained for each male were not unrelated and an overall rank would reflect the males' relative courtship ability. Table 5.4 gives the Spearman rank correlations between male courtship rank and the ranks of a number of variables.

There was no significant correlation between a male's overall courtship rank and the rank of the total number of eggs laid by the females mated to that male, or the rank of the total number hatching. Nor was overall courtship rank significantly correlated with male weight. However, earlier in this chapter it was found that male weight was correlated with the number of eggs hatched by the second female only. Although, there was no direct relationship between a male's courtship rank with the second female and the number of progeny left, there was a correlation between male weight and his courtship rank with the second female (table 5.4). Heavier males achieved mating faster and the female left more progeny. It was also reported earlier in the chapter that heavier males had shorter copulations and so, not surprisingly, there was a significant relationship between male courtship rank and copulation duration with the second female.

TABLE 5.4

Spearman rank correlations between male courtship ranks and female productivity, male weight and the duration of copulation.

Male total courtship rank with total eggs laid rank;	Sr=0.32, n=32, z=1.66, p>0.05
Male total courtship rank with total eggs hatched rank;	Sr=0.23, n=32, z=1.21, p<0.1
Male total courtship rank with male weight rank;	Sr=0.28, n=39, z=1.46, p>0.1

	With first female	With second female
Male courtship rank with eggs laid rank;	Sr=0.23, n=23, z=1.21, p<0.1	Sr=0.06, n=19, z=0.34, p>0.1
Male courtship rank with eggs hatched rank;	Sr=0.10, n=23, z=0.53, p<0.1	Sr=0.16, n=19, z=0.85, p<0.1
Male courtship rank with male weight rank;	Sr=0.11, n=39, z=0.56, p>0.1	Sr=0.49, n=39, z=2.53, p<0.01
Male courtship rank with copulation duration rank;	Sr=0.12, n=38, z=0.71, p>0.1	Sr=0.35, n=32, z=1.93 p>0.05

5.4. CONCLUSIONS.

1) The duration of copulation was inversely correlated with male weight for both first and second copulations. The second copulation was longer and the increase in duration was greatest for the smallest males.

2) The number of eggs laid during a female's lifetime and the number hatching were correlated with female wing length.

3) The numbers of eggs laid by each of the two females mated to a particular male were not correlated, but the numbers of eggs hatching were.

4) The first female mated did not lay any more eggs than the second female, but more eggs hatched as a consequence of a high level of egg fertility being maintained for a longer period. Male courtship duration with the second female was longer.

5) There was no direct relationship between male courtship rank and the number of eggs laid by or hatching for the two females mated to that male.

6) With the second female heavier males had faster courtships and left more progeny.

5.5. DISCUSSION.

GILBERT, RICHMOND and SHEEHAN (1981) analysed progeny production and sperm usage in D.melanogaster. The two primary components of productivity were explained in terms of an exponential growth in egg laying rate to a plateau reached after a few days and an exponential decay in sperm release. They suggested that the release of an individual sperm had a certain probability for a given time period and that this probability of release was independent of other stored sperm and of egg release. Initially, many sperm are stored, the rate of sperm release is high and so all eggs are fertilised and some sperm is wasted. At this stage progeny production is limited only by a female's ability to lay eggs. As the number of stored sperm declines, so too does the rate of sperm release. Examining the number of eggs laid and the number hatching should indicate if and when sperm is limiting, assuming the major cause of egg sterility is infertility and not low viability, which is probably the case (KAUFMANN and DEMEREC 1942, DAVID 1963, PYLE and GROMKO 1978). However, the situation is complicated by the fact that, after an initial exponential growth phase, egg laying rate declines with time in once mated females (e.g. DAVID 1963). The reasons for this decline are unclear and females allowed to remate maintain high egg laying rates (e.g. PYLE and GROMKO 1978). Components of male ejaculate are known to influence female egg laying rate during the growth phase (see GROMKO, GILBERT and RICHMOND in press for review) and there would certainly be an advantage gained by females who could match their egg laying rate to sperm availability and avoid wasting nutrients and energy in laying sterile eggs. If

this matching occurred, sperm could still be a limiting factor even when egg hatchability is 100%. However, in this experiment there was no evidence of a male effect upon female egg laying rate.

The results reported in this chapter are consistent with GILBERT et al.'s (1981) model of productivity. The number of eggs a female lays during her lifetime depends on her size and is independent of the male with whom she mates. However, the number of eggs hatching depends not only on the number laid but is also influenced by some characteristic of the female's mate. MAYNARD SMITH obtained a similar result with his inbred and outbred lines of D.subobscura (1956). Initially, the proportion of eggs hatching is high but for some females this proportion declines steadily with time, probably because sperm becomes limiting. Males mating for a second time fertilise fewer eggs. Perhaps, the egg fertility declines more rapidly for the second mated female because fewer sperm are stored initially, and, therefore, the rate of sperm release becomes limiting sooner. The number of sperm stored by female D.melanogaster shows a general decrease with successive matings of the male (PEACOCK and ERICKSON 1965, GILBERT et al. 1981). The decline in number stored is not due to a decrease in the number of sperm available for transfer, but is due to the depletion of the accessory fluid involved in sperm transfer and storage (LEFEVRE and JONSSON 1962). The observed difference in fertility between a male's first and second matings may be due to a simple quantitative effect; depleted males transfer less ejaculate and so have fewer sperm stored.

This hypothesis is lent support by the data on male size. Males depleted of ejaculate fertilise fewer eggs, probably because

the female stores fewer sperm from that mating. It, therefore, seems reasonable to argue that if the levels of fertilisation are similar, similar amounts of ejaculate may have been transferred (all else being equal). As there was no difference between the fertility of large and small males for the first mating they may well have been transferring similar amounts of ejaculate. RITCHIE (1984) found that although the regression lines between body size and the size of the accessory glands of male D.subobscura before and after copulation were displaced, their slopes were the same suggesting that large and small males had transferred similar amounts of accessory fluid. If this is the case and smaller males have smaller reproductive organs (RITCHIE 1984 and next chapter) then the effects of depletion might be greater for smaller males. This could explain why small males fertilised fewer eggs than large males when mating a second time.

That there was a correlation between the number of eggs fertilised for each of the two females mated to a particular male, even though egg fertility was only correlated with male size for the second mating, suggests that there may also be qualitative differences between the male ejaculates. Such differences could arise if there were differences between males in factors influencing sperm storage, release or abnormality. The activity of the enzyme esterase 6 in male D.melanogaster depends upon a structural gene and a regulatory gene and varies in males from natural populations. (TEPPER, TERRY, HOLMES and RICHMOND in press). The enzyme is transferred to the female during copulation and influences sperm release (the proportion released per unit time) and usage by females (GILBERT et al. 1981). For a given number of stored sperm a higher rate of release would mean the female ran out of sperm sooner, but a

lower rate might mean that not all eggs were fertilised during the early peak of egg production. I have argued that the second mating of a male may be less fertile because fewer sperm are stored initially, and , for a given rate of sperm release, females run out of sperm sooner. To maintain a similar pattern of sperm availability with fewer stored sperm requires a higher rate of sperm release. If sperm numbers are limiting, which seems to be the case for many second matings in this study, then increasing the rate of release would ensure that a high proportion of the eggs laid early in a female's lifespan were fertilised, but a lower proportion later on. GILBERT et al. (1981) found that with successive matings of a male D.melanogaster the initial number of sperm stored decreased, but the rate of sperm release increased. Obviously, patterns of sperm use by female Drosophila are complex and in this study it is only possible to speculate on what mechanisms are responsible for the differences in egg fertility observed. The likely candidates would seem to be variation in the initial number of sperm stored or the rate of sperm release. What has been established is that there are male effects upon egg fertility and that differences between males can result in substantial differences between singly mated females in the number of progeny they leave in the laboratory during their lifetime. Is this likely to be important in the field?

There are indications that there is variation in the fertility of wild males (see also chapter 6). For the two females mated to the wild male a4 brought straight from the field, egg hatchability had fallen to 0% for both females 35 days after mating (section 5.3.3). This male was one of only three wild males successfully mated to females on arrival in the laboratory. Unfortunately, little is known

about the longevity of females in the field but it seems likely that a proportion survive for at least a month. There is a peak emergence of D.subobscura in the field during November and from then until April the population ages continuously (BEGON 1976). Inseminated females are active in the wild from January onwards. The next generation emerges in May/June and then there appear to be further generations emerging in early July, early August, early September and then again in November. Between these peaks of emergence the population tends to age (BEGON 1976). Information on female longevity and egg-laying rate in the field would be useful and it ought to be possible to collect wild females and examine their egg-laying rate and egg hatchability on various substrates in the laboratory and even collect eggs from the field and score the proportion hatching. If wild females could be remated then it should be possible to separate male and female effects on egg sterility.

It is possible that females running out of sperm might remate. Indeed, if the first mating is sterile, females will remate (MAYNARD SMITH 1956, RITCHIE 1984). However, if fertile eggs are laid, the females tend not to remate (RITCHIE 1984) and this seems to be true even for females exhausted of sperm. MAYNARD SMITH (1956) ran 115 tests involving 19 such females and in only 6 of the tests did remating occur. In 3 of these cases the female died shortly after remating, in the other 3 fertile eggs were laid. However, by scoring larval genotypes LOUKAS, VERGINI and KRIMBAS (1981) estimated that 30% of wild females from a Mediterreanean population were carrying the sperm of more than one male. This may simply reflect geographical differences related to population density and availability of food or oviposition sites. Further information on

remating in D.subobscura or levels of multiple insemination in northern populations would be useful. If there are any costs associated with remating (discussed in THORNHILL and ALCOCK 1983) selection should still favour females who mate with high fertility males and so reduce the need for remating. Is there any evidence that females discriminate between high and low fertility males?

There was no relationship between a male's courtship rank in a single pair situation and the number of eggs fertilised for the first mating. The courtship durations of depleted males tended to be longer and egg hatchability was lower. Also, heavier depleted males had shorter courtships and fertilised more eggs than small depleted males. It seems that the mating speeds of depleted males might reflect their fertility, although there does not seem to be any relationship between the differences in male fertility prior to depletion and courtship duration. However, assessing male fertility by scoring the proportion of eggs hatching introduces considerable noise due to female effects. Although, we can now state that the proportion of eggs fertilised during a singly mated female's lifetime is influenced by differences between wild-type males, it would be useful to be able to assess male fertility by examining the male. In the next chapter I present a means of doing this. I also examine some of the reasons for the variation in male fertility observed and provide extensive data showing that higher fertility males do have shorter courtships. In chapter 7 more information on the duration of copulation will be presented and the reasons for the variation observed will be discussed. I shall also describe experiments examining the factors mediating the courtship success of high fertility males.

Chapter 6.

MALE REPRODUCTIVE ORGAN SIZE AND COURTSHIP SPEED.

6.1 INTRODUCTION.

The reproductive anatomy of a male Drosophila comprises a pair of testes each opening into a seminal vesicle joined to the ampullary end of the ejaculatory duct by the vas deferens. Also opening into the ejaculatory duct are a pair of accessory glands. The ejaculatory duct leads into the ejaculatory bulb which is joined to the genitalia. Sperm are produced by the testes and mature sperm are stored in the seminal vesicles. The accessory glands are responsible for the secretion of the proteinaceous accessory fluid. Constituents of this fluid are involved in sperm motility, storage and utilisation and affect the behaviour and reproductive physiology of the mated female (see GROMKO et al. in press for review). Secretions from the ejaculatory duct and bulb add to the seminal fluid.

In a series of consecutive matings it is the depletion of the accessory fluid that limits a male's ability to transfer sperm (LEFEVRE and JONSSON 1962). As both the accessory glands and seminal vesicles are depleted they decrease in size (LEFEVRE and JONSSON 1962, RITCHIE 1984). In the last chapter I demonstrated that males recently depleted of ejaculate (see also MARKOW et al. 1978) and small males mating for a second time (both characterised by having smaller accessory glands and seminal vesicles) had longer courtships and females mated to them left fewer progeny. I also referred to a paper demonstrating that young male D.melanogaster tend to have low fertility and poor mating success in a competitive situation (LONG et

al. 1980). It seems likely that younger males will have accumulated less accessory fluid and fewer mature sperm and consequently may well have smaller accessory glands and seminal vesicles. The size of a male's reproductive organs might be a good indicator of his fertility. In this chapter I look at the relationship between a male's courtship success and the sizes of three reproductive organs; the accessory gland, the testis and the seminal vesicle.

If there is a relationship between courtship speed and reproductive organ size, then this effect might might be due to the organs per se or some other variable correlated with organ size, such as 'vigour'. Large accessory gland size, for instance, may reflect a male's efficiency at collecting food and turning it into accessory fluid. It is possible that males who excel at this will also tend to build up large energy reserves and have high 'athletic ability'. Any association between courtship speed and reproductive organ size may simply reflect differences in vigour. Although this distinction makes no difference from the female's point of view, it would be interesting to distinguish between the two effects. Some measure of a male's vigour is therefore required.

In Drosophila energy is stored chiefly in the form of glycogen. The main deposits are in the fat body of the abdomen, and other large deposits occur in the halteres, at the base of the legs, along the sides of the thorax, in the scutellum and in the proventriculus and midgut cells (WIGGLESWORTH 1963). When flies are starved both glycogen and fat reserves are used up. During flight only glycogen is used, presumably because fat is less quickly converted into energy (WILLIAMS, BARNESS and SAWYER 1943, WIGGLESWORTH 1963). During continuous flight glycogen reserves from all the various reserves are

used. The fly is exhausted when there are insufficient glycogen reserves to ensure that the rate of mobilisation meets the fly's energy requirements. The speed at which the fly becomes exhausted depends on the levels of glycogen initially stored and by measuring flight duration an estimate of a fly's energy reserves can be obtained. Although this does not necessarily reflect a male's athletic ability during courtship, it does provide a crude measure by which the vigour of flies in different groups can be compared. Experiments measuring courtship vigour in terms of the rate of occurrence and duration of particular behaviours are reported in the next chapter.

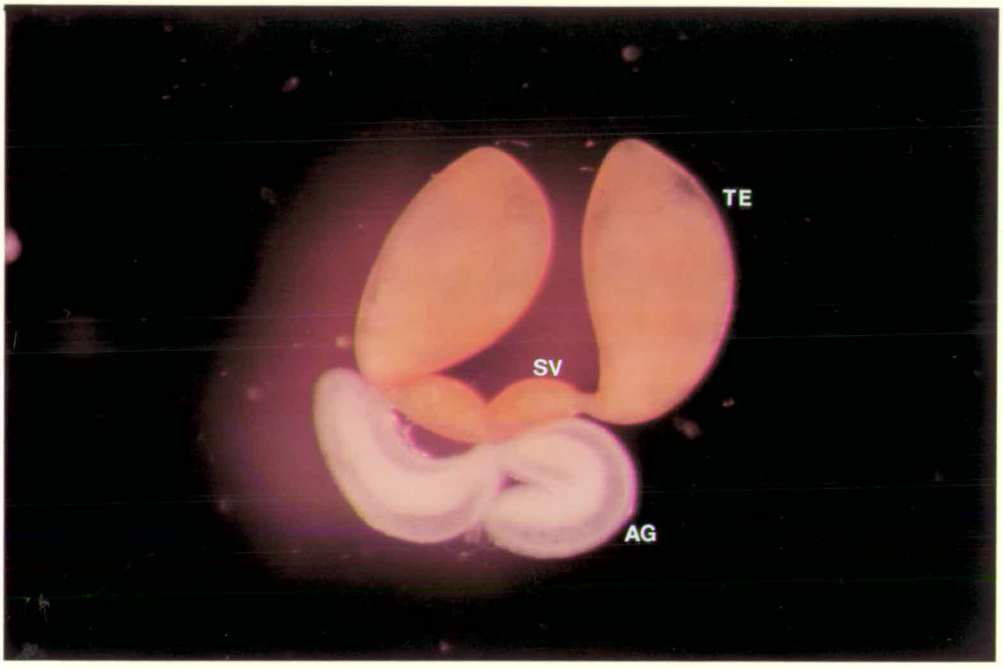
In this chapter I have attempted to manipulate reproductive organ size and vigour. The manipulations consisted of, fistly, looking at flies of different ages which might well have similar levels of vigour but different reproductive organ sizes, and, secondly, using flies of the same age but different backgrounds. For example, to reduce vigour without affecting reproductive organ size males were physically exhausted or starved for a short period. To keep organ size low but vigour high, males were fed on a glucose diet, and to keep both vigour and reproductive organ size low males were kept on a medium low in carbohydrates and protein. Comparisons were then made between groups and trends examined within to determine whether there was an association between courtship speed and reproductive organ size or vigour.

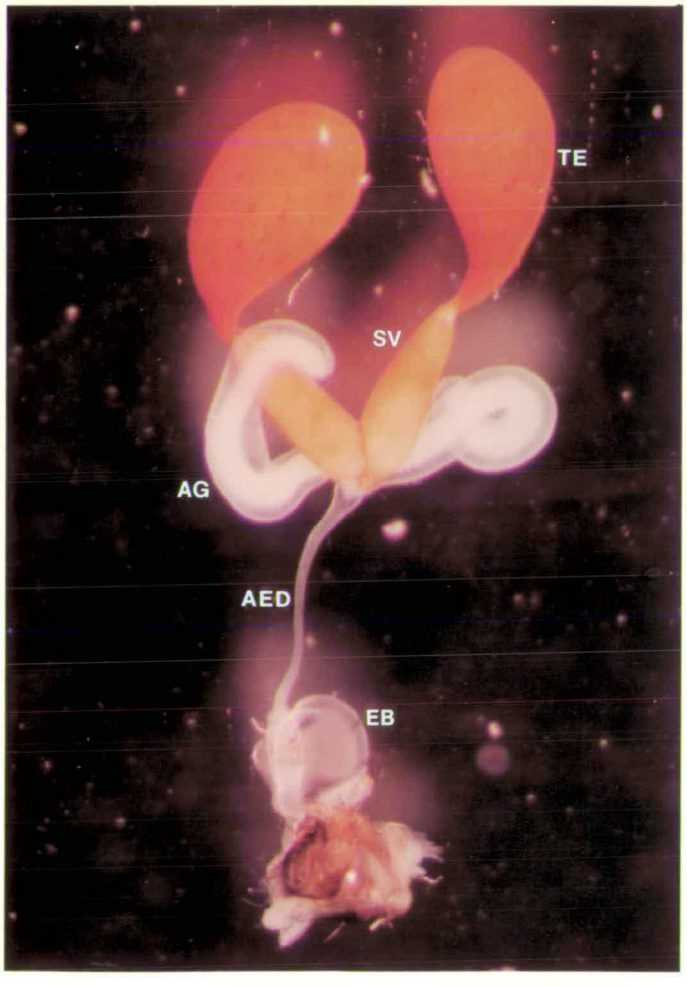
6.2 MATERIALS AND METHODS.

6.2.1. Measurement of variables.

Figure 6.1 shows the three paired reproductive organs (RO) measured in this chapter; the accessory glands (AG), the testes (TE) and the seminal vesicles (SV). Using fine forceps these structures were quickly and easily dissected out in a fixed volume of 70% alcohol on a glass slide under the binocular microscope. The rest of the fly was discarded and a coverslip put on the dissection. The reproductive organs were then drawn using a camera lucida. The introduction of a concave lens between the specimen and the drawing paper increased the size of the image relative to the paper allowing larger drawings to be made for measurement. At the start of each session of dissection and drawing the micrometer scale on the binocular eyepiece was drawn to check that the magnification of the drawings did not vary between sessions and to enable corrections to be made if necessary. The areas of the drawings were measured using a planimeter and converted to square millimeters. The figures obtained represent a flattened, cross-sectional area of each organ. In a pilot study using five day-old flies there were high correlations between the areas of the two accessory glands (n=25, r=0.8565), the two testes (n=30, r=0.8464) and the two seminal vesicles (n=30, r=0.6663). Consequently, only one of each pair was drawn and measured during the main experiment. This was an advantage because sometimes one of the pair was ruptured, awkwardly folded or obscured making it impossible to draw accurately. A wing was removed from each male and measured under the x50 magnification of the

Figure 6.1. The photographs (60x magnification) are of the reproductive organs of male D.subobscura. A cover-slip covered the preparation in the top photograph, but not the preparation in the lower photograph. In both photographs each of one of the paired testes (TE), seminal vesicles (SV) and accessory glands (AG) is labelled and the anterior ejaculatory duct (AED) and the ejaculatory bulb (EB) are labelled in the lower photograph.


TE
SV
AG


TE
SV
AG
AED
EB

binocular microscope.

The courtships between single males and single five day-old virgin females were observed in vials containing only damp cotton-wool. The courtship duration was recorded as the time from the first bout of orientation to mounting. As soon as mounting occurred the flies were separated before the male could transfer any ejaculate. If mounting had not occurred within 20 minutes of the start of courtship the flies were separated anyway. The courtship durations of each male with three different females were recorded and the median value was used as a measure of the courtship speed of that particular male.

To measure flight duration a small wooden splint was glued to the thorax of an anaesthetised fly. Copydex was used because it could be peeled off the thorax easily leaving the fly apparently undamaged (this was important for the 'exhaustion' treatment). Many flies could be simultaneously suspended by their splints from a horizontal rod so that each fly was in the proper flight position. Flight duration was recorded as the time between the start of flight and 'exhaustion'. Exhaustion was deemed to have occurred if flight could not be restarted within five seconds of stopping. Exhausted flies tended to hold the wings in the flight posture but could only make weak staccato movements.

6.2.2. Experimental treatments.

6.2.2.1. First experiment; age and poor food.

In this experiment the courtship speed, vigour and reproductive organ sizes of flies of different ages or flies aged on poor food were measured.

A range of males of different sizes were collected from high and low density vials shortly after eclosion and stored individually in vials containing standard drosophila medium seeded with active Baker's yeast. The flies were transferred to fresh vials every five days. For the zero age group only freshly eclosed flies with unfurled wings were collected and dissected. All dissections were done in the late morning. Batches of flies for the 15, 20, 25 and 30 day groups were assigned randomly.

The two day, five day well and poorly-fed and ten day-old flies were collected in eight batches (two for each treatment) so that four batches comprising one batch from each treatment were tested over a first four day period, and then the remaining four batches over a second four day period. For each male within a batch three courtship durations were recorded and then the male was dissected and the reproductive organs drawn and a wing measured. The flight durations were measured on other similarly treated groups of flies at a later date. The poorly-fed males were individually stored in vials containing a low nutrient medium (DAVID and CLAVELL 1965) with no active yeast added. Each male was transferred to a fresh vial every second day.

6.2.2.2. Second experiment; five day-old treatments.

In the second experiment eight batches of flies (two for each treatment) were again collected. The first batches from each treatment were tested over a four day period, and then the second batches over another four day period. For each male three courtship durations were recorded, then the flight durations and finally the male was dissected and his reproductive organs drawn and his wing measured. Measuring the flight duration on the same flies as the courtship duration and reproductive organ size enabled within group comparisons to be made between the variables. This procedure introduces the possibility that flight duration might underestimate the energy reserves available at the start of courtship in certain individuals or groups because they may use reserves up during courtship. However, it was thought that that the extra costs of longer courtships would be insubstantial compared to other effects and the results bear this out.

The treatments were:

Controls; the males were stored in vials containing standard drosophila medium seeded with active Baker's yeast for the full five days.

Glucose; After two days the males were transferred to vials containing only cotton-wool soaked in a glucose solution. Males were transferred to fresh vials containing glucose each day for the three days leading up to testing.

Starved; The day before testing the males were transferred to vials containing only damp cotton-wool.

Exhausted; The males were flown to exhaustion half an hour before the

experiment.

Before testing every male was put into a vial containing standard drosophila medium and active Baker's yeast just before artificial dawn and allowed to feed for 20 minutes. The males were then transferred to vials containing damp cotton-wool to await testing. This procedure ensured that all the males had similar crop contents at the start of courtship and so there should have been no variation in their ability to produce a drop during courtship or in the type of drop produced. Similarity of crop contents were also important for the measurements of flight duration. Glucose can be taken up from the crop and used to provide energy for flight very quickly (WIGGLESWORTH 1963). The flight durations of flies fed on glucose would be a measure both of stored glycogen and the amount of glucose stored in the crop at the start of flight, so it was important to standardise the latter across treatments.

6.2.3. Wild flies.

Wild flies were collected in the Dalkeith oak wood using banana bait during the early morning activity period. The bait was covered with fine muslin so that the flies, although attracted to the bait, could not feed. The flies were collected using a net and a pooter and stored in vials packed in ice and kept in darkness. Back at the laboratory the flies were stored in darkness in the fridge before being dissected in the late morning and their reproductive organs and crops drawn. A wing from each fly was removed and measured under the binocular microscope.

At a later date another group of wild flies was collected in the same manner and the duration of flight to exhaustion was recorded.

6.3. RESULTS.

6.3.1. Age and size of the reproductive organs.

The raw data are given in the tables in appendix B. For the two, five and ten day-old groups organ area was plotted against wing length and a regression line fitted to the untransformed data, a plot of $\log_{10}$(organ area x 100) against wing length and a plot of $\log_{10}$(organ area x 100) against $\log_{10}$(wing length x 100). The full logarithmic plot gave a linear relationship. This was expected because the relationship between organ area (y) and wing length (x) is likely to be of the form:

$$y = \text{constant} \cdot x^2$$

In a full logarithmic plot the constant is the y-intercept and the exponential is the regression coefficient. In the rest of this chapter the size variables are multiplied by 100 and log-transformed before being used in any analysis.

Within each data set $\log_{10}$(wing length x 100) was positively correlated with $\log_{10}$(organ area x 100) (table 6.1). The fitted regression lines for the different groups are plotted for each of the three reproductive organs in figures 6.2 to 6.4. Clearly the size of the accessory glands increased with age up to a plateau reached around day 20. The probability of getting this particular ordering due to chance alone (i.e. no effect of age) is 1 in 6! (1 in 720). Similarly, the size of the seminal vesicles increased with age, but the size of the testes remained constant. There was no effect of age on wing length (one way Anova; $F=0.56$, $p>0.1$). Figure 6.5 depicting camera lucida drawings of the reproductive organs of a young and an

TABLE 6.1

The Pearson correlations between the log-transformed reproductive organ areas and wing length and the regression equation for the log-transformed organ areas regressed on wing length for males of different ages, poorly-fed males and wild males.

		Relationship between log10 (organ area) and log10 (wing length)		
Group	N	Accessory gland	Testis	Seminal vesicle
0 day	21	r=0.4147 y=05.09+2.71x	r=0.4219 y=-4.14+2.44x	r=0.4183 y=07.56+3.56x
2 day	26	r=0.8112*** y=-7.03+3.61x	r=0.7759*** y=-4.44+2.52x	r=-0.6099*** y=-5.81+2.83x
5 day	36	r=0.6419*** y=-3.22+1.98x	r=0.5701*** y=12.87+1.79x	r=0.6892*** y=-5.37+2.66x4
10 day	36	r=0.5215*** y=-2.13+1.52x	r=0.4037* y=-1.52+1.19x	r=0.5244*** y=-3.14+1.68x
15 day	17	r=0.7127*** y=-4.31+2.52x	r=0.6164** y=-3.33+2.02x	r=0.3924 y=-3.04+1.70x
20 day	13	r=0.7918** y=4.47+2.63x	r=0.6301* y=-2.81+1.78x	r=0.7148** y=-4.26+2.26x
25 day	12	r=0.7133** y=-4.26+2.53x	r=0.4899 y=02.07+1.43x	r=0.2510 y=-0.94+0.78x
30 day	11	r=0.7842** y=-3.53+2.21x	r=0.0520 y=0.12+0.44x	r=0.7490** y=-4.2+2.25x
5 day poor food	41	5=0.6979*** y=-7.06+3.59x	r=0.3661* y=-3.89+2.22x	r=0.5950*** y=-4.64+2.3x
Wild	44	r=0.7000*** y=-6.61+3.50x	r=0.4712*** y=-2.48+1.62x	r=0.6504* y=-6.18+3.04x

*p<0.05 **p<0.01 ***p<0.001

Figures 6.2, 6.3 and 6.4. The fitted regression lines of $\log_{10}$(organ area x 100) regressed on $\log_{10}$(wing length x 100) are plotted for males of different ages, for five day-old poorly-fed males and for the wild males. The regression equations are given in table 6.1.

Figure 6.2. The relationship between accessory gland area and wing length for different groups of males.

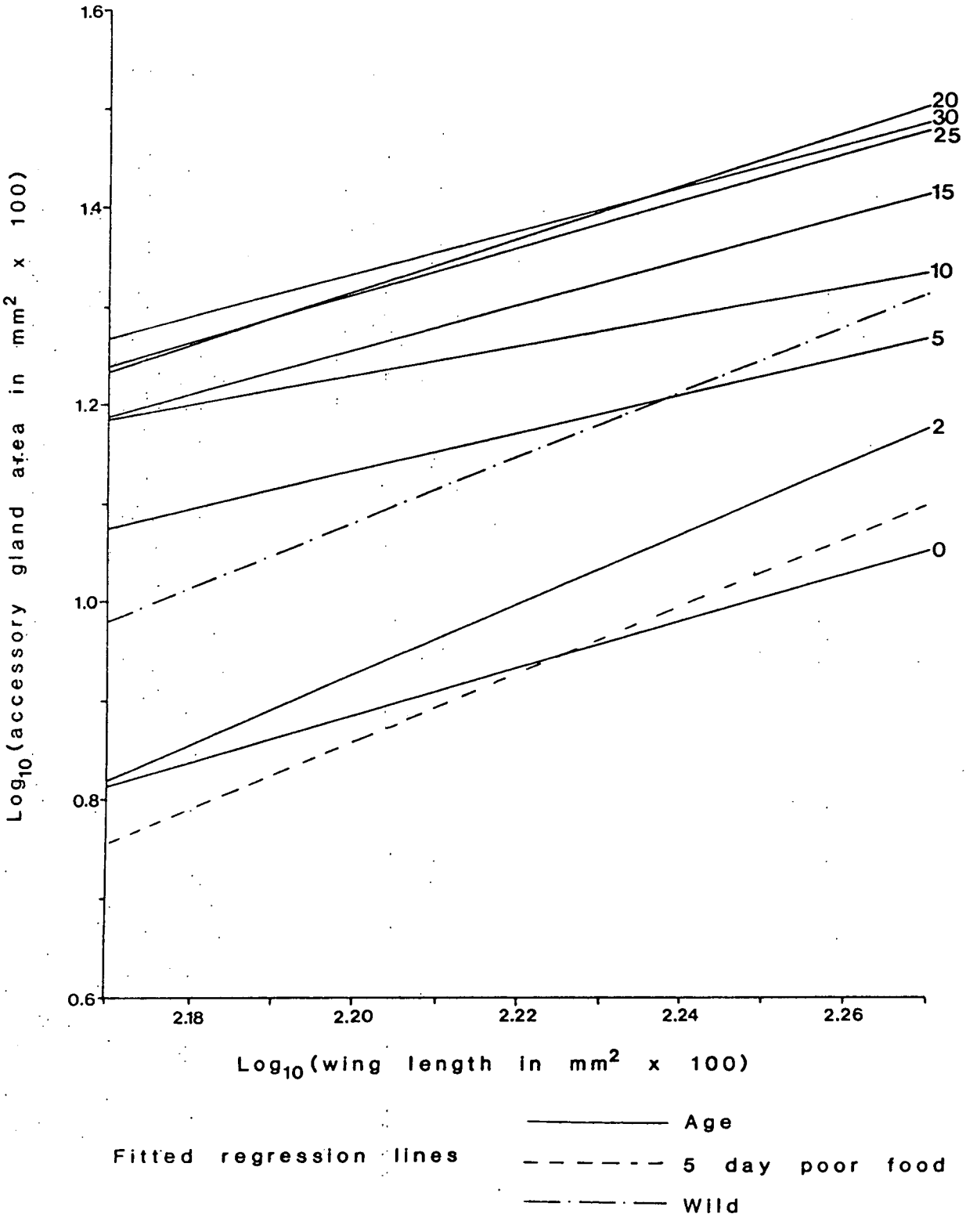


Figure 6.3. The relationship between testis area and wing length for different groups of males.

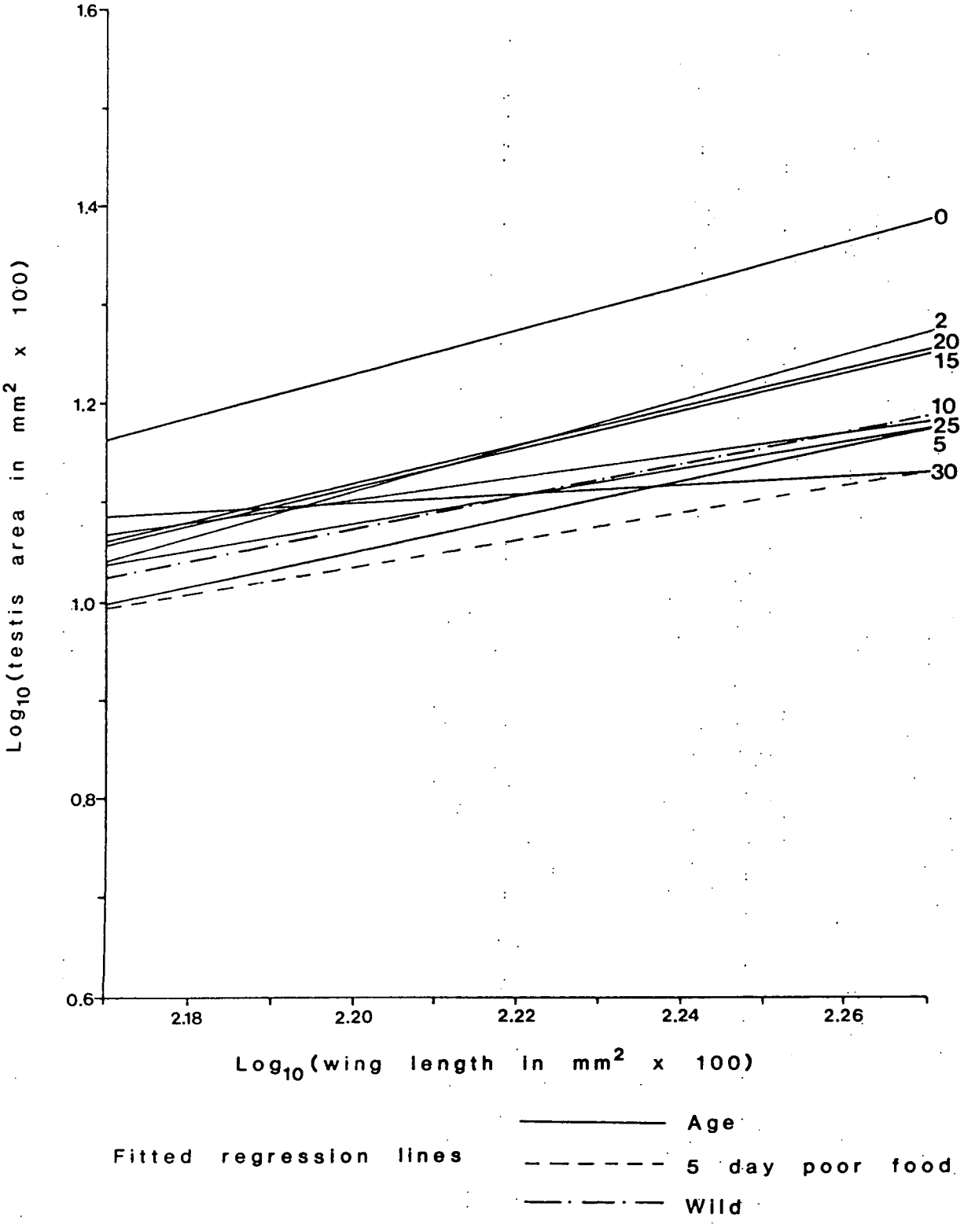
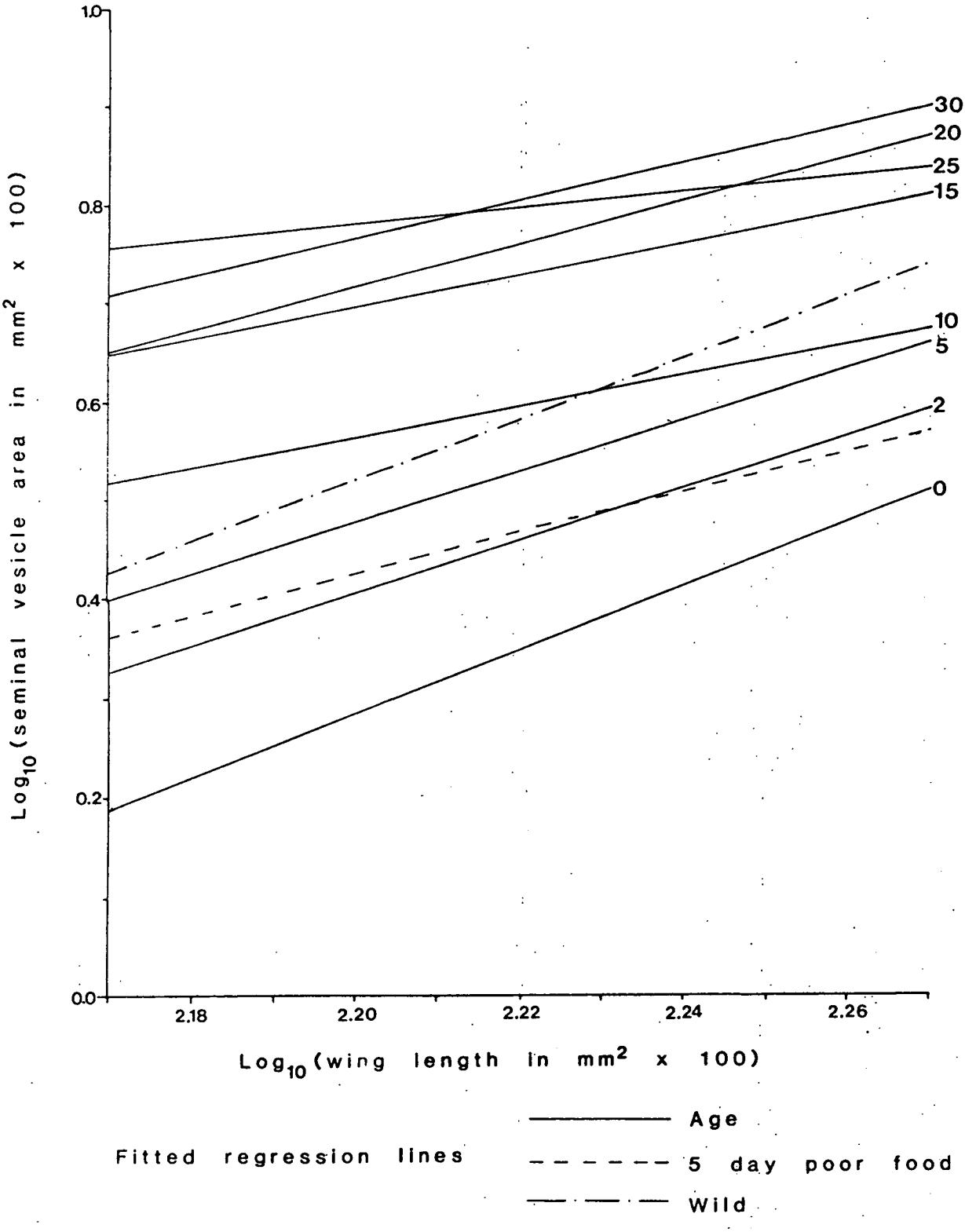


Figure 6.4. The relationship between seminal vesicle area and wing length for different groups of males.



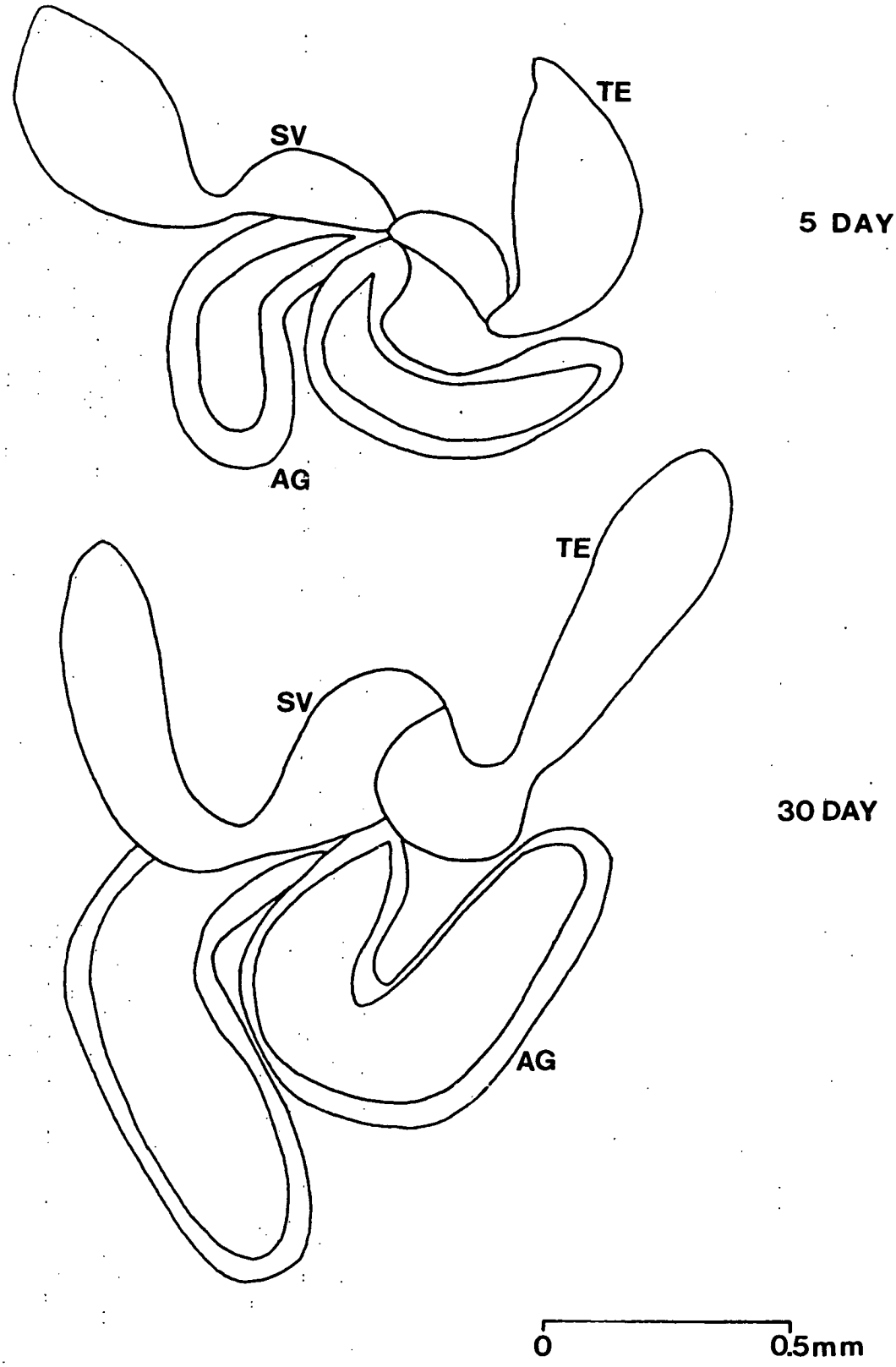


Figure 6.5. The camera lucida drawings are of the testes (TE), seminal vesicles (SV) and accessory glands (AG) of a five day-old male (wing length = 1.63mm) and a 30 day-old male (wing length = 1.65mm) of similar body size.

old male of similar wing length, illustrates the influence of age on accessory gland and seminal vesicle size. By 30 days of age the testes were becoming less turgid and more elongate and 'withered'.

These findings are consistent with the known functions of the three organs. Sperm are produced by the testes but, once mature, are stored in the seminal vesicles (LEFEVRE and JONSSON 1962). In the absence of copulation and sperm transfer one would expect the seminal vesicles to get larger as they accumulate mature sperm. The increase in size of the accessory glands might be due to an accumulation of accessory fluid or to a growth of the gland walls. A combination of the two seems probable.

Also given with the age data are values for the poorly-fed five day-old flies and the group of wild flies. The poorly-fed group had smaller accessory glands and seminal vesicles than the well-fed five day-old flies (see next section), but similar values for the testes. Nutritional background therefore seems to affect the production of both sperm and accessory fluid. The wild flies had accessory gland sizes broadly similar to the well-fed five day-old group and seminal vesicle sizes broadly similar to the ten day-old group. The testis sizes of the wild flies did not differ from the laboratory groups.

Table 6.2 gives the correlation coefficients and regression equations for $\log_{10}$(organ area x 100) on $\log_{10}$(wing length x 100) in the second experiment. Differences between the groups are discussed in the next section.

TABLE 6.2

The Pearson correlations between the log-transofrmed reproductive organ areas and wing length and the regression equations for the log-transformed organ areas regressed on wing length for the five day-old male treatments.

		Relationship between log10 (organ area) and log10 (wing length)		
Group	N	Accessory gland	Testis	Seminal vesicle
Control	33	r=0.6488*** y=-3.43+2.04x	r=0.6626*** y=-3.78+2.17x	r=0.5348*** y=-3.51+1.80x
Exhausted	32	r=0.6387*** y=06.67+3.53x	r=0.4290* y=-5.44+2.96x	r=0.5822*** y=5.70+2.84x
Glucose	29	r=0.7962*** y=011.7+5.68x	r=0.5329** y=-5.83+3.09x	r=0.6781*** y=-6.43+3.10x
Starved	26	r=0.5099** y=-5.15+2.81x	r=0.2345 y=-1.87+1.31x	r=0.5505** y=-4.05+2.04x

*p<0.05 **p<0.01 ***p<0.001

6.3.2. Organ area, vigour and courtship duration.

6.3.2.1. First experiment: age and poor food.

The raw data are given in the tables in appendix B. Table 6.3 summarises the results and analysis. The wing lengths were compared using a t-test and the log-transformed organ areas using an analysis of covariance. The proportion of unmated flies (value 20 minutes) was considered too high (>40%) in the poorly-fed group to justify the courtship times being transformed and analysed using a t-test and so the Mann-Whitney U test was used. The distribution of flight durations within the groups did not show a consistent pattern so the median and range is given for the different groups and groups were compared using the Mann-Whitney U test. The data on flight duration for the five day-old well-fed males is taken from the five day-old group in the second experiment.

Comparisons were made between the ten and five day groups, the five and two day groups, the five and five day poorly-fed groups and the two and five-day poorly-fed groups. The wing lengths of the different groups were not significantly different. However, the ten day-old flies had significantly larger accessory glands than the five day-old group, although there was no difference in the size of the testes, seminal vesicles or in the courtship durations. The five day-old flies had significantly larger accessory glands and seminal vesicles than the two day-old males, but smaller testes. There was no difference in the flight durations but the younger flies had longer courtships. The poorly-fed five day-old males had

TABLE 6.3

Comparisons of wing length (t-test), reproductive organ area (ANCOVA), courtship duration and flight duration (Mann-Whitney) for males of different ages and poorly-fed males.

Group	N	Mean wing length in mm ±95% c.l.	Adjusted mean log10 (organ area in mm^2 x 100)			Median courtship duration in mins. with range	Median flight duration in mins. with range
			Accessory gland	Testis	Seminal vesicle		
10 day	36	1.73±0.04	1.244	1.122	0.590	3.96 (00.92-20.00)	
10 x 5		t=0.82 p=0.412	F=12.22 p=0.001	F=1.29 p=0.260	F=3.03 p=0.086	w=1355.5 p=0.643	
5 day	36	1.70±0.05	1.176	1.104	0.568	4.37 (00.50-20.00)	66.0 (22-87) n=33
5 x 2		t=0.27 p=0.789	F=50.87 p<0.001	F=9.19 p=0.004	F=9.10 p0.004	w=1044.5 p=0.038	w=1229 p=0.554
2 day	26	1.71±0.04	0.984	1.154	0.473	8.08 (00.58-20.00)	63.0 (23-150) n=40
2 x P.F.		t=0.72 p=0.476	F=8.80 p=0.004	F=11.21 p=0.001	F=0.05 p=0.816	w=1651.5 p=0.042	w=2581.5 p<0.001
5 day poor food	41	1.69±0.03	0.910	1.038	0.466	16.08 (02.08-20.00)	16.0 (2-42) n=28
5 x P.F.		t=0.42 p=0.675	F=107.26 p<0.001	F=2.76 p=0.101	F=12.21 p=0.001	w=2060.0 p<0.001	w=718.5 p<0.001

significantly smaller accessory glands and seminal vesicles than the well-fed five day-old males, but similar sized testes. They also had significantly shorter flight durations and longer courtships. Compared to the two day-old flies, the poorly-fed group had smaller accessory glands and testes, shorter flights and longer courtships.

So two day-old males have small accessory glands and seminal vesicles relative to five day-old males and longer courtships. Poorly-fed five day-old males not only have smaller accessory glands and seminal vesicles, but also have fewer energy reserves and have the longest courtship durations.

6.3.2.2. Second experiment: the five day-old treatments.

The raw data are presented in the tables in appendix B. A summary of the results and analyses are given in table 6.4 . The statistical tests used were the same as those described in the previous section, except that the courtship times were log-transformed and analysed using a t-test. Comparisons were made between the control group and each of the treatment groups. In this experiment all the variables were measured for each fly.

The wing lengths of the control group and each of the treatment groups were not significantly different. The exhausted flies had larger testes and seminal vesicles than the controls, but a shorter flight time and they took longer to mate. The glucose group had smaller accessory glands, a shorter flight time and longer courtships than the controls. The only significant difference between the controls and the starved group was that the starved flies had shorter flight times. It is possible that the differences in flight duration

TABLE 6.4

Comparisons of wing length (t-test), reproductive organ area (ANCOVA), courtship duration (t-test on log-transformed data) and flight duration (Mann-Whitney) for the five day-old male treatments.

Group	N	Mean wing length in mm ±95% c.l.	Adjusted mean log10 (organ area in mm^2 x 100)			Median courtship duration in mins. with range	Median flight duration in mins. with range
			Accessory gland	Testis	Seminal vesicle		
Control	33	1.75± 0.04	1.099	1.037	0.486	2.66 (00.08-20.00)	66.0 (22-87)
C x E		t=0.73 p=0.471	F=1.84 p=0.180	F=5.42 p=0.023	F=15.81 p<0.001	t=2.16 p=0.034	w=1604.0 p<0.001
Exhausted	32	1.73±0.03	1.167	1.131	0.605	3.79 (01.08-20.00)	15.5 (0-41)
C x G		t=0.60 p=0.548	F=22.23 p<0.001	F=0.46 p=0.500	F=0.23 p=0.630	t=2.40 p=0.019	w=1225.5 p=0.009
Glucose	29	1.73±0.03	0.910	1.030	0.452	3.92 (00.84-20.00)	32.0 (3-103)
C X S		t=1.18 p=0.242	F=0.15 p=0.690	F=1.30 p=0.260	F=0.040 p=0.840	t=1.89 p=0.063	w=1300.0 p<0.001
Starved	26	1.78±0.08	1.088	1.038	0.479	4.67 (01.16-20.00)	35.0 (13-73)

between groups simply reflected the duration of the preceding courtships. However, there was less overlap between the control and treatment groups for flight durations than there was for courtship durations. Also, the courtship durations of the exhausted and glucose groups were the same ($t=0.36$, $p=0.7214$) but the flight times were significantly different ($W=769.5$, $p=0.0013$). The treatment effects outweigh any influence of courtship duration on subsequent flight time. The exhausted flies really did have low energy reserves as a consequence of the treatment rather than a tendency for longer courtships.

Table 6.5 summarises the findings in the two experiments. Because the two experiments were not directly comparable (the five day control courtship times differ), the groups are simply classified with respect to the five day-old controls within each experiment. In the first experiment it was found that poorer nutrition resulted in smaller accessory glands and seminal vesicles. When an energy source but not protein is available (glucose treatment) the growth of the seminal vesicles (and hence sperm maturation?) is normal, but the growth of the accessory glands is still poor (no amino acids available for the production of the proteinaceous accessory fluid). The between group comparisons suggest a negative association between courtship duration and both relative reproductive organ (specifically the accessory glands and seminal vesicles) size and the energy reserves available for flight. These relationships are further examined in the next section by looking for trends within the groups using correlation and partial correlation techniques.

TABLE 6.5

A simple classification for the groups in the two experiments of reproductive organ area, flight duration and courtship duration relative to the 5 day-old controls.

Group	Accessory glands	Testes	Seminal vesicles	Flight	Courtship
5 day	Large	Large	Large	Long	Fast
2 day	Small	V. Large	Small	Long	Medium
Exhausted	Large	V. Large	V. Large	Short	Medium
Glucose	Small	Large	Large	Medium	Medium
Starved	Large	Large	Large	Medium	Medium
Poor food	Small	Large	Small	Short	Slow

6.3.2.3. Further analysis; correlation and partial correlation.

The Pearson correlation matrices for each of the groups in the two experiments are given in tables 6.6 and 6.7. The size variables were log-transformed before the analysis as were the courtship times with the non-mating males given a value of 20 minutes. This is obviously an underestimation of the true courtship values for these males (assuming all males will eventually mate) but should not lead to erroneous conclusions as long as the proportion of non-mating flies is not too high. The poorly-fed five day-old group was not analysed because of the high proportion of non-maters.

In all the groups the log-transformed size variables were all significantly intercorrelated (except for wing length with testis area in the starved group). The degree to which flight duration and the logarithm of courtship duration were correlated with these variables and each other differed. The problem is to identify the spurious relationships and reveal those that may have biological significance. For instance, in the two day group courtship duration is negatively correlated with all the size variables. It seems likely that a number of these correlations are simply a consequence of inter-correlations between the size variables. The technique of partial correlation examines the relationship between variables whilst controlling for the effects of one or more other variables (SNEDECOR and COCHRAN 1980). For example, the partial correlation between courtship time and accessory gland size holding wing length constant ($r_{CTAG \cdot WL}$) would be a measure of the relationship between courtship time and accessory gland size at any given wing length.

Tables 6.6. and 6.7. The tables give the Pearson correlations between the variables measured for each group in the two experiments. The log-transformed variables used were wing length (WL), accessory gland area (AG), testis area (TE), seminal vesicle area (SV) and courtship duration (CD). Flight duration (FL) was included in the second experiment (table 6.7).

TABLE 6.6

2 DAY n=26

	LOG_{10} WL	LOG_{10} AG	LOG_{10} TE	LOG_{10} SV	LOG_{10} CT
LOG_{10} WL		0.811***	0.776***	0.610***	-0.471**
LOG_{10} AG	0.642***		0.602***	0.763***	-0.457**
LOG_{10} TE	0.570***	0.701***		0.513**	-0.368*
LOG_{10} SV	0.689***	0.623***	0.641***		-0.391
LOG_{10} CT	-0.187	-0.475**	-0.522***	-0.274	

5 DAY N=36

10 DAY N=36

	LOG_{10} WL	LOG_{10} AG	LOG_{10} TE	LOG_{10} SV	LOG_{10} CT
LOG_{10} WL		0.522***	0.403**	0.525***	-0.182
LOG_{10} AG	0.522***		0.565***	0.682***	-0.331*
LOG_{10} TE	0.533***	0.366***		0.496***	-0.257
LOG_{10} SV	0.858***	0.730***	0.451***		-0.278*
LOG_{10} CT	-0.261**	-0.409***	-0.341***	-0.336***	

2+5+10 DAY N=98

*p<0.05 **p<0.01 ***p<0.001

TABLE 6.7

CONTROLS N = 33

	LOG_{10} WL	LOG_{10} AG	LOG_{10} TE	LOG_{10} SV	LOG_{10} CT	FL
LOG_{10} WL		0.649***	0.662***	0.535***	0.001	0.163
LOG_{10} AG	0.639***		0.809***	0.495**	-0.212	0.164
LOG_{10} TE	0.429**	0.774***		0.536***	-0.221	0.235
LOG_{10} SV	0.588***	0.662***	0.527***		-0.055	0.133
LOG_{10} CT	-0.399*	-0.435**	-0.403*	-0.483**		0.093
FL	0.444**	0.456**	0.301*	0.403*	-0.631***	

EXHAUSTED N = 32
GLUCOSE N = 29

	LOG_{10} WL	LOG_{10} AG	LOG_{10} TE	LOG_{10} SV	LOG_{10} CT	FL
LOG_{10} WL		0.796***	0.532***	0.673***	-0.410*	0.249
LOG_{10} AG	0.508**		0.905***	0.804***	-0.604***	0.472**
LOG_{10} TE	0.234	0.598***		0.754***	-0.602***	0.319
LOG_{10} SV	0.551**	0.481**	0.375*		-0.514**	0.292
LOG_{10} CT	-0.304	-0.260	-0.218	-0.133		-0.326
FL	-0.325	-0.267	-0.206	-0.295		-0.326

STARVED N = 26
C+E+G N = 94

	LOG_{10} WL	LOG_{10} AG	LOG_{10} TE	LOG_{10} SV	LOG_{10} CT	FL
LOG_{10} WL		0.583***	0.502***	0.513***	-0.203*	0.199*
LOG_{10} AG	0.589***		0.721***	0.654***	-0.408***	0.185*
LOG_{10} TE	0.434***	0.667***		0.600***	-0.360***	0.067
LOG_{10} SV	0.506***	0.621***	0.568***		-0.267**	-0.055
LOG_{10} CT	-0.211**	-0.377***	-0.338***	-0.252**		-0.237*
FL	0.130	0.122	0.042	-0.076	-0.188*	

C+E+G+S N = 120

*p<0.05 **p<0.01 ***p<0.001

The determination of partial correlations is straightforward (SNEDECOR and COCHRAN 1980) but tedious when several variables are to be held constant. In this analysis an SPSS package was used.

Table 6.8 lists the partial correlations between $\log_{10}$(courtship time) and $\log_{10}$(organ area x 100) holding $\log_{10}$(wing length x 100) constant, and between $\log_{10}$(courtship time) and $\log_{10}$(wing length x 100) holding each of the reproductive organs constant. There is a negative correlation between courtship time and relative organ size in nearly all the groups, and these correlations are significant in a number of cases. When the groups are combined for each experiment courtship duration is significantly negatively correlated with the relative size of each of the reproductive organs. For the second experiment data are presented for the combined groups with and without the starved flies. This was done because it was felt that the starved treatment may have been perceived differently by flies of different sizes. Had all the flies been starved to a particular condition the treatment would have been comparable to the ad libitum feeding on yeast or glucose or the flight to exhaustion treatments. Hence the combined data set excluding the starved flies is probably more meaningful. The results indicate that in some groups and overall males with a relatively small reproductive organ have longer courtships. The next step is to obtain a measure of the combined influence of the three reproductive organ variables on courtship duration, and then to determine which of the correlations between courtship duration and reproductive organ size are a consequence of intercorrelations between the reproductive organs.

One approach to analysing the influence of variables 1,2 and 3 on variable 4 whilst controlling for variable 5 ($r_{4123 \cdot 5}$) is to

Partial correlations of $\log_{10}$ (courtship duration) with $\log_{10}$ (organ area) and $\log_{10}$ (wing length)

		Holding wing length constant			Holding organ area constant		
Group	N	$r_{CTAG.WL}$	$r_{CTTE.WL}$	$r_{CTSV.WL}$	$r_{CTWL.AG}$	$r_{CTWL.TE}$	$r_{CTWL.SV}$
2 day	26	-0.1460	-0.0041	-0.1486	-0.1923	-0.3166	-0.3189
5 day	36	-0.4711**	-0.5147***	-0.2046	0.1747	0.1583	0.0035
10 day	36	-0.2813*	-0.1933	-0.2183	-0.0118	-0.0930	-0.0442
2+5+10	98	-0.3320***	-0.2450**	-0.2345*	-0.0603	-0.0919	-0.0840
Control	33	-0.2798	-0.2959*	-0.0662	0.1867	0.2018	0.0364
Exhausted	32	-0.2552	-0.2798	-0.3344	-0.1751	-0.2735	-0.1626
Glucose	29	-0.5028**	-0.4967**	-0.3527	0.1464	-0.1330	-0.1017
Starved	26	-0.1290	-0.1592	0.0430	-0.2062	0.2662	-0.2785
C+E+G	94	-0.3580***	-0.3049***	-0.1935*	0.0428	-0.0280	-0.0803
C+E+G+S	120	-0.3194***	-0.2803***	-0.1716*	0.0140	-0.0761	-0.1005

*p<0.05 **p<0.01 ***p<0.001

Table 6.8. The table lists the partial correlations between courtship duration and each reproductive organ area holding wing length constant, and between courtship duration and wing length holding each reproductive organ area constant. All variables were log-transformed before the analysis. The correlation is between the variables represented by the letters occurring after r but before the full stop. The letters after the full stop represent the variable being held constant. The log-transformed variables used were courtship duration (CT), accessory gland area (AG), testis area (TE), seminal vesicle area (SV) and wing length (WL).

regress the dependent variable 4 and each of the independent variables 1, 2 and 3 on variable 5. The residuals from each regression (re) could then be used in a multiple regression of re_{45} on re_{15}, re_{25} and re_{35}. The resulting coefficient of determination would indicate what proportion of the variation in 4 is explained by the variables 1, 2 and 3 independent of the effects of variable 5. Similarly, to control for the effects of two variables the residuals from the multiple regressions of each variable on the two control variables would be used.

Table 6.9 gives the multiple correlations and multiple partial correlations for the different groups. It should be noted that multiple correlation coefficients can only be positive because they are computed from the coefficient of determination (r^2). The size of the three reproductive organs is significantly correlated with courtship time at any given wing length in three groups and overall in both experiments. The same trend is observed when the variable 'flight time' is included in the second experiment. In addition, this variable is significantly negatively correlated with courtship time in the exhausted group and overall. How much of this association is a result of flies with lower energy reserves taking longer to achieve copulation (as the between group comparisons suggest) and how much the result of longer courtships reducing the energy reserves available for subsequent flight is not known.

To summarise, relative reproductive organ size is negatively correlated with courtship time, but there is no association between wing length and courtship time independent of the reproductive organ effects.

Table 6.9. The table lists various correlations between the log-transformed variables for the different groups. The correlation is between the variables represented by the letters occurring after r and before the full stop, and the letters after the full stop represent the variables being held constant. The absence of a full stop indicates a multiple correlation. The variables used are courtship duration (CT), wing length (WL), flight duration (FL) and the three reproductive organ variables (RO; accessory gland area, testis area and seminal vesicle area).

Correlations between $\log_{10}$ (courtship duration) and $\log_{10}$ (organ area), $\log_{10}$ (wing length) and flight duration.

Group	N	r_{CTROWL}	$r_{CTRO.WL}$	$r_{CTWL.RO}$	$r_{CTROWLFL}$	$r_{CTRO.FL}$	$r_{CTRO.FLWL}$	$r_{CTFL.ROWL}$	$r_{CTWL.ROFL}$
2 day	26	0.4930*	0.1612	-0.1517					
5 day	36	0.5888***	0.566***	0.2097					
10 day	36	0.3450*	0.2933	0.0164					
2+5+10	98	0.4604***	0.3931***	0.0490					
Control	33	0.3178	0.3178	0.2104	0.3478	0.3401	0.3391	0.1499	0.2100
Exhausted	32	0.5263**	0.3728*	-0.1195	0.6935***	0.3742*	0.3421	-0.5320**	-0.0281
Glucose	29	0.6380***	0.5329**	0.0769	0.6043***	0.5797**	0.4878**	-0.0703	0.0554
Starved	26	0.3564	0.1897	-0.2392	0.3755	0.2280	0.1789	0.1264	-0.2171
C+E+G	94	0.4193***	0.3744***	0.0563	0.4593***	0.4050***	0.3752***	-0.2068*	0.0940
C+E+G+S	120	0.3951***	0.3416***	0.0213	0.4240***	0.3869***	-0.3423	-0.1678	0.0420

*p<0.05 **p<0.01 ***p<0.001

The next step was to calculate the higher order correlations to determine if any of the reproductive organs are spuriously correlated with courtship time through their association with another reproductive organ variable. The partial correlations between one variable and courtship time holding all other variables constant are given in table 6.10. The problem with this type of analysis with intercorrelated variables is that with each successive variable held constant, the effect of the variable of interest on the dependent variable is reduced by the removal of its relationship with the constant variable. The partial correlations with courtship duration in this table are negative for accessory gland and testis area but they are low and only significant in one case. When the groups are combined for each experiment, relative testis and accessory gland size are important in the first experiment, and relative accessory gland size and flight duration (probably largely due to the importance of flight in the exhausted group) in the second experiment. Seminal vesicle area and wing length are unimportant when all other variables are held constant.

6.3.3. Wild flies.

The relationships between wing length, the size of the reproductive organs and crop size are given in table 6.11. All the variables are significantly intercorrelated with the exception of testis size with wing length and with seminal vesicle size. The partial correlations reveal that both accessory gland and testis size are significantly correlated with crop size when all other variables are held constant. Males with fuller crops have larger accessory

Partial correlations with $\log_{10}$ (courtship duration) holding all other variables constant

Group	N	$r_{CTAG.}$	$r_{CTTE.}$	$r_{CTSV.}$	$r_{CTWL.}$	$r_{CTFL.}$
			Without flight			
2 day	26	-0.0742	-0.0032	-0.0781	-0.1517	
5 day	36	-0.2711	-0.3496*	-0.0536	-0.2097	
10 day	36	-0.1662	-0.0663	-0.0652	0.0164	
2+5+10	98	-0.2527**	-0.2193	-0.0065	0.0490	
Control	33	-0.1174	-0.1553	0.0272	0.2104	
Exhausted	32	-0.0049	-0.1298	-0.2456	-0.1195	
Glucose	29	-0.2225	-0.1991	-0.0007	0.0769	
Starved	26	-0.0607	-0.1225	0.1046	-0.2392	
C+G+E	94	-0.2200*	-0.1166	0.0121	0.0563	
C+E+G+S	120	-0.1963*	-0.1246	0.0046	0.0213	
			With flight			
Control	33	-0.1111	-0.1779	0.0262	0.2100	0.1499
Exhausted	32	0.1101	-0.1961	-0.2291	-0.0281	-0.5320**
Glucose	29	-0.1675	-0.2079	-0.0069	0.0554	-0.0703
Starved	26	-0.0562	-0.1150	0.1179	-0.2171	0.1264
C+G+E	94	-0.1765*	-0.1312	-0.0412	0.0940	-0.2068*
C+E+G+S	120	-0.1748*	-0.1269	-0.0312	0.0420	-0.1678

*p<0.05 **p<0.01 ***p<0.001

Table 6.10. The table lists the partial correlations between courtship duration and each variable holding all the other variables constant. All variables except flight duration were log-transformed before the analysis. The correlations are between the variables represented by the letters occurring after r and before the full stop. The variables used are courtship duration (CT), accessory gland area (AG), testis area (TE), seminal vesicle area (SV), wing length (WL) and flight duration (FL).

	LOG_{10} WL	LOG_{10} CROP	LOG_{10} AG	LOG_{10} TE
LOG_{10} CROP	0.715***			
LOG_{10} AG	0.720***	0.791***		
LOG_{10} TE	0.340	0.519**	0.384*	
LOG_{10} SV	0.651***	0.643***	0.835***	0.320

Correlations between the residuals of each variable regressed on log_{10} (WL)

	LOG10 CROP	LOG10 AG	LOG10 TE
LOG_{10} AG	0.599**		
LOG_{10} TE	0.422*	0.194	
LOG_{10} SV	0.330	0.703***	0.123

Partial correlations between LOG_{10}(crop) and each variable holding all other variables constant

AG rCPAG.WLTESV	TE rCPTE.AGWLSV	SV rCPSV.AGTEWL	WL rCPWL.AGTESV
0.478**	0.374*	-0.126	0.351

*p<0.05 **p<0.01 ***p<0.001

Table 6.11. The data are for a group of wild flies. The Pearson correlations between the log-transformed variables are given and so are the Pearson correlations between the residuals of each variable regressed on wing length and the partial correlations between crop area and each variable holding all other variables constant. The log-transformed variables used are crop area (CP), accessory gland area (AG), testis area (TE), seminal vesicle area (SV) and wing length (WL).

glands and testes. This is illustrated in figures 6.6 and 6.7 where the residuals from the regression of crop size on wing length are plotted against the residuals from the regressions of accessory gland and testis size on wing length.

The median flight duration of the group of wild flies was 36 minutes (n=19, range=9 to 105) and was similar to the median flight durations of the glucose-fed and starved flies.

Figures 6.6 and 6.7. Relative crop area (crop area with the effects of wing length removed) is plotted against relative reproductive organ area (reproductive organ area with the effects of wing length removed) for a group of wild males. CP = crop area in mm^2, WL = wing length in mm, AG = accessory gland area in mm^2 and TE = testis area in mm^2.

Figure 6.6. Relative crop area plotted against relative accessory gland area.

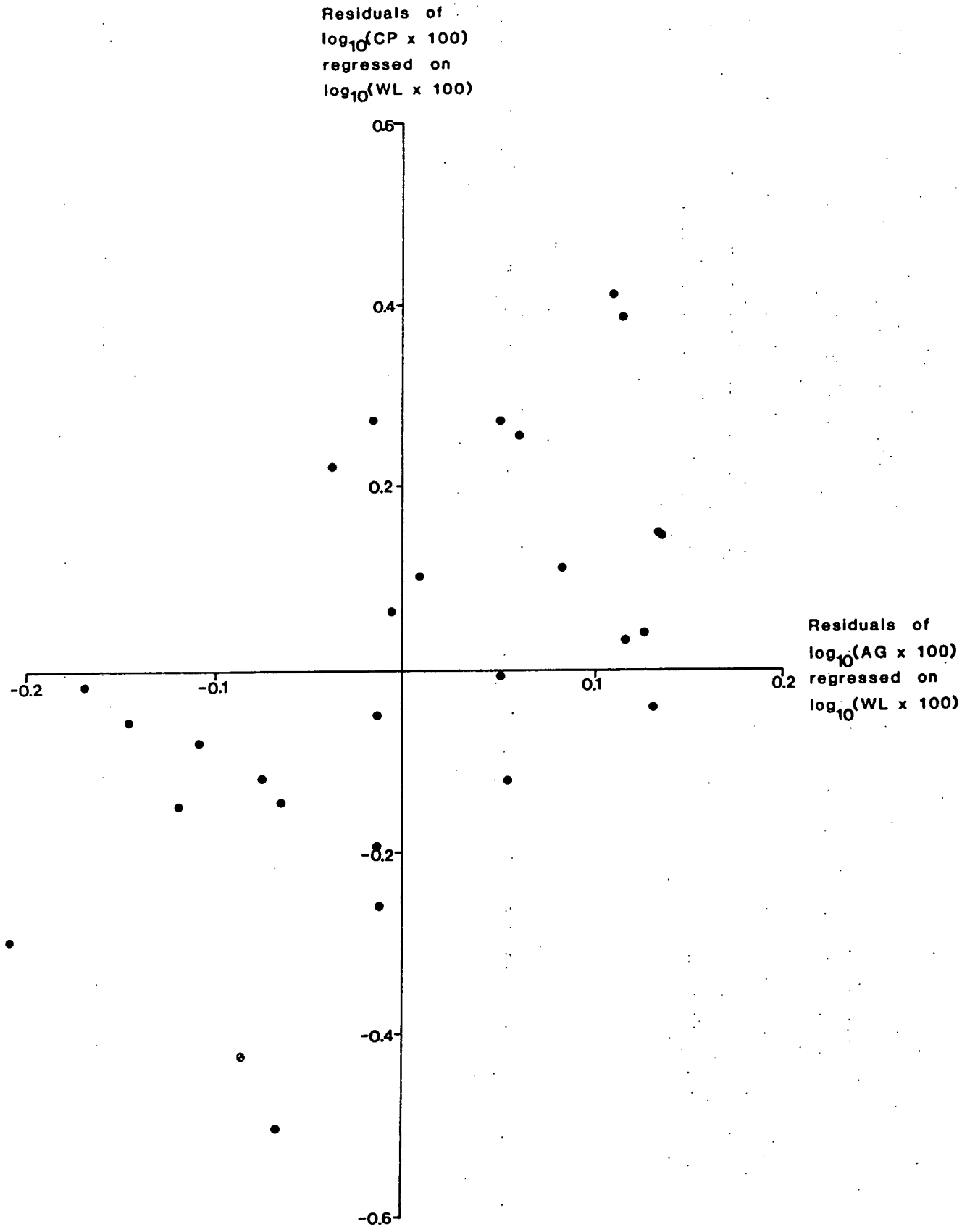
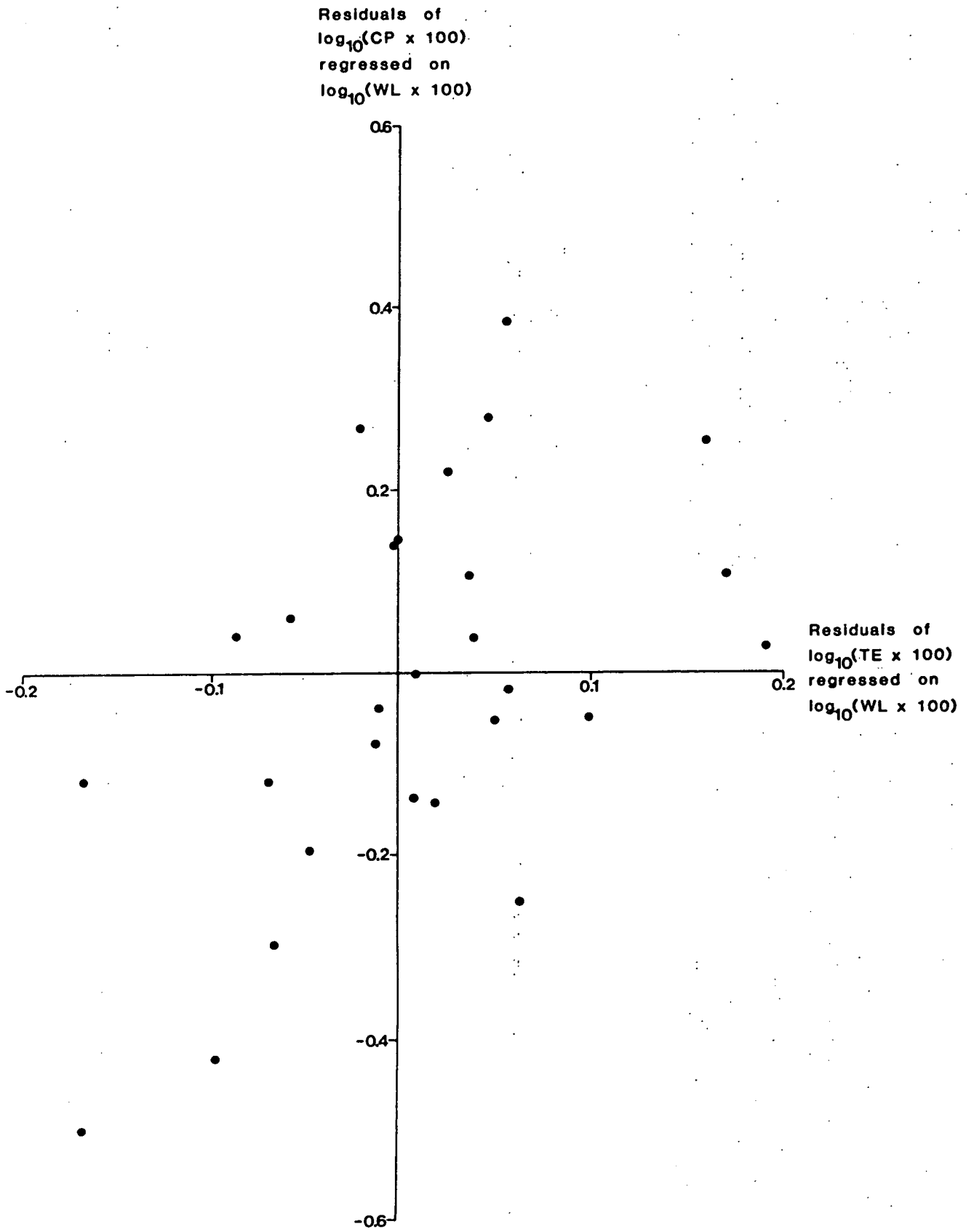


Figure 6.7. Relative crop area plotted against relative testis area.



6.4. CONCLUSIONS.

1) The size of the three reproductive organs and wing length are intercorrelated.

2) The accessory glands and seminal vesicles are larger in older flies.

3) Flies kept on a poor food medium had small accessory glands and seminal vesicles. If they were maintained on a glucose diet only the accessory glands were affected.

4) Wild flies tend to have a relative accessory gland size broadly similar to five day-old laboratory flies raised on good food and a relative seminal vesicle size resembling the ten day-old flies.

5) Younger flies have smaller accessory glands and seminal vesicles and have longer courtships than controls.

6) Flies exhausted of energy reserves for flight, but with similar reproductive organ sizes, have longer courtships than controls.

7) Starved flies have fewer energy reserves for flight than controls, but similar sized reproductive organs and tend to have longer courtships.

8) Flies kept on a glucose diet have small accessory glands and fewer energy reserves than controls and have longer courtships.

9) Poorly-fed flies have small accessory glands and seminal vesicles and few energy reserves for flight and have the longest courtships.

10) Within groups flies with relatively small reproductive organs have longer courtships. Wing length *per se* was not correlated with courtship time.

11) The individual reproductive organ sizes negatively correlated with courtship duration are the accessory gland and testis sizes.

12) Within the exhausted group there was a negative correlation between flight time after courtship and courtship duration.

13) Wild flies with larger crops have larger accessory glands and testes for any given wing length.

14) The energy reserves for flight of wild flies arriving at bait are similar to the laboratory glucose-fed and starved groups.

6.5. DISCUSSION.

The size of a male's accessory glands and seminal vesicles is affected by age, adult diet and previous copulations. The levels of glycogen stored are influenced by diet and exercise. Older flies have larger accessory glands and seminal vesicles. On a diet low in protein and energy little accessory fluid accumulates, few sperm mature and glycogen reserves are low. If the diet is low in protein but rich in glucose little accesssory fluid accumulates, but there is more sperm maturation and glycogen storage. Testis size appears to remain constant after eclosion and is not affected by these treatments. Three variables have been found to influence courtship duration; the amount of glycogen stored, relative accessory gland size and relative testis size. I will discuss each in turn.

Vigour, measured in terms of the time to exhaustion in sustained flight, seems to be important in flies with low glycogen reserves. Flies in the wild must fly to feeding and breeding sites patchily distributed in time and space. Generally, it is not known how long or frequent flights are or how much energy is expended before arrival. Some desert species travel considerable distances between oases, possibly partly relying on the wind (COYNE, BOUSSY, PROUT, BRYANT, JONES and MOORE 1982). The energy reserves of the wild flies collected in this study were similar to the starved and glucose-fed flies. Although this represents the energy available for courtship of flies arriving at the aggregation site, the flies had already flown an unknown distance to reach the aggregation site. Their energy reserves at the start of the activity period must have been greater. This observation, in conjunction with the observation

that wild flies have seminal vesicle sizes characteristic of ten day-old flies but accessory gland sizes characteristic of five day-old flies, suggests that the diet of wild flies is more limited in protein than glucose content.

Flies with relatively large accessory glands have faster courtships. This observation fits well with what is known about the influence of accessory fluid on male fertility and the relationship between male fertility and courtship success (discussed earlier). In the last chapter I argued that there was some quantitative influence on male fertility. The amount of accessory fluid available for transfer seems a likely candidate. Why males with smaller accessory glands have longer courtships is not yet known. It may be that females can assess the levels of accessory fluid present in males using a direct chemical cue and discriminate against males with smaller glands. MANE, TOMPKINS and RICHMOND (1983) have argued that males are able to detect cis-vaccenylacetate, which is produced and stored by the accessory glands, in other males and mated females. It is also possible that accessory gland size is related to some visual, auditory or tactile cue. Or possibly differences in accessory gland size are reflected in different courtship behaviours, courtship vigour (HALLIDAY 1976) or courtship persistence (RUTOWSKI 1979). The mechanisms mediating the observed effect are investigated in the next chapter.

The third influence on mating speed is relative testis size. This effect is perhaps, more surprising. Levels of mature sperm available for transfer are reflected by differences in seminal vesicle size. Again, the mechanisms mediating the effect are unknown and will be discussed in the next chapter.

Flies collected from the wild had similar reproductive organ sizes to the laboratory flies used in the courtship experiments. There was also an association between relative crop size and both accessory gland size independent of wing length, testis size and seminal vesicle size, and testis size independent of accessory gland size, wing length and seminal vesicle size. Flies with fuller crops tended to have larger accessory glands and larger testes. One possible explanation of the relationship between crop and accessory gland size is that flies with currently full accessory glands direct fewer nutrients and less energy into the production of accessory fluid and so have a lower turnover of food. This seems unlikely because the accessory glands of wild flies are not as large as the glands of the older laboratory flies and are probably not 'full'. Another possibility is that flies which are better at obtaining food (because they are better at locating it, monopolising it or collecting it) have more nutrients available for turning into accessory fluid and so have larger accessory glands. However, this does not explain the relationship between crop and testis size. Because the indications are that testis size does not change after eclosion (apart from the effects of senescence) it is tempting to speculate that relative testis size, or something correlated with it, in some way affects relative crop size, perhaps through the aggressive monopolisation of food. Male D.melanogaster aggressively defend food sources in the laboratory (JACOBS 1978, DOW and VON SCHILCHER 1975) and D.subobscura males aggressively displace each other from feeding sites in the field (unpublished personal data). However, the only studies to date concerning what determines the outcome of aggressive interactions in Drosophila have concentrated on

thorax or wing length (DOW and VON SCHILCHER 1975, PARTRIDGE and FARQUHAR 1983). It would be interesting to know whether testis size is important in aggressive disputes. Whatever the reasons for the association between relative crop size and relative accessory gland size in wild flies, the consequences are that males with fuller crops have larger accessory glands and testes. This is interesting in view of the work on courtship feeding and courtship success reported earlier in this thesis. If males with fuller crops are better able to regurgitate drops of food during courtship (chapters 2 and 3) then this would provide a mechanism by which females could discriminate between low and high fertility males. Females that mated with drop producers would be likely to mate with high fertility males and might leave more progeny as a result. The link between progeny production and relative reproductive organ size has not yet been fully established. In the next chapter I show that females mated to males with small accessory glands and seminal vesicles leave fewer progeny. I also investigate the mechanisms mediating the high courtship success of males with large reproductive organs.

Chapter 7.

MALE FERTILITY, PROGENY PRODUCTION AND MATING SUCCESS.

7.1. INTRODUCTION.

In the last chapter I reported a negative correlation between male courtship duration in single pair tests and the size of the accessory glands and testes. In this chapter I examine the relationship between the size of the reproductive organs and progeny production and attempt to identify some of the reasons why males with larger reproductive organs have faster courtships.

Three groups of males are used: five day-old males with large accessory glands and seminal vesicles and high vigour, two day-old males with small accessory glands and seminal vesicles and high vigour and poorly-fed five day-old males with small accessory glands and seminal vesicles and low vigour. In the first set of experiments I tested males in competition to determine whether the five day-old group's faster courtship speeds in single pair matings is reflected in a mating advantage in 'choice' experiments. The next question addressed is whether females gain any material benefit by mating with males possessing large reproductive organs. Do they lay more eggs and leave more progeny or do they, perhaps, have shorter copulations? Finally, I look for courtship differences between the groups. Males may differ in the proportion of time they spend courting, or in the rate at which they perform particular courtship behaviours, the duration of these behaviours or, possibly, the patterning of courtship. An analysis of detailed courtship recordings should provide some indication of whether certain males are courting more

vigorously.

It is possible that there are no differences in the courtship patterning or frequency at which males perform particular behaviours, but that the intensity of particular courtship stimulii varies. It is known that wing area contributes to the courtship success of male D.subobscura. The more of the wing that is removed, the greater is the reduction in courtship success (BROWN 1964). In D.melanogaster wing area is associated with the loudness of the courtship song produced (PARTRIDGE personal communication). However, male D.subobscura do not sing and the influence of wing area on courtship speed in this species probably lies in the visual stimulus presented by the wings during the wing display performed immediately prior to circling and attempted copulation. Indeed, BROWN (1964) argued that artificially reducing male wing area lessened the visual stimulation received by the female during wing display and made it more likely that she would move away before the male could circle and attempt copulation. In many Drosophila species in which wing waving displays feature prominently in the courtship, the males' wings are darkly coloured or patterned, for example the picture-winged Hawaiian species (SPIETH 1968) and D.tristis within the obscura group (BROWN 1964). Wing area appears to influence male courtship success and so too might the darkness or patterning of the wing.

The dark pigment, lipofuscin, is a product of cellular degeneration and accumulates in post-mitotic cells more or less linearly over time. It has been recorded in many organisms ranging from the bread-mould, Neurospora (MUNKRES and MINSSEN 1976), to the field mouse, Peromyscus polionotus (DAPSON et al. 1980). Measuring the amount of lipofuscin present in an individual can provide an

accurate assessment of physiological age (e.g. ETTERSHANK, MACDONNEL and CROFT 1983 for Scatophaga bullata). If this accumulation of age pigment results in a darkening of a fly's wings and physiological age also reflects the relative size of the reproductive organs, then wing darkness might be an important visual cue reflecting a male's fertility. I present the results from a few simple experiments designed to test whether the wings of male D.subobscura darken with age and whether darkness of wing does influence male courtship success.

Another possibility is that females are stimulated directly by chemicals associated with the male reproductive tract and that larger reproductive organs provide a greater chemical stimulus. SCHLEIN and GALUN (1984) showed that female Musca domestica were attracted to gauze-covered petri dishes containing macerated testes and accessory ducts, but not to macerated gut. They also found that males achieved copulation faster if the females had been exposed to macerated male reproductive tissue prior to courtship, but not if the females had been exposed to macerated gut. Furthermore, the effect of exposure to male reproductive tissue on subsequent courtship speed depended on the age of the tissue used. The testes and accessory ducts from two day-old males had no effect, but the influence on females increased with male age so that the greatest effect was recorded with extracts taken from six to ten day-old males.

MANE, TOMPKINS and RICHMOND (1983) showed that male courtship of virgin female D.melanogaster was inhibited if the abdomens of the virgin females were doused in cis-vaccenylacetate, a compound normally found in the accessory glands of males and transferred to females during copulation. Also, male D.melanogaster often direct

courtship towards very young males, but this is not the case if cis-vaccenylacetate is applied to the young males (RICHMOND personal communication). In this chapter I report the results of a crude experiment involving dousing young males with cis-vaccenylacetate to see if this increased their courtship speed with virgin females.

Finally, an important factor determining a male's courtship speed might be his ability to keep up with the female during the dance (MAYNARD SMITH 1956, this thesis). In a series of preliminary tests it proved very difficult to get low fertlity males to court a model (see chapter 4) and this line of investigation was abandoned.

7.2. MATERIALS AND METHODS.

The three groups of flies used in these experiments were five day-old, two day-old and poorly-fed five-day old flies. The five and two day-old flies were aged individually in vials containing standard drosophila medium seeded with active Baker's yeast. The poorly-fed flies were aged on a low nutrient medium (see chapter 6), but were given access to a good supply of active Baker's yeast half an hour before artificial dawn on the morning of courtship.

7.2.1. Competitive mating.

A single, marked, virgin five day-old male was introduced with either a marked, virgin two day-old male or a marked, virgin poorly-fed male to a five day-old virgin female in a vial containing only damp cotton-wool. The identity of the male achieving copulation was recorded. The males were marked whilst lightly anaesthetised with carbon dioxide on the evening of the fourth day by clipping either the right or the left wing. The wing clipped was varied within groups.

7.2.2. Copulation duration and progeny production.

A single virgin male was introduced to a five day-old virgin female in a vial containing damp cotton-wool and the duration of copulation recorded. The female was retained and the male was introduced to a second virgin female. Again, the duration of copulation was recorded and the female retained. A wing was removed

from each male and measured under the binocular microscope. The females were kept individually in vials containing charcoal medium with a suspension of yeast dried on the side of the vial (see chapter 5). Each day the females were transferred to fresh vials. The egg production and egg fertility of each female was scored for ten days after mating. The number of eggs laid and the number hatching over this ten day period correlates with a female's lifetime score (chapter 4- 1st female: eggs laid, r=0.7160; eggs hatched, r=0.7644: 2nd female: eggs laid, r=0.6052; eggs hatched, r=0.5158). Although using the ten day period is likely to underestimate the variance in female lifetime reproductive success (PARTRIDGE in preparation), particularly as one of the components reducing female progeny production in chapter 5 was egg hatchability declining earlier in a female's lifespan, it was felt that this disadvantage was outweighed by the considerable amount of time and work saved. One wing was removed from each female at the end of the experiment and measured.

7.2.3. Detailed courtship recording.

The courtships of single virgin males with a virgin female in vials containing damp cotton-wool were observed through a x2 magnifying lens and recorded on the Apple keyboard. Each courtship was observed for up to 3 minutes and then the flies were separated and the male's wing length measured. The males were weighed the evening before the courtship observation, then the following day the courtships of 3 large males (1 from each treatment) were sequentially observed, then three small males (1 from each treatment), then three large and so on. This procedure was continued for 4 mornings

observation until 30 courtships for each treatment had been recorded. This design should have ensured that there was no within or between day bias producing treatment differences.

The frequencies, rates, durations and proportion of time spent in particular behaviours were compared for the different groups using a Mann-Whitney U test (see chapter 2). Correlations between wing length and a number of variables were calculated using the Spearman rank correlation coefficient. To compare the large males within one treatment with the large males within another, the males were grouped by wing length (large> 1.75mm >small). Wing length was used in preference to weight because it does not fluctuate. This did mean that some males originally chosen as small on a weight basis were classified as large in the analysis, and vice versa for large males. However, this is unlikely to have introduced any bias into the results and the large and small group comparisons show the same trends as the overall group comparisons.

7.2.4. Wing darkness.

Males were stored individually in vials containing standard drosophila medium seeded with active Baker's yeast and transferred to fresh vials every 5 days. One wing was removed from males of different ages and fixed to a piece of transparent plastic using transparent sticky tape. Observers were asked to look at a pair of wings against a white background under the binocular microscope and say if one wing looked darker. The response and number of each pair of wings was recorded. The observers were asked to compare a particular patch on each wing (for instance, around the anterior

cross vein) rather than simply gain an overall impression which might be influenced by possible differences in wing size.

To examine the effect artificially darkening a male's wings had on his subsequent courtship success, two day-old virgin males were used. The males were weighed the evening before the experiment and pairs of males of similar weight were numbered and put to one side. The next day, an hour before courtship, each pair of males was anaesthetised using carbon dioxide and the wings of one male were dusted with carbon powder using a fine paint brush. Even after a certain amount of movement and grooming the wings of 'dusted' males were distinctly darker. Each male from a pair was introduced to a virgin five day-old female in a damp cotton-wool vial and the time from the first bout of courtship to copulation was recorded for each.

7.2.5. Dousing with cis-vaccenylacetate.

The procedure used was the same as that described by MANE et al. (1983) for D.melanogaster, except that twice the quantity of solution was applied as D.subobscura are bigger flies. To the abdomen of each anaesthetised two day-old male within one group (treated) 400 ng of cis-vaccenylacetate in 0.2 microlitres of acetone was applied using a micropipette and to the two day-old males within another group (controls) just 0.2 microlitres of acetone was applied. There was a third group of untreated two day-old males (untreated controls). Thirty minutes later the treated, treated control and untreated control two day-old males were put individually with a five day-old virgin female in vials containing damp cotton-wool and the courtship durations recorded.

7.3. RESULTS.

7.3.1. Competitive mating success.

In the 'choice' tests with two marked males and one female, the five day-old males mated significantly more often than both the two day ($n=25$, $\chi^2=4.50$, $p<0.05$) and poorly-fed ($n=25$, $\chi^2=10.58$, $p<0.05$) males.The proportion of two day-old and five day-old poorly-fed males mating in each test was not significantly different ($\chi^2=1.70$, $p>0.1$). These results are consistent with LONG et al.'s (1980) findings using D.melanogaster and with the courtship speeds reported in the last chapter.

7.3.2. Copulation duration.

In Figure 7.1 copulation duration is plotted against male wing length for the three treatments. In all 3 treatments a male's second copulation tended to be longer than his first, and both the two day and poorly-fed groups had longer first and second copulations than the five day group (table 7.1). There were no significant differences between the 2 day-old and poorly-fed flies.

For the second copulation of the five day-old males there was a significant negative correlation between male wing length and the log of copulation duration, a significant positive correlation between female wing length and log copulation duration and a significant correlation between the difference between male and female wing length and the log of copulation duration (table 7.2).

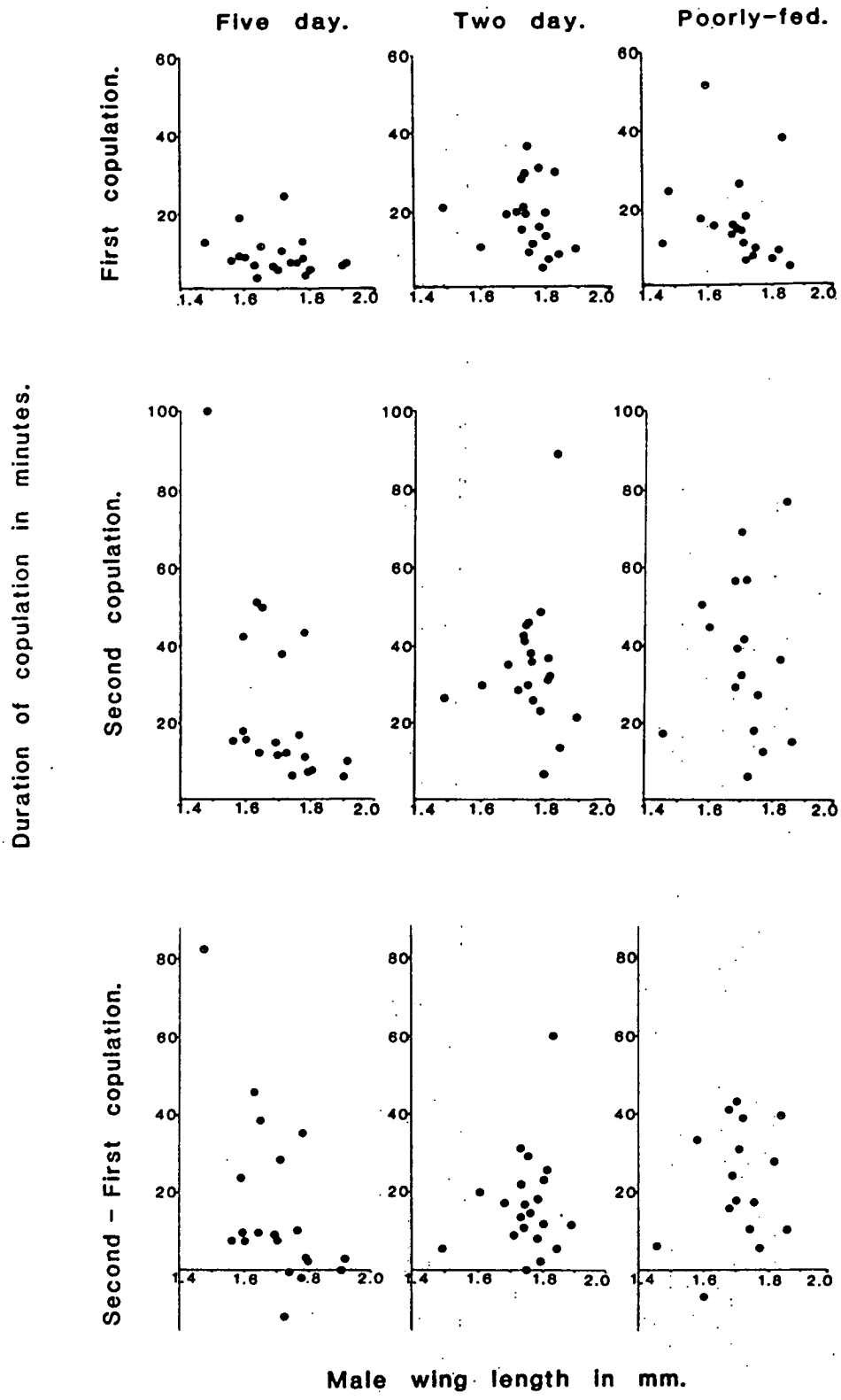


Figure 7.1. Copulation duration is plotted against male wing length for three groups of males; five day-old, two day-old and five day-old poorly-fed males. Shown are the duration of a male's first copulation, his second copulation starting within an hour of the end of the first and the difference between the two durations.

TABLE 7.1

Group comparisons of wing length (t-test) and copulation duration (Mann-Whitney and sign test).

Group	Mean wing length in mm ± 95% c.l.			Median copulation duration in minutes with range		
	Male	1st female mated	2nd female mated	1st female mated		2nd female mated
5 day	1.70+0.05	1.94+0.04	t=1.81	1.90+0.03	7.04(3.42-24.25)	p=0.02	14.75(5.50-99.92)
	n=20	n=18	p=0.08	n=20	n=19	sign	n=19
2x5	t=1.55	t=0.39		t=0.16	w=279	test	w=342
	p=0.130	p=0.70		p=0.87	p<0.001		p=0.028
2 day	1.75+0.04	1.95+0.03	t=2.7	1.90+0.03	18.87(4.42-35.92)	P<0.001	33.42(6.25-89.00)
	n=21	n=19	p=0.01	n=19	n=21	sign	n=21
2xP.F.	t=1.92	t=2.54		5=0.07	w=386	test	w=372
	p=0.064	p=0.016		p=0.94	p=0.273		p=0.946
Poor food	1.69+0.05	1.89+0.03	t=0.23	1.90+0.03	14.67(4.66-51.45)	p=0.08	34.12(5.66-76.66)
	n=19	n=18	p=0.82	n=18	n=20	sign	n=18
5xP.F.	t=0.34	t=1.74		t=0.21	w=522.5	test	w=418.5
	p=0.74	p=0.09		p=0.84	p=0.002		p=0.050

TABLE 7.2

Pearson correlations between wing length and copulation duration.

Group	Male wing length in mm and log10 (copulation duration in mins)		Female wing length in mm and log10 (copulation duration in mins)		Female–male wing length in mm and log10 (copulation duration in mins)	
	1st female mated	2nd female mated	1st female mated	2nd female mated	1st female mated	2nd female mated
5 day	-0.285 n=19	-0.636** n=19	0.269 n=18	0.482* n=19	0.323 n=18	0.736*** n=19
2 day	-0.218 n=21	-0.084 n=21	-0.045 n=19	0.227 n=21	-0.031 n=18	0.195 n=21
5 day poor food	-0.372 n=19	-0.043 n=21	0.199 n=18	0.099 n=21	0.120 n=17	0.092 n=15

*p<0.05 **p<0.01 ***p<0.001

The duration of a male's first copulation was significantly correlated with the duration of his second copulation in both the two day-old ($r=0.589, p<0.05$) and the poorly-fed groups ($r=0.623, p<0.05$), but not in the five day-old group ($r=0.279, p>0.05$).

7.3.3. The number of eggs laid and the number hatching.

In both the two day-old and the poorly-fed groups the first female to be mated laid significantly more eggs than the second female and a greater number hatched (table 7.3). The differences between the first and second females mated to the five day-old males were not significant.

For both the first and second females mated, the females mating with the five day-old males laid significantly more eggs than the females mating with the two day-old males and a greater number hatched. The second females mating with the poorly-fed males laid fewer eggs than the second females mating with the five day-old males and fewer eggs hatched. However, the differences between the first females mated in these two groups are not significant. The numbers of eggs laid by the females mating with the two day-old males were not significantly different from the numbers laid by the females mating with the poorly-fed males, nor were the numbers hatching. There were high correlations within all three treatments between the numbers of eggs laid by each female and the numbers hatching (table 7.4).

TABLE 7.3

Group comparisons of female productivity data.

(Mann-Whitney and sign test).

Group	Median and range in number of eggs laid: 1st female mated		Median and range in number of eggs laid: 2nd female mated	Median and range in number of eggs hatched: 1st female mated		Median and range in number of eggs hatched: 2nd female mated
5 day	271(52-469)	p=0.5	217(103-430)	258(49-458)	p=0.324	190(007-342)
	n=19	sign	n=19	n=19	sign	n=19
5x2	w=465	test	w=507	w=462	test	w=501
	p=0.044		p=0.0015	p=0.0051		p=0.0026
2 day	162(14-358)	p=0.026	116(0-312)	126(3-356)	p=0.016	13(0-276)
	n=21	sign	n=21	n=21	sign	n=21
2xPF	w=373.5	test	w=410.5	w=386.5	test	w=400.5
	p=0.081		p=0.434	p=0.159		p=0.297
Poor food	220(1-384)	p=0.042	123(0-378)	198.5(1-379)	p=0.012	96(0-369)
	n=20	sign	n=20	n=20	sign	n=20
5xPF	w=409.5	test	w=462	w=407	test	w=452
	p=0.415		p=0.022	p=0.456		p=0.048

TABLE 7.4

Pearson correlations between productivity data, male and female wing length and copulation duration.

Group	Male wing length and the number of eggs laid: 1st female	Male wing length and the number of eggs laid: 2nd female	Male wing length and the number of eggs hatched: 1st female	Male wing length and the number of eggs hatched: 2nd female
5 day	r=-0.308, =19	r=-0.257, =19	r=-0.167, n=19	r=-0.315, n=19
2 day	r=-0.090, n=20	r=-0.133, n=20	r=-0.122, n=20	r=-0.134, n=20
Poor food	r=-0.153, n=19	r= 0.460, n=19	r=0.147, n=19	r= 0.406, n=19

	Female wing length and the number of eggs laid: 1st female	Female wing length and the number of eggs laid: 2nd female	Female wing length and the number of eggs hatched: 1st female	Female wing length and the number of eggs hatched: 2nd female
5 day	r= 0.364, n=19	r= 0.441, n=19	r= 0.355, n=19	r= 0.287, n=19
2 day	r= 0.366, n=19	r= 0.059, n=20	r=-0.418, n=19	r= 0.071, n=20
Poor food	r= 0.203, n=18	r=-0.092, n=16	r=-0.130, n=18	r=-0.055, n=16

	Copulation during and the number of eggs laid: 1st female	Copulation during and the number of eggs laid: 2nd female	Copulation duration and the number of eggs hatched: 1st female	Copulation duration and the number of eggs hatched: 2nd female
5 day	r= 0.139, n=19	r= 0.210, n=19	r= 0.180, n=19	r= 0.077, n=19
2 day	r= 0.388, n=21	r=-0.316, n=21	r= 0.169, n=21	r=-0.356, n=21
Poor food	r= 0.073, n=20	r= 0.099, n=18	r=-0.021, n=18	

	The number of eggs laid and the number hatched: 1st female	The number of eggs laid and the number hatched: 2nd female	The number of eggs laid: 1st & 2nd female	The number of eggs hatched: 1st & 2nd female
5 day	r= 0.762***, n=19	r= 0.762***, n=19	r= 0.205, n=19	r= 0.140, n=19
2 day	r= 0.877***, n=21	r= 0.911***, n=21	r= 0.243, n=21	r= 0.248, n=21
Poor food	r= 0.875***, n=20	r= 0.943***, n=18	r= 0.037, n=18	r= 0.172, n=18

*p<0.05 ***p<0.001

7.3.4. Courtship behaviour.

A summary of the frequency, rate, duration and proportion of time spent in various courtship behaviours is given in table 7.5. Sequence diagrams illustrating the frequencies of the behavioural transitions are given in figures 7.2 to 7.4. Differences between the groups are discussed below.

7.3.4.1. Between group comparisons.

7.3.4.1.1. Two day-old and five day-old groups.

The comparisons significant at $p<0.05$ are given in table 7.6. The five day-old males produced more drops and danced more frequently than the two day-old flies. However, in this experiment the courtship durations for the two groups did not differ. The reasons for the failure to replicate the findings reported in chapter 6 are unclear. In the earlier experiment the courtship times of each male with three different females were recorded and the median value was used in the analysis. This procedure was adopted in order to reduce possible error introduced by differences in female receptivity. It could be these differences in receptivity that are responsible for the current findings. It might have been better to record the courtships of each male with three different females.

7.3.4.1.2. Five day-old and poorly-fed groups.

The poorly-fed males had significantly longer courtships than the five day-old males (table 7.6). The five day-old males produced drops at a higher rate, carried them for longer and had more taken by

Table 7.5. A summary of the courtship behaviour for the six groups is given. The number of times a behaviour occurred during courtship was measured as was the rate at which the behaviour occurred within a courtship and, for some behaviours, the median bout duration within a courtship and the proportion of a male's total courtship time that was spent performing that behaviour. For each group a median value is given with the range of values in parentheses.

TABLE 7.5

		Mean wing length mm	Number of courtships	DROPS: Total number Produced	Taken	% Taken	DROPS: Median number per courtship Produced	Taken	Median rate of production min^{-1}	Median duration carried secs.	COURTSHIP TIMES: Pairs mating within 3 minutes	% Mating	Median duration secs.
Five day	Large male	1.83±0.03	11	23	5	22	1.8 (1-5)	0.4 (0-1)	0.8 (0.5-3.1)	4.5 (1.7-6.9)	8	73	125.4 (19.2-190.0)
	Small male	1.68±0.02	19	30	9	30	1.2 (0-5)	0.4 (0-1)	0.9 (0.0-5.0)	4.6 (2.9-6.3)	13	68	74.4 (12.1-180.0)
Two day	Large male	1.81±0.02	18	20	13	65	1.1 (0-3)	0.8 (0-2)	1.1 (0.0-5.6)	4.3 (2.7-7.5)	14	70	57.0 (10.8-180.0)
	Small male	1.70±0.03	11	10	7	70	0.9 (0-2)	0.6 (0-2)	0.4 (0.0-3.6)	4.6 (3.3-4.9)	6	55	151.1 (17.0-180.0)
Five day poor food	Large male	1.80±0.02	17	3	1	33	0.1 (0-1)	0.0 (0-1)	0.0 (0.0-1.1)	3.2 (3.0-6.9)	5	29	177.2 (36.1-180.0)
	Small male	1.69±0.02	12	5	1	20	0.2 (0-3)	0.0 (0-1)	0.1 (0.0-4.5)	3.1 (2.5-3.2)	3	25	178.1 (13.3-180.0)

TABLE 7.5 (continued)

		ORIENTATION				ORIENTATION IN FRONT				FOLLOW			
		Frequency per courtship	Rate min^{-1}	Duration secs.	Proportion of total time	Frequency per courtship	Rate min^{-1}	Duration secs.	Proportion of total time	Frequency per courtship	Rate min^{-1}	Duration secs.	Proportion of total time
Five day	Large male	3.0 (1.0-7.0)	1.7 (0.8-6.2)	5.8 (2.0-31.6)	0.29 (0.06-0.70)	2.0 (1.0-6.0)	1.2 (0.5-3.5)	2.3 (1.7-3.7)	0.05 (0.02-0.30)	2.7 (0.0-7.0)	1.8 (0.0-4.1)	6.6 (2.7-14.1)	0.21 (0.00-0.97)
	Small male	2.4 (0.0-8.0)	2.0 (0.0-7.1)	5.0 (1.5-22.2)	0.24 (0.00-0.62)	1.3 (0.0-8.0)	1.2 (0.0-5.2)	1.6 (0.5-54.5)	0.05 (0.00-0.46)	1.9 (0.0-8.0)	1.7 (0.0-4.5)	6.7 (2.7-27.9)	0.25 (0.00-0.75)
Two day	Large male	2.5 (0.0-7.0)	2.5 (0.0-6.1)	3.6 (1.2-30.9)	0.25 (0.00-0.79)	1.3 (0.0-5.6)	1.5 (0.8-5.4)	1.8 (0.00-0.52)	0.10 (0.00-0.52)	1.2 (0.0-7.0)	1.4 (0.0-4.5)	7.6 (1.4-26.8)	0.19 (0.00-0.74)
	Small male	4.0 (1.0-11.0)	1.7 (0.7-4.6)	4.8 (1.2-32.3)	0.23 (0.07-0.45)	1.2 (0.0-4.0)	0.5 (0.0-3.6)	2.7 (1.2-36.6)	0.02 (0.00-0.34)	2.7 (0.0-12.0)	1.0 (0.0-5.0)	10.8 (2.5-59.0)	0.30 (0.00-0.45)
Five day poor food	Large male	3.0 (1.0-10.0)	1.3 (0.3-4.1)	3.7 (1.5-41.0)	0.12 (0.01-0.78)	1.1 (0.0-7.0)	0.4 (0.0-3.3)	1.9 (0.9-66.4)	0.01 (0.00-0.37)	3.7 (0.0-12.0)	1.3 (0.0-4.1)	5.3 (1.5-14.0)	0.24 (0.00-0.75)
	Small male	4.0 (0.0-11.0)	1.5 (0.0-4.5)	3.2 (2.1-5.0)	0.10 (0.00-0.49)	1.0 (0.0-4.0)	0.4 (0.0-4.5)	2.0 (1.3-4.0)	0.01 (0.00-0.10)	3.5 (0.0-11.0)	1.0 (0.0-3.7)	9.1 (5.0-21.6)	0.12 (0.00-0.59)

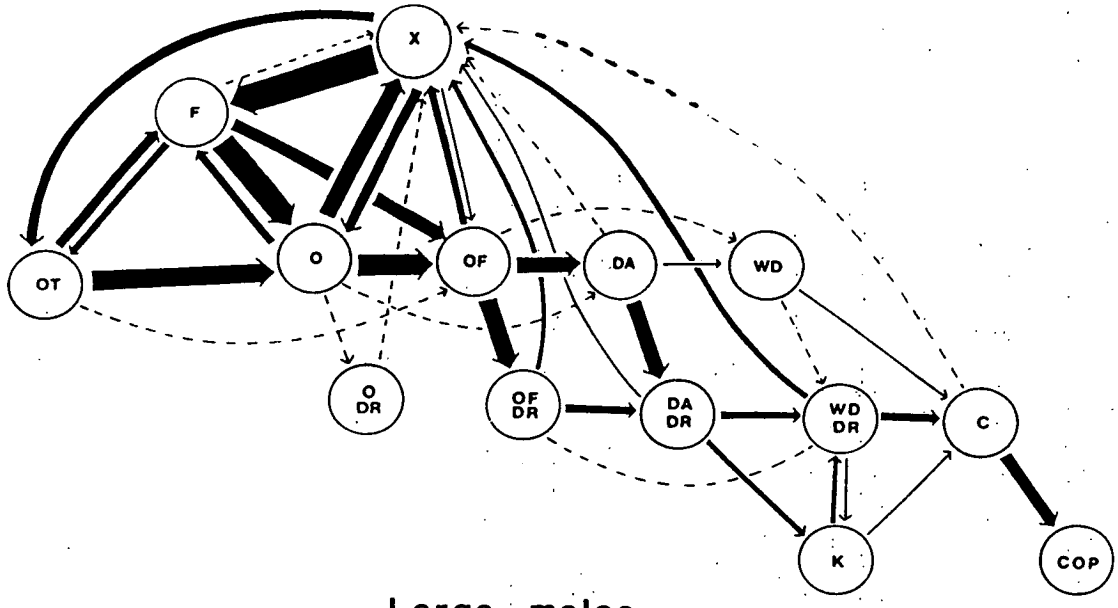
TABLE 7.5 (continued)

		DANCE				OTHER				WING DISPLAY		CIRCLE		FEMALE MOVE AWAY	
		Frequency court^{-1} ship	Rate$_{-1}$ min	Duration secs.	Proportion of total time	Frequency court^{-1} ship	Rate$_{-1}$ min	Duration secs.	Proportion of total time	Frequency court^{-1} ship	Rate$_{-1}$ min	Frequency court^{-1} ship	Rate$_{-1}$ min	Frequency court^{-1} ship	Rate$_{-1}$ min
Five day	Large Male	1.6 (1.0-2.0)	0.7 (0.0-1.5)	4.4 (0.6-6.2)	0.03 (0.00-0.12)	1.0 (0.0-3.0)	0.7 (0.0-1.4)	7.6 (2.2-74.6)	0.09 (0.0-0.60)	1.1 (0.0-2.0)	0.6 (0.0-3.1)	0.9 (0.0-1.0)	0.5 (0.0-3.1)	2.3 (1.0-8.0)	1.4 (0.4-4.7)
	Small male	1.1 (0.0-5.0)	0.9 (0.0-5.0)	3.5 (2.3-6.5)	0.05 (0.00-0.35)	0.7 (0.0-3.0)	0.3 (0.0-1.7)	9.8 (3.0-84.3)	0.02 (0.00-0.47)	0.8 (0.0-5.0)	0.7 (0.0-2.0)	0.8 (0.0-2.0)	0.8 (0.0-5.0)	1.8 (0.0-8.0)	1.3 (0.0-3.4)
Two day	Large male	1.0 (0.0-3.0)	1.1 (0.0-5.6)	3.5 (1.3-7.1)	0.09 (0.00-0.48)	0.2 (0.0-5.0)	0.0 (0.0-1.9)	14.8 (4.7-29.7)	0.00 (0.00-0.52)	0.9 (0.0-2.0)	1.0 (0.0-5.6)	0.9 (0.0-1.0)	1.0 (0.0-5.6)	1.5 (0.0-6.0)	0.7 (0.0-2.2)
	Small male	0.8 (0.0-2.0)	0.4 (0.0-3.5)	3.2 (1.8-6.6)	0.03 (0.00-0.12)	1.2 (0.0-1.3)	0.5 (1.8-44.6)	16.4 (0.00 0.59)	0.05 (0.0-1.0)	0.7 (0.0-1.0)	0.4 (0.0-3.5)	0.6 (0.0-1.0)	0.4 (0.0-3.5)	3.0 (0.0-12.0)	1.3 (0.0-5.0)
Five day poor food	Large male	0.3 (0.0-2.0)	0.0 (0.0-1.7)	2.6 (0.6-5.2)	0.00 (0.00-0.10)	2.6 (0.0-4.0)	1.0 (0.0-1.3)	26.1 (11.0-62.8)	0.57 (0.00-0.86)	0.6 (0.0-2.0)	0.3 (0.0-1.7)	0.3 (0.0-1.0)	0.0 (0.0-1.7)	2.7 (1.0-9.0)	1.3 (0.3-4.1)
	Small male	0.2 (0.0-2.0)	0.1 (0.0-4.5)	3.1 (2.6-3.1)	0.00 (0.00-0.19)	1.5 (0.0-4.0)	0.5 (0.0-1.3)	44.9 (13.8-17.4)	0.37 (0.00-0.95)	0.2 (0.0-3.0)	0.1 (0.0-4.5)	0.2 (0.0-1.0)	0.0 (0.0-4.5)	3.0 (0.0-10.0)	1.0 (0.0-3.3)

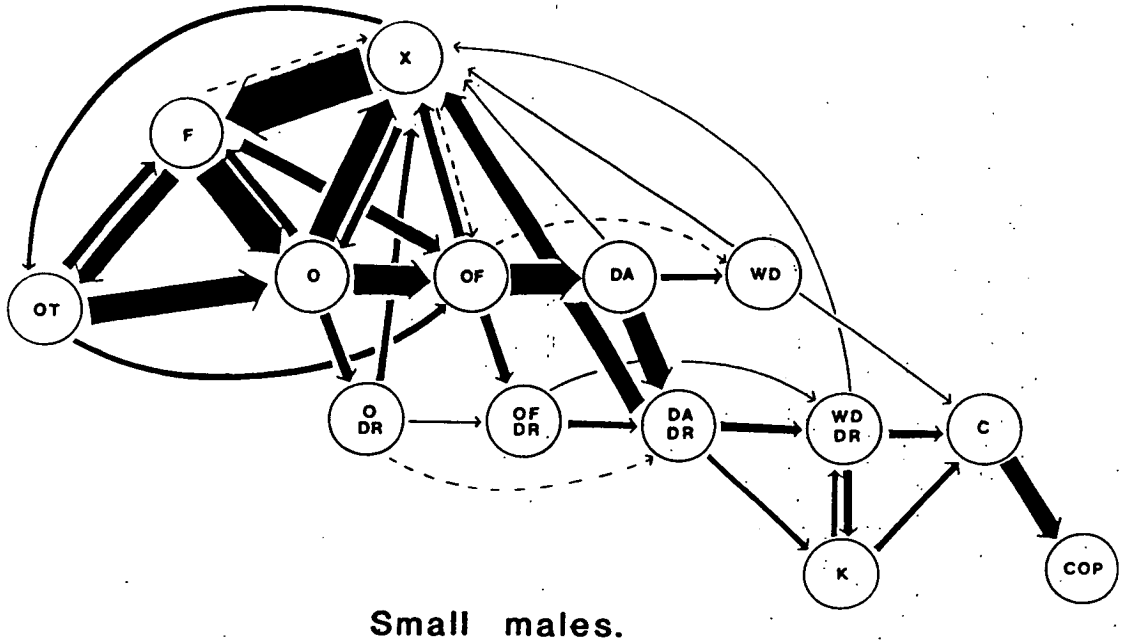
Figures 7.2, 7.3 and 7.4. The sequence diagrams depict the frequency of behavioural transitions during courtship for large and small five day-old, two day-old and five day-old poorly-fed males with five day-old females. The widths of the arrows are proportional to the frequency of the transitions. A width of 1.6mm represents 10 transitions. A single transition is represented by a dashed line and two transitions are represented by a single, solid line.

The behaviours shown in each diagram are other (OT), follow (F), female move away (X), orientation (O), frontal orientation (OF), dance (DA), wing display (WD), orientation with drop (ODR), frontal orientation with drop (OFDR), dance with drop (DADR), wing display with drop (WDDR), kiss (K), circle (C) and copulation (COP).

Figure 7.2. Five day-old males.

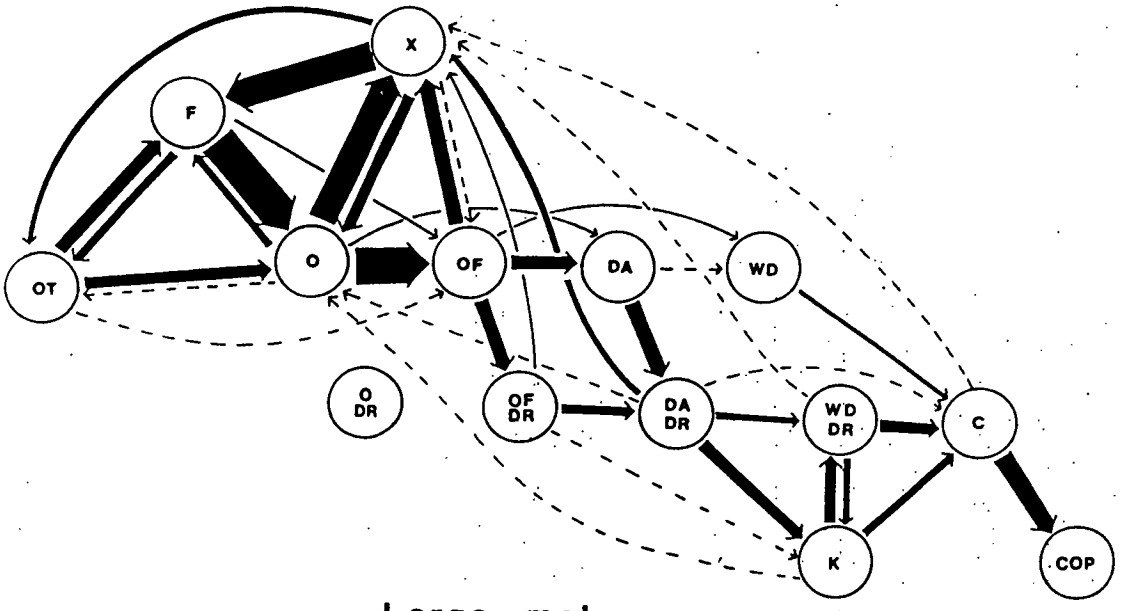


Large males.

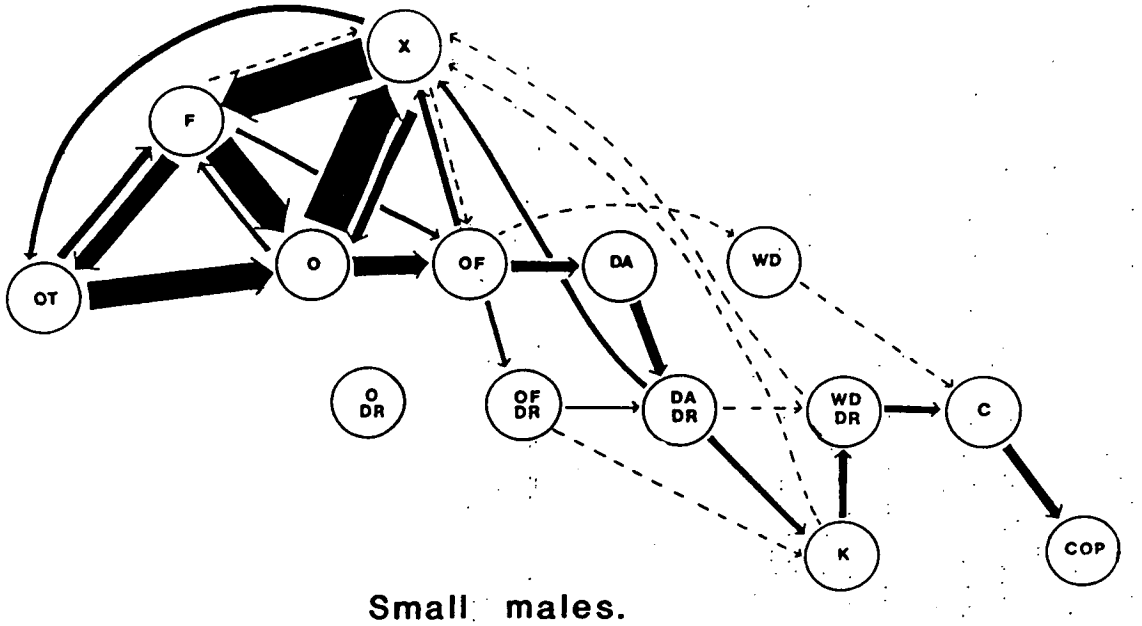


Small males.

Figure 7.3. Two day-old males.

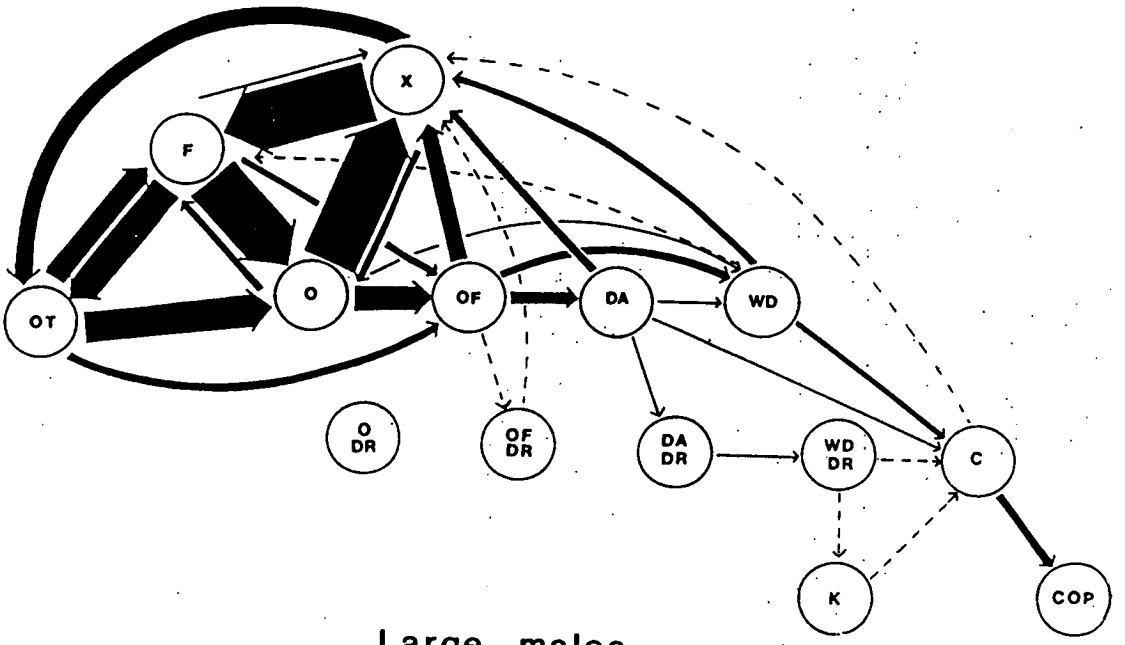


Large males.

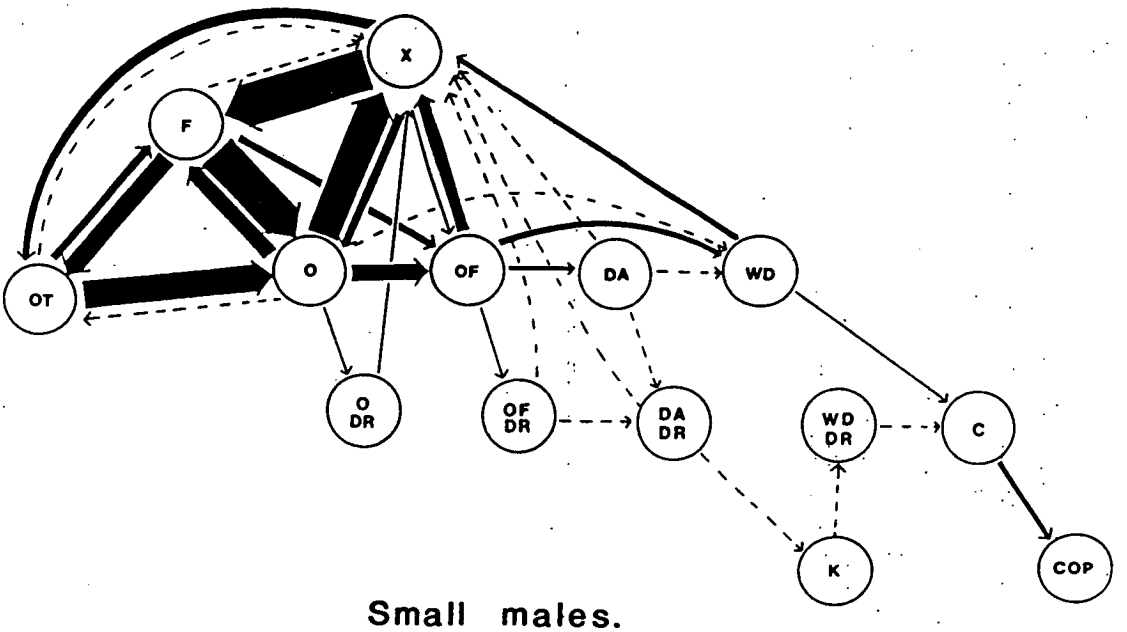


Small males.

Figure 7.4. Five day-old poorly-fed males.



Large males.



Small males.

TABLE 7.6

Five day compared to two day	z	p
Five day had higher frequency of drop production	-2.16	0.0308
Five day had higher frequency of DA	-2.33	0.0196
Five day compared to five poor food		
Five day had shorter courtships	-3.29	0.0010
Five day had higher frequency of drop production	-5.29	<0.0001
Five day had higher rate of drop production	-5.09	<0.0001
Five day had longer duration of drop carried	-2.08	0.0380
Five day had more drops taken by female	-3.41	0.0007
Five day had longer duration of O	-2.57	0.0100
Five day had higher ppn. of time in O	-3.00	0.0027
Five day had higher frequency of OF	-2.00	0.0460
Five day had higher rate of OF	-3.30	0.0010
Five day had higher ppn. of time in OF	-2.66	0.0079
Five day had higher frequency of DA	-4.25	<0.0001
Five day had higher rate of DA	-3.99	0.0001
Five day had higher ppn. of time in DA	-4.36	<0.0001
Five day had lower frequency of OT	-2.41	0.0160
Five day had shorter duration of OT	-3.43	0.0006
Five day had lower ppn. of time in OT	-2.91	0.0036
Five day had higher frequency of WD	-2.04	0.0410
Five day had higher rate of WD	-2.82	0.0048
Five day had higher frequency of C	-3.03	0.0025
Five day had higher rate of C	-3.39	0.0007
Two day compared to five day poor food		
Two day had shorter courtships	-3.12	0.0018
Two day had higher frequency of drop production	-3.91	0.0001
Two day had higher rate of drop production	-4.06	<0.0001
Two day had longer duration of drop carried	-2.22	0.0265
Two day had more drops taken by female	-4.38	<0.0001
Two day had higher rate of O	-2.42	0.0155
Two day had higher ppn. of time in O	-2.92	0.0030
Two day had higher rate of OF	-2.68	0.0072
Two day had higher ppn. of time in OF	-2.84	0.0045
Two day had higher frequency of DA	-2.78	0.0058
Two day had higher rate of DA	-3.41	0.0007
Two day had higher ppn. of time in DA	-3.68	0.0002
Two day had lower frequency of OT	-2.42	0.0154
Two day had shorter duration of OT	-2.66	0.0079
Two day had lower ppn. of time in OT	-3.23	0.0012
Two day had higher rate of WD	-2.44	0.0146
Two day had frequency of C	-2.87	0.0041
Two day had higher rate of C	-3.20	0.0013

Table 7.6. The table lists the between group comparisons that were significant at a two-tailed probability of <0.05. The groups were compared using the Mann-Whitney test and z is the standard normal variable and p is the two-tailed probability. O is orientation, OF is frontal orientation, DA is dance, OT is other, WD is wing display and C is circle.

the female than did the poorly-fed males. They also had longer bouts of orientation and more bouts of frontal orientation, dancing, wing display and circling. The poorly-fed males spent a greater proportion of their time in non-courtship behaviour (other) than did the well-fed males.

7.3.4.1.3. Two day-old and poorly-fed groups.

The two day-old males had faster courtship times than the poorly-fed males and produced drops at a higher rate, carried them for longer periods and had more taken by the female (table 7.6). The two day-old males also had higher rates of orientation, frontal orientation, dancing, wing display and circling than the poorly-fed males and spent a greater proportion of their time courting.

In summary, the poorly-fed males appeared to court less vigorously than both the five and two day-old males and took longer to achieve mating. The five day-old males produced more drops than the two day-old males and danced more frequently, but there was no difference in the courtship durations of the two groups.

7.3.4.2. Within group comparisons; the influence of male size.

The rank correlations significant at the 95% level between male wing length and a number of variables are given in table 7.7. There were no significant correlations within the five day-old group. Within the poorly-fed group smaller males had longer bouts of non-courtship behaviour. The influence of male size was greatest in the two day-old group. The smaller males had longer courtship durations. The larger males had higher rates of orientation, frontal orientation, wing display and circling.

Two day-old males	r	p
courtship duration	-0.3895	0.036
rate of O	0.4027	0.030
rate of OF	0.4036	0.030
ppn. time in DA	0.4150	0.026
rate of WD	0.4242	0.022
frequency of C	0.3890	0.038
rate of C	0.4230	0.022
Five day poor food males		
duration of OT	-0.4427	0.044

Table 7.7. The table lists the significant ($p<0.05$) Spearman rank correlations between the courtship variables and male wing length within the three groups. r is the Pearson correlation and p is the two-tailed probability. O is orientation, OF is frontal orientation, F is follow, DA is dance, WD is wing display, C is circle, MA is female move away and OT is other.

When comparisons were made between the large and small male groups within treatments a similar pattern emerged to the whole treatment comparisons (table 7.8) With the large groups there were few differences between the two day-old and five day-old treatments, but both these treatments had many differences with the poorly-fed treatment. With the small groups there were, again, few differences between the two day-old and five day-old treatments and many differences between the five day-old and poorly-fed treatments, however, there were few differences between the two day-old and poorly-fed small groups. This suggests that the courtship vigour of the small two day-old flies is intermediate between the five day-old and poorly-fed small flies and an examination of the courtship summary table (table 7.5) supports this view.

There would appear to be three groups in this experiment whose courtship vigour was low and courtship success poor; the small two day-old flies and the large and small poorly-fed flies.

7.3.4.3. Successul and unsuccessful courtships.

The courtships resulting in copulation within the 3 minute observation period were compared with those in which mating was not achieved for each treatment (table 7.9).

In the successful courtships within the five day-old group drop production , wing display and circling occurred at higher rates and more drops were taken by the female. Although the frequencies of orientation, frontal orientation and female move away were higher in the unsuccessful courtships the rates were not significantly different.

Table 7.8. The table lists the between group comparisons that were significant at a two-tailed probability of <0.05. The groups were compared using the Mann-Whitney test and z is the standard normal variable and p is the two-tailed probability. O is orientation, OF is frontal orientation, DA is dance, WD is wing display, OT is other, C is circle and MA is female move away.

TABLE 7.8

Five day large compared to two day large	Z	p
Five day large had higher frequency of drop production	-2.21	0.0274
Five day large had higher frequency of DA	-2.47	0.0135
Five day large had higher frequency of OT	-1.97	0.0494
Five day large had higher ppn. of time in OT	-1.96	0.0500
Five day large had higher rate of MA	-2.04	0.0410
Five day small compared to two day small		
Five day small had higher rate of OF	-2.09	0.0366
Five day large compared to five day poor food large		
Five day large had shorter courtships	-2.17	0.0298
Five day large had higher frequency of drop production	-4.45	<0.0001
Five day large had higher rate of drop production	-4.35	<0.0001
Five day large had more drops taken by female	-2.45	<0.0144
Five day large had higher ppn. time in O	-2.59	0.0097
Five day large had higher rate of OF	-2.39	0.0168
Five day large had higher ppn. in OF	-1.99	0.0471
Five day large had higher frequency of DA	-3.25	0.0012
Five day large had higher rate of DA	-2.33	0.0197
Five day large had higher ppn. time in DA	-2.69	0.0071
Five day large had higher duration of OT	-2.10	0.0357
Five day large had higher ppn. time in OT	-2.01	0.0439
Five day large had higher frequency of WD	-2.10	0.0354
Five day large had shorter rate of WD	-2.28	0.0224
Five day large had shorter frequency of C	-2.37	0.0179
Five day large had higher rate of C	-2.33	0.0198
Five day small compared to five day poor food small		
Five day small had courtships	-2.41	0.0159
Five day small had higher frequency of drop production	-2.98	0.0029
Five day small had higher rate of drop production	-2.78	0.0054
Five day small had longer duration of drop carried	-2.46	0.0139
Five day small had more drops taken by female	-2.23	0.0259
Five day small had longer duration of O	-2.31	0.0210
Five day small had higher rate of OF	-2.26	0.0238
Five day small had higher frequency of DA	-3.00	0.0027
Five day small had higher rate of DA	-2.99	0.0028
Five day small had higher ppn. time in DA	-3.13	0.0017
Five day small had shorter duration of OT	-2.72	0.0064
Five day small had higher ppn. time in OT	-1.96	0.0496
Five day small had higher rate of WD	-2.19	0.0284
Five day small had higher frequency of C	-2.35	0.0187
Five day small had higher rate of C	-2.43	0.0150

TABLE 7.8 (continued)

Two day large compared to five day poor food large	z	p
Two day large had shorter courtships	-3.12	0.0018
Two day large had higher frequency of drop production	-3.62	0.0003
Two day large had higher rate of drop production	-3.78	0.0002
Two day large had more drops taken by female	-3.66	0.0003
Two day had higher rate of O	-2.36	0.0182
Two day large had higher ppn. time in O	-2.23	0.0259
Two day large had higher rate of OF	-2.82	0.0047
Two day large had higher ppn time in OF	-2.34	0.0192
Two day large had higher frequency of DA	-2.10	0.0353
Two day large had higher rate of DA	-3.00	0.0027
Two day large had higher ppn. time in DA	-3.24	0.0012
Two day large had lower frequency of OT	-2.96	0.0031
Two day large had lower rate of OT	-2.06	0.0396
Two day large had lower ppn. time in OT	-3.23	0.0013
Two day large had higher rate of WD	-2.48	0.0133
Two day large had higher frequency of C	-2.86	0.0043
Two day large had higher rate of C	-3.22	0.0013
Two day large had lower frequency of MA	-2.33	0.0199
Two day large had lower rate of MA	-2.01	0.0443
Two day small compared to five day poor food small		
Two day small had longer duration drop carried	-2.39	0.0167
Two day small had more drops taken by female	-2.37	0.0177
Two day small had longer duration of OT	-2.31	0.0206

Table 7.9. The table lists the within group comparisons between the successful and unsuccessful courtships that were significant at a two-tailed probability of <0.05. The groups were compared using the Mann-Whitney test and z is the standard normal variable and p is the two-tailed probability. O is orientation, OF is frontal orientation, DA is dance, WD is wing display, F is follow, OT is other, C is circle and MA is female move away.

TABLE 7.9

Five day-old males	z	p
Males that mated had higher rate of drop production	-2.52	0.0119
Males that mated had more drops taken by female	-4.33	<0.0001
Males that mated had lower frequency of O	-2.82	0.0048
Males that mated had lower frequency of OF	-2.04	0.0414
Males that mated had higher frequency of WD	-3.03	0.0024
Males that mated had higher rate of WD	-4.00	0.0001
Males that mated had higher frequency of C	-4.78	<0.0001
Males that mated had higher rate of C	-4.32	<0.0001
Males that mated had lower frequency of MA	-2.84	0.0046
Two day-old males		
Males that mated had higher frequency of drop production	-2.17	0.0303
Males that mated had higher rate of drop production	-3.43	0.0006
Males that mated had more drops taken by female	-4.31	<0.0001
Males that mated had higher rate of O	-2.88	0.0040
Males that mated had higher rate of OF	-3.00	0.0029
Males that mated had longer duration of OF	-2.58	0.0099
Males that mated had lower frequency of F	-2.25	0.0242
Males that mated had shorter duration of F	-2.36	0.0185
Males that mated had higher frequency of DA	-3.36	0.0008
Males that mated had higher rate of DA	-4.00	0.0001
Males that mated had higher ppn. time in DA	-3.66	0.0003
Males that mated had lower frequency of OT	-3.01	0.0026
Males that mated had lower rate of OT	-2.27	0.0232
Males that mated had lower ppn. time in OT	-2.27	0.0233
Males that mated had higher frequency of WD	-2.92	0.0035
Males that mated had higher rate of WD	-3.54	0.0004
Males that mated had higher frequency of C	-4.87	0.0001
Males that mated had higher rate of C	-4.29	0.0001
Males that mated had lower frequency in MA	-2.13	0.0331
Five day poor food males	z	p
Males that mated had higher frequency of drop production	-2.22	0.0267
Males that mated had higher rate of drop production	-2.48	0.0131
Males that mated had more drops taken by female	-2.33	0.0196
Males that mated had higher rate of O	-2.79	0.0053
Males that mated had higher ppn. time in O	-2.64	0.0084
Males that mated had higher frequency of OF	-3.17	0.0015
Males that mated had higher rate of OF	-4.08	<0.0001
Males that mated had higher ppn. time in OF	-3.36	0.0008
Males that mated had higher frequency of DA	-3.84	0.0001
Males that mated had higher rate of DA	-4.14	<0.0001
Males that mated had higher ppn time in DA	-3.91	0.0001
Males that mated had lower frequency of OT	-3.55	0.0004
Males that mated had higher rate of OT	-2.84	0.0045
Males that mated had lower ppn. time in OT	-3.55	0.0004
Males that mated had higher frequency of WD	-4.12	<0.0001
Males that mated had higher rate of WD	-4.59	<0.0001
Males that mated had higher frequency of C	-4.87	<0.0001
Males that mated had higher rate of C	-5.00	<0.0001

Within the two day-old group, once again, drops were produced at a higher rate and more were taken in the successful courtships. The rates of orientation, frontal orientation, dancing, wing display and circling were higher in the successful courtships and the rates of other and following were lower as were the durations of following bouts.

Within the poorly-fed group the successful courtships had a higher rate of drop production and more drops were taken and higher rates of orientation, frontal orientation, dancing, wing display and circling and a lower rate and shorter durations of other.

When similar comparisons were made between the successful and unsuccessful courtships within the large and small groups within each treatment a similar pattern emerged. It seems that successful courtships tend to be characterised by having high rates of drop production, females taking the drop, orientation, frontal orientation, dancing, wing display and circling and low rates of other and following. However, in these comparisons it is not possible to distinguish the role of courtship differences between males from the role of variation in female receptivity in producing these effects. For example, the successful courtships may have higher rates of drop production because more receptive females tend to elicit drop production more often, or, perhaps, move around less enabling the male to court more easily. In the comparisons made in earlier sections the variation in female receptivity should have been the same in the different groups and courtship differences between the groups would have been a consequence of differences in male courtship behaviour. Comparing successful and unsuccessful courtships within a treatment might simply be an examination of

differences due to female receptivity. For this reason I have not described the successful and unsuccessful courtships in the same detail as the treatment comparisons.

7.3.5. Wing darkness

Without recourse to statistical tests it is clear from table 7.10 that the wings of older flies tend to appear darker to human observers. It would be interesting to follow this study up and devise a piece of apparatus to measure accurately the light absorbed by wings of different pigmentation. It would be necessary to focus an intense beam of paralell light (using an optic fibre) on to a known patch of wing (using a microscope) and measure the light transmitted with a photocell. It should then be possible both to make detailed measurements of changes in wing pigmentation with age on various nutritional backgrounds and also to assess the variation in wing darkness of wild flies and compare it to reproductive organ size. This technique might also allow the physiological age of wild flies to be estimated without the need for the tedious preparation and spectrofluorescent assays currently used (ETTERSHANK et. al. 1983).

Does wing darkness influence courtship duration? Table 7.11 shows that on the first two days of the experiment the young flies whose wings had been artificially darkened with carbon powder tended to mate faster than controls. However, on the third day the trend is reversed, apparently because of the reduced courtship success of the darkened group. It is possible that on this day the wings were too heavily darkened. This might mean that the males spent a greater

TABLE 7.10

A visual comparison of the relative darkness of wings of males of different ages. The table shows the male ages (in days) compared and, within a comparison, the number of pairs for which the males of each age were scored as having the darker wing.

Ages compared:	2 with 10	2 with 20	2 with 30	10 with 20	10 with 30	20 with 30
Observer A	1:16 (3)	3:13 (5)	1:15 (4)	1:14 (5)	4:13 (3)	5:13 (2)
Observer B	0:11 (9)	0:15 (6)	1:13 (7)	2:9 (6)	4:10 (6)	4:6 (10)
Observer C	1:15 (4)	2:16 (3)	1:17 (2)	6:9 (5)	7:10 (3)	0:16 (4)

Key

e.g. '2 with 10' Means comparing the wing of a 2 day-old male with the wing of a 10 day-old male.

1:16 (3) Means that in one pair the 2 day-old male wing was scored as darker, in 16 pairs the 10 day-old male was scored as darker and in 3 pairs no difference was seen.

TABLE 7.11

A comparison of the courtship durations of two day-old control males with two day-old males whose wings had been artificially darkened with carbon powder.

Day	The number of pairs in which the control male mated first	binomial test	'darkened' male mated first	Median and range of courtship durations of control males in mins. and secs.	Median and range of courtship durations of 'darkened' males in mins. and secs.
1	2	p=0.11	8	6.00 (4.05-30+)	5.20 (1.55-13.50)
2	1	p=0.022	9	6.40 (4.55-20.55)	4.35 (0.50-17.25)
3	9	p=0.012	2	4.55 (1.15-13.40)	13.20 (2.15-30+)

Courtships were observed for up to 30 minutes.

proportion of their time grooming or it is even possible that very dark wings increase courtship duration because in the field they are associated with very old senescent flies of lower fertility. To pursue this line of investigation further really requires a technique for taking accurate quantitative measures of wing darkness. Nonetheless, there are indications that artificially darkening a male's wings does influence the duration of courtship.

7.3.6. Dousing males with cis-vaccenylacetate

The application of acetone to male abdomens tends to increase the duration of courtship (table 7.12) and clearly this approach is not a useful one for determining whether there are quantitative differences in chemical cues associated with male D.subobscura that might be partly responsible for the differences in courtship success reported in earlier chapters. I should perhaps also point out that Professor JALLON has informed me that he has been unable to replicate MANE et al.'s (1983) dousing experiments with D.melanogaster females. Perhaps the particular experimental approach used is critical.

TABLE 7.12

The courtship durations of control two day-old males, two day-old males doused with acetone and two day-old males doused with acetone containing cis-vaccenylacetate (cVa).

	Courtship durations in minutes and seconds		
	Untreated males	Treated controls Acetone only	Treated males Acetone with cVa
	5.35	6.10	1.30
	10.30	30+	11.40
	6.40	30+	30+
	20.25	2.25	9.00
	11.20	30+	30+
	4.00	3.10	2.25
	2.10	24.55	0.55
	6.15	6.05	16.40
	1.05	0.30	1.00
	1.50	4.35	22.35
	5.10	30+	30+
	9.50	30+	30+
	2.10	0.55	30+
	1.50	13.30	5.55
	30+	30+	17.30
	2.10	3.20	30+
	5.20	15.20	30+
N	17	17	17
% males mating	94	65	59
Median duration	5.20	13.30	17.30

Mann-Whitney : Untreated with treated controls; w=251, p=0.113.
Untreated with treated males; w=236, p=0.036.
Treated controls with treated males; w=284, p=0.654.

'30+' represents the courtships in which copulation did not occur within the 30 minute observation period.

7.4. CONCLUSIONS.

1) Five day-old males had greater competitive mating success in a female 'choice' test than both two day-old and poorly-fed males.

2) Two day-old and poorly-fed males copulated for longer than five day-old males in both the first and second copulations.

3) In all three groups the second copulation was longer than the first.

4) The second female mated laid fewer eggs than the first female mated in both the two day-old and poorly-fed groups and fewer eggs hatched.

5) The first females mating with the two day-old males lay fewer eggs than the first females mating with the five day-old males and fewer eggs hatch.

6) The second females mating with the two day-old males and with the poorly-fed males lay fewer eggs than the second females mating with the five day-old males and fewer eggs hatch.

7) The five day-old males produced more drops than the two day-old males and danced more frequently, but there was no difference in the courtship duration.

8) Poorly-fed males courted less vigorously than both the two day-old and five day-old males and had longer courtships.

9) Small two day-old males courted less vigorously than large two day-old males and had longer courtships.

10) The successful courtships in all three groups tended to be characterised by high rates of drop production, orientation, frontal orientation, dancing, wing display and circling, more time was spent courting and females took the drop more often.

11) Older males had darker wings and artificially darkening a young male's wings using carbon powder did influence his courtship speed.

12) Applying a small drop of acetone to a male's abdomen tended to increase the duration of courtship.

7.5. DISCUSSION.

7.5.1. Copulation duration and progeny production.

In chapter 5 I reported that heavier five day-old male D.subobscura had shorter copulations, second copulations were longer than the first and the difference between first and second copulations was greater for smaller males. In this chapter the results show that younger males copulate for longer and so too do males aged on a medium low in carbohydrate and protein. In view of the data and arguments presented in chapter 6 concerning the size of the reproductive organs, these results suggest that males with smaller accessory glands tend to copulate for longer. This finding is consistent with a number of studies involving reciprocal crosses between strains or selected lines in which the authors argued that the duration of copulation in Drosophila is male determined (KAUL and PARSONS 1965, PARSONS and KAUL 1966, MACBEAN and PARSONS 1966 and 1967).

If males are simply transferring a proportion of the accessory fluid they have available then, unless rate of transfer is associated with gland size, males with smaller glands should finish copulating sooner. If all males transfer a 'fixed' quantity of accessory fluid then the duration of copulation in flies with glands of different sizes should be the same, unless males with smaller glands have less than the fixed quantity stored in the lumen. In this situation presumably males would have to wait whilst accessory fluid was released into the lumen from the vesicles of secretory cells in the gland wall. In the apical portion of the accessory gland are a

number of ovoid cells, the average number is 58 in D.melanogaster (GILL 1964), containing large vacuoles and which project into the lumen of the accessory gland. These cells are in an active secretory phase (see FOWLER 1973 for review). It is likely that the process of vesicular release will be slower than the transfer of fluid from the lumen since the contents of the lumen represent an accumulation of fluid produced and released by the secretory cells. The less fluid males have stored in the lumen, then the more will be required from the vesicular release to make up the fixed amount and the longer copulation would last. So, for instance, in the five day-old males there may be no variation due to male size in the duration of the first copulation because all the males have more fluid stored in the lumen than the fixed quantity required. However, after one copulation the small males will have less remaining than the large males. If the males now copulate again then there may be no change in the copulation duration of some of the larger males because they initially had enough fluid stored to transfer two fixed quantities. But the smaller the male the less he will have left over from the first copulation and the more fluid he will have to accumulate from vesicular release and the longer copulation will last. If even older flies had been used with even larger stores of accessory fluid then, perhaps all males would have two fast copulations before vesicular release became important.

Is there any evidence that males with different gland sizes transfer similar amounts of accessory fluid? Little work has been done in this area but one study is relevant. RITCHIE (1984) found that the reduction in size of the accessory glands of five day-old male D.subobscura after one copulation was the same for large and

small males suggesting that similar amounts of accessory fluid had been transferred. This type of experiment with older, younger, depleted and poorly-fed males for a series of copulations would provide data with which to test some of the assumptions of the model outlined above. Also, in chapter 5 I argued that large and small five day-old males transferred similar amounts of accessory fluid during the first copulation since the productivity of females mating with them was the same and probably depended on the amount of ejaculate transferred.

What determines the 'fixed' quantity to be transferred if such a quantity exists? In a series of experiments using Musca domestica (reviewed by LEOPOLD 1976) LEOPOLD and his co-workers were able to demonstrate that with each successive mating a male's copulation duration increased (LEOPOLD, TERRANOVA and SWILLEY 1970). If depleted males were injected with cyclohexamide, an inhibitor of protein synthesis, there was no recovery and copulation duration remained long (LEOPOLD et al. 1970). LEOPOLD, TERRANOVA, THORSON and DEGRUGILLIER (1971) argued that when the titre of the male secretions responsible reached a sufficient concentration in the female her receptivity was switched off and she withdrew her ovipositor and dislodged the male. They also argued that the target site for the male secretions was in the female's head because radiolabelled male accessory secretion was absorbed into the female's haemolymph approximately 20 minutes before copulation ended and appeared in the female's head (LEOPOLD et al 1971). Furthermore, mated females did not become unreceptive if they were decapitated (LEOPOLD, TERRANOVA and SWILLEY 1971). BALDWIN and BRYANT (1981) found that the difference in size betwen the male and female was an important

determinant of copulation duration and argued that this might be because the titre of accessory fluid is less in smaller males, relative to the size of the female, and so copulation duration is longer. It is interesting that, in the experiments reported in this chapter, in the five day-old male group female size and the difference in size between the male and female was correlated with copulation duration. Perhaps there is feedback control of copulation duration in D.subobscura, not neccesarily involving a target site in the female's head but possibly neural feedback from the sperm storage organs.

Other constraints would need to be considered in a model describing the control of copulation duration to explain the decline in female productivity with reduced male accessory gland size (discussed below). Clearly all males are not copulating for as long as it takes to transfer a fixed quantity of accessory fluid and some are probably transferring less than others. One simple constraint might be the amount of fluid available in the secretory cells. Once the vacuoles of these cells have been emptied then the factor limiting accessory fluid transferal will be its rate of synthesis. Males may well dismount at this stage and a recovery period involving the synthesis of accessory fluid will ensue. Alternatively, there may be further constraints on copulation duration imposed by the female. Perhaps females are more likely to reject males as copulation progresses. Much more work is required before an understanding of the control of copulation duration in D.subobscura is reached. The simple model outlined above does generate predictions that can be tested.

Although copulation duration appears to increase with decreasing accessory gland size in D.subobscura , this is not necessarily the case in other Drosophila species. For instance, RITCHIE (1984) found that in D.pseudoobscura smaller and depleted males tended to have shorter copulations (see also MAYR 1946) and the change in accessory gland size after one mating tended to be greater for larger males. These findings suggest that male D.pseudobscura might simply be transferring a proportion of their accessory fluid. If this is the case then one might predict that matings with smaller males were less fertile or, perhaps, females mating with smaller or depleted males would remate sooner to top up their sperm supply. There is some evidence that this is the case (RITCHIE 1984).

Clearly many factors have influenced the observed interspecific differences in copulation duration recorded for Drosophila (e.g. WHEELER 1947,SPIETH 1952). For example, in species in which females remate frequently selection may favour males that invest less time and energy in each female. When the female remates the male's sperm will probably be displaced (sperm displacement seems to be the rule in Drosophila- GROMKO et al. in press for review). Also, at any one time there will be more females available for mating and males might maximise their reproductive success by mating with as many females as possible. This evolutionary decline in male investment and increase in female remating frequency would presumably halt when the costs to the female of remating outweighed the benefits. It is of interest that male D.pseudobscura have larger reproductive organs than D.subobscura, copulate for a shorter period and the females remate (RITCHIE 1984). Male D.nebulosa have even larger reproductive organs, have very short, violent copulations and the females remate

even sooner. Equally, females might be selected to remate more frequently if males are transferring insufficient ejaculate to fertilise the eggs laid by a female during her lifespan or, perhaps, if valuable nutrients can be derived from the mating (PARTRIDGE in preparation).

The variation in copulation duration reported for D.subobscura in this study should serve to illustrate that it may not be particularly useful to describe a mean copulation duration for a species without giving the age of the flies used (SPIETH 1952) or the conditions under which the flies were aged. Nor may it be very productive to make interspecific comparisons using individuals of different ages (e.g. GRANT 1983), or flies raised at different temperatures or raised on different foods. There are also genetic influences on the duration of copulation in Drosophila (e.g. FOWLER 1973) in that it can be selected for (e.g. MACBEAN and PARSONS 1967). It seems likely that in D.subobscura the direct control of copulation duration depends on the size of the accessory glands and any selection for slow or fast copulating males might simply be changing the size of the reproductive organs in the selected lines.

Whatever the mechanisms involved, female D.subobscura mating with males with smaller accessory glands will tend to copulate for longer. Females are inactive whilst copulating in the laboratory and in the field (personal observation) and longer copulations will reduce the time available for other activities. Copulating females might also be more susceptible to predation than single females, although this is yet to be demonstrated for any insect species. Female Drosophila cannot fly or move as quickly when the male is mounted, nor can the male rapidly dismount. However, in the field

copulating females move off the aggregation site onto the underside of surrounding leaves where the pair sits motionless. Here there is little disturbance from other males who tend to move over the upper surface of leaves around the resource. It is also noticeable that one of the documented predators of Drosophila, the dung fly, Scatophaga stercoraria (SHORROCKS 1972), sits on the upper surface of leaves close to the resource from where it launches itself at flies on or around the resource. It may be that, although potentially a copulating female is more easily taken by a predator, her behaviour reduces the danger of predation in the field. However, it does seem likely that females that can receive the same quantity of ejaculate in a shorter period of time will have an advantage.

Are shorter copulations likely to be less fertile? Within the groups there was no correlation between copulation duration and the number of eggs laid by a female or the number hatching. In fact, the between group comparisons suggest the reverse. The groups of males with the smallest accessory glands have the longest copulations and the females they mate lay fewest eggs and leave fewest progeny. That the females mated to the young and poorly-fed males tend to lay fewer eggs than the females mated to the five day-old males, and fewer in the second copulation than in the first, suggests that female egg-laying rate as well as egg-hatchability depends upon the ejaculate received from males. DAVID (1963), COOK (1970), FITZ-EARLE (1971) and GILBERT et. al. (1981) have all reported that the quantity and quality of ejaculate transferred influences female egg-laying rate in D.melanogaster. It is also known that components of the accessory fluid do stimulate egg production (e.g. BAUMAN 1974 and review by CHEN 1984).

Female D.subobscura mated with recently mated five day-old males leave fewer progeny over their lifetime than females mated with virgin males, and fewer still if the depleted males are small (chapter 5). Females mated with young males and poorly-fed males (for the second mating) leave fewer progeny over the ten day period after mating than five day-old males. Females mating with males with larger accessory glands derive two material benefits; firstly, the period of copulation is shorter, secondly, they will leave more progeny.

7.5.2. Factors mediating the greater courtship success of high fertility males.

The males aged on the medium low in carbohydrates and protein courted less vigorously than both the five day-old and two day-old males and had poor courtship success both in terms of longer courtships in single pairs and a low frequency of mating in competitive tests. The poorly-fed males spent a lower proportion of their time courting and have lower rates of most courtship behaviours with the exception of following. These males have smaller accessory glands and seminal vesicles than the five day-old males and less glycogen stored.

It is possible that the poorly-fed males have low courtship vigour because they are physically incapable of courting more vigorously. The amount of energy they have available for flight is similar to the amount available to the 'exhausted' flies in the last chapter (poorly-fed males; mean flight duration=16.9$\pm$3.1 mins.:exhausted males; mean flight duration=16.0$\pm$3.7 mins.). In the

exhausted group there was a significant negative correlation between courtship duration and subsequent flight time, athough this could have been either because males with few energy reserves were unable to maintain high rates of courtship behaviour or because longer courtships used up more of a male's energy reserves. There was no evidence of an extra cost of longer courtships in the other treatments (chapter 5). Poorly-fed males may only be capable of bursts of courtship activity punctuated by recovery bouts.

Another possibility is that the poorly-fed males are physically capable of courting as vigorously as the five day-old and two day-old males but are putting less into courtship activity for other reasons. Females mating with poorly-fed males with their smaller accessory glands tend to leave fewer offspring than those females mating with the five day-old males. If there is a cost to courtship, energetic or an increased risk of mortality, then perhaps males with small accessory glands, whether because of nutrition, depletion or age, would benefit from diverting fewer resources into low fertility matings and more into the synthesis of accessory fluid enabling them to achieve a high fertlity mating sooner. DOW (1978) using D.melanogaster found that a measure of the intensity of courtship, the rate of licking, decreased with successive matings and the time to copulation increased. Because recovery was incomplete after 24 hours rest, he considered that the decline in courtship vigour was unlikely to be caused by physical fatigue but might be related to male fertility. RICHMOND and SENIOR (1981) found that it took mated 6 day-old male D.melanogater 24 to 48 hours to recover virginal levels of fertility.

If males are courting less vigorously because they have low fertility and the female reprents a low value resource, why were there few differences between the courtships of the five day-old and two day-old males. The two day-old males have small accessory glands, leave fewer progeny for any given mating and might, therefore, be expected to court less vigorously. In the competitive tests the two day-old males had poor mating success and in chapter 6 it was found that the two day-old males had significantly longer courtships than the five day-old males. Perhaps sampling error was responsible for the few courtship differences found in this chapter. There were differences in courtship vigour within the two day-old group. The smaller two day-old males tended to court less vigorously and took longer to achieve copulation. This was not the case for the smaller males within the five day-old group. It may be that in small, young males accessory fluid is limiting but less so in the larger two day-old males. However, there were two courtship differences between the five and two day-old groups that might be meaningful. The two day-old males produced fewer drops and danced less frequently. This is discussed below.

If the argument is that males with small accessory glands and low fertility might gain more by directing fewer resources into mating activity and more into the synthesis of accessory fluid enabling them to achieve a high fertility mating sooner, then why do low fertility males court at all? In two similar studies in which a male's courthip vigour declined with his sperm supply (HALLIDAY and HOUSTON 1978, RUTOWSKI 1979) the authors argued that it might be worth a male's while to court females at a low intensity whilst recouping his sperm supply so as not to pass up the opportunity to

mate with a highly receptive female. What are the energetic costs incurred by male D.subobscura during courtship and mating? There is the cost of the ejaculate transferred during mating. There is the cost of courtship. This has not been measured for any Drosophila species. Possibly, one of the more costly courtship behaviours in D.subobscura is the dance. MAYNARD SMITH (1956) found that the low fertility inbred lines in his experiment were unable to keep up during the dance and had poor courtship success. In this experiment the two day-old males danced less frequently than the five day-old males. Finally, there is the cost of the drop of food, representing energy as well as valuable nutrients for the production of accessory fluid, taken by the female during courtship. The importance of the drop to male courtship success and the possible scarcity of food in the wild was discussed in chapters 2 and 3 and in chapter 5 I reported that the progeny production of females mated to males taken straight from the wild was considerably lower than the progeny production of females mated to the same males given an abundant supply of food for a week. Both the poorly-fed and the two day-old groups produced drops less frequently than the five day-old males, although, almost certainly, all 3 groups had similar crop contents (the poorly-fed males were given access to Baker's yeast before the feeding period on the morning of courtship - see materials and methods). This might be one reason why low fertility males still court. They are giving themselves an opportunity to mate but rarely, in the case of the poorly-fed males, producing a drop, probably far and away the most costly courtship behaviour. It is also worth pointing out that the successful courtships in all 3 groups were characterised by high rates of drop production and the female taking

the drop more frequently. It certainly seems possible that low fertility males are avoiding the potentially high cost of drop production until the pay-off is higher, i.e. they have larger accessory glands. What will determine whether the male should or should not produce a drop?

If p1 is the increase in the probability of a low fertility male mating if he produces a drop and f1 is the number of progeny he will leave from that mating, and if p2 is the probability that the male will survive to a future encounter with a receptive female and f2 is the extra number of progeny he will leave with that female if he holds onto the drop from the first encounter, then;

$$\text{if } p1 \times f1 > p2 \times f2,$$

the low fertility male should produce the drop during the first encounter (PARKER personal communication). So is p1f1 likely to be greater than p2f2?

In chapter 2 (section 2.3.3.1.) I found that the probability of a normal medium-sized male mating with a fed female within a 10 minute observation period was 0.81 and that of a sealed male unable to produce a drop was 0.76 (p1 with fed females = 0.05). With starved females the probability of a normal male mating was 0.94 but only 0.18 if his proboscis had been previously sealed (p1 with starved females = 0.76). The mean number of progeny left over a 10 day period after mating by well-fed females (there is no data for starved females) mating with low fertility laboratory males was 198 for the poorly-fed males and 126 for the two day-old males (we will use a value of 160 for the low fertility males). The mean progeny production over the same period of females mating with high fertility males was 258 (this chapter). Although the treatment differences

producing this variation in male fertility involved more than the loss of a drop of food, we will use this data for now to give a value for f2 of 100. If we put this information into the model then we would predict that low fertility males should produce the drop with starved females if,

$$0.76 \times 160 > p2 \times 100.$$

Clearly, with the data used, low fertility males should always produce the drop with starved females. With fed females low fertility males should produce the drop if,

$$0.05 \times 160 > p2 \times 100.$$

So low fertility males should hold onto the drop if the probability of subsequently encountering a receptive female is greater than 0.08. The data used in this simulation probably bear little relation to the values the variables will take in the field but the relevant field data is unavailable. What might influence these values in the field?

The variable, p1, will depend not only on the nutritional status of the female, and the size and quality of the drop, but also on the size of the male. In chapter 2 (section 2.3.3.3.) I reported that sealing the male's proboscis made little difference to the courtship success of small males (p1 low) but was important for large males (p1 high). p1 will also be influenced by the nature of the mating system. The values of p1 used in the simulation were calculated from the performance of males in single pair tests with up to 10 minutes to reach copulation. What is really required is a measure of p1 for a low fertility male in the field. If a male's ability to secure a mating depends largely upon his success in aggressive encounters with other males and there tends to be a 'scrummage' of males around a receptive female then the production of

a drop may do little to enhance a male's chances of mating.

The variable, f1, will depend upon female egg-laying rate, her longevity and the probability of her remating. In laboratory stocks of D.subobscura the females rarely remate (MAYNARD SMITH 1956, RITCHIE 1984) but female longevity, egg-laying rate and remating frequency in the field are all unknown. From the limited information available on the nutritional status of wild Drosophila (chapter 3) it seems likely that f1 will be less than 200.

The variable, p2, will depend upon male longevity in the field and his encounter rate with receptive females (if the receptive females are inseminated females looking to remate then the degree of sperm displacement will be important). Again, both are unknown although field observation (personal unpublished data) suggests that the vast majority of male interactions are with other males and unreceptive females and encounter rates with receptive females are low. This also suggests that males and females may be long-lived and female remating infrequent.

The variable, f2, will depend upon the size and content of the drop, the male's efficiency at turning the drop into accessory fluid and how this extra quantity of accessory fluid converts into increased progeny production. So, like p1, f2 will depend upon male size. Small males producing small drops will have a low value of f2 and for large males producing large drops f2 will be higher. The value of f2 used in the simulation represented the extra number of progeny left by high fertility males over a 10 day period. If females live longer than 10 days in the field then this figure will tend to increase because a major component of the reduction in lifetime progeny production of females mating with low fertility

males was that egg hatchability declined earlier in the females' lifetime (chapter 5). However, the treatment differences producing the high and low fertility males examined in this chapter involved more than the loss of a drop of food and f2 is likely to be much lower than the value of 100 used in the simulation. f2 will also depend upon food availability in the field. If food is abundant so that males can give up a drop and then quickly replace it, f2 will tend to be low. The reduction in a male's future fertility associated with the production of a drop will also depend upon the male's chances of losing the drop when he produces it. If he can produce the drop but not give it up to the female (see discussion chapter 3) then, p1 might still be high, but f2 will tend to be lower because males who produce the drop in the first encounter may retain it.

From this discussion it seems likely that p1 x f1 will tend to be greater in the field than p2 x f2, but that this may depend upon food availability. In areas or at times of year when food is scarce, then both the males and females will tend to be starved (see chapter 3). This will tend to increase p1, but reduce f1 and f2 because female egg-laying rate will be low, and may also tend to reduce p2 if male mortality is influenced by food abundance. When or where food is abundant, p1 will tend to be reduced but f1, f2 and possibly p2 will tend to be higher. It may well be the case that low fertility males will tend to produce the drop when food is scarce but retain it when food is abundant (as it is in the laboratory). The cost to males of producing the drop when food is abundant might involve an increase in foraging time and, possibly, an increased risk of mortality if there is a high risk of predation at feeding sites.

Clearly more information is required before the model can be used to explain the observation that low fertility males in the laboratory produce drops during courtship less frequently than high fertility males. The model will also need to be refined to include other variables, such as the influence on male survival of losing a drop. Another factor that ought to be considered is whether the advantages of drop production vary with female receptivity or the stage of courtship. For instance, during orientation or with females of low receptivity there may be a high risk of losing the drop but a low probability of going on to copulate. Perhaps with highly receptive females, or later in a courtship sequence when a female has indicated her 'readiness' to copulate by initiating the dance (the female's level of sexual excitement is closer to her acceptance threshold), the ratio of the risk of drop loss to the probability of mating may be lower.

Other cues, such as wing darkness, may also be important in mediating the higher courtship success of high fertility males and it does seem likely that a male's physical capacity to court vigorously will be important in the field. In chapter 5 exhausted males had similar accessory gland sizes to the five day-old males and therefore a female would represent a similar resource value (although because of their lower energy reserves the cost in terms of an instantaneous risk of mortality might be greater). However, the exhausted males took longer to mate than the five day-old males, perhaps because they were incapable of courting as vigorously. The poor courtship success of exhausted and poorly-fed males might be explained partly in terms of a physical limit to courtship vigour. The poor courtship success of depleted, young, glucose-fed and, again, poorly-fed males

might be explained partly in terms of reduced courtship vigour because of the lower resource value of the female and the diversion of resources into the production of accessory fluid for later copulations. Both processes might be important in wild males. The flight durations of wild males overlap those of the exhausted and poorly-fed groups and the sizes of the accessory glands overlap those of the 'low fertility' groups (see chapter 5).

Chapter 8.

FINAL DISCUSSION.

In this thesis I have shown that male D.subobscura provide females with resources at mating; that the resources provided by the male increase the number of progeny left by the females; that males vary in their ability to provide resources; and that the quality of the resources provided by the males influences a measure of male mating success. The resources fall into two categories; nutrients that increase the number of eggs laid by the female, and a 'high quality' ejaculate that ensures that a high proportion of the eggs laid are fertile.

The nutrients are provided in the form of collected food regurgitated from the crop (chapter 2). Females that take this food lay more eggs immediately after mating (chapter 3) and a male's ability to provide food depends upon his size and recent feeding history. Small males produce small drops and starved males produce drops less frequently and the drops produced are 'watery'. When females were starved for a period, males who failed to provide a drop, or who provided inferior drops, took longer to obtain a mating. This was because the female was more likely to move away from these males during courtship. I argued that if food is scarce in the wild, as the crop sizes of wild flies suggests (chapter 3), males who produce a drop as they intercept females approaching aggregation sites may well slow the female down by eliciting a feeding response and give themseves a chance to court. If males are starved to the extent that they are unable to produce drops then smaller males may have an advantage because their agility makes them better able than

large males to keep up with a moving female (chapter 4).

A number of Lepidopteran species provide females with nutrients in the form of accessory fluid transferred at mating (e.g. BOGGS and GILBERT 1979), and MARSHALL (1982) has argued that a male's willingness to invest should be determined by three variables: the degree of paternity certainty, the probability of obtaining future matings, and the effects of investment on reproductive gain as a result of that investment. Female remating is rare in stocks of laboratory D.subobscura derived from Scottish populations (MAYNARD SMITH 1956, RITCHIE 1984). Furthermore, the great majority of male:female interactions in the field are between males and inseminated females and the males do not court these females (unpublished personal data). This suggests that females rarely remate in the field and paternity certainty may be high, although if females take the male's food offerring but do not mate, paternity certainty will be reduced. The male's probability of obtaining future matings is low (for the reasons outlined above) and so the ratio of benefit to the male of investing weighed against the cost to future matings will tend to be high, particularly if food is scarce (see above). As Drosophila go, we might expect male D.subobscura to be 'willing investors'.

This view is supported by the low proportion of males producing drops in two other species within the group; D.pseudoobscura and D.obscura (chapter 3). Females of both these species remate (RITCHIE 1984, personal data). In a species from the willistoni sub-group, D.nebulosa, males indulge in what may be a 'low cost' form of courtship feeding. They produce anal drops which they deposit on the substrate in front of the female who consumes them. Females of this

species remate frequently (RITCHIE 1984). A review of the literature on the courtship of various Hawaiian species (chapter 3) suggests that courtship feeding using regurgitated food and anal drops may be widespread but that the extent to which feeding occurs may vary considerably. For instance, the proboscideal contact between a male and female D.truncipenna may last up to 60 seconds whereas D.spectabilis simply use their proboscis to moisten their fore-tarsii before waving them at the female. If these behaviours do involve food or food odours then there is tremendous scope for comparative studies as well as for examining the intraspecific variation. Information on the frequency of remating, adult nutritional ecology, longevity, the degree of male investment and how these variables vary spatially and temporally might indicate what factors are neccesary for a high degree of male nutritional investment.

It may be that it pays males to invest because the females' ability to lay eggs is otherwise severely limited. This may be the case in a number of Lepidopteran species where the egg production of adult females is often limited by nutrients that are carried over from the larval stages (MARSHALL 1982). To an extent the male and female interests coincide in that the more the male invests the greater is his and the female's reproductive success. However, if males have valuable resources, females should be expected to remate to gain more of the resource and selection should therefore favour males who limit their investment to the eggs they will fertilise. It seems likely there will be conflict between the sexes over the male's investment and deception and counter-measures to this deception in the other sex should evolve. Females could deceive males by accepting their food and then not mating or by remating rapidly.

Males could prevent the females remating by using plugs and they could deceive females by 'fooling' them about the extent of their investment. So, for instance, selection in Lepidoptera may favour males who transfer a large, absorbable spermatophore that females can use for egg production (BOGGS 1981). A large spermatophore may also increase the period to a female's next mating in species which remate because there is evidence that a female's willingness to remate depends upon the size of the spermatophore in her bursa (BOGGS 1981). The distension of the bursa is probably the cue the female uses to assess the size of the spermatophore and BOGGS argues that this results in an opposing selection pressure on the males to produce a large, unabsorbable spermatophore.

In cases where male investment is high it may be that the female behaviour is forcing the male to invest. The cannibalistic female mantids and midges described in chapter 2 are an extreme example. In Hylobittacus apicalis females copulate for as long as the male's food offerring lasts (reviewed in chapter 2). If the food offerring is small or unpalatable the females break off copulation and remate. However, if the male manages to copulate for 20 minutes he is able to transfer a full complement of sperm and the female's receptivity is switched off for a short period during which she lays eggs. Males dismount at this stage and struggle with the female for what's left of the food offerring. The males usually win and use the food to try and obtain further matings. Finally, in Mormon crickets (GWYNNE in press) the males transfer a large, nutritious spermatophlax at mating and, at high densities when food is scarce, females compete for matings with males and males reject poor quality females. The reason for this role reversal is that intraspecific

competition for food is intense and few males, at any one time, have a large spermatophylax available for transfer. Males with a small or medium-sized spermatophylax will not mate because a mated female soon finishes eating it and starts on the ampulla before it has had a chance to empty of sperm.

The other material benefit male D.subobscura provide females with is a sufficient quality or quantity of ejaculate to ensure that a high proportion of the eggs laid by a female are fertile (chapters 5 and 7). Females might also derive nutrients from the ejaculate and this could be tested using radiolabelling experiments. The ability of males to ensure high egg hatchability varied and an important factor influencing this variability was the amount of accessory fluid a male had available for transfer reflected by the size of his accessory glands. Male accessory gland size depended on age and diet.

A further benefit associated with mating with males of high fertility is that the duration of copulation is short. This will tend to increase the time available to the female for other activities and may reduce any risk of predation. It is not clear whether a copulating female is more likely to be eaten than a solitary female but this might be tested in the laboratory using the dungfly, Scatophaga stercoraria, a documented predator (SHORROCKS 1972). The risk of attack and probability of surviving an attack could be measured for a range of flies and it might be possible to gather data on the influence of size, courtship activity, position on resource and copulation on predation. Presumably female D.subobscura rarely remate because the advantages of remating are outweighed by the disadvantages. The advantages might include nutrients for egg

production and keeping the sperm supply 'topped up'. These advantages might be small if the nutrients are not very important for egg production and if the females' egg laying rate is low. It would be interesting to measure potential disadvantages.

In chapter 7 I discussed the control of copulation duration in D.subobscura and this suggested further work on the amount of ejaculate transferred in D.subobscura and other species and how this varied with male and female body size, remating frequency and recent matings. It would be interesting to explore the question of how much time and ejaculate a male should invest in a female and the range of copulation durations, remating frequencies and reproductive organ sizes make Drosophila a good genus to study.

In chapters 5 and 6 I showed that high fertility males had shorter courtships than low fertility males. This was not due to an association between low fertility and a shortage of energy reserves because both variables were considered and controlled for. The amount of glycogen a male had stored was an important determinant of courtship duration if reserves were low and the amount stored depended upon recent flight and diet. The technique used to assess the glycogen stored was to fly the flies to exhaustion. This technique could also be used to measure the energetic costs of courtship. Sensitivity might be increased if wing beat frequency (using a strobe) as well as flight duration was recorded and the number of wing beats to exhaustion assessed. Body size would need to be considered. Males could be induced to court for different periods of time and their subsequent flight duration recorded. It might even be possible to assess the energetic costs of individual courtship behaviours. For instance, the cost of the dance by using a dancing

model (chapter 4).

The relative size of the reproductive organs also influenced the duration of courtship. Males with larger reproductive organs for any given wing length obtained matings faster. When the effects of the reproductive organs were analysed separately it was the accessory glands and testes that were important. The influence of relative accessory gland size on male fertility has already been discussed but the influence of the testes is more surprising and warrants further investigation.

Some possible reasons for the differences in courtship duration of high and low fertility males were examined in chapter 7. Males with low energy reserves and low fertility courted less vigorously than high and low fertility males with high energy reserves. The courtships of the high and low fertility males with high energy reserves were similar with the exception that the low fertility males produced the drop less frequently. Conditions that favoured low fertility males who retained the drop were discussed using a model. Comparative studies or various manipulations, such as keeping the adults on different planes of nutrition, might be used to test the model.

It is also neccesary to consider whether the various male attributes that are important in determining courtship duration in the laboratory are likely to be important in the field. Some attempt has been made to do this by looking at the nutritional status of wild flies (chapter 3) and their fertility (chapter 6). A group of wild males had low energy reserves and accessory gland sizes overlapping the low fertility laboratory males. Wild males with fuller crops also had larger accessory glands and testes for any given wing

length. The association between crop and accessory gland size in wild flies might be explained if males differed in their ability to gather food and hence their ability to invest in accessory fluid. Another possibility is that once the size of the accessory glands passes a 'threshold' quantity sufficient to ensure one high fertility mating, males divert fewer nutrients into the production of accessory fluid. The turnover in the crop may be less and males would be better able to produce a drop during courtship. Information on the nutritional status of flies in different areas and through a season would be interesting. Measures of crop size, size and state of the ovaries, glycogen and fat storage could all be used. The preliminary results reported in chapter 5 on the fertility of wild males mated to laboratory females could be followed up and, similarly data on the fecundity and egg-laying rate of wild females would be very useful.

Another possibility would be to collect wild males and observe their courtship behaviour with females in the laboratory. Preliminary experiments (unpublished personal data) suggest that a good proportion of wild males do not produce the drop during courtships in the laboratory. Instead these males perform a 'lunge-chase' courtship not seen in the laboratory stocks. Males attempt intromission without any frontal display and the courtship is very similar to the courtship of lines selected by PINSKER and DOSCHEK (1980) to mate in the dark. It would be interesting to examine the conditions resulting in this courtship behaviour. They may depend upon the environment. For instance, at high densities, males displaying at the front of the female risk the danger of another male mounting when the female accepts before they can circle. This occurs in laboratory stocks (personal observation). The

patterning of courtship may also depend upon a male's phenotype. For instance, small or starved males unable to produce large drops of food may well benefit from the lunge-chase approach under certain conditions in the field. Males may adopt either behaviour or they may use only one form of courtship. Further work on the success rate and costs of the two different patterns of courtship for different males under different conditions would be interesting.

Finally, the influence of male interference and competition for access to females needs to be considered. Males use lunging and chasing behaviour to displace each other from resources in the field (unpublished personal data) and may be observed grappling and shoving in head-to-head combat. The losers of these interactions leave the resource. So that at any one time there are a few males on the resource and a pool of males around. The males on the resource patrol patches and the encounter rate with other flies is higher on the resource than off it. As density increases the spacing behaviour breaks down, a large number of males are moving over the resource and a lower proportion of male:male interactions are aggressive (personal observation). The influence of male size, fertility and nutritional status on the outcome of aggressive interactions and the effect this has on male mating success might be difficult to measure in the field, but could be complemented with laboratory studies of flies in vials and larger arenas. If high fertility males put less into courtship is this also true of aggressive interactions? If not, should females assess males around the feeding site rather than moving onto the resource where their 'choice' of mate might depend entirely upon the outcome of male combat? Male interference during courtship should also be measured and might depend upon other

variables such as male agility.

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APPENDICES.

TABLE A

Male	♀ 1 ♀ 2	Ten day totals of the number of eggs laid and hatched																				Day dead or lost
		0 - 10		10 - 20		20 - 30		30 - 40		40 - 50		50 - 60		60 - 70		70 - 80		80 - 90		90 - 100		100 - 110		110 - 120		
		L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	
2	1	90	89	90	89	92	89	19	19																	L 40
	2	304	302	186	106	46	0																			L 22
3	1	153	153	76	75	63	61	149	149	128	127	54	54	7	6	39	39	51	51	2	2	1	1	0	0	D 112
	2	0	0	0	0	72	64	73	58	117	101	59	50	3	0	4	0	4	0	2	0	2	0			D 106
7	1	61	20																							L 10
	2	243	243	239	239	64	63	6	5	8	8	9	4	0	0	2	2	3	0							D 87
9	1	165	95	137	0	163	0	52	0																	D 40
	2	2	2	17	17	6	6	4	3	38	38	15	15	25	16	0	0									D 71
25	1	250	248	241	235	140	111	191	65	175	43	74	1	80	0	4	4	7	5							D 88
	2	129	129	124	123	14	14	2	2																	D 40
29	1	197	195	104	104	53	53	58	58	113	111	17	17	72	68	33	32	5	5	22	22	2	2			D 107
	2																									
33	1	215	213	213	163	105	22	60	24	217	28	206	96	69	9	2	0									L 78
	2	102	80	195	157	114	82	73	58	102	22	51	12	24	1	12	2	14	4	28	5					D 100

TABLE A (ii)

Male	♀ 1 / ♀ 2	Ten day totals of the number of eggs laid and hatched																				Day dead or lost
		0 - 10		10 - 20		20 - 30		30 - 40		40 - 50		50 - 60		60 - 70		70 - 80		80 - 90		90 - 100		100 - 110		110 - 120		
		L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	
41	1	72	71	0	0	2	1	0	0	1	1															L 50
	2	53	1	3	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			D 108
42	1	213	212	228	228	101	101	130	130	78	78	5	5	3	3	2	1	7	5	4	2	2	1			D 109
	2	142	142	143	143	83	81	33	33	37	37	8	8	12	12	6	6	5	5	3	2	3	3	0	0	D 111
44	1	256	255	268	265	116	115	150	112	221	221	153	153	144	144	59	59	29	29	1	1	0	0			D 110
	2	311	310	98	87																					L 12
49	1	189	189	206	206	120	119	165	163	203	202	188	188	108	108	72	72	39	39	25	25	47	47			D 109
	2	4	0																							D 8
63	1	254	254	280	271	155	150	109	106	23	23	90	78	52	10	45	2	3	0	4	0	3	0			D 110
	2	135	131	204	204	110	108	6	6																	D 40
69	1	52	0																							RM 10
	2	0	0	120	0																					RM 20
70	1	73	72	111	111	87	85	104	101	99	95	30	27	1	1											D 61
	2	189	189	100	98	121	119	3	2	51	49	54	52	2	1	1	1	1	1							D 81

TABLE A (iii)

Male	♀ 1 ♀ 2	Ten day totals of the number of eggs laid and hatched																				Day dead or lost
		0 - 10		10 - 20		20 - 30		30 - 40		40 - 50		50 - 60		60 - 70		70 - 80		80 - 90		90 - 100		100 - 110		110 - 120		
		L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	
71	1	155	153	332	228	263	256	177	171	170	166	255	240	161	44	71	4	42	1	5	0	30	0			D 110
	2	255	255	198	198	134	132	28	28																	D 39
85	1	308	308	387	386	241	240	241	115	278	24	171	17	155	22	99	11	12	0	2	1	1	0			D 107
	2	236	236	196	195	137	125	179	80	121	3	74	0	71	0	23	0	61	0	30	0	2	0			D 109
87	1	142	140	168	167	141	141	107	106	198	180	116	106	77	70	107	77	29	9	1	1	0	0			D 101
	2	6	6	0	0	2	2	1	1	0	0															D 43
92	1	129	128	105	104	55	55	49	44	8	8	28	28	3	3	13	13	75	69	20	17	1	1			D 107
	2	87	86	46	46	5	5																			D 30
102	1	194	194	136	136	92	91	64	63	82	82	16	16	4	4											L 68
	2	138	137	117	117	44	44	74	73	66	66	138	131	57	35	94	45	34	16	13	2	1	0			D 107
103	1	182	182	299	299	140	140	199	199	163	163	139	139	61	61	70	70	45	45	39	39	2	2			D 107
	2	190	189	248	248	255	255	258	257	140	140	137	137	53	52	57	56	20	19	1	1	0	0			D 106
104	1	0	0	13	0	57	0	13	0																	L 38
	2	38	0	95	0	155	0	240	0	135	0	64	0	45	0	42	0	4	0	5	0	4	0	0	0	D 112

TABLE A (iv)

Male	♀ 1 / ♀ 2	Ten day totals of the number of eggs laid and hatched																				Day dead or lost
		0 - 10		10 - 20		20 - 30		30 - 40		40 - 50		50 - 60		60 - 70		70 - 80		80 - 90		90 - 100		100 - 110		110 - 120		
		L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	
108	1	3	3	28	0	144	0	80	0	10	0	51	0	43	0	5	0	52	0	5	0	0	0			D 109
	2	0	0	0	0	43	8	37	0	73	1	54	0	37	0	75	0	82	0	9	0	1	0			D 107
121	1	99	98	91	91	25	25	9	9																	D 40
	2	0	0	28	1	151	0	52	0	20	0	39	0	2	1	4	0	6	0	3	0	0	0			D 108
122	1	240	239	189	188	128	127	71	71	86	86	63	60	43	42	30	28	7	7	10	10	2	2			D 105
	2	239	235	198	34	159	135	162	42	76	48	0	0													L 51
124	1	78	77	2	2	6	6	3	3	58	58	10	10	18	18	57	56	1	1	3	1	3	0			D 108
	2	191	190	211	207	100	99	166	109	5	5															D 43
127	1	127	127	104	102	10	10	7	6	25	23	4	4	1	1	0	0									D 75
	2	33	28	178	174	97	89	158	109	58	22	6	0													L 52
143	1	276	271	197	192	130	123	115	110	122	104	60	58	23	24	4	3	1	1	3	2	2	1			D 107
	2	130	127	160	152	81	75	134	121	92	65	116	55	106	42	14	10	66	39	5	3	2	1	26	23	
144	1	140	138	161	156	81	80	108	107	10	10	9	9	31	31	2	2	67	64	6	6	3	3			D 107
	2	324	306	281	176	233	91	226	20	222	6	0	0													D 51

TABLE A (v)

Male	♀ 1 ♀ 2	Ten day totals of the number of eggs laid and hatched																				Day dead or lost
		0 - 10		10 - 20		20 - 30		30 - 40		40 - 50		50 - 60		60 - 70		70 - 80		80 - 90		90 - 100		100 - 110		120 - 130		
		L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	
145	1	0	0	0	0	3	3	9	7	1	0	14	12	1	0	5	3	2	0	1	1	1	0			D 106
	2	15	14	53	52	55	54																			L 29
147	1	144	143	92	91	69	68	59	59	67	67	40	40	42	42	3	3	2	2							D 88
	2	114	114	173	173	69	69	156	144	154	77	178	19	99	6	51	35	42	42	4	1					D 95
149	1	224	223	193	193	107	104	172	171	59	59	144	142	68	56	40	40	36	36	3	3	1	1			D 101
	2	85	84	318	317	98	98	156	156	239	239	233	233	79	79	63	0	58	0							D 90
162	1	0	0	11	0	1	0	79	0	1	0															D 42
	2	17	0	0	0	34	0	137	0	35	0	0	0	125	0	5	0	102	0	9	0	5	0			D 105
164	1	0	0	2	2	11	11	17	17	1	1															
	2	181	179	277	256	225	142	304	72	306	1	195	6	62	7	3	0	1	1							D 82
166	1	93	93	121	121	59	59	112	111	41	41	38	36	10	10	70	69	48	48	0	0	1	1			D 105
	2																									
167	1	37	37	60	60	9	9	64	63	33	33	19	18	11	10	2	0	38	29	7	7	2	2			D 105
	2	2	2	30	30	1	0	2	2	3	3	2	2	2	2	3	2	3	2	3	0	1	0			D 105

Male	♀ 1 ♀ 2	Ten day totals of the number of eggs laid and hatched																				Day dead or lost
		0 - 10		10 - 20		20 - 30		30 - 40		40 - 50		50 - 60		60 - 70		70 - 80		80 - 90		90 - 100		100 - 110		110 - 120		
		L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	
168	1	263	261	272	269	74	71	100	99	86	85	57	55	14	14	4	4	3	3	2	2	2	2			D 105
	2	287	286	249	248	278	153	362	0	270	0	272	0	180	0	88	0	0	0							D 81
169	1	0	0	0	0	18	0	65	0	140	0	6	0	2	0	5	0	3	0	3	0	1	0			D 105
	2	294	289	269	250	275	264	261	237	159	151	60	57	32	32											D 64
170	1	155	154	183	182	24	24																			L 26
	2	17	17																							L 4
171	1	47	46	13	13	2	2	96	96	3	3	2	2	1	1	0	0	26	15	10	9	43	25	4	2	
	2	133	130	255	254	62	61	207	154	163	43	145	11	78	2	21	0	14	0	2	0	2	0			D 106
173	1	221	219	164	164	176	176	249	245	235	235	207	185	147	68	100	91									L 78
	2	152	152	115	112	67	66	50	49	59	58	39	37	15	13	10	9	25	24	5	5	3	2			D 105
182	1	172	170	168	148	195	192	145	123	205	140	142	71	133	41	3	1	1	1							D 86
	2																									
183	1	301	300	191	191	129	128	209	190	98	8	58	49	10	10	0	0	0	0	0	0	0	0			D 104
	2	21	21	179	177	193	193	316	305	108	7	0	0	54	0	128	0	79	0	146	0	45	0			D 103

TABLE A (vii)

Male	♀	Ten day totals of the number of eggs laid and hatched																Day dead or lost
		0 - 10		10 - 20		20 - 30		30 - 40		40 - 50		50 - 60		60 - 70		70 - 80		80 - 90		90 - 100		100 - 110		110 - 120		
		L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	
186	1	139	138	198	189	90	79	22	10	2	0															D 44
	2	64	63	13	13	2	2	82	0	11	11	43	43	12	12	2	2	4	4	2	2					D 92
187	1	154	154	73	72	13	13	4	4	0	0															D 47
	2	261	260	226	224	231	86	184	11	168	1	149	0	54	3	93	1	14	0	3	0	0	0			D 106

Appendix B. The tables list the size measurements, courtship an flight durations for the males in the different groups used i chapter 6. The variables listed are:

WL wing length in mm.
AG accessory gland area in mm^2.
TE testis area in mm^2.
SV seminal vesicle area in mm^2.
AGE in days.
CD courtship duration in minutes.
CP crop area in mm^2.
FL flight duration in minutes.

ROW	WL	AG	TE	SV	AGE
1	1.75	0.077	0.1741	0.0318	0.0
2	1.78	0.108	0.2582	0.0355	0.0
3	1.72	0.113	0.2318	0.0309	0.0
4	1.67	0.076	0.1609	0.0191	0.0
5	1.66	0.074	0.2018	0.0177	0.0
6	1.73	0.094	0.2468	0.0205	0.0
7	1.69	0.070	0.1623	0.0177	0.0
8	1.67	0.088	0.1909	0.0159	0.0
9	1.75	0.095	0.1909	0.0259	0.0
10	1.82	0.087	0.2405	0.0264	0.0
11	1.71	0.110	0.2273	0.0300	0.0
12	1.74	0.094	0.2400	0.0282	0.0
13	1.77	0.095	0.1964	0.0309	0.0
14	1.79	0.082	0.1586	0.0223	0.0
15	1.64	0.085	0.1655	0.0227	0.0
16	1.76	0.105	0.2268	0.0255	0.0
17	1.70	0.086	0.1591	0.0205	0.0
18	1.73	0.128	0.2114	0.0218	0.0
19	1.64	0.068	0.1873	0.0264	0.0
20	1.78	0.124	0.2386	0.0250	0.0
21	1.71	0.128	0.2523	0.0427	0.0

ROW	WL	AG	TE	SV	CD	AGE
1	1.51	0.071	0.1195	0.0182	20.00	2.
2	1.60	0.094	0.1314	0.0186	12.92	2.
3	1.74	0.081	0.1500	0.0223	17.42	2.
4	1.65	0.091	0.1127	0.0295	4.66	2.
5	1.49	0.066	0.1123	0.0232	20.00	2.
6	1.59	0.093	0.1414	0.0273	8.58	2.
7	1.66	0.064	0.1295	0.0127	20.00	2.
8	1.48	0.061	0.1355	0.0277	9.25	2.
9	1.75	0.124	0.2027	0.0395	0.58	2.
10	1.81	0.113	0.1491	0.0436	1.58	2.
11	1.80	0.170	0.1695	0.0418	2.16	2.
12	1.82	0.157	0.1827	0.0491	20.00	2.
13	1.77	0.125	0.1359	0.0368	7.08	2.
14	1.84	0.123	0.1986	0.0295	0.66	2.
15	1.78	0.173	0.1550	0.0268	3.50	2.
16	1.80	0.186	0.1727	0.0436	3.75	2.
17	1.66	0.132	0.1405	0.0390	20.00	2.
18	1.52	0.087	0.0818	0.0277	6.42	2.
19	1.67	0.105	0.1423	0.0386	5.42	2.
20	1.79	0.122	0.1486	0.0345	7.58	2.
21	1.77	0.129	0.1609	0.0355	5.08	2.
22	1.80	0.114	0.2236	0.0382	20.00	2.
23	1.92	0.178	0.2423	0.0536	4.50	2.
24	1.80	0.141	0.1414	0.0405	4.16	2.
25	1.70	0.094	0.1614	0.0273	12.66	2.
26	1.82	0.124	0.1941	0.0291	1.75	2.

ROW	WL	AG	TE	SV	CD	AGE
1	1.48	0.108	0.0941	0.0236	4.66	5.
2	1.47	0.093	0.0850	0.0309	8.25	5.
3	1.69	0.117	0.1245	0.0400	2.58	5.
4	1.53	0.127	0.1000	0.0273	4.33	5.
5	1.67	0.172	0.1468	0.0509	3.16	5.
6	1.90	0.243	0.1827	0.0391	0.50	5.
7	1.92	0.226	0.1377	0.0623	11.25	5.
8	1.83	0.200	0.1668	0.0509	4.25	5.
9	1.74	0.173	0.1500	0.0532	2.05	5.
10	1.55	0.127	0.1068	0.0205	13.42	5.
11	1.84	0.204	0.1986	0.0500	3.58	5.
12	1.69	0.181	0.1318	0.0332	0.50	5.
13	1.60	0.147	0.1100	0.0250	20.00	5.
14	1.64	0.131	0.1477	0.0318	6.84	5.
15	1.72	0.170	0.1409	0.0491	3.58	5.
16	1.64	0.149	0.1291	0.0291	1.66	5.
17	1.54	0.092	0.0909	0.0264	20.00	5.
18	1.90	0.197	0.1632	0.0514	4.00	5.
19	1.85	0.200	0.1409	0.0455	3.25	5.
20	1.89	0.198	0.1350	0.0541	3.92	5.
21	1.90	0.219	0.1491	0.0514	4.42	5.
22	1.71	0.190	0.1873	0.0523	1.33	5.
23	1.88	0.160	0.1282	0.0318	2.00	5.
24	1.57	0.186	0.0945	0.0259	9.00	5.
25	1.47	0.145	0.1301	0.0281	3.00	5.
26	1.55	0.159	0.0895	0.0247	7.50	5.
27	1.66	0.142	0.1434	0.0477	9.00	5.
28	1.75	0.158	0.2029	0.0355	4.33	5.
29	1.75	0.163	0.1263	0.0435	5.92	5.
30	1.48	0.178	0.1109	0.0351	2.16	5.
31	1.86	0.154	0.1066	0.0452	15.92	5.
32	1.76	0.208	0.1447	0.0381	6.50	5.
33	1.76	0.191	0.1823	0.0677	5.00	5.
34	1.74	0.157	0.1267	0.0322	12.16	5.
35	1.72	0.095	0.0649	0.0335	18.66	5.
36	1.70	0.111	0.1087	0.0217	13.92	5.

ROW	WL	AG	TE	SV	CD	AGE
1	1.49	0.138	0.1259	0.0391	2.25	10.
2	1.64	0.151	0.1373	0.0386	11.42	10.
3	1.55	0.173	0.1227	0.0327	2.08	10.
4	1.52	0.220	0.1100	0.0414	12.75	10.
5	1.88	0.204	0.1550	0.0500	1.66	10.
6	1.88	0.231	0.1727	0.0605	13.66	10.
7	1.77	0.261	0.2109	0.0527	1.50	10.
8	1.82	0.177	0.1491	0.0582	0.92	10.
9	1.83	0.310	0.2200	0.0505	3.92	10.
10	1.66	0.207	0.1296	0.0432	1.84	10.
11	1.81	0.193	0.1323	0.0445	10.42	10.
12	1.57	0.122	0.1086	0.0255	15.33	10.
13	1.75	0.183	0.1578	0.0359	4.00	10.
14	1.60	0.129	0.0991	0.0314	9.42	10.
15	1.70	0.174	0.1291	0.0400	3.66	10.
16	1.50	0.184	0.1386	0.0295	1.08	10.
17	1.83	0.232	0.1332	0.0635	0.92	10.
18	1.85	0.203	0.1236	0.0414	9.92	10.
19	1.75	0.243	0.2150	0.0545	2.16	10.
20	1.82	0.250	0.1814	0.0568	6.50	10.
21	1.88	0.224	0.1077	0.0473	2.25	10.
22	1.89	0.204	0.1700	0.0464	7.75	10.
23	1.77	0.159	0.1182	0.0214	3.25	10.
24	1.59	0.242	0.1268	0.0477	9.75	10.
25	1.71	0.164	0.1032	0.0468	1.33	10.
26	1.68	0.151	0.1041	0.0309	20.00	10.
27	1.62	0.168	0.1268	0.0373	20.00	10.
28	1.53	0.145	0.1282	0.0427	13.58	10.
29	1.68	0.205	0.1436	0.0368	2.33	10.
30	1.75	0.153	0.1759	0.0386	14.92	10.
31	1.91	0.245	0.1214	0.0495	3.25	10.
32	1.91	0.206	0.1305	0.0436	4.42	10.
33	1.82	0.211	0.1614	0.0445	2.16	10.
34	1.74	0.219	0.1327	0.0518	4.75	10.
35	1.83	0.206	0.2232	0.0491	1.75	10.
36	1.75	0.142	0.1173	0.0391	11.58	10.

ROW	WL	AG	TE	SV	AGE
1	1.50	0.149	0.0986	0.0414	15.
2	1.67	0.220	0.1577	0.0518	15.
3	1.75	0.273	0.1473	0.0600	15.
4	1.62	0.184	0.1318	0.0536	15.
5	1.65	0.161	0.1227	0.0323	15.
6	1.84	0.227	0.1545	0.0427	15.
7	1.72	0.270	0.1695	0.0395	15.
8	1.60	0.130	0.0959	0.0409	15.
9	1.70	0.213	0.1682	0.0686	15.
10	1.75	0.233	0.1977	0.0645	15.
11	1.78	0.202	0.1427	0.0614	15.
12	1.83	0.274	0.2095	0.0845	15.
13	1.67	0.215	0.1618	0.0536	15.
14	1.82	0.207	0.1609	0.0854	15.
15	1.49	0.147	0.1491	0.0550	15.
16	1.61	0.220	0.1564	0.0650	15.
17	1.65	0.232	0.1345	0.0686	15.

ROW					
1	1.62	0.198	0.1323	0.0436	20.
2	1.87	0.346	0.1855	0.0682	20.
3	1.86	0.374	0.2050	0.0890	20.
4	1.59	0.185	0.1373	0.0550	20.
5	1.87	0.316	0.1427	0.0700	20.
6	1.73	0.246	0.1491	0.0750	20.
7	1.75	0.268	0.1777	0.0777	20.
8	1.75	0.281	0.2159	0.0686	20.
9	1.83	0.203	0.1286	0.0559	20.
10	1.51	0.203	0.1168	0.0418	20.
11	1.74	0.287	0.1414	0.0754	20.
12	1.60	0.219	0.1250	0.0635	20.
13	1.83	0.324	0.1818	0.0727	20.

ROW	WL	AG	TE	SV	AGE
1	1.83	0.244	0.1827	0.0809	25.
2	1.67	0.199	0.1232	0.0468	25.
3	1.61	0.225	0.1523	0.0900	25.
4	1.82	0.333	0.1568	0.0705	25.
5	1.87	0.415	0.1573	0.0659	25.
6	1.69	0.205	0.0841	0.0518	25.
7	1.48	0.195	0.1168	0.0573	25.
8	1.76	0.250	0.1491	0.0523	25.
9	1.71	0.270	0.1282	0.0600	25.
10	1.83	0.283	0.1395	0.0764	25.
11	1.78	0.361	0.1500	0.0827	25.
12	1.82	0.251	0.1359	0.0591	25.

ROW					
1	1.680	0.282	0.1409	0.0635	30.
2	1.870	0.397	0.1609	0.0840	30.
3	1.880	0.279	0.1900	0.0994	30.
4	1.530	0.191	0.1282	0.0695	30.
5	1.790	0.235	0.1195	0.0627	30.
6	1.570	0.200	0.1132	0.0454	30.
7	1.800	0.273	0.1318	0.0858	30.
8	1.900	0.306	0.0918	0.0895	30.
9	1.710	0.266	0.1282	0.0691	30.
10	1.770	0.286	0.1432	0.0745	30.
11	1.800	0.251	0.1136	0.0709	30.

Five day-old poorly-fed males.

ROW	WL	AG	TE	SV	CD
1	1.49	0.072	0.1118	0.0264	17.75
2	1.58	0.060	0.0723	0.0259	20.00
3	1.55	0.044	0.0532	0.0155	20.00
4	1.60	0.064	0.0818	0.0209	8.66
5	1.82	0.095	0.1068	0.0295	15.50
6	1.70	0.128	0.1241	0.0395	12.33
7	1.74	0.117	0.1227	0.0436	20.00
8	1.80	0.098	0.1159	0.0318	10.33
9	1.72	0.108	0.1364	0.0314	20.00
10	1.74	0.084	0.1386	0.0318	20.00
11	1.74	0.083	0.1241	0.0418	20.00
12	1.79	0.109	0.1373	0.0418	20.00
13	1.71	0.109	0.1077	0.0336	20.00
14	1.85	0.086	0.1223	0.0355	4.33
15	1.72	0.070	0.1359	0.0295	20.00
16	1.66	0.070	0.0927	0.0241	20.00
17	1.64	0.072	0.1055	0.0250	15.42
18	1.46	0.064	0.0886	0.0209	20.00
19	1.71	0.073	0.1191	0.0255	20.00
20	1.58	0.060	0.0759	0.0309	8.66
21	1.57	0.068	0.1386	0.0268	17.75
22	1.62	0.062	0.1091	0.0309	20.00
23	1.72	0.105	0.1136	0.0232	11.58
24	1.82	0.149	0.1605	0.0414	4.33
25	1.82	0.174	0.1718	0.0359	18.66
26	1.75	0.089	0.1355	0.0355	20.00
27	1.78	0.184	0.2086	0.0500	2.84
28	1.76	0.150	0.1977	0.0409	11.25
29	1.88	0.087	0.1364	0.0273	2.08
30	1.78	0.145	0.0203	0.0432	3.66
31	1.81	0.129	0.1714	0.0500	12.50
32	1.72	0.085	0.1091	0.0241	16.08
33	1.60	0.059	0.1300	0.0232	20.00
34	1.52	0.068	0.1186	0.0314	20.00
35	1.44	0.054	0.0636	0.0236	11.25
36	1.56	0.064	0.0932	0.0259	20.00
37	1.58	0.089	0.0955	0.0291	10.16
38	1.76	0.127	0.1941	0.0436	5.75
39	1.77	0.128	0.1314	0.0327	4.92
40	1.73	0.136	0.1927	0.0359	4.75
41	1.78	0.067	0.0764	0.0236	15.00

Wild males: organ area.

	WL	AG	TE	SV
1	1.86	0.240	0.1750	0.0591
2	1.83	0.213	0.1964	0.0514
3	1.91	0.299	0.1900	0.0968
4	1.81	0.192	0.2150	0.0477
5	1.83	0.198	0.1664	0.0550
6	1.66	0.172	0.1386	0.0373
7	1.58	0.142	0.1159	0.0345
8	1.78	0.194	0.1450	0.0455
9	1.79	0.189	0.1550	0.0518
10	1.77	0.171	0.1436	0.0341
11	1.67	0.123	0.1041	0.0368
12	1.80	0.181	0.1350	0.0414
13	1.75	0.133	0.1036	0.0277
14	1.79	0.232	0.1755	0.0550
15	1.61	0.132	0.1132	0.0414
16	1.89	0.158	0.1505	0.0464
17	1.72	0.103	0.1500	0.0305
18	1.75	0.230	0.2127	0.0709
19	1.65	0.099	0.1245	0.0291
20	1.76	0.105	0.1182	0.0300
21	2.03	0.305	0.1427	0.0659
22	1.52	0.073	0.1345	0.0218
23	1.82	0.195	0.1641	0.0582
24	1.51	0.142	0.1145	0.0350
25	1.75	0.143	0.1095	0.0332
26	1.92	0.277	0.1023	0.0673
27	1.72	0.175	0.1459	0.0541
28	1.65	0.123	0.0855	0.0382
29	1.76	0.178	0.1309	0.0418
30	1.80	0.209	0.1564	0.0455
31	1.89	0.163	0.1777	0.0455
32	1.61	0.195	0.1250	0.0500
33	1.90	0.238	0.1550	0.0423
34	1.80	0.219	0.1277	0.0491
35	1.88	0.195	0.1536	0.0614
36	1.84	0.282	0.1418	0.0555
37	1.79	0.217	0.2023	0.0427
38	1.83	0.198	0.1800	0.0550
39	1.68	0.195	0.1073	0.0532
40	1.70	0.177	0.1318	0.0341
41	1.79	0.173	0.1482	0.0477
42	1.89	0.307	0.1682	0.0727
43	1.81	0.149	0.1200	0.0477
44	1.78	0.182	0.1218	0.0495

Wild males: organ and crop area.

ROW	WL	CP	AG	TE	SV
1	1.89	0.604	0.175	0.1505	0.0464
2	1.72	0.355	0.117	0.1500	0.0305
3	1.75	0.498	0.228	0.2127	0.0709
4	1.65	0.241	0.109	0.1245	0.0291
5	1.76	0.244	0.110	0.1182	0.0300
6	2.03	0.959	0.287	0.1427	0.0659
7	1.52	0.144	0.071	0.1345	0.0218
8	1.51	0.131	0.139	0.1145	0.0350
9	1.82	0.351	0.194	0.1641	0.0582
10	1.75	0.175	0.143	0.1095	0.0332
11	1.92	0.729	0.277	0.1023	0.0673
12	1.72	0.524	0.198	0.1459	0.0541
13	1.65	0.091	0.121	0.0855	0.0382
14	1.76	0.901	0.171	0.1309	0.0418
15	1.80	1.101	0.217	0.1564	0.0455
16	1.61	0.335	0.176	0.1250	0.0500
17	1.90	1.144	0.238	0.1550	0.0423
18	1.73	0.463	0.224	0.1477	0.0341
19	1.88	0.584	0.195	0.1536	0.0614
20	1.84	0.959	0.284	0.1418	0.0555
21	1.79	0.994	0.217	0.2023	0.0427
22	1.83	0.589	0.198	0.1795	0.0550
23	1.68	0.862	0.194	0.1073	0.0532
24	1.70	0.365	0.177	0.1318	0.0341
25	1.79	0.925	0.173	0.1482	0.0477
26	1.89	2.075	0.299	0.1682	0.0727
27	1.81	0.456	0.165	0.1200	0.0477
28	1.78	0.607	0.182	0.1218	0.0495

Five day-old males: Controls.

ROW	WL	AG	CD	FL	TE	SV
1	1.73	0.171	0.00	53.	0.1500	0.0391
2	1.85	0.132	6.92	78.	0.1136	0.0364
3	1.51	0.133	1.00	66.	0.1180	0.0200
4	1.63	0.144	4.00	82.	0.1095	0.0286
5	1.69	0.129	1.25	44.	0.1168	0.0341
6	1.64	0.105	2.92	83.	0.0900	0.0232
7	1.89	0.171	7.75	77.	0.1573	0.0386
8	1.79	0.169	5.50	57.	0.1527	0.0505
9	1.91	0.177	3.00	39.	0.1250	0.0436
10	1.75	0.151	1.00	58.	0.1445	0.0400
11	1.63	0.104	3.58	34.	0.1036	0.0318
12	1.75	0.130	5.25	55.	0.1295	0.0314
13	1.55	0.125	1.66	69.	0.0905	0.0318
14	1.79	0.217	2.00	68.	0.1855	0.0582
15	1.89	0.171	0.58	65.	0.1591	0.0373
16	1.72	0.116	20.00	74.	0.1155	0.0409
17	1.66	0.125	0.75	40.	0.0904	0.0300
18	1.53	0.099	10.50	87.	0.0936	0.0277
19	1.78	0.136	20.00	87.	0.1104	0.0318
20	1.91	0.119	2.16	76.	0.1214	0.0477
21	1.83	0.173	5.42	56.	0.1077	0.0332
22	1.55	0.069	7.33	22.	0.0586	0.0255
23	1.79	0.117	2.58	81.	0.1159	0.0364
24	1.88	0.167	2.33	72.	0.0955	0.0355
25	1.87	0.174	1.75	75.	0.1682	0.0327
26	1.56	0.102	3.50	57.	0.0841	0.0214
27	1.57	0.137	20.00	42.	0.0977	0.0336
28	1.87	0.147	4.92	68.	0.1500	0.0355
29	1.88	0.160	6.42	39.	0.1400	0.0186
30	1.81	0.153	20.00	48.	0.1305	0.0391
31	1.70	0.137	2.42	66.	0.1305	0.0364
32	1.89	0.199	4.66	75.	0.1586	0.0395
33	1.82	0.123	3.50	59.	0.1334	0.0364

Five day-old males: Exhausted.

ROW	WL	AG	CD	FL	TE	SV
1	1.72	0.237	1.08	41.	0.2591	0.0514
2	1.74	0.155	5.16	31.	0.1309	0.0309
3	1.80	0.176	2.92	18.	0.1264	0.0386
4	1.62	0.123	20.00	5.	0.1009	0.0327
5	1.74	0.123	2.08	28.	0.1073	0.0455
6	1.59	0.109	20.00	7.	0.1177	0.0309
7	1.80	0.119	1.50	27.	0.1186	0.0473
8	1.71	0.137	20.00	3.	0.1427	0.0227
9	1.73	0.115	2.08	12.	0.1282	0.0414
10	1.76	0.134	20.00	0.	0.1414	0.0414
11	1.73	0.175	3.50	10.	0.1818	0.0409
12	1.68	0.192	7.08	8.	0.2491	0.0550
13	1.62	0.101	8.42	21.	0.1050	0.0341
14	1.73	0.142	3.33	16.	0.1455	0.0423
15	1.67	0.088	20.00	0.	0.0923	0.0309
16	1.73	0.170	2.42	33.	0.1341	0.0450
17	1.60	0.136	2.08	7.	0.1414	0.0414
18	1.87	0.229	1.66	28.	0.2791	0.0500
19	1.74	0.148	5.00	18.	0.2023	0.0477
20	1.50	0.086	13.08	5.	0.0682	0.0291
21	1.71	0.169	6.00	17.	0.1409	0.0405
22	1.78	0.174	2.08	13.	0.1345	0.0395
23	1.77	0.142	2.08	23.	0.0986	0.0414
24	1.76	0.184	1.25	25.	0.1795	0.0505
25	1.79	0.179	3.58	15.	0.1882	0.0523
26	1.74	0.189	4.00	6.	0.1614	0.0509
27	1.68	0.162	2.33	6.	0.1491	0.0459
28	1.83	0.232	7.66	27.	0.2059	0.0627
29	1.80	0.140	2.75	15.	0.1441	0.0473
30	1.82	0.174	8.50	21.	0.0909	0.0582
31	1.77	0.157	20.00	6.	0.1136	0.0477
32	1.74	0.162	4.33	19.	0.1273	0.0391

Five day-old males: Glucose.

ROW	WL	AG	CD	FL	TE	SV
1	1.79	0.129	0.84	32.	0.1818	0.0436
2	1.86	0.204	2.25	81.	0.1632	0.0386
3	1.79	0.105	20.00	15.	0.1036	0.0359
4	1.72	0.110	2.50	44.	0.1155	0.0332
5	1.87	0.117	5.84	13.	0.1408	0.0355
6	1.79	0.165	2.00	66.	0.1614	0.0427
7	1.70	0.058	20.00	3.	0.0637	0.0236
8	1.74	0.113	3.42	28.	0.1205	0.0395
9	1.64	0.088	20.00	6.	0.1386	0.0314
10	1.70	0.098	4.42	20.	0.1445	0.0409
11	1.73	0.111	1.25	86.	0.1241	0.0364
12	1.60	0.066	10.42	67.	0.0859	0.0277
13	1.63	0.088	20.00	103.	0.1082	0.0295
14	1.60	0.068	4.66	8.	0.0868	0.0182
15	1.80	0.130	3.92	93.	0.1300	0.0305
16	1.75	0.119	2.58	103.	0.1350	0.0373
17	1.71	0.117	3.16	88.	0.1223	0.0332
18	1.82	0.121	11.33	95.	0.1409	0.0291
19	1.79	0.117	6.42	38.	0.0791	0.0327
20	1.68	0.104	20.00	10.	0.1286	0.0309
21	1.73	0.096	2.84	25.	0.1236	0.0273
22	1.79	0.177	1.08	55.	0.1777	0.0414
23	1.69	0.081	7.58	24.	0.1209	0.0341
24	1.70	0.088	20.00	11.	0.0959	0.0300
25	1.82	0.097	3.84	14.	0.1105	0.0332
26	1.60	0.051	20.00	9.	0.0832	0.0218
27	1.77	0.146	3.33	39.	0.1523	0.0377
28	1.58	0.064	3.16	16.	0.1000	0.0227
29	1.78	0.162	1.92	49.	0.2077	0.0414

Five day-old males: Starved.

ROW	WL	AG	CD	FL	TE	SV
1	1.78	0.139	1.16	20.	0.1409	0.0355
2	1.70	0.135	10.00	37.	0.1223	0.0445
3	1.73	0.171	6.75	29.	0.1791	0.0364
4	1.80	0.159	1.58	19.	0.1155	0.0327
5	1.90	0.152	4.92	48.	0.1582	0.0418
6	1.69	0.090	5.16	66.	0.0809	0.0282
7	1.86	0.175	1.92	57.	0.1036	0.0395
8	1.76	0.163	3.50	36.	0.0818	0.0295
9	1.76	0.145	5.00	36.	0.1195	0.0341
10	1.73	0.136	5.92	73.	0.1100	0.0336
11	1.82	0.163	5.33	38.	0.1073	0.0386
12	1.77	0.145	2.25	63.	0.1409	0.0322
13	1.87	0.188	2.00	46.	0.1100	0.0432
14	1.68	0.169	5.25	46.	0.1514	0.0264
15	1.76	0.146	3.00	18.	0.1545	0.0377
16	1.75	0.143	3.92	21.	0.1014	0.0477
17	1.80	0.186	5.50	34.	0.1255	0.0359
18	1.80	0.179	3.33	28.	0.1364	0.0400
19	1.79	0.182	2.00	27.	0.1473	0.0364
20	1.72	0.084	2.50	20.	0.0727	0.0273
21	1.93	0.152	1.66	13.	0.1200	0.0386
22	1.69	0.081	20.00	55.	0.0882	0.0305
23	1.79	0.168	5.84	16.	0.1155	0.0382
24	1.68	0.128	20.00	49.	0.0814	0.0291
25	1.94	0.171	20.00	15.	0.1159	0.0377
26	1.71	0.166	4.42	34.	0.1295	0.0336

Flight duration.

	Two day-old males.		Five day-old poorly-fed males.	
	WL	FL	WL	FL
ROW				
1	1.53	68.	1.88	11.
2	1.78	67.	1.61	9.
3	1.82	81.	1.93	10.
4	1.76	55.	1.72	37.
5	1.86	85.	1.86	23.
6	1.69	80.	1.47	2.
7	1.72	123.	1.85	12.
8	1.76	62.	1.75	21.
9	1.84	150.	1.85	21.
10	1.54	89.	1.65	11.
11	1.76	23.	1.84	6.
12	1.85	102.	1.59	24.
13	1.70	42.	1.49	29.
14	1.87	56.	1.80	10.
15	1.73	67.	1.88	27.
16	1.81	85.	1.67	19.
17	1.71	51.	1.66	15.
18	1.79	108.	1.92	42.
19	1.59	48.	1.53	18.
20	1.59	59.	1.77	30.
21	1.74	34.	1.50	22.
22	1.83	58.	1.80	22.
23	1.50	24.	1.65	16.
24	1.70	81.	1.79	16.
25	1.67	46.	1.68	18.
26	1.64	57.	1.90	21.
27	1.85	57.	1.65	29.
28	1.86	91.	1.76	24.
29	1.87	77.	1.93	12.
30	1.89	67.	1.87	4.
31			1.80	5.
32			1.56	16.
33			1.72	4.
34			1.45	4.
35			1.84	14.
36			1.84	7.
37			1.78	13.